UNIVERSITY OF CYPRUS
DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

Advanced systems for the enhancement of the environmental performance of wineries - wastewater purification combining biological, advanced chemical and reverse osmosis treatment

Doctoral Dissertation

Ioannou Lida

November 2013

UNIVERSITY OF CYPRUS
DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

Advanced systems for the enhancement of the environmental performance of wineries - wastewater purification combining biological, advanced chemical and reverse osmosis treatment

By

LIDA IOANNOU
Chemical Engineer, Aristotle University of Thessaloniki, Greece
MSc. Environmental Engineering, University of Cyprus, Cyprus

Submitted to the Department of Civil and Environmental Engineering of the University of Cyprus in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Nicosia, November 2013

UNIVERSITY OF CYPRUS

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

APPROVAL PAGE

DOCTOR OF PHILOSOPHY DISSERTATION

This is to certify that this dissertation was prepared

By	Lida Ioannou
Titled	Advanced systems for the enhancement of the environmental performance of wineries - wastewater purification combining biological, advanced chemical and reverse osmosis treatment

complies with the University regulations and meets the standards of the University for originality and quality.

This dissertation was successfully presented and defended to the examining committee on Monday 11th of November 2013.

Academic/Research Advisor	Despo Fatta-Kassinos, *Nireas - International Water Research Center, Director*
Examination Committee	Michael Petrou, *Professor (Chairman of the Committee)*
	Symeon Christodoulou, *Associate Professor*
	Evan Diamadopoulos, *Professor*
	Constantina Kapnisi-Christodoulou, *Assistant Professor*

Ph.D. examination committee

Academic/Research Advisor

Dr. Despo Fatta-Kassinos
Nireas - International Water Research
Center, Director
Department of Civil and
Environmental Engineering
University of Cyprus

Members of the Committee

Dr. Michael Petrou
Professor
Department of Civil and
Environmental Engineering
University of Cyprus
(Chairman of the Committee)

Dr. Evan Diamadopoulos
Professor
Department of Environmental
Engineering
Technical University of Crete

Dr. Symeon Christodoulou
Associate Professor
Department of Civil and
Environmental Engineering
University of Cyprus

Dr. Constantina Kapnisi-Christodoulou
Assistant Professor
Department of Chemistry
University of Cyprus

Dedicated to my family

ACKNOWLEDGMENTS

One of the joys of completion is to look over the journey past and remember all the friends and family who have helped and supported me along this long but fulfilling road.

I would like to thank first of all and from the bottom of my heart, my supervisor *Dr. Despo Fatta-Kassinos*, Director of Nireas - International Water Research Center who is not only my mentor but a dear friend. I would like to thank her for the support and confidence in my ability to fulfill this Ph.D thesis, as well as for the assignment of this so interesting topic, her scientific guidance, help and unlimited interest during the implementation of my research work. I appreciate her contribution of time, ideas and funding to make my Ph.D experience productive and stimulating. The joy and enthusiasm she has for her research was contagious and motivational for me, even during the tough times of the Ph.D pursuit.

I would also like to warmly thank the other members of my examination committee, Professor *Dr. Michael Petrou*, Associate Professor *Dr. Symeon Christodoulou*, Assistant Professor *Dr. Constantina Kapnisi-Christodoulou*, and Professor *Dr. Evan Diamadopoulos* for the honor to participate in the committee of my Ph.D thesis, and for their support, guidance and helpful suggestions.

I am also very grateful to visiting Professor and senior scientist of Gaia- Laboratory of Environmental Engineering and Nireas - IWRC, *Dr. Costas Michael* for his scientific advice and knowledge, his encouragement, the many insightful discussions and suggestions, and his humor during the difficult times.

I would also like to give a heartfelt, special thanks to Professor *Dr. Evan Diamadopoulos* and Assistant Professor *Dr. Nikos Xekoukoulotakis* who offered me the opportunity to work in their laboratories and helped me with the reverse osmosis experiments and the HPLC analysis. Many thanks to the staff and students at the Laboratories of “Environmental Technology and Management” and “Environmental Organic Chemistry - Micropollution” of the Department of Environmental

Engineering, at the Technical University of Crete (TUC); especially, *Katerina Drosou, Nikos Vakondios, Dr. Theodora Velegraki, Dr. Constantina Tyrovola* and *Elisavet Koukouraki*. Thank you for welcoming me as a friend and helping me to develop various ideas during the work implementation.

Many thanks to Assistant Professor *Dr. Constantina Kapnisi-Christodoulou* who offered me the opportunity to work in her laboratory in the Department of Chemistry, of the University of Cyprus; and to *Christiana Georgiou* for helping me with the HPLC analysis.

I would also like to thank the personnel of the S.K. Euromarket Ltd, for the construction of the two winery wastewater treatment plants (SBR and MBR), as well as the two solar pilot plants. Special thanks to *Stathis Kyriakou* (owner and director of the company) and *Georgia Konstantinidou* (Chemical Engineer) for their help and support.

I wish to express my appreciation to the personnel of the "*Tsiakkas*", "*SODAP*" and "Stylianoudakis" wineries for the permission of collecting wastewater samples, as well as the *State General Laboratory* of Cyprus for the permission of using the TOC analyzer. Many thanks to *Costas Tsiakkas*, manager of "Tsiakkas" winery for his help and the generosity he showed to me.

I gratefully acknowledge the funding sources that made this Ph.D work possible. This research was co-funded by the LIFE financial instrument of the European Commission through grant LIFE08/ENV/000455, and part of the work has been supported by the activities of the International Research Center "NIREAS" (Strategic Infrastructure Project ΝΕΑ ΥΠΟΔΟΜΗ/ΣΤΡΑΤΗ ΙΙ/0308/09), which is co-funded by the Republic of Cyprus and the European Regional Development Fund. I would like to thank both organizations for their generous financial support towards the laboratories I worked in.

I would like to thank all the present and past members of GAIA-Laboratory of Environmental Engineering and NIREAS-International Water Research Center, for their help and support, as well as for our excellent collaboration. My warmest thanks to *Dr. Evroula Hapeshi*, *Dr. Irene Michael*, *Dr. Marlen Ines Vasquez*, *Constantinos Yiannapas*, *Toumazis Toumazi*, *Popi Karaolia*, *Maria Tarapoulouzi* and *Raphaela Andreou* for their support and for providing a pleasant and productive working atmosphere. Each person listed played a special role in my time here.

I also thank my friends for their support and valuable friendship. ‘Thank you for your encouragement, support and most of all your humor.’

Of course the acknowledgments couldn’t be complete without giving thanks to my *parents* and my sister *Elpida*. My hard-working parents have sacrificed their lives for my sister and myself and provided unconditional love and care. Especially my mom has instilled many admirable qualities in me and given me a solid foundation to build my upon life. They have taught me to be a hard worker and a self-respect person, persistent and independent. My sister is a great role model of strength and character, and I truly thank her for all the advice’ and support provided to me. I know I always have my family to count on when times are rough.

Last but certainly not least, I would like to express my gratitude to my husband, *Panayiotis Ttofa*. His love, patience, support and unwavering belief in me, have enabled me to complete this long dissertation journey. He is my biggest fan and supporter. He was taking care of whatever needed tending without complaining during the implementation of my Ph.D thesis, so as I could focus on its completion. I could not have completed this journey without *Panayiotis* by my side. ‘There are no words that can express my appreciation for all you’ve done and been for me. Thank you with all my heart and soul.’

Lida Ioannou
University of Cyprus
November, 2013

ABSTRACT

The presence of high organic and inorganic compounds, and the spatio-temporal dynamics of winery wastewater among and within wineries, makes the treatment of winery wastewater challenging. The various wine processing systems applied at each winery, generate wastewater with specific properties, and therefore, establishing a general agreement on the most suitable cost-effective technology for the treatment of this wastewater stream does not seem feasible. Several winery wastewater treatment technologies are available, but the development of alternative ones is essential, in order to increase their efficiency and decrease the investment and operational costs.

Five underlying objectives were set to fulfill the overall target of this dissertation. The first was to investigate and identify the environmental impacts related to the wine production process, using Cyprus wineries as a case study, and the various actions that could be implemented by wineries, in order to minimize or eliminate these impacts. The second objective was to investigate the application of an advanced chemical oxidation process (AOP), and specifically the solar photo-Fenton process, as post-treatment, for the removal and the possible mineralization of the organic content of winery effluents, which have been pretreated by a biological process, i.e. (a) a Sequencing Batch Reactor (SBR), and (b) a Membrane Bioreactor (MBR), at a bench- and a pilot-scale setup. The results obtained by the bench- and the pilot-scale experiments indicated that solar photo-Fenton is very efficient for the treatment of both biologically pretreated flows, yielding, after the end of the treatment, high removal of the organic content, as well as complete reduction of the toxicity towards *D. magna*, and significant reduction of the phytotoxicity towards the three plant species examined. The combined MBR + solar Fenton process seems to be a more effective technology for winery wastewater treatment than the combined SBR + solar Fenton, since the first one can reduce the organic pollutants in the winery effluent to values well below those included in the Cypriot discharge limits (residual COD < 30 mg L^{-1}, TN < 1.6 mg L^{-1}, TP < 0.4 mg L^{-1} and TSS < 10 mg L^{-1}).

The third objective was the monitoring of the efficiency of the solar Fenton as post-treatment of the most efficient biological treatment (i.e. MBR) at an industrial scale, which led to the conclusion that no significant differences with regard to the organic content removal, as well as toxicity reduction should be expected, when upscaling the process from the pilot to industrial level. The fourth objective of this thesis was to investigate the efficiency of a membrane separation process, and specifically reverse osmosis (RO), for the treatment of raw winery wastewater, as an alternative to the biological and advanced treatment. RO achieved high levels of purification of the winery wastewater, and resulted in a water fraction (permeate), which can be discharged in aquatic systems or to be reused in winery cleaning processes. Taking into consideration that the RO does not really destroy the pollutants, but merely concentrates them into smaller volumes of wastewater, i.e. concentrate, solar photo-Fenton oxidation was applied for its treatment before its disposal to the environment. This resulted in a COD reduction of 75±2.5%, while the toxicity towards all species examined was significantly reduced.

The last objective of this thesis was to establish the overall ownership cost of an MBR, a solar Fenton system, and an RO system, the combination of which (MBR + solar Fenton and RO + solar Fenton) may represent an integrated system for complete management of winery wastewater, based on a typical medium-size winery that produces an average of 50 m^3 of wastewater per day. The total cost of the full-scale

MBR unit was estimated to be 2.1 € m^{-3}, of the solar Fenton unit was 1.83 € m^{-3} and of the integrated RO system was 2.55 € m^{-3}.

ΠΕΡΙΛΗΨΗ

Η ταυτόχρονη παρουσία υψηλών συγκεντρώσεων οργανικών και ανόργανων ενώσεων, καθώς και η διαχρονική διακύμανση των συγκεντρώσεων τους που παρατηρείται σε κάθε οινοποιείο, αλλά και από οινοποιείο σε οινοποιείο, καθιστά την επεξεργασία αυτών των αποβλήτων μια μεγάλη πρόκληση. Επίσης, τα ποιοτικά χαρακτηριστικά των αποβλήτων οινοποιείου είναι συγκεκριμένα για κάθε οινοποιείο λόγω των διαφορετικών επεξεργασιών που λαμβάνουν χώρα μέσα σε αυτό και ως εκ τούτου, μια γενική συμφωνία σχετικά με την καταλληλότερη και την οικονομικά αποδοτικότερη τεχνολογία για την επεξεργασία των αποβλήτων αυτών δεν είναι μέχρι σήμερα εφικτή. Η ανάπτυξη εναλλακτικών τεχνολογιών για την επεξεργασία των υγρών αποβλήτων οινοποιείου είναι απαραίτητη, παρά τη διαθεσιμότητα διαφόρων τεχνολογιών, με στόχο την αύξηση της αποτελεσματικότητάς τους και την ταυτόχρονη μείωση του κόστους επένδυσης και λειτουργίας.

Η παρούσα διατριβή έχει πέντε κύριους ερευνητικούς στόχους. Ο πρώτος ήταν να διερευνηθούν και να προσδιοριστούν οι περιβαλλοντικές επιπτώσεις που σχετίζονται με τη διαδικασία παραγωγής κρασιού, μελετώντας την περίπτωση των οινοποιείων της Κύπρου και να προσδιοριστούν στη συνέχεια οι διάφορες δράσεις που μπορούν να εφαρμοστούν από τα οινοποιεία, προκειμένου να ελαχιστοποιήσουν ή ακόμα και να εξαλείψουν τις επιπτώσεις αυτές. Ο δεύτερος στόχος ήταν να μελετηθεί η εφαρμογή μιας προχωρημένης διεργασίας οξείδωσης, και συγκεκριμένα της ηλιακής οξείδωσης Fenton, ως μετ-επεξεργασία, για την απομάκρυνση και την πιθανή ανοργανοποίηση του οργανικού περιεχομένου των υγρών αποβλήτων οινοποιείου, τα οποία έχουν ήδη υποστεί βιολογική επεξεργασία με (α) sequential batch reactor (SBR) και (β) με βιοαντιδραστήρα μεμβρανών, σε εργαστηριακή και πιλοτική κλίμακα. Τα αποτελέσματα από τα εργαστηριακής και πιλοτικής κλίμακας πειράματα έδειξαν ότι η ηλιακή οξείδωση Fenton είναι πολύ αποδοτική στην επεξεργασία και των δύο βιολογικά επεξεργασμένων αποβλήτων, πετυχαίνοντας μετά το τέλος της επεξεργασίας σημαντική μείωση του οργανικού φορτίου, εξάλειψη της τοξικότητας ως προς το μικροοργανισμό *D. magna* και σημαντική μείωση της φυτοτοξικότητας ως προς τα τρία φυτά που μελετήθηκαν. Η συνδυασμένη επεξεργασία με τη χρήση βιοαντιδραστήρα μεμβρανών ακολουθούμενη από ηλιακή οξείδωση Fenton, φαίνεται να είναι πιο αποδοτικό σύστημα για την επεξεργασία των υγρών αποβλήτων οινοποιείου, από την συνδυασμένη επεξεργασία με τη χρήση του sequential batch reactor (SBR) ακολουθούμενη από ηλιακή οξείδωση Fenton, καθώς η πρώτη μπορεί να μειώσει το οργανικό περιεχόμενο των υγρών αποβλήτων σε τιμές αρκετά μικρότερες από τα επιτρεπόμενα όρια (υπολειμματικό COD < 30 mg L^{-1}, TN < 1.6 mg L^{-1}, TP < 0.4 mg L^{-1} και TSS < 10 mg L^{-1}).

Ο τρίτος στόχος ήταν η εφαρμογή της ηλιακής οξείδωσης Fenton σε βιομηχανική κλίμακα, ως μετ-επεξεργασία της βιολογικής επεξεργασίας με τη χρήση βιοαντιδραστήρα μεμβρανών, η οποία οδήγησε στο συμπέρασμα ότι δεν υπάρχουν σημαντικές διαφορές στη μείωση τόσο του οργανικού φορτίου όσο και της τοξικότητας, όταν αυξάνεται η κλίμακα σε βιομηχανικό επίπεδο. Ο τέταρτος στόχος ήταν να εξεταστεί η απόδοση μιας διεργασίας διαχωρισμού με μεμβράνες, και συγκεκριμένα της αντίστροφης όσμωσης, προκειμένου να υπάρξει μια ολοκληρωμένη εικόνα για την επεξεργασία αυτών των βιομηχανικών αποβλήτων. Η αντίστροφη όσμωση κατάφερε να επιτύχει υψηλού επιπέδου επεξεργασία των υγρών αποβλήτων οινοποιείου, και οδήγησε στη δημιουργία ενός διηθήματος (permeate), το οποίο μπορεί να διατεθεί με ασφάλεια σε υδάτινα συστήματα ή να επαναχρησιμοποιηθεί στο οινοποιείο στις διάφορες διαδικασίες καθαρισμού. Λαμβάνοντας υπόψη ότι η αντίστροφη όσμωση δεν καταστρέφει στην πραγματικότητα τους οργανικούς ρύπους αλλά τους συμπυκνώνει εν μέρη σε μικρότερους όγκους, δηλαδή στο συμπύκνωμα, στη συνέχεια εφαρμόστηκε η διεργασία οξείδωσης (ηλιακή οξείδωση Fenton) για την επεξεργασία αυτού του ρεύματος, επιτυγχάνοντας απομάκρυνση του χημικά απαιτούμενου οξυγόνου κατά 75±2.5%, καθώς και σημαντική μείωση της τοξικότητας.

Ο τελευταίος στόχος της διδακτορικής διατριβής ήταν να προσδιοριστεί το συνολικό κόστος ιδιοκτησίας ενός βιολογικού συστήματος μεμβρανών (MBR), ενός ηλιακού συστήματος Fenton και ενός συστήματος αντίστροφης όσμωσης (RO), ο συνδυασμός των οποίων (MBR + ηλιακό σύστημα Fenton και RO + ηλιακό σύστημα Fenton) μπορεί να αποτελέσει ένα ολοκληρωμένο σύστημα πλήρους διαχείρισης των υγρών αποβλήτων οινοποιείου, για ένα μεσαίου μεγέθους οινοποιείο που παράγει κατά μέσο όρο 50 m^3 υγρών αποβλήτων την ημέρα. Η εκτίμηση του ολικού κόστους του βιολογικού συστήματος μεμβρανών υπολογίστηκε ίση με 2.1 € m^{-3}, του ολοκληρωμένου συστήματος solar Fenton 1.83 € m^{-3} και του συστήματος αντίστροφης όσμωσης ίση με 2.55 € m^{-3}.

TABLE OF CONTENTS

CHAPTER 7: TREATMENT OF WINERY WASTEWATER……………………227

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMATICS

ABBREVIATIONS

AAS	*Atomic Absorption Spectrometry*
ABB	*Air Bubble column Bioreactor*
ACN	*Acetonitrile*
AOPs	*Advanced Oxidation Processes*
APHA	*American Public Health Association*
ASBR	*Anaerobic Sequential Batch Reactor*
ASP	*Activated Sludge Process*
ATH	*Allylthiourea*
BATNEEC	*Best Available Technology Not Entailing Excessive Cost*
BDL	*Below Detection Limit*
BOD	*Biochemical Oxygen Demand*
CA	*Cellulose Acetate*
Capex	*Capital Expenditure*
CAS	*Conventional Activated Sludge*
CFCs	*Chlorofluorocarbons*
CIP	*Cleaning In Place*
CMBR	*Complete-Mix Biofilm Reactor*
COD	*Chemical Oxygen Demand*
COP	*Cleaning Out of Place*
CP	*Concentration polarization*
CPCs	*Compound Parabolic Collectors*
CW	*Constructed Wetland*
DAD	*Diode Array Detector*
DO	*Dissolved Oxygen*
DOC	*Dissolved Organic Carbon*
DOM	*Dissolved Organic Matter*
EC	*Electrical Conductivity*
EEM	*Excitation-Emission Matrix*
EPA	*Environmental Protection Agency*
EPA	*Environmental Protection Authority*
FAO	*Food and Agriculture Organization*
FBB	*Fluidized-Bed Bioreactor*
FBBR	*Fixed Bed Biofilm Reactor*

FC	*Folin-Ciocalteu*
FL	*Fluorescence*
FS	*Flat Sheet*
GHG	*Green House Gas*
GI	*Seed Germination Inhibition*
HF	*Hollow Fibre*
HPLC	*High Performance Liquid Chromatography*
HPLC-DAD	*High Performance Liquid Chromatography with Diode Array Detector*
HRT	*Hydraulic Retention Time*
IEX	*Ion Exchange*
JLR	*Jet-Loop activated sludge Reactor*
KHP	*Potassium hydrogen phthalate*
LC	*Liquid Chromatography*
LCAs	*Life Cycle Assessment*
LOD	*Limit of Detection*
LTAS	*Long-Term Aerated Storage*
MBR	*Membrane BioReactor*
MCBR	*Membrane Coagulation BioReactor*
MF	*Microfiltration*
MLSS	*Mixed Liquor-Suspended Solids*
MLVSS	*Mixed Liquor Volatile Suspended Solids*
MSBR	*Membrane Sequencing Batch Reactor*
MSDS	*Material Safety Data Sheets*
MT	*Multi-Tubular*
MWCO	*Molecular Weight Cut-Off*
NDIR	*Non-Dispersive Infrared Detector*
NF	*Nanofiltration*
NHE	*Normal Hydrogen Electrode*
NP	*Normal Phase*
NPOC	*Non-Purgable Organic Carbon*
OIV	*International Organization of Vine and Wine*
OLR	*Organic Load Rate*
OMW	*Olive Mill Wastewater*
Opex	*Operating Expenditure*
OUR	*Oxygen Uptake Rates*
PAC	*Powered Activated Carbon*

PBB	*Packed-Bed Bioreactor*
PMB	*Phosphomolybdenum Blue*
POPs	*Persistent Organic Pollutants*
PVC	*PolyVinyl Chloride*
RBC	*Rotating Biological Contactor*
RAS	*Returned Activated Sludge*
RF	*Rough Filtration*
RI	*Root growth Inhibition*
RP	*Reversed Phase*
RO	*Reverse Osmosis*
RSD	*Relative Standard Deviation*
RWE	*Red Winery Effluents*
SBBR	*Sequencing Batch Biofilm Reactor*
SBR	*Sequencing Batch Reactor*
SEC	*Size-Exclusion*
SI	*Shoot growth Inhibition*
SOLR	*Specific Organic Loading Rate*
SNPV	*Specific Net Value*
SPE	*Solid-Phase Extraction*
SRT	*Sludge Retention Time*
SS	*Suspended Solids*
SWRO	*SeaWater Reverse Osmosis*
TC	*Total Carbon*
TDS	*Total Dissolved Solids*
TFC	*Thin Film Composite*
TKN	*Total Kjeldahl Nitrogen*
TN	*Total Nitrogen*
TOC	*Total Organic Carbon*
TP	*Total Phosphorus*
TPC	*Total Phenolic Content*
TPh	*Total Polyphenols*
TS	*Total Solids*
TSS	*Total Suspended Solids*
TUC	*Technical University of Crete*
TVS	*Total Volatile Solids*
UASB	*Upflow Anaerobic Sludge Blanket*

UCY	*University of Cyprus*
UF	*Ultrafiltration*
UPW	*Ultrapure Water*
UV	*Ultraviolet*
UV/Vis	*Ultraviolet/Visible radiation*
VCOs	*Volatile Organic Compounds*
VFAs	*Volatile Fatty Acids*
VS	*Volatile Solids*
VSS	*Volatile Suspended Solids*
WEI	*Water Exploitation Index*
WG	*syntethic Wastewater by diluting Grape juice*
WSI	*Water Stress Index*
WV	*syntethic Wastewater by diluting Wine*
WW	*Wastewater*
WWE	*White Winery Effluents*
WWTP(s)	*Wastewater Treatment Plant(s)*
WWWTPs	*Winery Wastewater Treatment Plants*

MAIN SYMBOLS

$\hat{y}_i$	*Model predicted values*
$\overline{UV}$	*Average solar ultraviolet radiation*
$\bar{y}$	*Average of the experimental values*
y_i	*Experimental value*
A	*Membrane area*
b	*Slope of the calibration curve*
C	*Concentration*
C_0	*Initial concentration*
C_E	*Energy cost*
C_f	*Concentration in the feed*
C_p	*Concentration in the permeate*
E^0	*Redox potential*
E_a	*Activation energy*
$f(C)$	*Function of the compound concentration*
h_{VB}^{+}	*Valence hole*
$h\nu$	*Light irradiation*
k_{app}	*Apparent pseudo-first-order rate constant*
k	*Kinetic rate constant*
l	*Length*
J	*Flux*
n	*Number of experiments performed*
Rj	*Solute rejection coefficient*
P	*Pressure*
P_E	*Power cost*
P_{in}	*Inlet pressure*
P_{out}	*Outlet pressure*
R	*Organic substrate*
r	*Degradation rate*
r_0	*Initial rate of degradation*
R^2	*Correlation coefficient*
R_C	*Concentration factor*
sa	*Standard deviation of the y-intercept of the regression line*
T	*Temperature*

t	*Time*
$t_{30W,n}$	*Normalized illumination time*
t_{exp}	*Actual experimental time*
t_R	*Retention time*
t_W	*Number of working hours a day*
W	*Power required to pump water in the system*
V	*Volume*
V_i	*Irradiated volume of the collector*
V_T	*Total volume of the collector*
V_t	*Capacity of the plant*
λ	*Wavelength*
ΔP	*Pressure drop*
λ_{ex}	*Excitation wavelength*
λ_{em}	*Emission wavelength*

TERMINOLOGY

Solar photo-Fenton experiments:	bench-scale experiments performed under controlled conditions of temperature, with the irradiation provided by a solar simulator
Solar Fenton experiments:	pilot- and industrial-scale experiments performed under real conditions of temperature, with the irradiation provided directly by the natural sunlight

CHAPTER 1: INTRODUCTION

The grapevine is one of the oldest cultivated plants that, along with the process of making wine, have resulted in a rich geographical and cultural history of development (Johnson and Robinson, 1994; Jones, 2007). Wine is the product of the total or partial alcoholic fermentation of fresh grape juice resulting from a number of production methods, depending on the type of wine desired (Arcese et al., 2012). Today, wine is an integral component of the culture of many countries and a form of entertainment in others (Bisson et al., 2002).

The wine production process is a complex activity. It starts with the planting of vines, and then various activities for the production of juice from the grapes take place. The bottling, the distribution, and sale of the product on the market complete the process (Vinci et al., 2008). Wine preparation also includes numerous additives (e.g. stabilizers, preservatives, etc.) (Arcese et al., 2012). The main stages of the production process of a winery are as follows (Vlyssides et al., 2005; Arcese et al., 2012):

1. Grape reception: It takes place for almost 15 working days from the end of August to the beginning of October.

2. Must production: The grapes come through a press and produce the must and the solid residues. The produced amount of must is about 80 liters per 100 kg of grapes.

3. Fermentation: Yeast primarily turns the sugar in the wine into carbon dioxide, heat and alcohol. This takes place in the fermentation vessels and lasts about 15 days for each tank from the day they are filled with 80% must.

4. Decanting: This takes place after the fermentation and lasts about 2 days at the beginning of September for red grapes, and about 5 days at the beginning of October for white grapes. During the decanting, the supernatant wine is separated from the produced wine lees, and is fed by pumps to empty tanks that are filled up to 100% for further stabilization. The wine lees are 5% v/v, and are led to alcohol production.

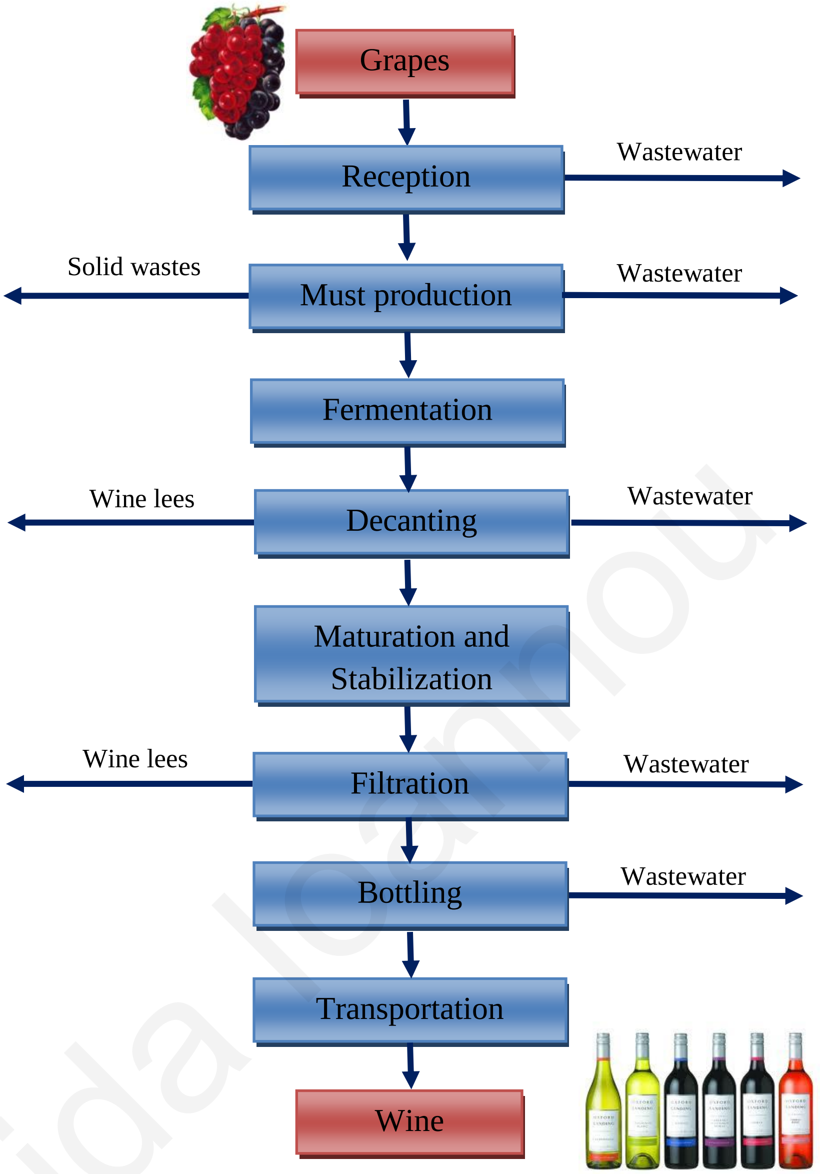
5. Maturation-stabilization: It takes place in the tanks and lasts about 15 days for each tank from the day it is filled 100% with decanted wine.

6. Filtration: The produced wine is filtered in order to improve its quality (e.g. removes any fining agents or any other undesirable elements in the wine), and takes place through a diatomaceous earth filter and is decanted into empty tanks. This stage takes place for a 10-day period at the beginning of December.

7. Bottling: This must be done carefully to ensure the wine is not coming into contact with air.

8. Transportation-disposal: The produced wine is sold either in bulk or as bottled, which is charged from tanks to transportation trucks or in the packaging unit. This stage lasts approximately a semester, from the end of January up to the end of July.

The wine making procedure is also presented in Schematic 1.1.



Schematic 1.1: Wine making procedure

Sources: Vlyssides et al., 2005; Arvanitoyiannis et al., 2006b; Pérez-Serradilla and Castro, 2008; Arcese et al., 2012

Nowadays, the world’s wine markets are going through a fascinating period of structural adjustment. For many centuries wine has been a European product (Anderson et al., 2003). That is still the case today, as more than two-thirds of the world wine production, consumption and trade involves Europe, and most of the rest involves Argentina, Australia, Canada, Chile, New Zealand, South Africa, the United States and Uruguay (Foster and Spencer, 2002; Anderson and Norman, 2003).

In most countries, where viniculture is well developed, grapes utilised for wine making are the basis for a healthy economic outlook (Gimeno et al., 2007), while traditionally the countries producing wine were also the countries consuming it, with only about one-tenth of global sales being across national borders, and most of that is with near neighbours (Wittwer et al., 2003). According to the International Organization of Vine and Wine (OIV, 2013), the 2012 world production of wine amounted globally to 248 million hectolitres (hl), which is lower than that of the last known five-year average (2007-2011), as shown in Figure 1.1. On the other hand, the world wine consumption shows an increasing trend until 2007-2008, while a significant decrease follows the next years, probably due to the crisis in global market, reaching the value of 238 million hl in 2012.

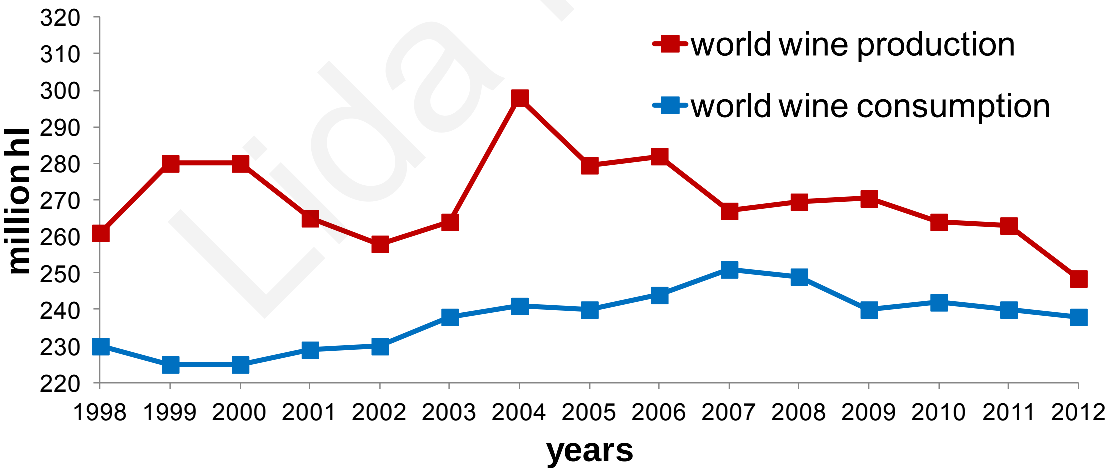


Figure 1.1: World wine production and consumption during 1998-2012

Source: International Organization of Vine and Wine (OIV), 2013

Europe, with a total wine production in 2012 of 164 million hl, leads the wine sector with 66% of production, followed by America (19%), Asia (6%), Oceania (5%) and Africa (4%). On the other hand, in the period of 2000-2012, Europe was the only continent where there was a decrease in wine production, as shown in Figure 1.2.

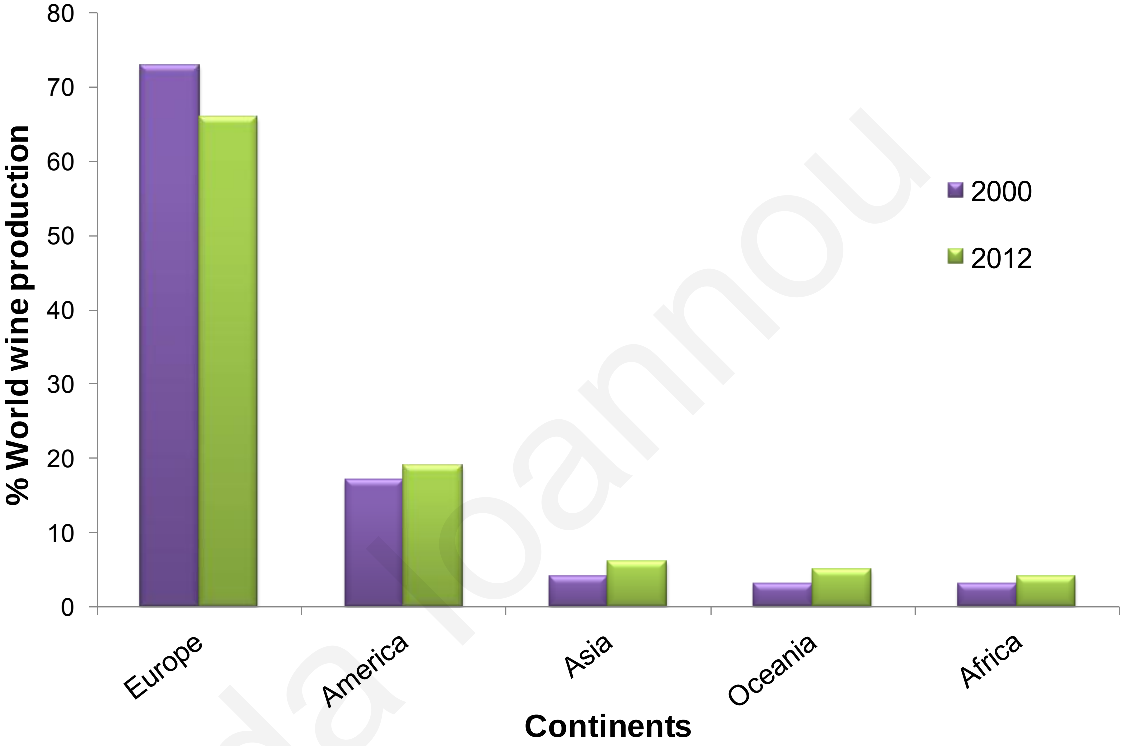


Figure 1.2: Distribution of the world wine production per continent in the period of 2000 and 2012

Source: International Organization of Vine and Wine (OIV), 2013

The ability to produce wine is not available to every country, as it requires a distinctive environment to successfully grow wine grapes. Vines can only be grown successfully between 30 and 50 degrees north and south of the equator, where their distinctive annual growth cycle can be accommodated. Vines can survive in winter through dormancy.

However, the temperature must reach 10 °C in spring before shoots grow and 20 °C in summer for flowering. Frost in spring is damaging to grape growing, as is rain in the autumn harvesting period. Vine growing is suited to regions with a winter rain climate, access to sunlight, with irrigation water for summer drought, and where protection from wind is provided. Soils should be gravely, well drained and not overly fertile (Anderson et al., 2001).

The so-called characteristics of a wine or its quality are heavily influenced by the conditions under which the grapes are grown. In fact, countries within the European Union place considerable restrictions on the classification of wine, including the climatic conditions and the natural environment that influence wine grape production. Another important factor affecting wine production is the development and adoption of technological innovation in both grape growing and wine making (Foster and Spencer, 2002).

France is the biggest wine producer with an average production of 46.2 million hl, which represent 17.5% of the global production. Italy follows roughly with 45.8 million hl (17.3% of global), while the third biggest producer in Europe and worldwide is Spain, which in 2010 produced 36.1 million hl (13.7% of global). Greece is the fifteenth biggest producer country in the world (3.4 million hl and 1.29% of global), while Cyprus is the forty-second wine producer country, with a wine production of 146520 hl in 2010, as shown in Figure 1.3.

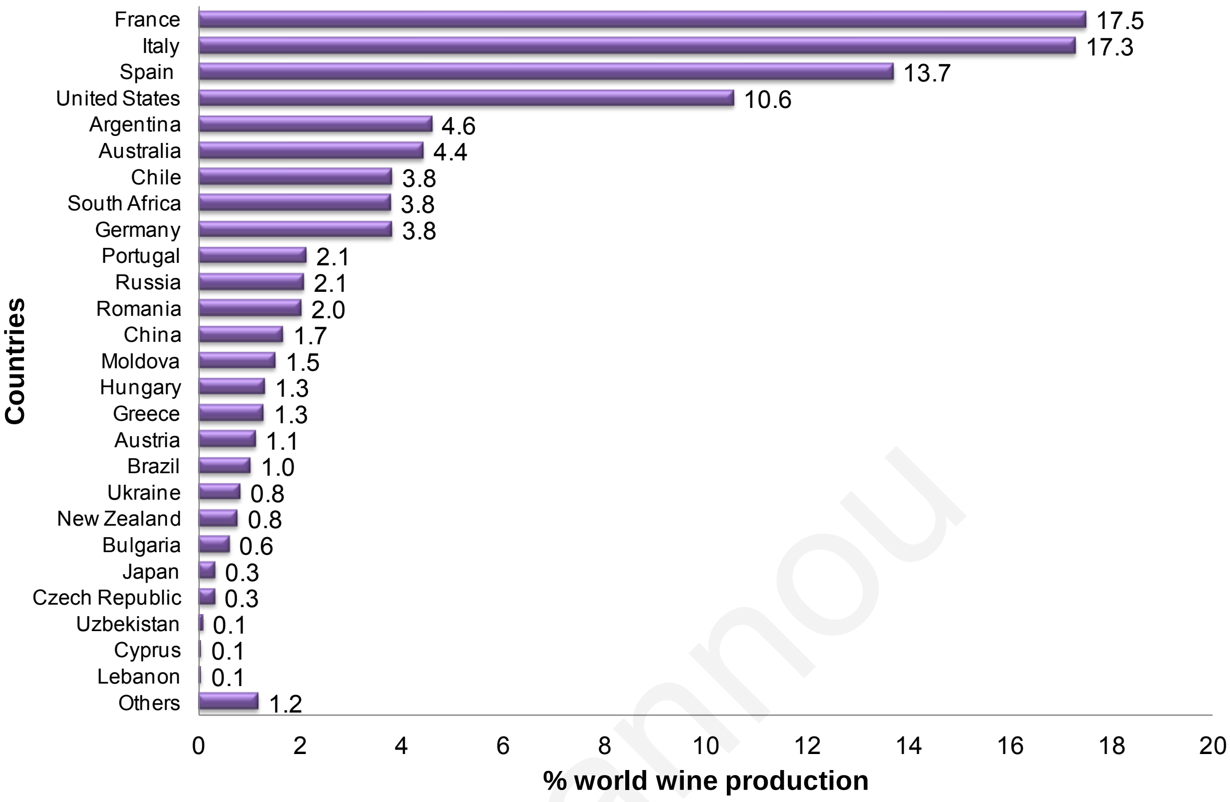


Figure 1.3: World wine production per country in 2010

Source: Wine Institute (www.wineinstitute.org/resources/statistics), 2013

Winemaking is one of the most important agricultural activities mainly in Mediterranean countries. Thus, Spain and Italy account for 15.1 and 11.0% of the total area of vineyards in the world, respectively; while 11.4% of the total area of vineyards belongs to France, as shown in Figure 1.4. Vineyards, globally, cover approximately 18.2 × 10^6 acres according to the latest data of the Food and Agriculture Organization (FAO), (2013). Interestingly however, is the fact that Greece amounts for 1.6% of the total area of vineyards, while Cyprus for 0.2% (OIV, 2013).

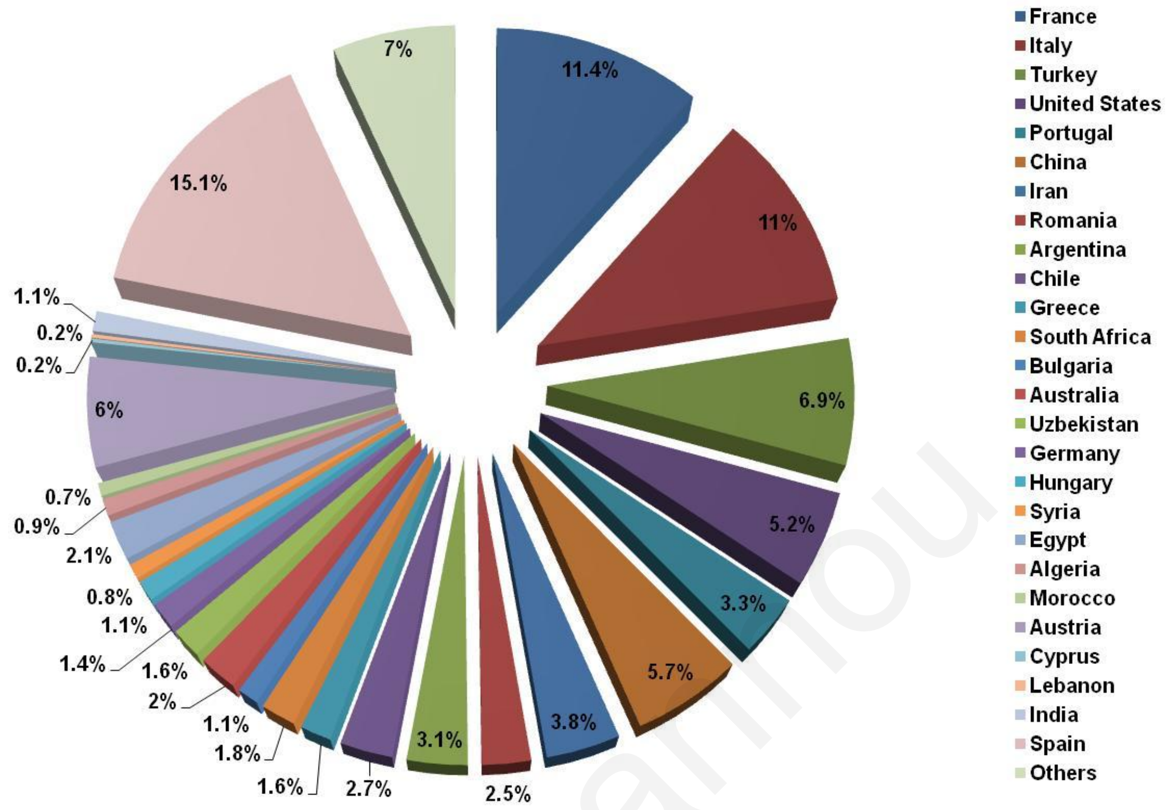


Figure 1.4: Share of particular vineyards area in the total vineyards area in the world

Source: International Organization of Vine and Wine (OIV), 2013

Analyzing the evolution of the area of vineyards during the last twelve years (2000-2012), it was found that there was an increase in global surface of vineyards in all continents except Europe, as shown in Figure 1.5. In Europe this reduced tendency (by 7%) refers mainly to Bulgaria, Hungary and Spain with a decrease of 22%, 13% and 12%, respectively. France follows with a reduction in the area under vines in the order of 7%, Greece and Italy 6%, and Portugal reduced its vineyards area by 3% (International Organization of Vine and Wine (OIV), 2013).

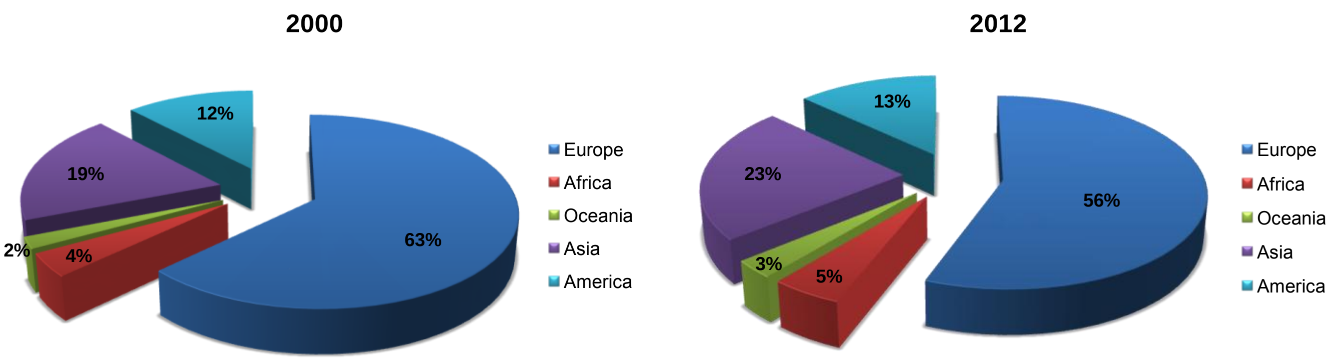


Figure 1.5: Evolution of the last twelve years of the total area of vineyards worldwide

Source: International Organization of Vine and Wine (OIV), 2013

The world production of grapes in 2011 significantly increased compared to the last five years, to 69.2 million tonnes of grapes, as shown in Figure 1.6; despite the fact that according to the International Organization of Vine and Wine, (2013) the world total area of vineyards has decreased since 2007 by 2.3%. This situation can be explained by an increase in the efficiency, the more productive grape varieties and the existence of more favourable climatic conditions and techniques of continuous improvement, as well as the improvement of the winemaking practices.

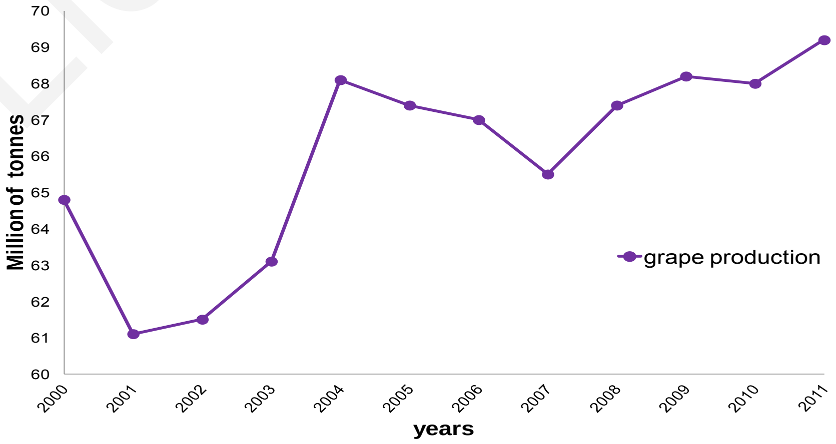


Figure 1.6: World production trend of grapes

Source: International Organization of Vine and Wine (OIV), 2013

As observed in Figure 1.7, Europe leads the grape production sector with 41% of production in 2011, while is antagonised by Asia (29%) and America (21%). It must be noted, that despite the fact that Europe holds the 41% of world production of grapes, it produces 66% of world wine (Figure 1.2), which confirms somehow the increased efficiency in the wine production (i.e. the high efficiency of the winemaking practices, as well as the highly productive grape varieties).

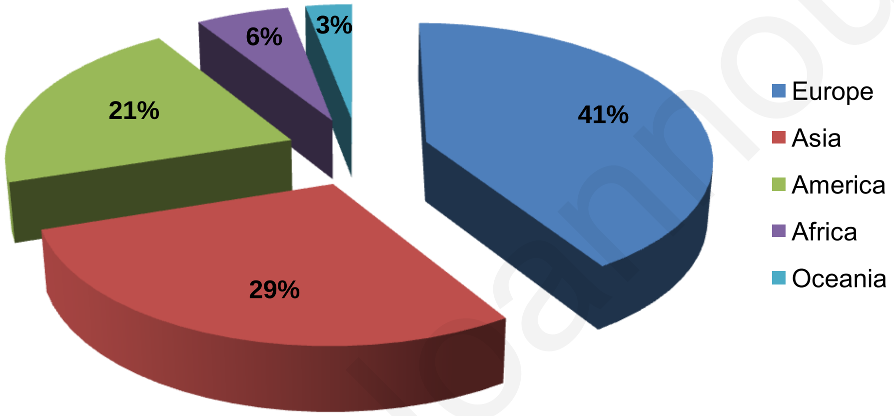


Figure 1.7: Distribution of the world grape production in 2011

Source: International Organization of Vine and Wine (OIV), 2013

Table 1.1 provides a list of the wine-producing countries all over the world and information on their wine production volume and consumption, as well as their vineyards area for the year 2010, according to the latest data of International Organization of Vine and Wine (2013) and Wine Institute (2013). Interesting is the fact that Cyprus is the forty-second biggest wine producer country in the world with a production of 146520 hl in 2010, the forty-fifth biggest wine consumer country with 140000 hl in 2010, while Cypriot vineyards area is the thirty-eighth biggest in the world (33600 acres).

Table 1.1: Global wine production, consumption and vineyards area in 2010

Rank	**Country**	**Production (hl)**	**Vineyards (acres)**	**Consumption (hl)**
1	France	46269000	2038600	28920000
2	Italy	45800000	1919000	24500000
3	Spain	36609700	2476200	10600000
4	United States	26653180	945000	29120410
5	Argentina	16250000	563400	9714000
6	Australia	10730000	378000	5300000
7	Germany	9320000	247100	20205000
8	South Africa	9220000	323700	3460000
9	Chile	8840000	494200	3118000
10	Portugal	5872000	600400	4477000
11	Russia	5400000	104000	11500000
12	Romania	4957400	453000	5097000
13	China	4250000	1167500	9290000
14	Moldova	4100000	370600	270000
15	Greece	3365600	284200	2955000
16	Hungary	3343700	187500	2310000
17	Brazil	2450000	227300	3550000
18	Austria	2313700	108000	2400000
19	Ukraine	2000000	175400	2050000
20	New Zealand	1900000	91400	921000
21	Bulgaria	1426000	195200	800000
22	Croatia	1278000	85000	1180000
23	Switzerland	1270000	37100	2905000
24	Uruguay	1120000	20100	895000
25	Mexico	980000	64100	550000
26	Japan	850000	46000	2390000
27	Macedonia	850000	49300	130000
28	Georgia	800000	120000	750000
29	Slovenia	740000	39800	730000
30	Algeria	700000	170800	330000
31	Canada	560000	27300	3360000
32	Czech Republic	540000	39800	1980000
33	Peru	520000	38500	162000
34	Slovakia	346330	35800	649000
35	Morocco	300000	123500	310000
36	Tunisia	260000	71500	234000
37	Uzbekistan	250000	296500	200000
38	Kazakhstan	200000	23500	250000
39	Turkmenistan	180000	69200	220000
40	Albania	170000	21300	182000
41	Lebanon	150000	32600	140000
42	Cyprus	146520	33600	140000
43	Luxembourg	135000	3500	258000
44	Belarus	100000	3000	535000
45	Madagascar	90000	6200	87000
46	Bolivia	70000	12400	26000
47	Lithuania	70000	2100	190000
48	Latvia	60500	2500	128000
49	Israel	60000	14800	70000
50	Other countries	90000	3335200	32599410
	World total	**263956630**	**18174700**	**232137820**

Sources: International Organization of Vine and Wine, 2013; Wine Institute, 2013.

In Cyprus, winemaking is one of the most traditional industries. Cyprus viti-viniculture is characterised by small vineyards and highly self-owner growers. Wine grape plantations occupy more than 8.5% of the total cultivated agricultural land, and the value of their production accounts for slightly over 3.5% of the crop production. The vine growing area in Cyprus has declined vastly during the last years. According to the National Vineyard Register, there were 13000 hectares covered with wine grapes by the end of 2008, a minor share of which is semi-abandoned. This situation reflects partly the industry's shift towards a micro-market with emphasis in quality rather than quantity (Cypriot Wine Products Council, 2011).

The Cypriot grape production at the end of the 2011 harvesting period was 308077 hl, an increased production compared to that of 2010 (288874 hl). Out of this volume, 120283 hl of wine grapes was disposed exclusively for the production of wine in 2011, as shown in Figure 1.8.

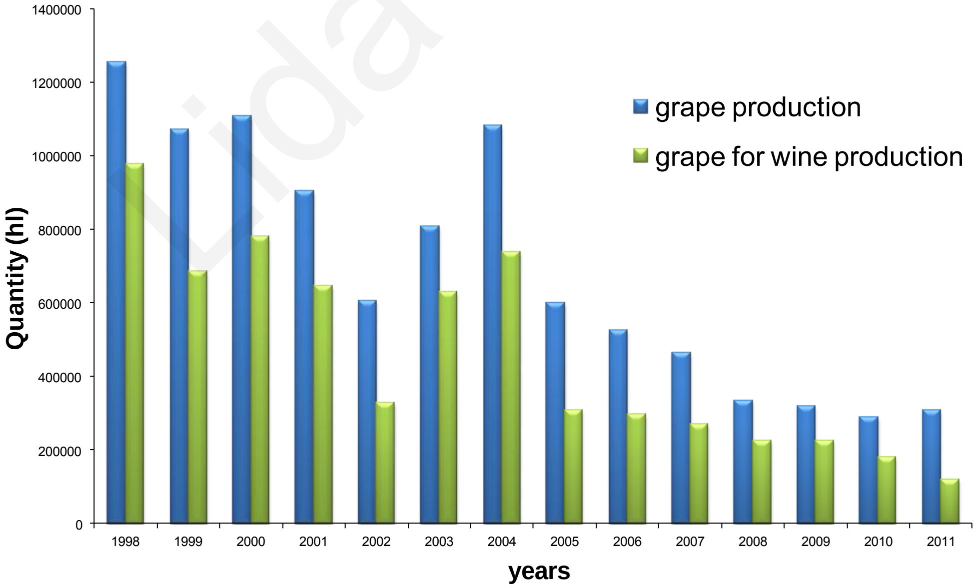


Figure 1.8: Cypriot annual grape production and grapes for wine production

Source: Cypriot Wine Products Council (WPC), 2011

According to the Cypriot Vineyard Register, during 2011 the wine-making grapes plantations covered 9058 hectares. From that, 6640 hectares concern indigenous varieties (e.g. local mavro and xynisteri), while 2418 hectares are international varieties (e.g. Cabernet sauvignon, Shiraz, etc.). Viticulture in Cyprus was significantly reduced during the last five years, mainly due to the implementation of the measure of permanent abandonment of vineyards. Moreover, the structure of vineyards changes dramatically due to the need for replanting with varieties that produce wine of higher quality. The viniculture potential configuration (in hectares) during the period 1999-2011 is presented in Figure 1.9.

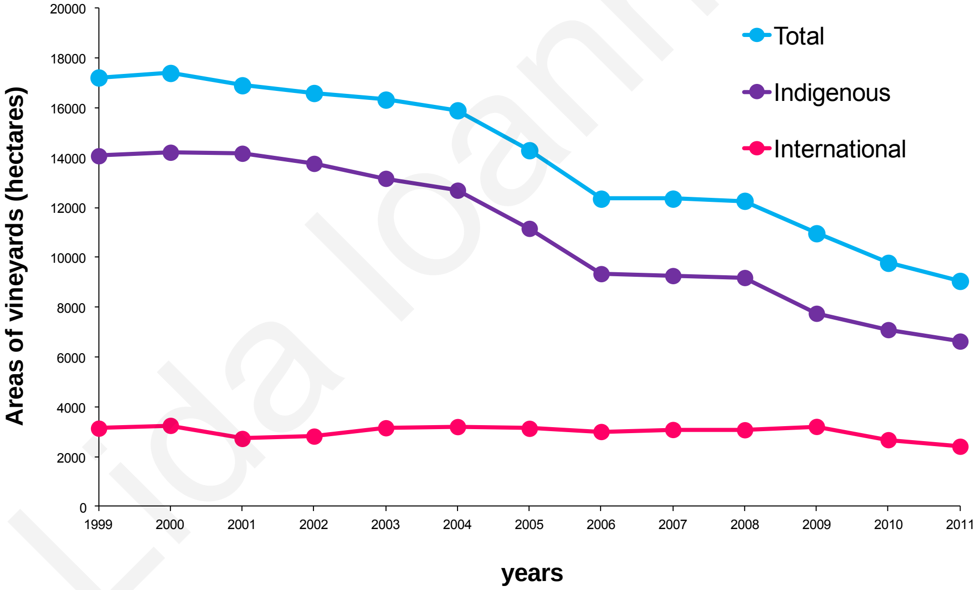


Figure 1.9: Vineyards area in Cyprus, during the period 1999-2011

Source: Cypriot Wine Products Council (WPC), 2011

Wine sales in local market have reached 10-12 million liters annually during the last years, contrary to the exports of Cypriot wines that continue facing problems. Based on the statistics of the Cypriot Wine Products Council, it is easier to maintain and enlarge the local market of Cypriot wines, than their use in foreign markets. This is due to the fact that imported wines are not necessarily better than the Cypriot ones, and also because Cyprus is a country which regularly host a tourist population of around 2.5 million per year (Cypriot Wine Products Council, 2011). Figure 1.10 shows the Cypriot wine sales in the domestic and international market, during the period 1998-2011.

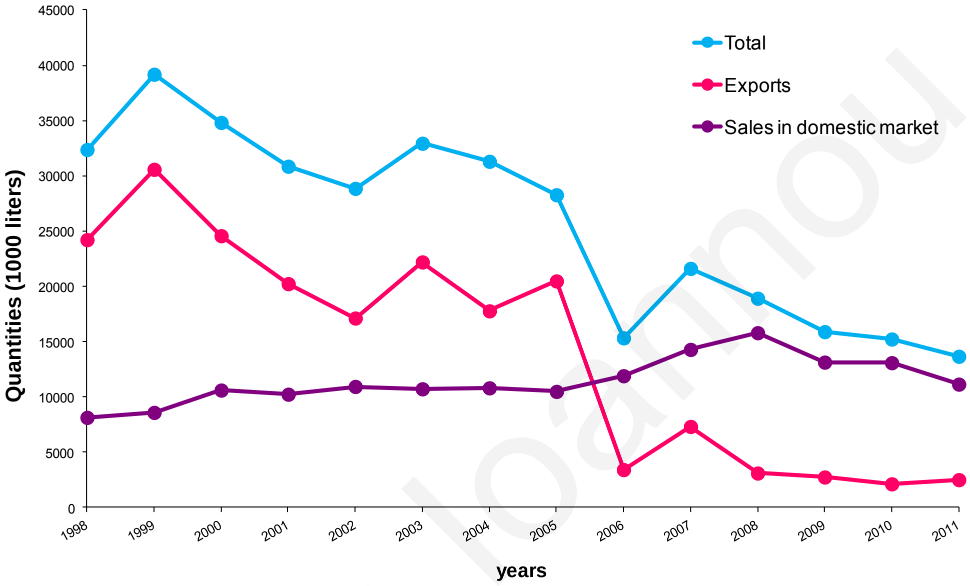


Figure 1.10: Cypriot wines sales in domestic and international markets

Source: Cypriot Wine Products Council (WPC), 2011

It should be mentioned that according to the latest data of the Cypriot Wine Products Council, Cypriot wine exports show a significant increase of 18%, which amounted to 2519386 liters of wine in 2011 compared to 2140417 liters in 2010. The highest increase was recorded for the exports of bulk wines, which amounted to 1471398 liters, compared to 1109771 liters in 2010 (33% increase). Bottled wine exports amounted to 96062 liters in 2011, representing an increase of 22%, while the smallest increase occurred in exports of wine to Tetra Pak package, which was only 9%. Russia absorbed the 76% of exports of wine of Tetra Pak package, while the largest percentage of exports of bottled wine was destined for United Kingdom (66%), and 65% of bulk wine exports absorbed by the Germany, as shown in Figure 1.11.

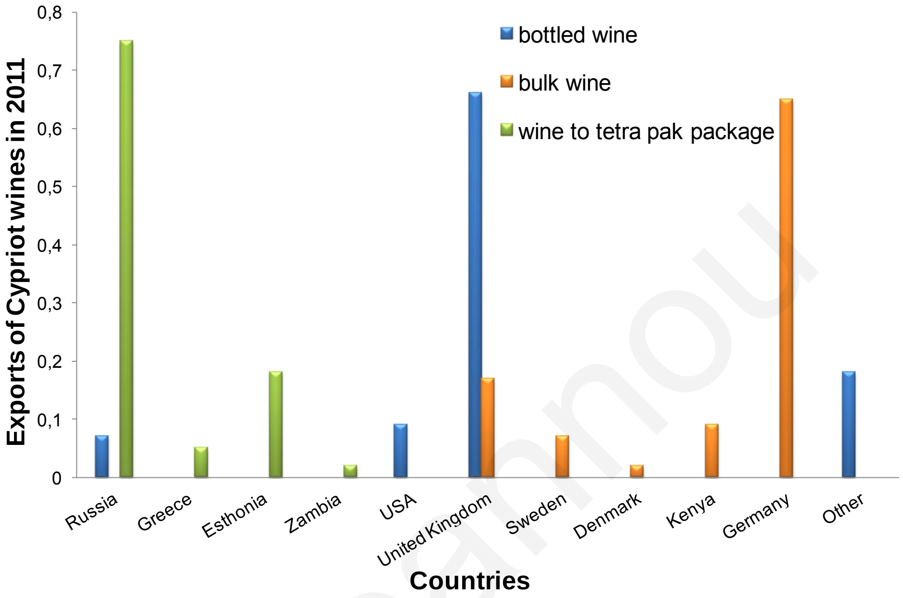


Figure 1.11: Cypriot wine exports in 2011

Source: Cypriot Wine Products Council (WPC), 2011

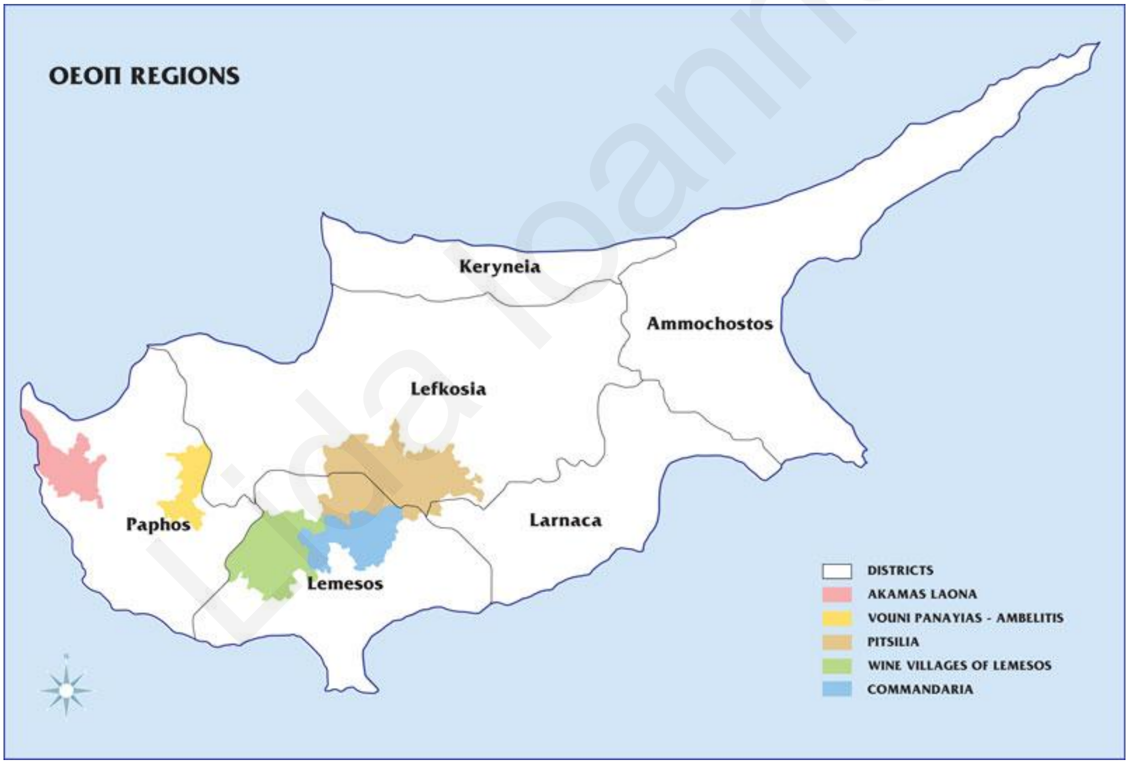
The Wine Products Council (Determination of Regions and Criteria of wine Production of Denomination of Controlled Origin) Decree 706/2004, determines the regions of the Republic of Cyprus, the name of which can be used in the presentation of denomination of controlled origin wine. These regions and their communities are the following:

- **Akamas Laona:** Drousia, Inia, Kathikas, Kritou Terra, Pano and Kato Arodes.
- **Vouni Panayias - Ampelitis:** Galataria, Koilinia, Panayia, Statos - St. Fotios.
- **Pitsilia:** St. Irene, St. Ioannis, St. Theodoros, Agridia, Agros, Alithinou, Alona, Apliki, Askas, Gouri, Dymes, Kampi, Cannavia, Kato Mylos, Kourdali,

Kyperounta, Lagoudera, Lazania, Levadia, Odou, Palaichori, Pelendri, Platanistasa, Potamitissa, Polystypos, Saranti, Spylia, Sykopetra, Farmakas, Fikardou, Fterikoudi, Chandria.

- **Wine villages of Limassol:** St. Ambrosios, St. Therapon, Arsos, Basa, Boyni, Gerobasa, Dora, Kissousa, Koilani, Kouka, Lofou, Malia, Mandria, Omodos, Pano Kybides, Pachna, Pera Peri, Potamiou, Trimiklini, Trozena.

A map of Cyprus, that outlines the abovementioned wine regions of the country is presented in Schematic 1.2.



Schematic 1.2: Map of Cyprus outlining the wine regions of the country

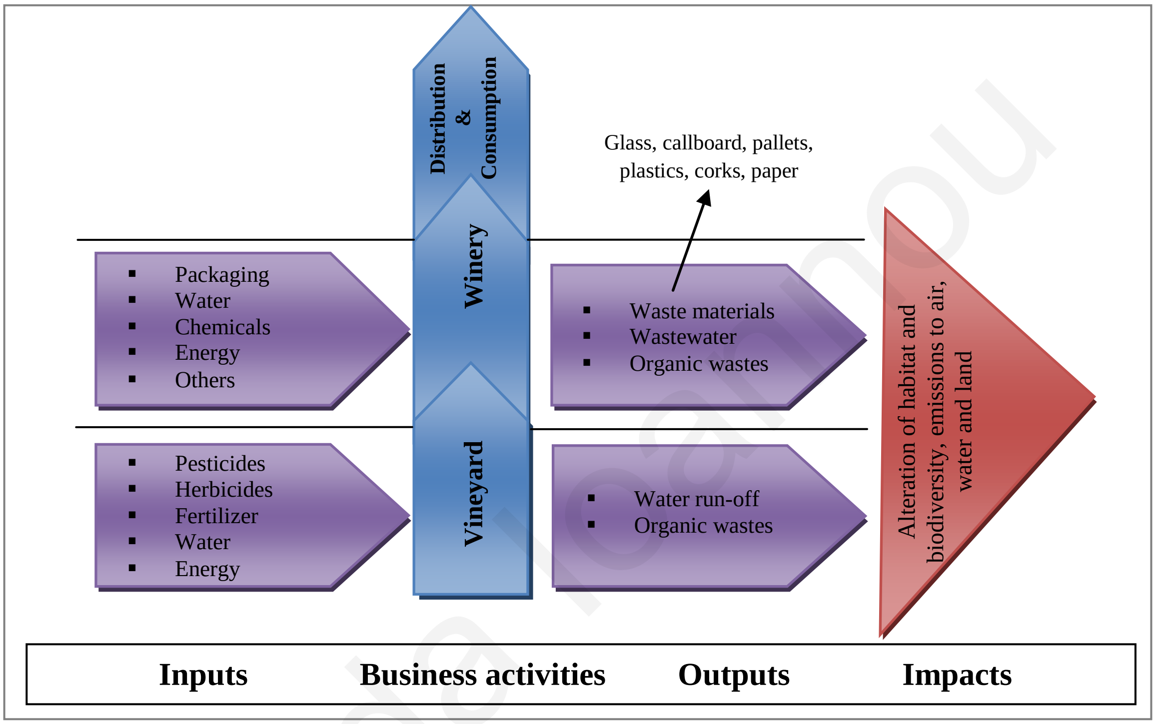
Source: www.cypruswinepages.com

CHAPTER 2: THE ENVIRONMENTAL IMPACTS OF THE WINE PRODUCTION

Wine production is one of the foremost agro-industries in many countries all over the world, with increasing impact on the local economies (e.g. Mediterranean countries, Australia, Chile, United States, South Africa, etc.) (Ganesh et al., 2010). Wine production has been traditionally seen as an environmentally friendly process. However, it requires a considerable amount of resources such as water, energy, fertilizers and organic amendments, and on the other hand produces large volumes of waste streams (Ruggieri et al., 2009; Lucas et al., 2010; Arcese et al., 2012). These waste streams include solid organic waste (grape marc, skins, pips, etc.), wastewater, greenhouse gases (CO_2, volatile organic compounds, etc.), and packaging waste (Lucas et al., 2010; Arcese et al., 2012).

The types of the environmental impacts associated with growing wine grapes and producing wine are depicted in Schematic 2.1. Environmental impacts associated with cultivating vineyards are agricultural in nature, while those in the winery are related to food processing.

The various activities taking place in the vineyards, involve fertilisers (both synthetic and natural), pesticides, water for irrigation and energy to power equipment in the field (Schematic 2.1). There are natural pesticides and pest control approaches; however, the majority of vineyards use synthetic chemical pesticides in the form of insecticides, herbicides and fungicides. These chemicals, while varying in terms of level of toxicity, are detrimental to air, water and soil quality. They can also induce harmful impacts on vineyard workers and neighbours, as well as on animals sharing the local habitat. A major issue with synthetic fertilisers in soils inclined to leaching to groundwater is the potential to contaminate local water supplies through inappropriate or excessive application. Water shortages can also be an issue in many areas. Energy issues associated with the use of fossil fuels in operating farm equipment relate to greenhouse gas (GHG) emissions, depletion of natural resources and air quality impacts (Silverman et al., 2005).



Schematic 2.1: Typical environmental impacts of the vineyards and the wine production

Source: Silverman et al., 2005

The activities taking place during the wine production, involve energy (cooling during the fermentation process, maintaining storage temperature, pumping, running equipment, etc.), water (barrels, tanks and equipment are continuously cleaned to minimise contamination levels), chemicals (caustic soda, sulphur gas, refrigerants, etc.), and packaging materials (glass, corks, wood pallets, glues, cardboard, metal and plastic foil), as shown in Schematic 2.1. Winery operational water issues relate to the treatment of wastewater containing organic matter, nitrates and phosphorous. The impacts potentially caused by the use of chemicals vary, depending on the chemicals used in the winery, and can include spills and various air, soil and water quality impacts. Packaging materials impact natural resource stocks and pose landfill issues (EPA, 2002; Silverman et al., 2005).

Although the environmental performance of the wine industry does not receive as much media attention as industries often characterized as ‘dirty’, such as the chemical industry, wine production faces a number of serious environmental issues. Wine industry managers work to limit the use of toxic pesticides and herbicides, of fertilizer, of scarce water supplies and of energy along with trying to reduce the amount of contaminated wastewater run-off, organic wastes and non-hazardous packaging materials (Baughman et al., 2000). Winery operations need either to treat wastewater on-site or send effluent to municipal treatment plants. Additionally, wineries must also pay attention to land use issues involving habitat destruction and endangered species (Marshall et al., 2005).

The management and disposal of the generated waste, as well as the overall environmental management of wineries are of great importance to the winery operators, and so far, in order to achieve these, several methods and processes have been used worldwide (Bustamante et al., 2008; Mosse et al., 2011).

The main potential overall environmental impacts of wineries according to EPA, (2006) are:

- pollution of groundwater and surface water, degradation of soil and damage to vegetation arising from liquid and solid waste reuse and disposal practices;

- odor and air emissions resulting from the management of raw materials, wastewater, solid and semi-solid by-products from the winemaking process; and
- noise from pumps, chillers, crushers and other winery equipment, as well as vehicle noise, particularly during vintage.

Some of the potential environmental impacts of the various constituents of the liquid and solid wastes from the winemaking process are summarised in Table 2.1.

Table 2.1: Potential environmental impacts of winery wastes (liquid and solid)

Constituent	Indicators	Effects
Organic matter	BOD, COD, TOC	• Depletes oxygen when discharged into water, leading to the death of fish and other aquatic organisms. • The odors generated by anaerobic decomposition cause nuisance if waste is stored in open lagoons or applied on land.
Alkalinity/Acidity	pH	• Can induce the death of aquatic organisms at extreme pH values. • Affects microbial activity in biological wastewater treatment processes. • Affects the solubility of heavy metals in the soil and availability and/or toxicity in waters. • Affects crop growth.
Nutrients	nitrogen, phosphorous, potassium, sulphur	• Cause eutrophication or algal bloom when discharged to water or stored in lagoons; algal blooms can cause undesirable odors in lagoons. • Nitrogen as nitrate and nitrite in drinking water supply can be toxic to infants. • Toxic to crops in large amounts.
Salinity	EC, TDS, chloride	• Imparts undesirable taste to water. • Toxic to aquatic organisms. • Affects water uptake by crops.
Metal contamination	cadmium, chromium, copper, nickel, lead, zinc, mercury	• Toxic to plants and animal.
Solids	TSS	• Reduces soil porosity, leading to reduced oxygen uptake that can reduce light transmission in water, thus compromising ecosystem health. • Odor generated from anaerobic decomposition.

Sources: EPA, 2006; Strong and Burgess, 2008; EPA, 2011

*BOD: Biological Oxygen Demand; TOC: Total Organic Carbon; COD: Chemical Oxygen Demand; EC: Electrical Conductivity; TDS: Total Dissolved Solids; TSS: Total Suspended Solids

The main environmental problems related to the operation of wineries are divided into six categories: wastewater, consumption of water and energy, solid waste, use of chemicals and air emissions. These are presented in detail below.

2.1 Production of wastewater

Winery wastewater originates from a number of sources that include (Petrucciolli et al., 2000; Malandra et al., 2003; EPA, 2006; Mosteo et al., 2008; Vlyssides et al., 2005; Mosse et al., 2011):

- cleaning of tanks
- hosing down of floors and equipment
- rinsing of transfer lines
- barrel washing
- spent wine and product losses
- bottling facilities
- filtration units
- laboratory wastewater
- stormwater diverted into, or captured in the wastewater management system.

Winery wastewater is a major waste stream resulting from the numerous cleaning operations that occur during each production phase as mentioned above, and the effluent produced contains various contaminants (Daffonchio et al., 1998; Brown and Pollard, 2009). Volumes and pollution loads greatly vary in relation to the working period (vintage, racking, bottling) and the winemaking technologies used (e.g. in the production of red, white and special wines) (Artiga et al., 2005; Bustamante et al., 2008; Lucas et al., 2010). It is estimated that a winery produces between 1.3 and 1.5 kg of residues per liter of wine produced, 75% of which is winery wastewater (Lucas et al., 2010). Up to six winery wastewater production periods can be defined, as summarized in Table 2.2.

Table 2.2: Description of wastewater production periods at wineries

Period	**Duration**	**Description***
Pre-vintage	1 - 4 weeks	Bottling, caustic washing of tanks, non-caustic equipment in readiness for vintage.
Early vintage	2 - 3 weeks	Wastewater production is rapidly rising to peak vintage flows and has reached 40% of the maximun weekly flow; vintage operations dominated by white wine production.
Peak vintage	3 - 14 weeks	Wastewater generation is at its peak; vintage operations are at maximum.
Late vintage	2 - 6 weeks	Wastewater production has decreased to 40% of the maximum weekly flow; vintage operations dominated by production of red wines; distillation of ethanol spirit may coincide with this period.
Post-vintage	6 - 12 weeks	Pre-fermentation operations have ceased; effect of caustic cleaning is at its greatest and wastewater quality may be poor.
Non-vintage	10 - 20 weeks	Wastewater generation is at its lowest, generally less than 30% of maximum weekly flows during vintage; wastewater quality is highly dependent on day-by-day activities.

Sources: EPA, 2006; van Schoor, 2005

* The demarcation between one period and another, and the timing or existence of each period, vary between wineries and regions.

Most of the wineries all over the world consist of small to medium size enterprises, which cannot afford the appropriate management of the produced waste (Silverman et al., 2005). Some wineries in urban areas channel their wastewater to local sewage treatment facilities, which causes heavy penalties, due to the low pH and the high COD content of the waste stream. In contrast, rural wineries often have very little or no treatment operation for wastewater which is often applied onto fields for irrigation, leading to adverse environmental effects (Strong and Burgess, 2008).

The environmental impact of wastewater from the wine industry is noticeable, due to the high organic load, the large volumes and the aforementioned pronounced seasonal variability (Chatzilazarou et al., 2010). Each winery is unique in the winery wastewater generation (highly variable, 0.5-14 L per litre of wine produced) and disposal (Oliveira and Duarte, 2010). Winery wastewater can cause eutrophication (nutrient enrichment) of water resources (e.g. natural streams, rivers, dams and wetlands) (van Schoor, 2005). In surface waters, eutrophication is particularly serious because it can lead to algal blooms and the subsequent oxygen-consuming degradation processes, which finally may result in the death of the total aquatic biocenosis (Brentrup et al., 2004). Furthermore, winery effluents can cause salinity, contamination with a wide range of chemicals, loss of soil structure and increased susceptibility to erosion, as shown in more detail in Table 2.1.

In addition, application of winery effluent to soil without proper monitoring, perilously affects the groundwater quality by altering its physicochemical properties such as color, pH, electrical conductivity (EC), etc., due to leaching of organic and inorganic ions (Mohana et al., 2009). The high acidity of winery wastewater can affect the plant vigor by reducing the availability of plant nutrients (particularly phosphorous and calcium); and decreasing populations of useful microbes (EPA, 2006). In addition, the inhibitory effects of winery wastewater on plant growth can be attributed to the high percentage of organic compounds and salts, while its high electrical conductivity, which makes water uptake by seeds difficult, causes retardation of germination (Melamane et al., 2007).

Moreover, numerous phenolic compounds are present in wines as a result of their extraction from the skin, flesh, and seeds of grapes. Phenolic compounds form a relatively small portion of the organic load of the wastewater, but can cause environmental damages if released untreated (Strong and Burgess, 2008). Some phenolic compounds are harmful pollutants that are toxic to human, animals and many microorganisms, even at relatively low concentration (Nair et al., 2008). They are particularly resistant to degradation, but some bacteria (Melamane et al., 2007; Pepi et al., 2010; Saravanan et al., 2008) and fungi (Asses et al., 2009; Strong and Burgess, 2008) can tolerate and degrade those compounds. Moreover, if high levels of biological oxygen demand (BOD) in untreated winery wastewater are allowed to flow to streams, rivers, ponds, lakes and other surface water, the dissolved oxygen in the waterways may be quickly consumed. As the dissolved oxygen (DO) in the waterways is depleted, aquatic and amphibious life suffocates (Chapman et al., 1995).

The wastewater in wineries is high in organic content, containing predominantly sugars, organic acids (e.g. acetic, tartaric, propionic), esters and phenolic compounds (Malandra et al., 2003; Mosse et al., 2011). COD concentration ranges from 320 to 27200 mg L^{-1} or even 49105 mg L^{-1} (mean value: 10843 mg L^{-1}) depending on the harvest load and process activities (Petruccioli et al., 2002; Eusebio et al., 2004; Artiga et al., 2005; Bustamante et al., 2005; Mosteo et al., 2008; Mosse et al., 2011). Usually in this type of wastewater the concentration of nitrogen and phosphate is relatively low (Kalyuzhnyi et al., 2001; Ganesh et al., 2010). The winery effluents are very biodegradable and the period where the ratio BOD_5/COD is higher is the vintage, because of the presence of molecules such as sugar and ethanol (Ganesh et al., 2010).

In an attempt to better understand the complex nature of winery wastewater, a summary of compositional data from a number of wineries have been compiled as shown in Table 2.3.

Table 2.3: Summary of reported winery wastewater characteristics

Parameter	Unit	Min	Max	Mean	References
Chemical Oxygen Demand (COD)	mg L^{-1}	320	49105	10843	[2-16]
Biochemical Oxygen Demand (BOD_5)	mg L^{-1}	125	22418	9006	[2-4,6,9,11,15]
Total Organic Carbon (TOC)	mg L^{-1}	41	7363	2037	[2,8,13,14]
pH	-	3.5	5.7	4.6	[1,2,5-7,9-13,15,16]
Electrical Conductivity (EC)	mS cm^{-1}	3	5.1	4.1	[1,12]
Total Solids (TS)	mg L^{-1}	748	18332	7727	[2,9,11]
Total Volatile Solids (TVS)	mg L^{-1}	661	12385	5599	[9,11]
Suspended Solids (SS)	mg L^{-1}	170	5137	1179	[2-4,6,7,9-12,15]
Total Phosphorous (TP)	mg L^{-1}	3.3	176	49.1	[3,4,6,7,9,11,15]
Total Nitrogen (TN)	mg L^{-1}	21.3	415	140.2	[6,8,11,15]
Total phenolic compounds (TPh)	mg L^{-1}	5.8	1450	275.4	[2-4,9,11-14,16]

References: 1. Chapman et al., 1995; 2. Beltran et al., 2000; 3. Petrucciolli et al., 2000; 4. Petrucciolli et al., 2002; 5. Malandra et al., 2003; 6. Eusebio et al., 2004; 7. Andreotolla et al., 2005; 8. Artiga et al., 2005; 9. Bustamante et al., 2005; 10. Colin et al., 2005; 11. Vlyssides et al., 2005; 12. Arienzo et al., 2009a; 13. Mosteo et al., 2006; 14. Mosteo et al., 2008; 15. Zhang et al., 2008; 16. Lucas et al., 2009b.

Even though the main characteristics of winery wastewater are well known, it turns out to be quite difficult to establish a criterion to define in advance the polluting load, since it depends on the winemaking mode and the technologies adopted (Brucculeri et al., 2005). The wastewater can be treated as it is produced or can be stored for treatment over several months (Ganesh et al., 2010). There are numerous treatment options for winery wastewater, all of which aim at achieving a significant reduction in the concentration of organic matter and solids, and some may reduce inorganic load additionally (Mosse et al., 2011). Winery wastewater can be treated using various physicochemical, biological, advanced oxidation and membrane filtration and separation processes, which are described in detail in Chapter 3.

2.2 Water consumption

The main use of water within the winery itself (excluding vineyards) is for cleaning and sanitation, and sometimes as coolant. Water is used to wash down floors and areas throughout the winery, to clean the equipment including the receiving lines, the presses, the tanks, and the bottling lines, and to wash the barrels at various stages of the winemaking process. Water is also used for humidification in the cellars and barrel storage areas, and on other non-production uses at the winery (e.g. cleaning of offices, etc.) (Galitsky et al., 2005).

Wineries differ widely in their consumption of water, while bottling is responsible for about 40% of the wastewater produced. The average water use per unit production in the winery is about 2.0 kL ton^{-1} grape crushed, while with a wise water use is about 0.4 kL ton^{-1} crushed. This is equivalent to an average water use of 2.0 L per 750 mL bottle of wine. Bottling onsite significantly increases the average water consumption. This can range from 1.4 L per bottle for wineries that do not bottle onsite, to 2.5 L per bottle for wineries that do bottle onsite (Oliveira et al., 2009). However, according to Walsdorff et al. (2007) comparing water consumption between cellars can be erroneous, as it greatly depends on various parameters, such as type of tanks, processing equipments, etc; and can therefore only be used as an indication of a trend.

The demands for freshwater by industry, as well as by agriculture are causing groundwater resources to be depleted and surface water resources to be abstracted in ways which compromise freshwater ecosystem health (Smakhtin, 2008). Pressure on freshwater resources is also intensifying rapidly with climate change, population growth, continuous economic development, and the expansion of biofuel crops, raising the concern of governmental and non-governmental organizations alike. For all these reasons, many businesses are seeking to demonstrate good corporate citizenship by measuring, reporting and addressing negative impacts arising from water use in their operations and product life cycles (Chapagain and Orr, 2009).

The water footprint of a product is typically the sum of all water consumed in the various stages of production, and therefore the same as its virtual water content (Ridoutt and Pfister, 2010). The assessment of product water footprints has raised the awareness of the extent and magnitude that local businesses and consumers are intervening in the hydrological cycle throughout the world (Chapagain and Hoekstra, 2008). This is viewed as a positive development because, in many places, freshwater has become a scarce and overexploited natural resource leading to a wide range of social and environmental concerns (Falkenmark, 2008).

The reduction in the amount of water consumed in a winery will have several environmental and economic benefits, including conservation of water resources, and consequently, lower wastewater discharge volumes. This can potentially allow for less costly wastewater treatment equipment (Vancouver, 1997; Galitsky et al., 2005).

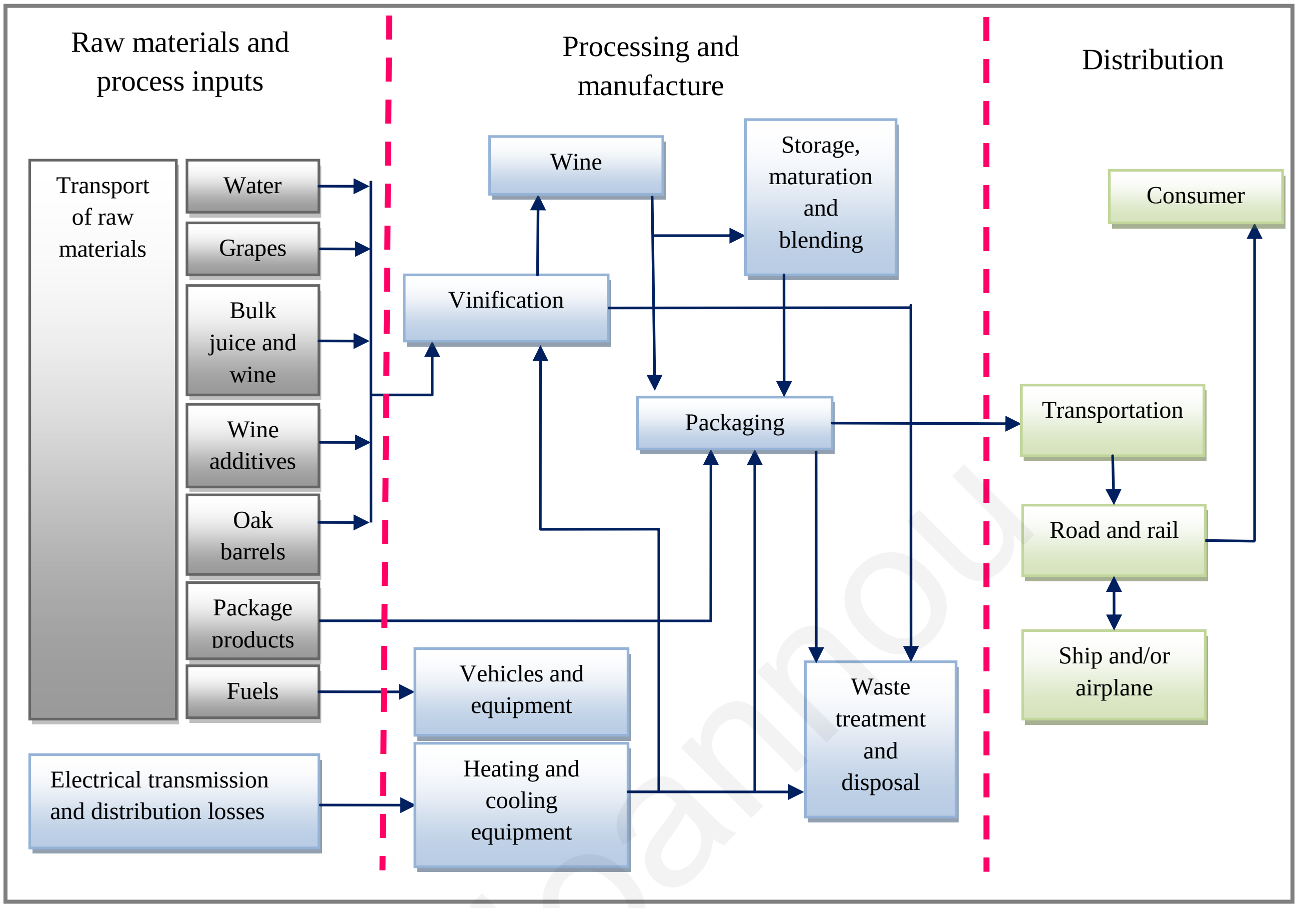
However, water conservation should not compromise winery sanitation or safety considerations, and should only be used in conjunction with initiatives intended to reduce the pollutant loadings in the effluent (Vancouver, 1997).

2.3 Energy consumption

A number of stages in the wine making process are energy intensive. Winemaking requires significant energy inputs for grapes crushing, must pressing, juice filtering, cooling/heating of fermentation tanks, and wine bottling. Refrigeration is another main energy-using application. Energy usage has a direct correlation to the operating costs of a company (Galitsky et al., 2005).

The highest consumption of energy by the winery takes place during the harvesting period (August up to November). The exact annual consumption depends on the exact temperature and weather conditions affecting the power used by the cooling units. It has been suggested that it takes about 0.71 GJ of electricity to make a kiloliter of wine (McCorkle, 2009).

In order to better understand the processes that contribute to the energy use of a winemaking enterprise, one of the most effective methods is the map of the supply chain, so all energy and fuel related inputs to be contained, as presented in Schematic 2.2. Winery energy inputs include fuels consumed for the transportation of the raw materials (e.g. grape, package products, etc.), as well as the energy consumed through the wine making process and the waste/wastewater treatment, and the fuels consumed for the distribution of the final product.



Schematic 2.2: Winery supply chain showing fuel and energy inputs

Sources: Forsyth et al., 2008; Smyth and Nesbitt, 2012

The vast majority of the energy used in the winery is related to the production. In Figure 2.1 the distribution of energy consumed in the production activities of wineries is illustrated (Smyth and Nesbitt, 2012). Heating, cooling and ventilation are by far the biggest segment (44%) of energy use within the winery, and therefore represent the area where the greatest energy savings could be made. Bottling activities (at 13%) reflect the use of significant mechanical power via the automated bottling machinery or lines, some refrigeration in the disgorging process and the use of compressed air systems. Lighting, at 22%, is the second largest area of energy usage within the winery. This is primarily due to the extensive use of inefficient lighting systems being used for prolonged periods of operation. Grape processing and sterilization/cleaning activities require a similar level of energy input (8%). Finally, miscellaneous (3%) is a very broad category that includes a range of equipment necessary to provide a suitable working environment for the production processes, and includes monitoring devices, security devices, shutter doors, laboratory equipment, etc.

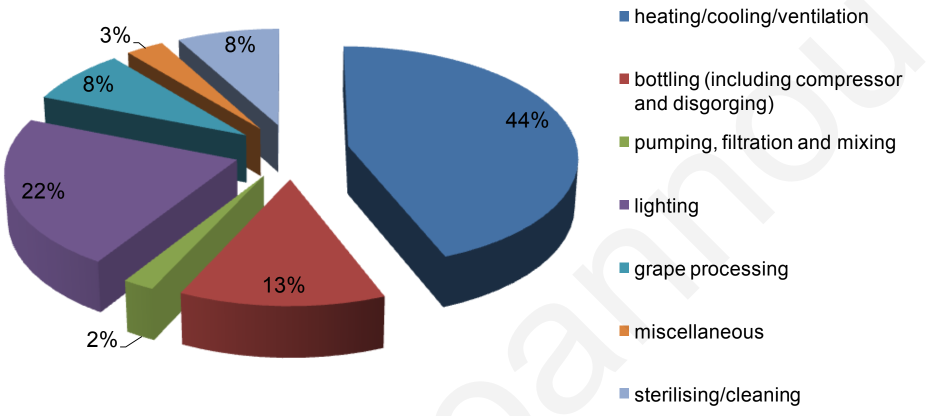


Figure 2.1: Distribution of energy consumed in wine production

Source: Smyth and Nesbitt, 2012

A reduction in energy consumption is also an important consideration in a pollution prevention program and in lowering the operational cost as well. While energy conservation measures reduce the amount of pollution created in the production or use of energy (e.g. CO_2, NO_X, SO_2, ash, etc.), pollution prevention measures reduce the energy requirements for waste handling and treatment (EPA, 2006). The emission of gases during the wine production process that contribute to climate change is very small compared to the national emissions of each country. According to Smyth and Nesbitt, (2012) the wine industry is estimated to produce only 0.08% of global greenhouse gas

(GHG) emissions. Nevertheless, there is scope for further decrease of this contribution (Berg and Lindholm, 2005), and this energy reduction can be achieved by the implementation of conservation measures for electricity, thermal energy and fuels consumed within the winery (Vancouver, 1997).

2.4 Solid waste

Wineries produce a range of solid wastes including:

Organic solid wastes:

- grape marc - the grape material (mainly skin, pulp and seeds), which is left over after grape crushing and pressing
- grape stalks - separated from the grapes during the crushing process
- lees - the material which accumulates in the bottom of grape juice or wine fermentation tanks, and
- filtered solids - generally diatomaceous earth and bentonite clay, filter media (Arvanitoyannis et al., 2006a; Bustamante et al., 2005; Strong and Burgess, 2008).

Non organic solid wastes:

- general refuse - empty chemical containers, cardboard, plastic containers, wooden pallets
- packaging wastes - produced mainly by the bottling process include paper, scrap plastic and glass (EPA, 2006).

As more than 20% of the wine production is organic solid waste, the latter constitutes a serious environmental problem that has to be solved urgently (Arvanitoyannis et al., 2006b). The primary concern is the high organic matter related to the skins and lees, which could lead to a depletion of the dissolved oxygen in surrounding streams, rivers and other surface water resources. For these reasons, the winery organic solid wastes should be treated following the appropriate management practices, as presented below (Bustamante et al., 2008).

Most of the solid wastes generated in a cellar (80-85%) are organic wastes. In Figure 2.2, an approximate distribution of the organic solid wastes generated in the wine industry is presented (Ruggieri et al., 2009). Grape marc corresponds to 56% of organic solid wastes and is produced during grape press and is constituted mainly by peels and seeds. The rest of wastes are lees (22%), which are generated in the clarification of wine fermentation process; stalk, (11%) constituted by branches and leaves of the grapevine, and wastewater sludge (11%) from wastewater treatment.

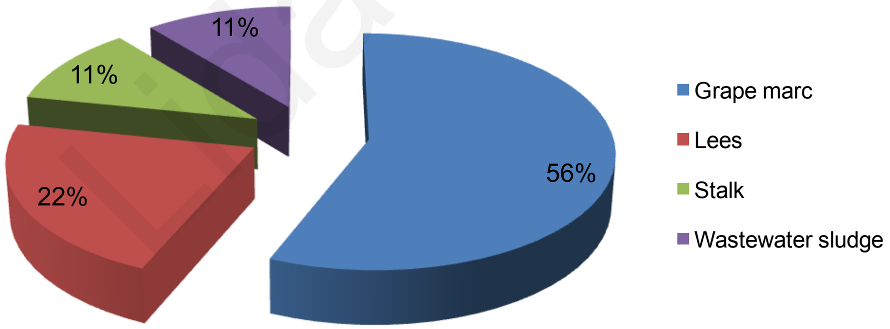


Figure 2.2: Approximate distribution of the organic waste produced in the wine industry

Source: Rugierri et al., 2009

Moreover, organic solid waste can cause salinity, terrestrial eutrophication, loss of soil structure, while its high acidity can affect the plant growth (EPA, 2004), as presented in more detail in Table 2.1. Additionally, the odors generated by anaerobic decomposition can cause nuisance if waste is applied on land (EPA, 2006). A summary of the qualitative characteristics of the main organic solid wastes (grape stalk, grape marc and wine lee) from a number of wineries are presented in Table 2.4.

Table 2.4: Summary of reported winery solid waste characteristics

Parameter	**Unit**	**Grape stalk**	**Grape marc**	**Wine lee**	**References**
		Mean values			
pH		4.6	4.7	5.2	[1-8]
Electrical Conductivity (EC)	dS cm^{-1}	3.1	2.5	5.3	[2-8]
Organic matter	gr kg^{-1}	920	915	759	[7,8]
Total organic carbon (TOC)	gr kg^{-1}	221.3	404.3	409.5	[3-8]
Total phenolic compounds (TPh)	mg kg^{-1}	2052	536.9	6998.5	[3,4,7,8]
Total Nitrogen (TN)	gr kg^{-1}	10,.9	18.4	27.8	[2-8]
Total Phosphorous (TP)	gr kg^{-1}	0,94	1.2	4.9	[7,8]

References: 1. Ferrer et al., 2001; 2. Bertran et al., 2004; 3. Nogales et al., 2005; 4. Bustamante et al., 2007; 5. Marhuenda-Egea et al., 2007; 6. Romero et al., 2007; 7. Bustamante et al., 2008; 8. Bustamante et al., 2009.

Regarding the organic solid wastes, according to the literature, there are various biological and chemical processes that can be implemented to treat these wastes in a sustainable way in order to provide economic and resource benefits (Brown and Pollard, 2009). Some of the treatments include: combustion, solid-state fermentation, incineration, composting, aerobic and anaerobic digestion, electrodialysis and pyrolysis

(Arvanitoyannis et al., 2006b). Each method has its own advantages and disadvantages, making it crucial for wineries to assess their facilities and ensure that the method they use will be most efficient and economically feasible for their business (Bertran et al., 2004).

Some of the organic wastes have been traditionally recovered by using them as raw materials in other industrial sectors (Kammerer et al., 2005; Ruggieri et al., 2009); while some other winery waste can be potentially used as soil conditioner or for fertiliser production (Arvanitoyannis et al., 2006b).

Grape marc is the primary solid waste generated from wine production (includes skin, stalks and seeds), and if not treated properly can initiate various environmental hazards, from water pollution to foul odors, as well as an increase in the spread of diseases (Arvanitoyannis et al., 2006a; Mcmanus, 2008). Grape marc represents a rich source of various high-value products such as ethanol, tartrates and malates, citric acid, grape seed oil, hydrocolloids and dietary fibre (Kammerer et al., 2004; Bustamante et al., 2007; Strong and Burgess, 2008). Grape marc can easily be recycled back to the soil as compost, since it has a high organic and nutrient content. It can also be used as a fuel for heating or as animal feed. Lees can be collected separately and taken to a recycling company for tartrate recovery, but this alternative is costly to small businesses, as income generated is insignificant (Walsdorff et al., 2007).

In addition, certain winery solid wastes produced have economic value in other industries and can be managed sustainably by recycling it into other products (Bustamante et al., 2008; Ruggieri et al., 2009). For example, grape seed extracts are high in antioxidant activity (high content of phenol and flavanol, which serve as inhibitors of neoplastic processes) that can be used as a dietary supplement for disease prevention; providing economic advantage for wineries since exploitation is a cheap source of extraction for pharmaceutical companies, and reduces the amount of waste for wineries to deal with (Bustamante et al., 2007).

2.5 Use of chemicals

The importance of cleanliness and sanitation within the winery cannot be overemphasized. Cleanliness and disinfection are of utmost importance in the production of consistenly high quality wines (Sipowich, 2008).

The word ‘chemicals’ in a winery, includes not only the chemicals used for cleaning and disinfection (e.g. caustic soda, citric acid, chlorine, soap, etc.), but also pesticides and fertilizers. These chemicals must be used safely and efficiently, and must be managed with consideration of the potential on and off site environmental impacts, including effects on local residents and other agricultural activities, issues of contamination of land and water, and impacts on flora and fauna (Galitsky et al., 2005).

The excessive application of fertilizers (including quantity and frequency of application) usually exceeds the functional ability of the soil to retain and transform the nutrients, and synchronizes the availability of nutrients with crop needs. In many cases, the saturation of the soil with nitrogen or phosphate, have led to losses of nitrates into shallow groundwater and saturation of the soil with phosphate, which may also move into groundwater (Breeuwsma and Silva, 1992; Zalidis et al., 2002). Fertilizers contain nitrogen and phosphorous that can contribute to the over-enrichment or excess fertility of surface waters, resulting in excessive growth of algae, phyto- and zooplankton, and deterioration of water quality with respect to potability and the survival of fishes or other microorganisms (Viets and Lunin, 1975).

On the other hand, the extensive use of pesticides in agricultural land influences both the biotic and abiotic processes within the soil. As a result, several soil functions are degraded including the food web support, the retention and transformation of toxicants and nutrients, soil resilience, and the ability of soil to protect surface and ground water (Mourato et al., 2000). Moreover, pesticides cause the destruction of part of the soil flora and fauna, which in turn causes both physical and chemical deterioration. Additionally, effects on non-target organisms in soil occur frequently (Pimentel and Levitan, 1986). Pesticides may also cause severe yield reductions in crops, which follow in rotation due to the presence of residual herbicides. Concerning the water regime, the main problem derives from the leaching and drainage of pesticides into the surface and ground water. Additionally, the reduction of the soil's ability to remove other pollutants, due to the alteration of soil properties by pesticides and the degradation of soil's toxicant retention and transformation function, allows the movement of those pollutants to adjacent water bodies (Zalidis et al., 2002).

Moreover, there are also other chemicals that are used in the winemaking process (as shown in Table 2.5). These chemicals include sterilizing agents, sources of nitrogen in order to help fermentation, anti-microbial agent that regulate the growth of harmful yeast in wine, and stabilizers in order to ensure that refermentation will not occur during the sweetening of the wine (Cornellus, 1997).

Table 2.5: Basic chemicals used in the wine production process

Chemicals	Description
Bentonite	Clarification agent, that prevents hazing in wine.
Calcium carbonate / Potassium bicarbonate	Reduces acid in juice (added before fermentation).
Potassium (or Sodium) metabisulfite	This chemical produces sulfites in wine; sulfites help to protect the wine against oxidation and refermentation. It is also used as a sterilizing agent.
Sulfur dioxide	An anti-microbial agent that regulates the growth of harmful yeast and bacterial growth in the wine.
Citric acid	It is used to increase the total acidity of wine.
Diammonium phosphate (DAP)	This is a nitrogen source for the yeast, and helps with fermentation by limiting the number of “stuck” fermentations that will occur.
Potassium sorbate	A stabilizer, a yeast inhibitor to ensure that refermentation does not occur during sweetening.
Yeast nutrient / Fermaid	This is “food” for the yeast.

Source: Cornellus, 1997

Reducing the use of chemicals and inorganic fertilizers is one of the foremost goals of maintaining sustainability and responsibility in a winery (Barlow et al., 2009). Every winery should identify the chemicals used for cleaning/disinfection, the pesticides and fertilizers, and monitor the quantities used. It should also take some measures to limit the chemicals use and/or use of environmentally friendly cleaning products and organic fertilizers. A good option is the maintenance of an archive with all the specifications of chemical products used in winery and vineyards.

2.6 Air Emissions

Grape juice fermentation produces a host of atmospheric emissions, aside from carbon dioxide, including sulphur dioxide, ethanol, and volatile organic carbons (VOCs) (Weber and Bahner, 1999; Musse et al., 2007). Typical examples of the air emissions produced during the wine production process are presented in Table 2.6.

Table 2.6: Identification of air emissions produced in the wine production process

Process categories	Air emissions *
Raw material reception and crushing area	SO_2
Separation processes	SO_2, VOCs
Tank-based processes	CO_2, VOCs, ethanol
Heat/transfer	CFCs, ammonia, CO_2, GHG
Packaging/loading	VOCs, SO_2

Source: Musse et al., 2007
*VOCs: Volatile Organic Compounds
CFCs: ChloroFluoroCarbons
GHG: GreenHouse Gasses

Moreover, winery wastewater is generally stored before being treated (e.g. biological treatments), and odors are produced at this stage. Given the high organic concentration of these effluents, their long-term storage induces the formation of malodorous compounds through anaerobic fermentation. Malodorous compounds cause major harmful olfactory effects, which represent an important problem for winery wastewater management at this time (Bories et al., 2005). Various VOCs have been identified in winery wastewater: esters, mercaptans, aldehydes (Guillot et al., 2000).

Few studies have dealt with odor treatment of winery wastewater. During the storage of winery wastewater in tanks, treatment by acidification reduces odor intensity by a factor of ten. In the study of Bories et al. (2005), the preventive treatment of odors by nitrate addition was tested on an industrial scale in winery ponds. When nitrate (an electron acceptor) is added, catabolism leads to an anaerobic respiration phenomenon (denitrification). The organic compounds are oxidised to CO_2, and the nitrate is reduced to N_2 (odorless compounds), without volatile fatty acids (VFAs) formation. The results make it possible to consider using nitrate for the curative treatment of pond odors (Bories et al., 2005).

Composting of winery organic solid wastes presents some associated environmental impacts, like the generation of polluted or odorous gaseous emissions (Haug, 1993). Ammonia is one of the main compounds responsible for the generation of offensive odors and atmospheric pollution, when composting organic wastes with high nitrogen content. Ammonia is a colorless, toxic, reactive, and corrosive gas with a very sharp odor. Although the detection and recognition thresholds for ammonia are relatively high (17 ppmv and 37 ppmv, respectively) (Pagans et al., 2006), ammonia gas is the main compound found in exhaust gases from composting, except for carbon dioxide (Beck-Friis et al., 2001), in concentrations well over the threshold limit (Elwell et al., 2002). Ammonia gas can cause adverse effects on vegetation and can be converted to N_2O, a powerful greenhouse gas (GHG) (Krupa, 2003).

A few studies have been conducted on the ammonia emissions derived from the composting process, and concluded that temperature, pH, and initial ammonium content are the most important parameters affecting the amount of nitrogen emitted as ammonia; since high temperature and pH favour ammonia volatilization by displacing NH_4^+/NH_3 equilibrium to ammonia. Simultaneously, it is widely reported that high temperature inhibit the nitrification process (Grunditz and Dalhammar, 2001), and thereby, the possibility for ammonia volatilization is high. Beck-Friis et al. (2001) observed that ammonia emissions had started when thermophilic temperatures (>45 ^oC) and high pH (~9) co-existed in the compost environment, resulting in a loss of nitrogen within 24-33% of the initial nitrogen content. Similarly, Cronje et al. (2002) determined that the nitrogen losses for organic mixtures with an initial pH<6.2 were below 4% of the initial nitrogen content. Nevertheless, it must be emphasized that pH control is in practice very difficult during a composting process, whereas temperature control can be conducted once the sanitisation requirements are fulfilled. In other works, the strategy of using an intermittent aeration are tested and proved to be effective in decreasing the ammonia emissions (Elwell et al., 2002); however, this causes an oxygen limitation in the aerobic process and a loss of biological activity.

Finally, according to Colman and Paster, (2007) even though the production and transport of wine only makes up to 0.08% of global greenhouse gas (GHG) emissions or about 2 kg of greenhouse gas per 750 mL bottle, the industry has a great deal at stake. Given this scale, the industry may not be an obvious target for direct legislative action aimed at reducing emissions. However, vineyards and wineries are directly dependent upon several industries with larger carbon footprints such as power generation, transportation, glass production, as well as the fertilizer/pesticide manufacturing industries.

2.7 Life cycle analysis of the wine production

Viticulture (grape growing), viniculture (making wine), manufacturing glass bottles, various transportation links, refrigeration and recycling of glass bottles are processes within wine’s life cycle that necessitate the transformation of materials and energy, and thus result in emissions that contribute to various environmental impacts.

Life cycle assessment (LCA) is an internationally recognized environmental accounting tool which offers a standardized framework and methodology for quantifying the environmental impacts of a product or a production system (ISO, 2006). As its name suggests, LCA seeks to encompass the entire “life cycle” of a product into its accounting framework, from the extraction of raw materials used to make a product, through to the product’s end-of-life activities. Formalized life cycle assessments of wine, as well as other analyses that have employed “life cycle thinking” provide insight into the relative importance of each of these life cycle phases, as well as the nature of the associated environmental impacts. In each of the following studies, the functional unit of study was one 750 mL bottle of wine.

In Italy, Notarnicola et al. (2003) performed an LCA on the viticultural, vinicultural, and bottle production stages of wine with the intent to identify the environmental hotspots for four different bottles of wine - a high quality red and white wine, and a low quality red and white wine. All systems performed similarly, whereby the most burdensome phase of wine’s life cycle was grape growing, followed by glass bottle production and lastly wine making. On the vineyard, pesticide application contributed the majority of all toxicity-related emissions, while the use of nitrogen and phosphorous fertilizers were important contributors to eutrophication and acidification impact categories. Glass bottle production is an energy-intensive process and this contributed heavily to wine’s total energy use, as well as to global warming, human toxicity, smog-forming and acidifying emissions. Wine making was shown to be an important process for both ozone depleting and smog-forming emissions, due respectively to electricity use at the winery, and emissions of volatile organic compounds (VOCs) that are released from wine during fermentation.

A Spanish wine LCA conducted by Aranda et al. (2005) encompassing all life stages from the vineyard to recycling of the glass bottle, indicated a different life cycle hotspot for wine. Unlike Notarnicola et al. (2003) this study did not account for field-level pesticide or fertilizer emissions on the vineyard, but did include the transport of wine to retail. The transport phase, which consisted of truck transport within Europe, and container shipping overseas, resulted in the largest contributions to total life cycle impacts. Transport-related impacts were due mainly to the combustion of diesel as a fuel. Vineyard activities were the second most burdensome life cycle phase, resulting from the energy requirements of fertilizer and pesticide manufacture, and the use of an electric irrigation system. Winery processes caused the least amount of environmental impact, though impacts within this life cycle stage were dominated by the production of the glass bottle. Recycling was reported to contribute no environmental impacts to wine's total life cycle, since these authors interpreted recycling as providing a net energy benefit to the overall system, when compared to the alternative of manufacturing glass bottles from virgin materials.

Though not a formalized LCA, Ardente et al. (2006) used a life cycle framework to determine the energy use and related emissions associated with all life cycle phases (excluding disposal) of a bottle of red wine in Italy. Bottle production contributed approximately half of total energy consumption and CO_2 emissions, but refrigeration requirements during wine making, as well as the manufacture of fertilizers and pesticides were also identified as important potential improvement options. Interestingly was the fact that the modeling of bulk wine transport to domestic markets reduced the resulting energy requirements of the system by more than 50%, since glass bottles were excluded from much of the transport distance.

LCA has also been used to compare the environmental impacts of growing grapes and making wine in a small-scale organic and semi-industrial, conventional vineyard in Italy

(Pizzigallo et al., 2006). Despite lower yields in the organic system (approximately 20% lower), the overall life cycle emissions for organic grapes were lower than for grapes grown conventionally. Due to more mechanized farming practices, fuel and steel consumption were respectively 2 and 6 times greater on the semi-industrial farm, which counteracted the benefits of higher yields in this system. The results of this LCA must be viewed with the knowledge however, that production-related emissions for fertilizers were calculated only for the conventional system, and field-level fertilizer emissions in both systems were excluded entirely. If emissions related to the production and application of organic fertilizers had been calculated, a potentially much different environmental profile would have resulted. In both systems, the production of glass bottles was a life cycle hotspot, despite the organic wine's use of lighter bottles.

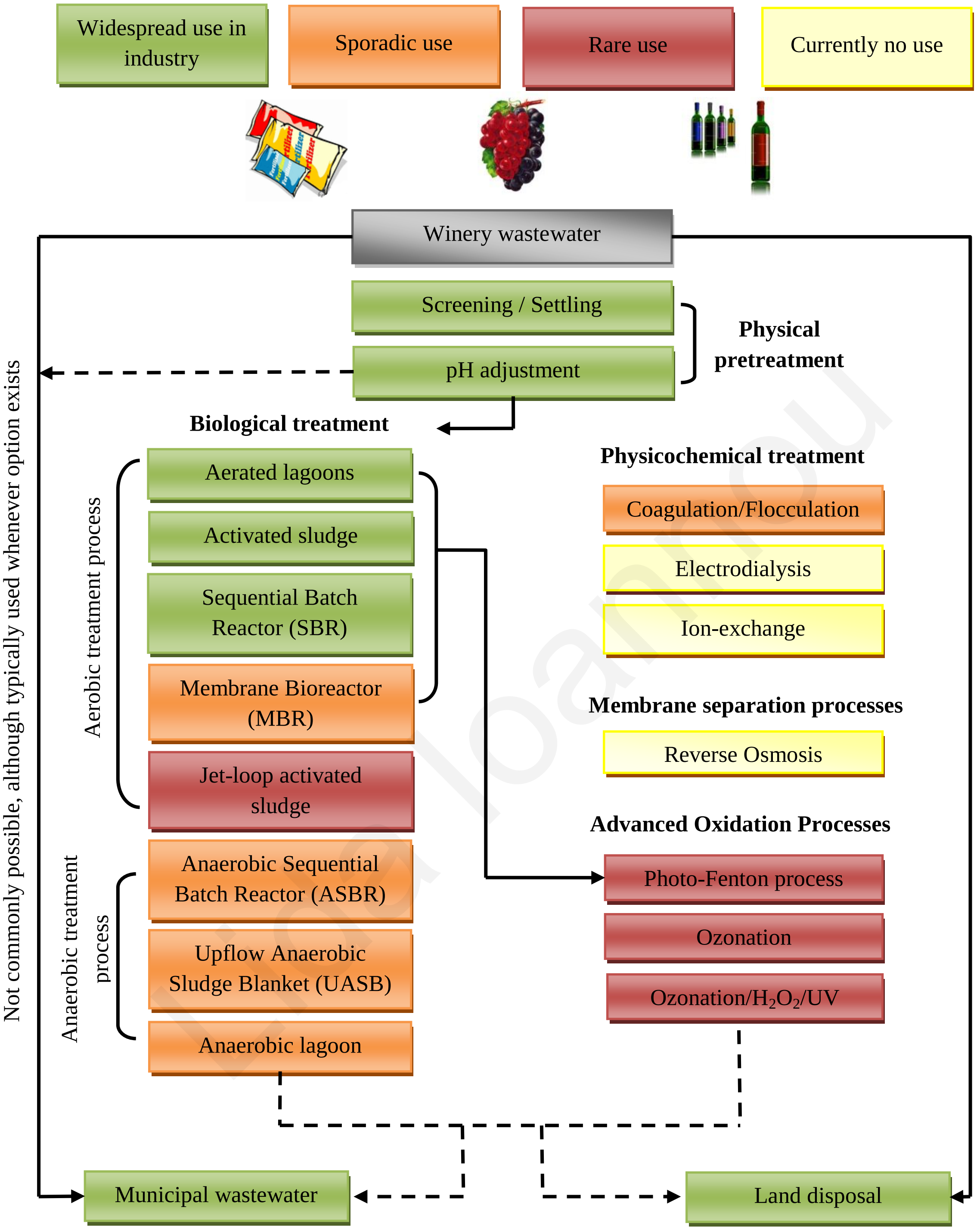
Niccolucci et al. (2006) compared these same vineyards to those of Pizzigalo et al. (2006), using Ecological Footprint Analysis. Per bottle of wine, the Ecological Footprint of the conventional system was nearly double than that of the organic wine. Finally, Nicoletti et al. (2001) also compared the life cycle impacts of organic and conventional wine. In contrast to the aforementioned studies, the organic production system was associated with higher environmental emissions in all impact categories except human toxicity and ecotoxicity. To a large extent, this was a function of grape yields since the organic system's yields were 30% lower than its conventional counterpart. In addition, authors noted higher rates of acidifying and eutrophying emissions from manure, which is used as an organic fertilizer, in comparison to synthetic fertilizers used on the conventional vineyard.

CHAPTER 3: TECHNOLOGIES CURRENTLY APPLIED FOR THE WINERY WASTEWATER TREATMENT

The combination of high organic and inorganic compounds, and the spatio-temporal dynamics of winery wastewater among and within wineries, makes the treatment of winery wastewater challenging, both at individual winery and industry levels (Mosse et al., 2011).

The high concentration of ethanol and sugars in winery wastewater justifies often the choice of a biological treatment (Bolzonella and Rosso, 2007). But the various wine processing systems applied at each winery, generates wastewater with specific properties, and therefore, meeting a general agreement on the most suitable cost-effective alternative for biological treatment of this wastewater does not seems feasible. Several winery wastewater treatment technologies are available, but the development of alternative ones is essential to increase their efficiency, and to decrease the investment and exploration costs (Coetzee et al., 2004). Hence, criteria should be considered in the selection of the adequate technology, such as maximization of removal efficiency, flexibility in order to deal with variable concentration and loads, moderate capital cost, easy to operate and maintain, small footprint, ability to meet discharge requirements for winery wastewater, and also low sludge production.

Schematic 3.1 provides an overview of the treatment methodologies that may be used for the treatment of winery wastewater, and their relative frequency of use (Mosse et al., 2011).



Schematic 3.1: Processes applied for the treatment of winery wastewater

The technologies that are used nowadays for the treatment of winery wastewater have been divided into five subcategories: physicochemical, biological, membrane filtration and separation, advanced oxidation processes (AOPs), and combined technologies. For each subcategory the main findings are being discussed along with the procedure for assessing their treatment efficiency.

3.1 Physicochemical processes for the treatment of winery wastewater

Chemical pretreatment may involve pH adjustment or coagulation and flocculation. The acidity or alkalinity of wastewater affects both wastewater treatment and the environment. Coagulation and flocculation are physicochemical processes commonly used for the removal of colloidal material or color from water and wastewater (Gregory, 2005). In wastewater treatment, coagulation constitutes the step where particles are destabilized by a coagulant, and this may include the formation of small aggregates. On the other hand, the subsequent process in which larger aggregates (flocs) are formed by the action of shear is then known as flocculation (Simate et al., 2011). After small particles have formed larger aggregates, colloidal material can then be more easily removed by physical separation processes such as sedimentation, flotation, and filtration.

Coagulation/flocculation has limitations such as high operational cost due to the chemical consumption, as well as the increased volume of generated sludge. To overcome those problems, electrocoagulation (EC) seems to be a better alternative than the conventional coagulation, as it can remove smallest colloidal particles and produce just a small amount of sludge (Kurniawan et al., 2006).

More specifically, in the case of winery wastewater, there are various physicochemical methods that have been successfully applied for their treatment, such as sedimentation with the addition of flocculants (Rytwo et al., 2011), coagulation/flocculation (Braz et al., 2010; Rizzo et al., 2010), and electrocogualation (Kirzhner et al., 2008; Kara et al., 2013), as shown in Table 3.1.

Table 3.1: Physicochemical processes applied for the treatment of winery wastewater

Winery wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
pH= 3.8-4.0 COD= 1500-17000 mg L^{-1} BOD= 1500-2500 mg L^{-1} TP= 13 mg L^{-1}	Winery wastewater after pH adjustment to 6.0 (Israel)	Electrocoagulation (EC) followed by aquatic emergent plants treatment technology (*Hydrocotyle umbellate* and *Eichhornia crassipes*)	COD, BOD and TP removal	EC of winery wastewater was conducted at 2A, and applied voltage up to 10 V. A 10 min batch treatment of winery wastewater produced 16.4-27.9% reduction of BOD, 28.2-41.9% of COD, and 89.2% of the TP. The effluent of EC with reduced organic load was then purified with aquatic emergent plants treatment technology. This facilitated further treatment by aquatic plants. At 1:1 dilution (with fresh water), 97.5 and 95.6% of the BOD were removed, after 23 days of treatment, with the floating *Hydrocotyle umbellate* and *Eichhornia crassipes* plants and aeration, respectively. Emergent plants proved also to be exceptionally effective in removing 98.2% of the COD.	Kirzhner et al., 2008
pH= 4.25-4.56 COD= 31369-38391 mg L^{-1} TSS= 3490-7660 mg L^{-1} VSS= 2820-6360 mg L^{-1} TP= 41-53 mg L^{-1} Turbidity= 319-782 NTU TPh= 1.06-1.35 mg L^{-1}	Winery wastewater from white and red wine production (Portugal)	Coagulation / flocculation process using four different coagulants ($FeSO_4$, $Al_2(SO_4)_3$, $FeCl_3$ and $Ca(OH)_2$) combined with long-term aerated storage (LTAS)	Effect of pH and coagulant dosage on COD, TSS, VSS and turbidity removal	Coagulation/flocculation allowed, within optimal conditions of pH=5.0 and coagulant dosage (30 mL), an efficient removal of turbidity (92.6%) and TSS (95.4%). However, it revealed small capacity to remove the COD load of winery wastewater, either from white or red wine production (31.5-37.9% COD removal). As an alternative a previous and economical aerobic biological process was evaluated in articulation with the coagulation/flocculation process. The LTAS was studied with two different aeration periods (2.4 h day^{-1} and 4.0 h day^{-1}) trying to reach a better significant COD removal with reduced operational costs. LTAS possibilities obtained a COD degradation of 75% in 11 weeks for an aeration period of 4 h day^{-1}. Through the combination of the LTAS process followed by the $Ca(OH)_2$ coagulant an overall removal efficiency of 84.5% was obtained for COD, 96.6% for turbidity, 98.7% for VSS, and 99.1% for TSS.	Braz et al., 2010

pH= 6.8 COD= 1550 mg L^{-1} TSS= 750 mg L^{-1} Turbidity= 180 NTU	Winery wastewater (Italy)	Coagulation using a natural organic coagulant (chitosan)	Effect of coagulant (chitosan) dosage and pH on TSS, turbidity and COD removal	The optimum chitosan dosage in terms of turbidity removal from winery wastewater was found to be 20 mg L^{-1}, which allowed reaching a residual turbidity of 36 NTU. Further increases in coagulant dosage resulted in a decreased efficiency of the process. A similar trend was observed for TSS removal. No significant difference on the performance of the coagulation process by chitosan was observed as the pH was changed; thus no chemicals addition was required to adjust pH. The efficiency of the chitosan coagulation was found to be high in terms of TSS (80%), turbidity (92%), and organic matter removal (73% in terms of COD).	Rizzo et al., 2010
Raw effluent: pH= 4.9 TSS= 1400 mg L^{-1} Conductivity= 723 mS cm^{-1} Turbidity= 130.3 NTU **pH corrected effluent:** pH= 7.0 TSS= 1600 mg L^{-1} Conductivity= 1472 mS cm^{-1} Turbidity= 163.3 NTU	Two winery wastewater flows **Raw effluent:** before pH adjustment and **pH corrected effluent:** after neutralization of pH (Israel)	Two-step sedimentation reactor with the addition of organo-sepiolite and sepiolite particles as flocculants	COD, TSS and turbidity removal	The surface charge of the remaining dispersed particles decreased to about 50% after the first step and to about 30% of the initial values after the second step. Almost complete TSS removal (98%) of both effluents was achieved for 8 cycles. The turbidity reduction was also considerable (44%), especially in the first cycle. COD reduction was only 20-40% of the initial values. The results show that ten cycles of effluent may be treated by each dose of flocculant. Thus, 8000 L of winery effluents might be cleaned with 1 kg of organosepiolite, and 1 kg of sepiolite. Considering an average TSS of 1500 mg L^{-1} effluent, a total amount of about 12 kg sludge will be transferred into sediment by 1 kg of organo-sepiolite and 1 kg of sepiolite.	Rytwo et al., 2011
pH= 5.2 COD= 25200-28640 mg L^{-1} TSS= 1240 mg L^{-1} Conductivity= 3.5 mS cm^{-1} Color= 6500 Pt-Co Turbidity= 2490 NTU	Winery wastewater (Turkey)	Electrocoagulation (EC) using aluminum (Al) and iron (Fe) electrodes	Effect of pH on COD, color and turbidity removal	The maximum removal efficiencies were found at pH 7 for Fe electrode and pH 5.2 for Al electrode with current density of 300A m^{-2} for both electrodes, and with an operating time of 90 and 120 min for Fe and Al electrodes, respectively. The removal efficiencies were found as 46.6% (COD), 80.3% (color) and 92.3% (turbidity) for Fe electrode, while they were 48.5% for COD, 97.2% for color and 98.6% for turbidity when Al electrodes were used.	Kara et al., 2013

3.1.1 Assessment of the performance of the physicochemical processes

Several physicochemical processes for the treatment of winery wastewater streams, such as coagulation/flocculation, electrocoagulation, and sedimentation with the addition of flocculants have been applied, worldwide, as shown in Table 3.1.

Coagulation is a chemical process typically used in wastewater treatment, in order to remove suspended solids, as well as to improve the removal of COD. By means of this process the tendency of small particles in an aqueous suspension to attach to one another can be significantly improved. The interaction among the particles results in the formation of larger aggregates that can be removed from the water by sedimentation and/or filtration. The effectiveness of this process depends on the coagulating agent, its dosage, the initial pH, the initial concentration, and the nature of the organic compounds present in the wastewater (Braz et al., 2010).

The pretreatment of winery wastewater by coagulation, using a natural organic coagulant, was investigated as a possible alternative to conventional metal based coagulants, in order to produce a potentially reusable organic sludge. Chitosan was chosen as a model natural organic coagulant, and the coagulation process was optimized investigating different coagulant doses and pH values. The efficiency of the chitosan coagulation was found to be high in terms of TSS (80%), turbidity (92%), as well as COD removal (73%) for winery wastewater (Rizzo et al., 2010). According to Braz et al. (2010), a similar and even higher efficiency on TSS (95.4%) and turbidity (92.6%) removal was also achieved by the application of coagulation/flocculation in winery wastewater. However, in this study, only a small part of COD (<38%) was removed by this process. The combination of long-term aerated storage (LTAS) followed by coagulation/flocculation enhanced the overall removal efficiency to 85% for COD, 99% for TSS, and 97% for turbidity (Braz et al., 2010). The study of Rytwo et al. (2011) presents a very effective two-step procedure with organo-sepiolite and sepiolite modified with crystal violet that changes the colloidal properties of winery effluents, inducing sufficient TSS (98%) and turbidity (44%) reduction, while COD reduction was only 20-40% of the initial values. The effluents after this process reached relatively low TSS (<70 mg L^{-1}, about 98% removal) and turbidity (<80 NTU, approx. 44% reduction) values, allowing implementation of filtering techniques and even disposal to regular wastewater treatment plants (WWTPs).

The removal of pollutants from effluents by electrocoagulation (EC) has become an attractive method in recent years. EC is a process whereby metallic hydroxide flocs are created within the wastewater due to the action of soluble anodes, usually iron or aluminium. The main advantages of EC are its simple and easy to operate equipment. Wastewater treated by EC often gives clear, colorless, and odorless water. In addition flocs formed by EC settle easily and they are readily de-waterable. A 10 min batch EC treatment of winery wastewater was significantly reduced the COD (up to 42%), while a moderate reduction of BOD (28%) was also observed (Kirzhner et al., 2008). In a recent study of Kara et al. (2013) the treatment of winery effluents by EC process using two different electrodes (aluminum (Al) and iron (Fe)) was investigated. The removal efficiencies of COD, color and turbidity for Fe and Al electrodes were found to be dependent on initial pH, applied current density and operating time. The optimal operating conditions were seen at pH 7 for Fe electrode and pH 5.2 for Al electrode, with current density of 300A m^{-2} and with an operating time of 90 and 120 min for Fe and Al electrodes, respectively. When Fe electrodes were used under optimal conditions, the removal efficiencies of COD, color, and turbidity were calculated as 46.6, 80.3, and 92.3%, respectively. On the other hand, when Al electrodes were used, the removal efficiencies were found as 48.5% for COD, 97.2% for color, and 98.6% for turbidity. According to these results the color and turbidity can be removed successfully from winery effluents, but COD concentration is still too high for discharge. The residual COD concentrations were 13810 and 15200 mg L^{-1} for Al and Fe electrodes, respectively; while the initial COD concentration of these effluents was in the range of 25200-28640 mg L^{-1}. Thus, the EC process, according to Kara et al. (2013) can be applied as a pretreatment step, followed by a biological or an advanced oxidation process, in order to achieve a sufficient removal of winery effluents' organic load.

From all the above, it can be noted that the physicochemical processes (i.e. coagulation/flocculation, electrocoagulation) have been found to be an effective pretreatment step for winery wastewater, and more specifically, for lowering the TSS (80-99%), the turbidity (44-98.6%), as well as the organic content (20-73%) to more acceptable levels for further treatment by other biological, membrane separation and advanced oxidation processes.

3.2 Biological processes for the treatment of winery wastewater

The biological process is based on the activity of a wide range of microorganisms, converting the biodegradable organic pollutants in the wastewater (Simate et al., 2011). Biological treatment is particularly well suited to the treatment of winery wastewater, because of the fact that the majority of the organic components in the waste stream are readily biodegradable. One of the greatest difficulties that biological treatment systems face is the variable nature of wastewater composition and the quantity (Mosse et al., 2011). This variability means that treatment plants must be able to handle fluctuations in influent composition and volumes, and allow a series of start-up and shut-down activities, which is a challenge when working with biological systems. Biological treatment of wastewater can be either aerobic (with air/oxygen supply) or anaerobic (without oxygen) (Metcalf and Eddy, 2008). The biological treatment of winery wastewater according to the international literature is based on activated sludge, jet-loop reactor (JLR), sequencing batch reactor (SBR), full-scale fixed bed biofilm reactor (FBBR) system, anaerobic digestion, upflow anaerobic sludge blankets (UASB), rotating biological contactor (RBC), aerated lagoons, and membrane bioreactor (MBR), as presented in Table 3.2.

Table 3.2: Biological processes (aerobic and anaerobic) applied for the treatment of winery wastewater

Winery wastewater initial quality characteristics	**Matrix**	**Technology characteristics**	**Measure of treatment efficiency**	**Main findings**	**References**
I. Aerobic biological processes					
pH= 7.0-12.9 COD= 2000-9000 mg L^{-1} BOD_5= 1200-6000 mg L^{-1} TSS= 200-1200 mg L^{-1} TP= 5.0-10.0 mg L^{-1} TN= 25.0-70.0 mg L^{-1} TPh= 5.8-33.6 mg L^{-1}	Winery wastewater (Italy)	Activated sludge reactor	COD, TN and TSS removal	The COD removal efficiency of the full-scale, long-term, activated sludge treatment of winery wastewater, for the entire period was very high (average 98%). The COD levels of the biologically treated effluents ranged from 50 to 130 mg L^{-1}, the concentration of nitrogen (organic and ammoniacal) always remained below 10 mg L^{-1}, and the pH levels varied between 8.3 and 8.6. The effluent was also clear, and had a content of material in suspension (TSS) below 60 mg L^{-1} and a nitrite concentration which never went above 0.20 mg L^{-1}. The plant proved to be flexible enough to withstand large variations in the hydraulic and pollutions load. Other important factors that render the running of the plant economically valid are: the small amount of sludge produced (0.065 kg TSS kg^{-1} COD); the use of settling agents, pH correctors and nutrients not needed; the low level of manpower needed to supervise the process; and the vertical design, which reduces the area occupied.	Fumi et al., 1995
COD= 5200-17900 mg L^{-1} BOD= 2500-11340 mg L^{-1} TSS= 522-5800 mg L^{-1} TVS= 742 mg L^{-1} TKN= 61-260 mg L^{-1} TP= 5.5-68 mg L^{-1}	Winery wastewater (France)	Sequential Batch Reactor (SBR)	COD, BOD_5, TKN and TP removal	The process achieved 93% removal for total COD, 95% for soluble COD, and 97.5% for BOD_5, while total nitrogen and total phosphorous removal was 50% and 88%, respectively. These results, together with the low capital costs, and the moderate operating costs showed that the process is well-suited to the depollution of wastewater from small wineries (producing 7300 hl yr^{-1}).	Torrijos and Moletta, 1997

pH= 3.5-7.5 COD= 800-11000 mg L^{-1} BOD_5= 500-6900 mg L^{-1} TSS= 200-1300 mg L^{-1} TP= 4.0-35.0 mg L^{-1} TN= 0.001-2.0 mg L^{-1} TPh= 5.8-33.6 mg L^{-1}	Winery wastewater from two different wineries (Italy)	Activated sludge systems: (a) Air bubble column bioreactor (ABB), (b) fluidized-bed bioreactor (FBB) and (c) packed-bed bioreactor (PBB)	COD, P-PO_4 removal, hydraulic retention time and MLSS (mixed liquor suspended solids)	The highest efficiency was obtained with the ABB; and more specifically, the COD removal efficiency was 92.2% (COD effluent=450 mg L^{-1}), the hydraulic retention time and MLSS were 0.8 d and 5800 mg L^{-1}, respectively; while the P-PO_4 removal efficiency was 78%. In the case of FBB system the efficiency removal was lower than ABB, and specifically it was 88.7% for COD and 71% for P-PO_4, while the hydraulic reaction time was 2.2 d and the MLSS 600 mg L^{-1}. On the other hand, 91.1% of the COD and 85% of P-PO_4 were removed with PBB; while the hydraulic retention time was 1.2 d and the MLSS was 80 mg L^{-1}.	Petruciolli et al., 2000
pH= 7.5 COD= 800-12800 mg L^{-1} TSS= 200-1300 mg L^{-1} TP= 5.0-75.0 mg L^{-1} P-PO_4= 0.3-35 mg L^{-1} N-NH_3= 0.001-2.0 mg L^{-1} N-NO_3= 0.1-0.9 mg L^{-1} TPh= 13.1-247.0 mg L^{-1}	Winery wastewater from three different wineries (Italy) after adjustment of the pH to 7.5	Jet-loop activated sludge reactor (JLR)	COD, TP, TPh removal and hydraulic retention time	The COD percentage removal was always higher than 90% with the organic load in the final effluents ranging between 110 and 300 mg L^{-1}; while the TP removal efficiency was, in general, 85% or more. The TPh removal was more than 75%; however, it decreased to approximately 65% when wastewater from a red wine making process was used with a content of TPh of 247 mg L^{-1}. Probably, the high TPh removal efficiency recorded in this study was due not only to the presence, in the activated sludge, of microorganisms able to degrade phenolic compounds (e.g. *Pseudomonas*), but also to the favorable highly oxidizing conditions created by the strong turbulence, and aeration characteristic of the jet aeration system.	Petruciolli et al., 2002
COD= 8000 mg L^{-1}	Synthetic winery wastewater (after centrifugation)	Rotating biological contactor (RBC)	COD removal and retention time	The efficiency of the RBC was satisfactory, reaching 43% COD reduction with a retention time of 1 h. Considering the seasonal fluctuations in effluents discarded by wineries, the RBC could therefore be an effective primary treatment system to lower the COD to more acceptable levels for further treatment by constructed wetlands or other biological, chemical or physicochemical processes.	Malandra et al., 2003

pH= 5.77 COD= 3828 mg L^{-1}	Winery wastewater (South Africa)	A pilot-scale rotating biological contactor (RBC)	COD removal, pH increase, retention time and biofilm recovery	On average, the RBC reduced the influent COD of the winery effluent by 23% (from 3828 to 2910 mg L^{-1}), and increased the pH by 0.95 units (from 5.77 to 6.13) at an average retention time of 1 h. After 30 days, the biofilm covered approximately 35% of the microscope slides submerged in the wastewater. Considering the winery wastewater variations and the short retention timed of 1 to 4 h, the RBC could be an effective primary treatment system to treat the peaks of high COD and acidity experienced during the harvest season, and to lower the COD to more acceptable levels for secondary treatment, by constructed wetlands, or other biological, chemical or physicochemical processes.	Coetzee et al., 2004
pH= 4.0-5.0 COD= 3100-27200 mg L^{-1} BOD_5= 210-8000 mg L^{-1} TSS= 170-490 mg L^{-1} VSS= 130-420 mg L^{-1} TP= 16.6-65.7 mg L^{-1} TN= 21.3-64.0 mg L^{-1}	Winery wastewater from white wine production (Portugal)	Jet-loop bioreactor (JLR)	COD removal, MLVSS (mixed liquor volatile suspended solids) and cell counts	The operation of a JLR for the treatment of winery wastewater for more than 1 year, induced selection of the best adapted microorganisms, maintaining a high degree of conversion and productivity, and revealing good adaptation of the microbial inocula initially developed. The resulting microbial consortium had a high capacity for COD removal (>80% biodegradation) in winery effluents. Moreover, determinations of MLVSS and cell counts also confirmed the good microbial growth obtained in the JLR. Values of 500 mg L^{-1} MLVSS and 0.2×10^{10} cell mL^{-1} measured at start-up, increased significantly during biotreatment with JLR; reaching values of 6000 mg MLVSS L^{-1} and 3.0×10^{10} cell mL^{-1} after 160 days of continuous process.	Eusebio et al., 2004

pH= 3.9-8.2 COD= 7130±3533 mg L^{-1} TSS= 692±815 mg L^{-1} P-PO_4= 6.4±7.2 mg L^{-1} N-NH_4= 21.2±24.8 mg L^{-1} N-Org= 25.1±28.8 mg L^{-1} T= 17±0.9 ^{o}C	Winery wastewater during the harvest period - grap samples after sieve and after homogenization (Italy)	Full-scale fixed bed biofilm reactor (FBBR)	COD removal	The two-stage FBBR applied for the treatment of winery wastewater achieved a high removal efficiency of total COD equal to 91% on average, reaching an effluent COD concentration equal to 212 mg L^{-1} on average, for most of the operational period. The difficulty in reaching lower COD concentration in the effluent wastewater was due to the non-biodegradable soluble fraction of COD (about 10% of total COD on average during the whole year), that cannot be removed by biological process or settling; as demonstrated from the results of respirometric tests. The 1st stage FBBR contributed for the most part to the oxidation of biodegradable COD, while the 2nd stage was built only for the refining of 1st stage effluent in the case of presence of slowly biodegradable COD or in the case of flow rate peaks.	Andreottola et al., 2005
COD= 1000-4000 mg L^{-1} TP= 1-4 mg L^{-1} TN= 5-20 mg L^{-1}	Synthetic winery wastewater - diluted white wine and nutrients (NH_4Cl, KH_2PO_4 and $NaHCO_3$) with tap water	Membrane biological reactor (MBR) - Zenon ZW-10	COD removal	A membrane biological reactor, Zenon ZW-10 (average pore size 0.04 μm and nominal surface area of 0.9 m^2), with 220 L volume, was used during 50 days for treating an influent similar to the streams generated in wineries. MBR technology applied for the treatment of winery wastewater obtained a high removal efficiency of total COD equal to 97%; reaching an effluent COD concentration equal to 60±40 mg L^{-1}. The accumulation of solids in terms of volatile suspended solids (VSS) in the reactor caused a decrease of the oxygen capacity of the system during the experimental period. Moreover, more frequent maintenance cleaning was required, when the MBR was operated at higher biomass concentration; while increases of biomass concentration did not enhance the COD in the effluent.	Artiga et al., 2005

COD= 468-4240 mg L^{-1} BOD_5= 203-2120 mg L^{-1} TSS= 315-700 kg day^{-1} Flow= 1100-2100 m^3 day^{-1}	Winery wastewater (France)	Aerobic activated sludge wastewater treatment plant	BOD_5 and COD removal	The oxygenation power needed during the grape harvest periods required the installation of an efficient aeration system, particularly in the first reactor. Hydraulic optimization of the treatment line could be achieved by the installation, with a constant volume, of two reactors instead of one (41% COD removal instead of 16%). In addition, the installation of a secondary clarifier between these two reactors improved the treatment quality, reaching reductions of 50% on BOD_5, and on COD as well.	Beck et al., 2005
COD= 2515-5480 kg day^{-1} TN= 259-367 kg day^{-1} TP= 29-40 kg day^{-1}	Mixed winery wastewater and municipal wastewater (Italy)	Full-scale activated sludge system	COD, TN removal and activated sludge production	The wastewater treatment plant considered in this work operated an extended-oxidation process during vintage (four months per year), and a pre-denitrification/oxidation process during the rest of the year. The experimentation showed that good performances, in terms of COD and nitrogen removal, could be obtained in both cases: 90% and 60%, for COD and TN removal, respectively. Thanks to the high solid retention times applied to the system (up to 48 days) the waste activated sludge production was low (0.20 kg MLVSS kg^{-1} COD removed), and respiration was the main process for carbon removal.	Brucculeri et al., 2005
pH= 3.6-4.3 **Feed 1st lagoon:** COD= 8700 mg L^{-1} **Feed 2nd lagoon:** COD= 18700 mg L^{-1}	Winery wastewater (Chile)	Pilot-scale fed-batch aerated lagoons	COD removal	Two pilot-scale aerated lagoons, operating in fed-batch mode were used for the treatment of winery wastewater. A lagoon of 27.2 m^3 working volume was gradually filled with wastewater with an average COD content of 8700 mg L^{-1}. Given that with the flow-rate used (790 L day^{-1}) this first lagoon completed its working volume after 30 days of starting, part of the liquid contained in the first lagoon was transferred to another adjacent second lagoon of 10.8 m^3 working volume. Therefore, the experiment continued in the second lagoon for another additional 24 days using an influent with a COD content of 18700 mg L^{-1} at a flow-rate of 170 L day^{-1}. A maximum COD removal efficiency of 91% was achieved after the 21st day of operation, and this value was maintained virtually constant.	Montalvo et al., 2010

pH= 2.5-6.7 COD= 5050-13448 mg L^{-1} TSS= 958-1230 mg L^{-1} TP= 14.8-39.5 mg L^{-1} TN= 34.0 mg L^{-1} Conductivity= 4.4-5.6 mS cm^{-1}	Winery wastewater (Spain)	Membrane bioreactor (MBR) pilot plant and full-scale conventional activated sludge (CAS) system	COD and TSS removal	Despite the high pollution loads of the influent wastewater, the average COD removal was 97 and 95% for MBR and CAS, respectively. The average effluent TSS concentrations reported were 2 and 26 mg L^{-1} for the MBR and CAS, respectively; confirming a higher TSS efficiency removal for the MBR process (99%). The results indicate that both CAS and MBR systems, can achieve high removal efficiencies in winery wastewater treatment, and their effluents are suitable for urban, agricultural and recreational reuse according to the quality criteria defined by the Spanish Royal Decree (1620/2007) for water reuse.	Valderrama et al., 2012
II. Anaerobic biological process					
COD= 7000-7500 mg L^{-1}	Synthetic winery wastewater - diluted white and red wine in tap water	Anaerobic digestion at bench scale	COD removal	White winery effluents (WWE) were more easily degradable (92% average soluble COD removal) than red winery effluents (RWE) (85% average soluble COD removal). With both effluents, the reactor promptly reacted to volumetric organic load (Bv) stress (tripling from 4 to 12 g COD L^{-1} day^{-1}). Following the Bv perturbation, COD removal decreased to 50%. However, after two weeks the COD removal values were as before perturbation.	Daffoncio et al., 1998
pH= 5.4-7.2 COD= 1000-17000 mg L^{-1} BOD$_5$= 100-10300 mg L^{-1} VSS= 110-8600 mg L^{-1} TP= 1.0-7.0 mg L^{-1} N-NH$_3$= 6.0-14.0 mg L^{-1} TPh= 480-1200 mg L^{-1}	Wine vinasses from distillation of low quality red and white wine - diluted with tap water	Laboratory upflow anaerobic sludge blanket (UASB)	Organic load rates (OLR), hydraulic retention time, COD and color removal and reduction of polyphenol content	The start-up and operational performance of two laboratory UASB reactors (working volume of 2.6 L) treating diluted vinasse were investigated at four difference temperatures: a) 35 ^{o}C, (run 1, without recycle), b) 19-21 ^{o}C, (run 2, without recycle), c) 18-20 ^{o}C (run 3, with recycle 1:1), and d) 9-10 ^{o}C, (run 4, with recycle 1:2.6). The reactors for run 1 and 2 were seeded with flocculants mesophilic sludge. For runs 3 and 4, the reactor seed sludge was upgraded by addition of 30% of psychrotrophically adapted granular sludge. A successful start-up of the reactors for all runs was achieved in 2-3 months. The maximum applies OLR were 15.9, 6.5, 12.5 and 7.2 g COD L^{-1} d^{-1} for runs 1-4, respectively. Hydraulic retention times at these loadings were around or less than 1 day. The total COD removal achieved under these OLR	Kalyuzhnyi et al., 2000

				was higher than 85% for the first 3 runs, and higher than 60% for run 4 with substantial decolorization of effluents; while the reduction of polyphenol content varied between 45-67%.	
COD= 8600 mg L^{-1}	Winery wastewater (Belgium)	Anaerobic sequencing batch reactor (ASBR)	COD removal, hydraulic retention time, kinetics of COD and VFA	The reactor was operated at an organic loading rate approximately 8.6 g COD L^{-1} day^{-1}, with soluble COD removal efficiency greater than 98%, hydraulic retention time of 2.2 days, and a specific organic loading rate of 0.96 g COD g^{-1}VSS day^{-1}. The kinetics of COD and volatile fatty acids (VFA) removal were investigated for winery wastewater, and for simple compounds such as ethanol, which is a major component of winery effluent, and acetate, which is the main VFA produced. The comparison of the profiles obtained with the 3 substrates shows that, overall, the acidification of the organic matter and the methanization of the VFA follow zero order reactions, in the operating conditions of this study.	Ruiz et al., 2002
pH= 5.5-7.5 COD= 2595 mg L^{-1}	Filtered winery wastewater (South Africa)	Upflow anaerobic sludge blankets (UASBs)	COD removal and hydraulic retention time	Three UASBs were evaluated for the treatment of winery wastewater: the first was seeded with granular sludge enriched with *Enterobacter sakazakii*, and reached 90% COD removal within 17 days at hydraulic retention time of 24 h; the second was seeded with brewery granules and achieved 85% COD removal within 50 days, while the third was seeded with just sludge, and showed the typical problems encountered with conventional sludge seeding and had continuously to be re-seeded.	Keyser et al., 2003

COD= 8000-22000 mg L^{-1} BOD_5= 13000 mg L^{-1} TSS= 1000 mg L^{-1}	Winery wastewater (France)	Anaerobic digestion	COD removal, biogas production and methane content in the biogas	Anaerobic digestion shows good reliability for this wastewater because generally it has low nitrogen and phosphorous, in regard of carbon content for aerobic treatment. The working pH values were 6.5 to 8, and the addition of soda helped the process. The COD removal yields for anaerobic digestion was between 65 to 95%, and the biogas production was between 400 to 600 L kg^{-1} of COD removed. The methane content in the biogas was between 60 to 70%.	Moletta, 2005
COD= 8000 mg L^{-1} Feed flow= 30 L h^{-1}	Synthetic winery wastewater - diluted wine and nutrients	Hybrid upflow sludge bed-filter anaerobic reactor (USBF)	COD removal, methane concentration in the biogas and DOC concentration in the effluent	The results presented here demonstrate the reliability of this technology for the treatment of wastewater from seasonal processes, such as winery wastewater, during a long period of time (four years). Both effluent and biogas were of good quality. Dissolved organic carbon concentration in the effluent was always lower than 100 mg L^{-1} under normal operation, while methane concentration in the biogas was in the range 70-74%, making it suitable for energy recovering. A COD removal of 96-98% and a negligible accumulation of intermediates (such as VFA) were also obtained.	Molina et al., 2007
pH= 8.0-11.0 COD=18000-21000 mg L^{-1} TSS= 150-200 mg L^{-1} TOC= 4400-5140 mg L^{-1}	Simulated winery wastewater - diluted wine and nutrients	Anaerobic fixed bed reactor	Organic load rate (OLR) and COD removal	Anaerobic fixed bed reactors with small floating supports offer great promise as high-rate systems for the treatment of high COD wastewater which typically contains readily biodegradable organics, such as winery wastewater. A maximum OLR of 42000 mg L^{-1} day^{-1} at 80% COD removal efficiency was attained in the reactor with the supports of smallest size and highest specific surface area.	Ganesh et al., 2010

3.2.1 Assessment of the performance of the biological processes

Various biological processes for the treatment of winery wastewater have been worldwide applied. They are considered environmentally friendly and, in most cases, cost-effective. Nevertheless, biological treatment is not able to remove adequately the organic matter present at high concentration levels in winery wastewater, and as a result some particularly toxic compounds may degrade so slowly as to be considered persistent if not recalcitrant. In addition, in part due to the lack of an effective monitoring parameter for the living biomass, the biological systems rarely are able to maximize efficiency. Winery effluents are generally acidic, high in COD and color, and may contain phenolic compounds that can inhibit biological treatment systems (Strong and Burgess, 2008). Thus, care needs to be taken in the selection of the microorganisms employed and in their adaptation to treating winery wastewater. Moreover, the control of biological processes is difficult, as bacterial growth is influenced by a great number of factors.

Biological wastewater treatment processes are based on the use of three types of microbial aggregates: static biofilms (e.g. in trickling filters), particulate biofilms (e.g. in upflow anaerobic sludge blanket reactors (UASB)), and flocs (e.g. in activated sludge processes) (Malandra et al., 2003; Moletta, 2005). At the broadest level, biological treatment processes can be divided into aerobic and anaerobic. It should be mentioned, that this division is important because of the differing microbial activities that occur with varying levels of oxygen availability (Metcalf and Eddy, 2008).

i) Aerobic microbiological treatment technologies

Aerobic treatment relies on oxygen to facilitate microbially-mediated breakdown of organic matter present in wastewater. Heterotrophic microorganisms utilize the carbon as an energy source, and typically convert it to biomass and CO_2 (Metcalf and Eddy, 2004). While this process is highly effective, it results in the production of large volumes of sludge (microbial biomass) that in turn requires management. Furthermore, aerobic treatment is generally tolerant of fluctuations in influent waste stream composition (Metcalf and Eddy, 2003).

There is a range of aerobic treatment systems in use in wineries, as shown in Table 3.2. The biological treatment of winery wastewater started more than twenty years ago, with activated sludge providing a simple, flexible and economical treatment for the highly variable flow and characteristics of winery effluents, achieving even 98% COD removal (Fumi et al., 1994), 85% P-PO_4 removal (Petrucciolli et al., 2000), and 50% BOD_5 removal (Beck et al., 2005). Moreover, a significantly high efficiency in the COD removal (90%) has been observed with the co-treatment of municipal and winery wastewater in a full-scale activated sludge treatment plant (Brucculeri et al., 2005). According to Torrijos and Moletta (1997) sequencing batch reactor (SBR) seems to be very effective process for the treatment of winery wastewater; yielding a 95% soluble COD elimination, 97.5% of BOD_5, and a nitrogen and phosphorous removal of 50% and 88%, respectively.

Moreover, jet-loop activated sludge reactors (JLR) appear to be promising for the treatment of winery wastewater, reaching high yields (80-90%) of organic matter removal, as well as of phenolic compounds (75% removal) (Petrucciolli et al., 2002; Eusebio et al., 2004). Although the aerobic treatment of winery wastewater using JLR was found to be technically feasible, settleability required improvement, even though it was often within acceptable limits (Petruccioli et al., 2002). The rotating biological contactor (RBC) was an effective primary biological system for lowering the COD of winery wastewater (up to 43%) to more acceptable levels for treatment by other biological, chemical or physicochemical processes (Malandra et al., 2003; Coetzee et al., 2004). The two-stage fixed bed biofilm reactor (FBBR) configuration used in the study of Andreottola et al. (2005), ensured a simple management, with high efficiency also in the case of higher fluctuations of flow and loads (91% COD removal on average), and also can guarantee a good settleability of the sludge, without bulking problems.

Additionally, winery wastewater treatment processes can rely on aerobic lagoons (fed-batch) being quite simple; and removal of organic compounds can be reasonably effective, achieving 91% COD removal in pilot studies (Montalvo et al., 2010). Fed-batch operation involves the slow addition of highly concentrated wastewater into an aerated lagoon with no effluent removal until the tank is full. The aerated lagoon usually contains a large volume of highly active and dense microorganisms at the beginning of operation with slow feeding. Concentrated or toxic wastewater is diluted inside the reactor, resulting in less inhibition and higher COD removal rates (Costa et al., 2004). Recently, the use of membrane bioreactors (MBRs) has been considered as a suitable option for winery wastewater treatment, as they combine efficiently the BOD, suspended solids, nitrogen and phosphorous removal, as well as the microbial decontamination. The advantages of the MBR system over conventional biological treatment processes include maximum flexibility of the biology according to the influent loadings, small footprint, a reduced sludge production and a compact system with better solids removal (99%), and disinfection. As a consequence, the effectiveness of MBR systems for the treatment of winery wastewater was found to be high (>97%) (Artiga et al., 2005; Valderrama et al., 2012).

Overall, aerobic treatment systems are commonly used for the treatment of winery wastewater, because of their high efficiency and ease of use. Also the versatility of the process suits different sized operations. In addition, the fundamental process is well established, which simplifies troubleshooting (Strong and Burgess, 2008). Disadvantages include the higher capital cost for aeration equipment (to maintain the dissolved oxygen (DO) levels needed to maintain aerobic conditions in the treated wastewater for aerobic growth), the higher operating cost (particularly energy for pumps or aerators), higher maintenance requirements, and possibly monitoring requirements for checking the DO level in the liquid (LaPara and Alleman, 1999). Moreover, it should be noted that the residual COD of the winery wastewater of aerobic biological treatment most often ranges from 212 to 5440 mg L^{-1}, a range that is higher than the international environmental limits, and as a consequence, a further treatment before its disposal into the environment may be required.

ii) Anaerobic microbiological treatment technologies

As shown in Table 3.2, winery wastewater may also be treated using anaerobic treatment processes. Anaerobic digestion is carried out in the absence of molecular O_2 by a series of anaerobic microorganisms, mainly bacteria, which have lower growth rates than aerobic microorganisms. The processes involved in the anaerobic digestion are: (a) hydrolysis, in which organic polymers (proteins, lipids, carbohydrates) are converted to organic monomers (amino acids, fatty acids, glycerol, sugars); (b) acidogenesis and acetogenesis, in which organic monomers are converted to acetate, carbon dioxide and hydrogen gas; and (c) methanogenesis, in which acetate, carbon dioxide and hydrogen gas are converted to methane (Metcalf and Eddy, 2003).

The major advantages of using anaerobic treatment are the nil energy requirements; the fact that the anaerobic microbes are slower to grow, meaning that the biomass (i.e. sludge) yields are much lower, the fact that the anaerobic organisms can be preserved unfed for long periods of time (exceeding one year) without any serious deterioration of their activity, and the fact that the methane is produced as a waste gas, which can subsequently be recovered as an energy source (Lettinga, 1995). The most significant disadvantage of the anaerobic process is that it produces a variety of volatile fatty acids (VFAs), which are responsible for the malodor of winery wastewater (Bories et al. 2005). Odor emission can be controlled by the addition of nitrate salts to winery wastewater, to act as an alternative electron acceptor and thus prevent the formation of VFAs (Bories et al. 2007).

According to the results reported in the literature, anaerobic sequencing batch reactor (ASBR) can achieve a COD removal efficiency greater than 98%, with hydraulic retention time of 2.2 days, and a specific organic loading rate of 0.96 g COD g^{-1} VSS day^{-1} (Ruiz et al., 2002). Additionally, an upflow anaerobic sludge blanket (UASB) proved to be an effective option for the treatment of winery wastewater (>90% COD removal), not involving the production and subsequent removal of excess sludge, which greatly contributes to the total operating costs (Keyser et al., 2003). Moreover, UASB reactors were found able to reach a substantial decolorization of winery wastewater, while they were able to reduce the phenolic compounds up to 67% (Kalyuzhni et al., 2000). According to Daffoncio et al. (1998) and Moletta, (2005) the removal yield for anaerobic digestion of winery wastewater can be very high, up to 85-95% COD removal. The biogas production was found to be between 400 and 600 L kg^{-1} COD removed, with 60 to 70% methane content (Moletta, 2005).

Moreover, the upflow sludge bed-filter anaerobic reactor (USBF) presented very short start-up periods after a short and long shut-down of the wastewater treatment plant, and rapidly turned back to normal operation after suffering a complete destabilization due to organic overload (Molina et al., 2007). A COD removal of 96-98% and a negligible accumulation of intermediates (such as VFA) were obtained; while methane concentration in the biogas was in the range 70-74%, making it suitable for energy recovering (Molina et al., 2007). Finally, the anaerobic fixed-bed reactors can offer great promise as high-rate systems for the treatment of winery wastewater, reaching a maximum removal efficiency of 80% (measured as COD) (Ganesh et al., 2010).

It should be mentioned, that also in the case of the anaerobic biological treatment, the residual COD of the winery effluents ranged from 240 to 4400 mg L^{-1}, or even 7700 mg L^{-1}, leading to the conclusion that a further post-treatment is required before its disposal in the environment.

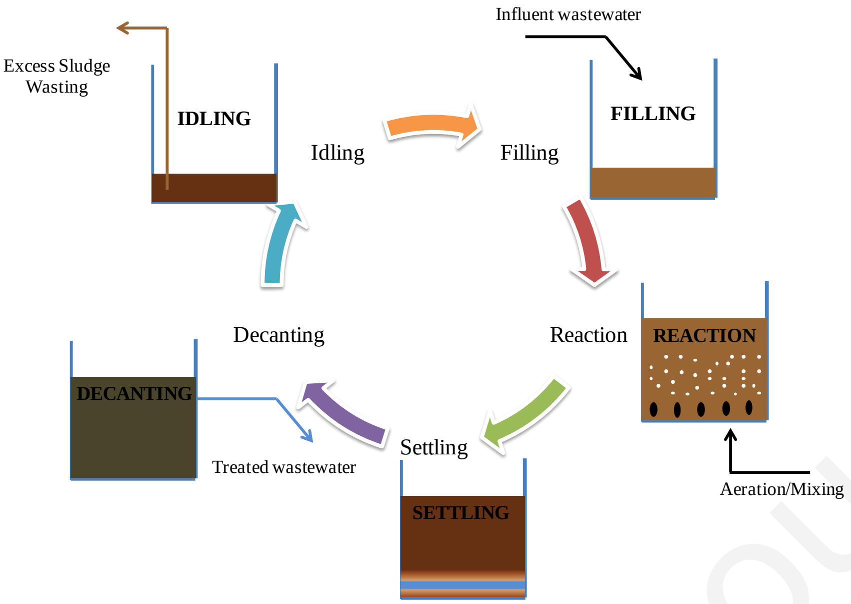
3.2.2 Biological processes applied in this thesis

In this thesis, two biologically pretreated winery flows were further subjected to advanced oxidation treatment. The first flow was a winery effluent obtained from a Sequential Batch Reactor (SBR), and the second one from a Membrane Bioreactor (MBR). The operating principles of these two biological processes (SBR and MBR), the main characteristics, their successful applications for the treatment of various wastewater streams, as well as their advantages and disadvantages are presented in detail in the following sections.

3.2.2.1 Sequential Batch Reactor (SBR)

Sequential or Sequencing Batch Reactor (SBR) is the name given to wastewater treatment systems based on activated sludge, operated on a sequence of fill-and-draw cycles. These systems normally include a biological nutrient removal process. The unit operations involved in an SBR are equivalent to those of conventional activated sludge systems. Aeration and sedimentation-clarification therefore take place. The difference between the systems is that in conventional systems, these two processes take place in two different tanks whereas in SBR systems, they occur sequentially in the same tank (Mace and Mata-Alvarez, 2002).

SBR is designed to operate under non-steady state conditions. The SBR operates in a true batch mode with aeration and sludge settlement both occurring in the same tank (Norcross, 1992). The process consists of repetition of a cycle including five separate steps: filling, reaction, settling, decanting and idling, as shown in Schematic 3.2.



Schematic 3.2: Major stages of the SBR operation cycle

Filling - During the fill phase, the tank receives influent wastewater. The influent brings food to the microbes in the activated sludge, creating an environment for biochemical reactions to take place. Mixing and aeration can vary during the fill phase to create the following two different scenarios (Wilderer et al., 2001; Poltak, 2005):

Anoxic Filling (or Mixed Filling) - The influent wastewater is distributed throughout the settled sludge through the influent distribution manifold to provide good contact between the microorganisms and the substrate. Most of this period occurs without aeration to create an environment that favors the procreation of microorganisms with good settling characteristics. Aeration begins at the beginning of the next period.

Aerated Filling - Mixed liquor is drawn through the manifold, mixed with the influent flow in the motive liquid pump, and discharged, as motive liquid, to the jet aerator (Norcross, 1992). This initiates the feast period. Feast is when the microorganisms have been in contact with the substrate, and a large amount of oxygen is provided to facilitate the substrate consumption. Nitrification and denitrification occurs at the beginning of this stage, and this period ends when the tank is either full or when a maximum time for filling is reached.

Reaction - This phase allows for further reduction or "polishing" of wastewater parameters. During this period aeration continues until complete biodegradation of BOD and nitrogen is achieved. After the substrate is consumed, famine stage starts. During this stage some microorganisms will die because of the lack of food and will help reduce the volume of the settling sludge. The length of the aeration period determines the degree of BOD consumption (Chang and Hao, 1996; Poltak, 2005).

Settling - Aeration is discontinued at this stage and solids separation takes place leaving clear, treated effluent above the sludge blanket. During this clarifying period no liquids should enter or leave the tank to avoid turbulence in the supernatant (Norcross, 1992). This phase is a critical part of the cycle, because if the solids do not settle rapidly, some sludge can be drawn off during the subsequent decant phase, and thereby degrade effluent quality (Wilderer et al., 2001; Poltak, 2005).

Decanting - This period is characterized by the withdrawal of treated effluent from approximately two feet below the surface of the mixed liquor by the floating solids excluding decanter. This removal must be done without disturbing the settled sludge (Wilderer et al., 2001).

Idling - The period between Decant and Fill is termed Idle. The time in this stage can be used to waste sludge or perform backwashing of the jet aerator. The wasted sludge is pumped to an anaerobic digester, in order to reduce the volume of the sludge to be discarded. The frequency of sludge wasting ranges between once each cycle to once every two to three months depending upon system design (Chang and Hao, 1996; Poltak, 2005; Al-Rekabi et al., 2007).

SBR systems contain either two or more reactor tanks that are operated in parallel, or one equalization tank and one reactor tank. The type of tank used depends on the wastewater flow characteristics (e.g. high or low volume). The majority of the aeration equipment of SBRs consist of jet, fine bubble, and coarse bubble aeration systems (Poltak, 2005).

SBRs have been sucessfully used to treat municipal and industrial wastewater (Mahvi, 2008) including textile (Shaw et al., 2002), piggery (Obaja et al., 2003), brewery (Ling and Lo, 1999), winery (Torrijos and Moletta, 1997), malting (Schwarzenbeck et al., 2004), coke (Maranon et al., 2008), dairy wastewater (Garrido et al., 2001), grey water (Shin et al., 1998) and landfill leachate (Neczaj et al., 2005). A brief review of successful SBR applications for the treatment of the most relevant effluents to winery wastewater is presented in Table 3.3.

Table 3.3: Applications of Sequencing Batch Reactor technology (SBR) for the treatment of various wastewater streams

Wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
pH= 6.1-9.5 COD= 1038-4524 mg L^{-1} BOD_5= 671-4200 mg L^{-1} TOC= 677-1720 mg L^{-1} TSS= 450-1044 mg L^{-1} TKN= 28-343 mg L^{-1} TP= 6.3-56 mg L^{-1} NH_3-N= 0-4 mg L^{-1}	Brewery wastewater (Canada)	Bench-scale aerobic sequencing batch reactors (SBRs), in both suspended-growth and attached-growth modes	Effect of hydraulic retention time (HRT) on TSS, COD, TOC and BOD_5 removal	Experiments were conducted over a wide range of HRT, from 0.56 to 6.06 days. In the suspended-growth SBRs, the HRT had little effects on the TOC percentage removal (>96%); while in the case of attached-growth SBRs, the TOC removal increased with increased HRT, from 82 to 94%. Moreover, the overall treatment efficiency of suspended-growth reactors (97.3% for TSS, 97.1% for COD, and 99% for BOD_5) was significantly better than that of the attached-growth reactors (85.9% for TSS, 89.6% for COD, and 96.6% for BOD_5).	Ling and Lo, 1999
pH= 8.1 total COD= 10-20 g L^{-1} soluble COD= 2.9-6.6 g L^{-1} TS= 6.5-12.4 g L^{-1} VS= 5.1-10.1 g L^{-1} TKN= 0.8-1.1 g L^{-1} TN= 0.8-1.1 g L^{-1} NH_3-N= 0.53-0.54 g L^{-1}	Dairy wastewater (USA)	SBR combined with complete-mix biofilm reactor (CMBR)	Effect of hydraulic retention time (HRT) on COD, TS, VS, TKN and TN removal	A single-stage SBR system was tested with 10000 mg L^{-1} COD influent at three HRTs of 1, 2, and 3 days, and 20000 mg L^{-1} COD influent at four HRTs of 1, 2, 3, and 4 days. A 1-day HRT was found sufficient for treating 10000 mg L^{-1} COD wastewater, with the removal efficiency of 80.2% COD, 63.4% TS, 66.2% VS, 75% TKN, and 38.3% TN from the liquid effluent. A 2-day HRT was found sufficient for treating 20000 mg L^{-1} COD dairy wastewater (67% COD) if complete ammonia oxidation is not desired. However, 4-day HRT needs to be used for achieving complete ammonia oxidation. A two-stage system consisting of an SBR and a complete-mix biofilm reactor was capable of achieving complete ammonia oxidation, and comparable COD (86%), total solids (69%), and nitrogen (92%) removal; while using at least 1/3 less HRT as compared to the single SBR system.	Li and Zhang, 2002

pH= 8.4 COD= 3969 mg L^{-1} BOD_5= 1730 mg L^{-1} TS= 11210 mg L^{-1} TSS= 2580 mg L^{-1} VSS= 1960 mg L^{-1} TP= 171 mg L^{-1} TN= 1700 mg L^{-1} NH_4^+-N= 1500 mg L^{-1} PO_4^{3-} -P= 144 mg L^{-1}	Piggery wastewater (Spain)	SBR	Effect of temperature on TN, TP, NH_4^+-N, PO_4^{3-}-P and COD removal	The SBR was operated with 3 cycles per day, temperature 30 °C, sludge retention time (SRT) 1 day, and hydraulic retention time (HRT) 11 days. With a wastewater containing 1500 mg L^{-1} ammonium and 144 mg L^{-1} phosphate, a removal efficiency of 99.7% for nitrogen and 97.3% for phosphate was obtained. Experiments set up to evaluate the effect of temperature on the process showed that it should be run at temperatures higher than 16 °C to obtain good removal (>95% TN and TP). Even with the recalcitrant character of the substrate COD, a reduction of the COD of around 70.2% was observed. The SBR proved to be a very flexible tool, and was particularly suitable for the treatment of piggery wastewater, characterized by high nutrient content and by frequent changes in composition, and therefore affecting process conditions.	Obaja et al., 2003
total COD= 1700 mg L^{-1} soluble COD= 470 mg L^{-1} BOD_5= 600 mg L^{-1} MLSS= 950 mg L^{-1} TKN= 45 mg L^{-1} NH_4^+-N= 3 mg L^{-1} Settleable matter= 15 mL L^{-1}	Malting wastewater (Germany)	SBR	Effect of granular and flocculent sludge on COD removal	At an organic loading rate (OLR) of 3.2 kg m^{-3} day^{-1} total COD an average removal of 50% in total COD, and 80% in soluble COD could be achieved. A comparison of granular and flocculent sludge grown under the same operating conditions showed no significant difference in removal efficiency, although granules exhibited a higher metabolic activity in terms of specific oxygen uptake rate. Two distinct mechanisms of particle removal were observed for granular sludge: during initial granule formation, particles were incorporated into the biofilm matrix. For mature granules, a high level of protozoa growth on the granule surface accounted for the ability to remove particulate COD.	Schwarzenbeck et al., 2004

COD= 1100-1700 mg L^{-1} VSS= 1500-2900 mg L^{-1} NH_4^+-N= 401-750 mg L^{-1} TPh= 185-253 mg L^{-1} SCN= 210-487 mg L^{-1}	Coke wastewater (Spain)	SBR at pilot scale	Effect of hydraulic retention time (HRT) on COD, NH_4^+-N, SCN and phenols removal	The treatment of coke wastewater was studied using a pilot plant composed of a stripping unit, a homogenization tank, and a biological reactor operated in sequencing batch mode. NH_4^+-N removal by stripping is influenced by the HRT employed, efficiencies of 90% being obtained for HRT of 66 h. After stripping and subsequent neutralization with H_2SO_4, the biodegradation of pollutants in an SBR led to removal efficiencies higher than 69%, 98%, and 90% for COD, phenols and SCN, respectively, even for the lower HRT (58 h). Increasing the hydraulic retention time, higher removals were achieved, especially in COD (up to 90% for HRT of 225 h).	Maranon et al., 2008

One of the advantages of these batch SBR systems is that they can easily be adapted for continuous variations of pollutant concentrations (Mace and Mata-Alvarez, 2002). Other advantages of SBRs are that equalization, primary clarification, biological treatment, and secondary clarification can be achieved in a single reactor vessel. These advantages can reduce the treatment area and the cost. The SBR process is versatile as it can be used, in addition to BOD removal and nitrification, for nitrate removal and nitrogen and phosphorous removal (up to 97%), mainly by altering the length or operation of the aeration cycle, thus enabling anaerobic, anoxic, or aerobic conditions to occur in accordance with the treatment objectives. Moreover, the SBR system can remove heavy metal such as Zn^{2+}, Cu^{2+}, Pb^{2+} and Ni^{2+} (Obaja et al., 2003; Metcalf and Eddy, 2008). In Table 3.4 the advantages and disadvantages of SBR systems are summarized.

Table 3.4: Advantages and disadvantages of SBR systems

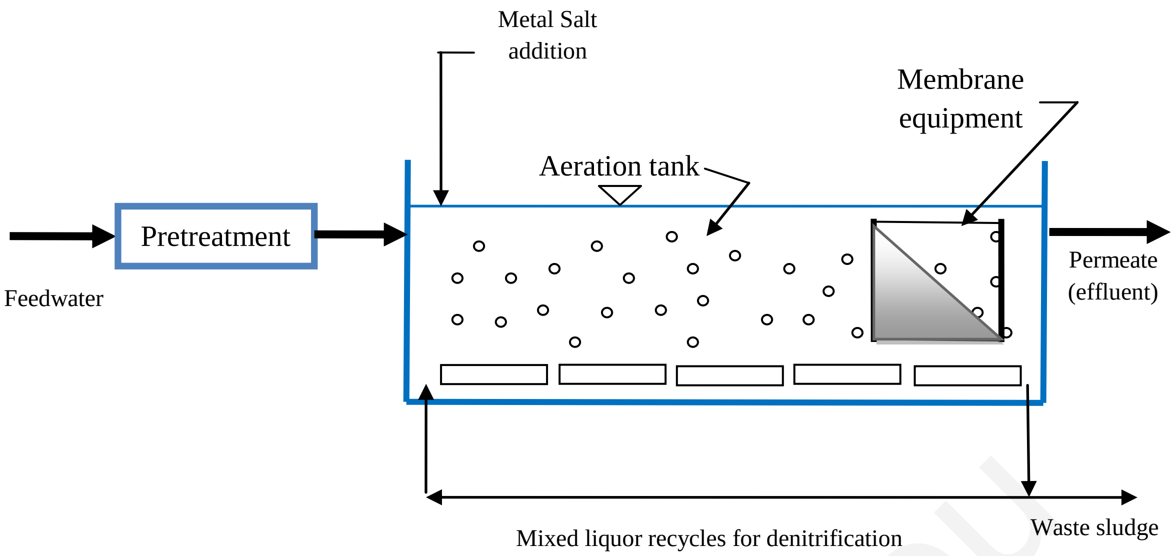
Advantages	Disadvantages
▪ Compact facility ▪ Final clarifiers and returned activated sludge (RAS) pumping are not required ▪ Operation is flexible ▪ Nutrient removal can be accomplished by operational changes ▪ Quiescent settling enhances solid separation (low effluent TSS) ▪ Economical process for small wastewater treatment plants ▪ Less land required than conventional methods ▪ Easier to control filamentous growth and settling problems	▪ Plant capacity expansion is more difficult ▪ Process control is more complicated ▪ Batch discharge may require equalization prior to filtration and disinfection ▪ Redundant units required for continuous flow operation ▪ Equalization units after SBR may be required, depending on the downstream processes

Sources: Ndon and Dague, 1997; Mace and Mata-Alvarez, 2002; Linlin et al., 2005; Mohan et al., 2005; Poltak, 2005; Mahvi, 2008; Metcalf and Eddy, 2008

3.2.2.2 Membrane Bioreactor (MBR)

One of the newer and most promising technologies for utilization in water reuse systems is the Membrane Bioreactor (MBR). Membrane bioreactors combine biological treatment with an integrated membrane system to provide enhanced organics and suspended solids removal. Membranes replace sedimentation and depth filtration for separating the biomass in suspended growth systems from the treated water (Metcalf and Eddy, 2008). According to Hai et al. (2011), MBR is an absolute barrier to solids and microorganisms, thus providing a removal system of a high MLSS concentration, which can reach 30 g L^{-1} in industrial applications.

The MBR process is a suspended growth activated sludge system that utilises microporous membranes for solid/liquid separation in lieu of secondary clarifiers (Simate et al., 2011). Membrane bioreactors come in several different configurations because most MBRs are of proprietary design and have distinctively different features. The typical arrangement shown in Schematic 3.3 includes submerged membranes in the aerated portion of the bioreactor and internal mixed liquor recycles (Metcalf and Eddy, 2008).



Schematic 3.3: Description of the membrane bioreactor (MBR) system

The performance of the MBR process is determined by the configuration of the membranes, which is determined by the geometry (planar or cylindrical), mounting and orientation in relation to the flow of water. Membranes can be immersed into the biological tank of the wastewater treatment plant (as shown in Schematic 3.3), or can be located in a different container directly linked to the tank. The feed to the aeration tank is pressurized and circulated through the tank module with the use of a pump. A valve assists in the accumulation of the wastewater constituents on the membranes. Floc forming and dispersed microorganisms are kept in the MBR system which biodegrade and transform pollutants.

The commercial configuration of MBR membranes has three principal set-ups, the flat sheet (FS), the hollow fibre (HF), and the multi-tubular (MT). The choice of membrane and system configuration is optimised when the factors of minimizing clogging and deterioration are considered (Simate et al., 2011).

Membranes can be classified according to their material of construction. The constituent material of MBR is ceramic or polymeric, and organic. The main materials of membranes include polypropylene, cellulose acetate, aromatic polyamides, and thin-film composite. Composite membranes have thin cellulose acetate bonding, polyamide or another active layer (mainly 0.15-0.25 μm), and a thicker porous substrate which provides stability (Gregory, 2005).

The highest efficiency has been noted in MBR with HF configuration operating in the ultrafiltration (UF) range, with an efficiency higher than 90%, as it is most efficient in comparison with nanofiltration (NF) and microfiltration (MF) range in the removal of biodegradable organics, hardness, heavy metals, nitrates, synthetic organic compounds and viruses (Metcalf and Eddy, 2003).

The initial concentration and removal efficiency of pollutants are not directly associated, but chemical and physical properties of pollutants are highly associated to their removal efficiency.

The primary removal of particles from wastewater in MBR is achieved by sieving with the application of hydraulic force on the wastewater through the membrane. All large molecules are gathered by the membrane as they cannot pass through, and can be collected. In this way the separation of suspended solids is not restricted only to the sludge settling characteristics, but to the filtration characteristics of the MBR as well, which will define the separation efficiency. The membrane has to be strong to hold the hydraulic pressure exerted by the wastewater shear force and the pore size. The membrane material is the parameter to determine the filtration efficiency. Because the membrane units have small pore sizes, usually ranging from 0.04 to 0.4 μm, highly clarified product water is produced that is low in BOD, TSS, turbidity, and bacteria (Metcalf and Eddy, 2008). It also concentrates the biomass, which results in a reduced tank size and increase in the efficiency of the bio-treatment process. This moreover, removes the need for sedimentation removal of solids. The treated water is of high purity in respect to dissolved constituents such as organic matter, and ammonia, which are significantly removed (Metcalf and Eddy, 2003; Radjenovic et al. 2007).

MBR systems have mostly been used to treat various industrial wastewater (Yang et al., 2006), including winery (Artiga et al., 2005), beverage (Jakopović et al., 2008), brewery (Dai et al., 2010), dairy (Bae et al., 2003), food industry (Katayon et al., 2004), piggery (Shin et al., 2005), olive mill (Dhaouadi and Marrot, 2008), hospital (Wen et al., 2004), saline (Artiga et al., 2008), coke plant (Zhao et al., 2009), as well as household (Abegglen et al., 2008) and municipal wastewater (Xing et al., 2001). In Table 3.5 the applications of the MBR systems for the treatment of agricultural and food industries effluents are presented.

Table 3.5: Applications of membrane bioreactor technology (MBR) for the treatment of various wastewater streams

Wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
pH= 6.5-7.5 BOD= 2604 mg L^{-1} TSS= 413 mg L^{-1} MLSS= 8100 mg L^{-1} TKN= 136 mg L^{-1} TP= 56.8 mg L^{-1} Temperature= 22-26 ^{o}C	Dairy wastewater (Korea)	Membrane sequencing batch reactor (MSBR)	BOD, TSS, TKN and TP removal	A membrane separation process was coupled to a sequencing batch reactor (SBR) for biological nutrient removal. This combined system was named membrane sequencing batch reactor (MSBR). MSBR was used for the treatment of dairy industry wastewater and optimized to increase the treatment efficiency. BOD removal was high (97-98%) and stable. TSS-free effluent was obtained by membrane separation (100% TSS removal). Since nitrogen was mainly consumed as nutrient for synthesis of new cells due to the low influent concentration, the removal rate reached 96%. Phosphorous removal was relatively low because of the limit of the biological process, i.e. removal efficiency ultimately depends on the amount of excess sludge wasting. A TP removal rate of 80% was reached after system optimization.	Bae et al., 2003
pH= 3.9 COD= 880 mg L^{-1} BOD= 680 mg L^{-1} TS= 2480 mg L^{-1} VTS= 2110 mg L^{-1} MLSS= 4340-10780 mg L^{-1} Turbidity= 2321 NTU Temperature= 30.5 ^{o}C	Food industry wastewater (Nestle factory) (Malaysia)	MBR with microfiltration hollow-fibre membrane	Influence of initial MLSS concentration and position of the membrane module on TSS and turbidity removal and flux	Two experimental procedures consisting of low (4340-5390 mg L^{-1}) and high (6330-10780 mg L^{-1}) MLSS concentrations were set up. The process at low MLSS concentrations provided a higher degree of treatment in terms of suspended solids (99.2%) and turbidity (99.7%) removal. The mean flux value of process at low MLSS concentrations (5.03 L m^{-2} h^{-1}) was found higher than those at high MLSS concentrations (2.27 L m^{-2} h^{-1}). Furthermore, the effectiveness of MLSS concentrations was greatly influenced by the position of the membrane modules. Higher turbidity and suspended solids removal, as well as higher flux values were obtained with a horizontally positioned membrane module compared to a vertically placed membrane module.	Katayon et al., 2004

pH= 5.21 COD= 117.6 g L^{-1} TSS= 7.65 g L^{-1} N-NO$_3^-$ =41 mg L^{-1} N-NH$_4^+$= 32 mg L^{-1} Total salinity= 18.46 g L^{-1} TPh= 6.32 g L^{-1} VFAs= 9.78 g L^{-1}	Diluted solutions of olive mill wastewater (OMW) (Tunisia)	External ceramic microfiltration MBR	COD, TSS and phenolic compounds removal	The used reactor, equipped with an external ceramic microfiltration membrane gave stabilized permeate flux, around 92 L h^{-1}m^{-2}, with zero suspended solids and no phenolic compounds. No fouling problems occurred during the experiments. The short backpulse method adopted allowed the use of the MBR in a continuous way. The COD removal achieved ranged from 97 to 99%. However, the COD remained quite high 285 to 3339 mg L^{-1}, and its abatement improvement could have been achieved by enhancing the oxygen transfer to the mixed liquor contained in the MBR.	Dhaouadi and Marrot, 2008
Beverage wastewater: pH= 7.06 COD= 722 mg L^{-1} BOD=232 mg L^{-1} TOC= 194 mg L^{-1} **Vegetable oil wastewater:** pH= 6.83 COD= 677 mg L^{-1} TSS= 210 mg L^{-1} Oil and grease= 172 mg L^{-1} Conductivity= 1470 μS cm^{-1}	Food industry wastewater - from beverage and vegetable oil production (Croatia)	MBR combined with activated sludge process	COD, TOC, BOD$_5$ and oil and grease removal	In beverage production, MBR was found able to achieve effective and stable organic compounds removal from the wastewater (94% for COD, 94% for TOC, and 98% for BOD$_5$) during the experiment. The average percent of the COD removal for treatment of wastewater from vegetable oil production was 75%, while oil and grease was removed with an average of 86%. The best treatment efficiency was achieved by treatment of mixed wastewaters from all sources, where COD was removed with 91%, and oil and grease with 95% efficiency. Activated sludge was successfully kept in the bioreactor by immersed membrane so that stable concentration of activated sludge biomass could be maintained in spite of large fluctuations in the composition of the wastewater.	Jakopovic et al., 2008
pH= 7.94-8.12 COD= 30200-40000 mg L^{-1} TS= 165020 mg L^{-1} TSS= 13600 mg L^{-1} Conductivity= 27.2 mS cm^{-1} Ash= 544 mg L^{-1} Color= 76894-82345 Co-Pt unit	Molasses-based distillery wastewater (India)	Bench-scale MBR	Effect of different organic load rates on COD, color and TSS removal	Organic loading rates ranging from 3 to 5.71 kg m^{-3} day^{-1} were investigated and the performance was analyzed in terms of the COD removal. Up to 41% COD removal was obtained over 245 days of reactor operation. The molecular weight profiles of the untreated and treated effluent suggested that the degradation of low molecular weight compounds occurred in the reactor, while the high molecular weight compounds comprising the color imparting melanoidins remained unaffected. Up to 100% suspended solid retention (average 87%) was obtained, and the system could be operated up to 2 weeks	Satyawali and Balakrishnan, 2008

				without significant flux drop.	
pH= 7.4 COD= 2372 mg L^{-1} TN= 351 mg L^{-1} NH_3-N= 266 mg L^{-1} TPh= 566 mg L^{-1} Turbidity= 198 NTU Alkalinity= 438 mg L^{-1} Temperature= 34 ^{o}C	Coke plant wastewater (China)	Bench-scale anaerobic-anoxic-oxic MBR (A_1/A_2/O-MBR) system	COD, phenol, NH_3-N, TN removal and acute toxicity (using luminescent bacteria *Photobacterium phosphoreum*)	When the total hydraulic retention time of the MBR system was 40 h, the average effluent COD, phenol, NH_3-N, TN concentrations and acute toxicity were 264 mg L^{-1}, 0.2 mg L^{-1}, 0.8 mg L^{-1}, 112 mg L^{-1} and 0.17 mg L^{-1}, with removal of 89.8%, >99.9%, 99.5%, 71.5% and 98.3%, respectively. Hydrophobic/hydrophilic fractionation indicated that the hydrophobic acids were the most abundant fraction of dissolved organic matters in influent and effluent; accounting for 70.3% and 67.2% of total DOC, and 75.0% and 76.2% of total color intensity, in influent and effluent, respectively. The hydrophilic substances of the oxic supernatant could be rejected effectively by the membrane. Fluorescence excitation-emission matrix (EEM) analysis suggested that humic substance-like matters were potentially refractory and color causing matters in coke plant wastewater.	Zhao et al., 2009
Influent UASB: COD= 2000-2500 mg L^{-1} UASB Flow rate= 208 m^3 h^{-1} **Influent MBR:** COD= 500-1000 mg L^{-1} NH_3-N= 20-30 mg L^{-1} TP= 0.6-14 mg L^{-1} TN= 19.5-41.1 mg L^{-1}	Brewery wastewater (China)	MBR combined with up-flow anaerobic sludge bed reactor (UASB)	COD, NH_3-N and TP removal	The brewery applied UASB process as pretreatment, achieving a COD removal between 60 to 75%; and then the UASB effluent entered the MBR system for more intensive treatment. MBR removal efficiency was high, and specifically for COD was 96%, for NH_3-N 92%, and for TP up to 96%. The quality of the effluent water in this system met the water quality standards for reuse of urban recycling and landscaping water.	Dai et al., 2010

The advantages offered by MBR have been well documented in the literature, such as excellent effluent quality, low sludge production, high treatment efficiency and small footprint (Liang et al., 2007; Gil et al., 2010). However, just like other membrane separation processes, membrane fouling remains the most serious problem for the widespread application of MBR systems (Gil et al., 2010). In Table 3.6 the advantages and disadvantages of MBR systems are summarized in more detail.

Table 3.6: Advantages and disadvantages of MBR systems

Advantages	Disadvantages
▪ Secondary clarifiers and tertiary filtration processes are eliminated (i.e. footprint is reduced) ▪ Unlike secondary clarifiers, the quality of solids separation is not dependent on the mixes liquor suspended solids (MLSS) concentration or characteristics. Since elevated mixed liquor concentrations are possible, the aeration tank volume can be reduced, further reducing the plant footprint ▪ No reliance upon achieving good sludge settleability, hence quite amenable to remote operation ▪ Can be designed with long sludge age, hence low sludge production ▪ Produces a MF/UF quality effluent suitable for reuse applications or as a high quality feed water source of RO treatment ▪ The resultant small footprint can be a feature used to address issues of visual amenity, noise and odor ▪ Good retention of all microorganisms and viruses ▪ Maintenance of high biomass concentration and real compactness ▪ Simple operation	▪ High capital costs for the membrane modules ▪ Limited data on membrane life, thus a potential high recurring cost of periodic membrane replacement ▪ Higher energy costs due to membrane scouring as compared to conventional suspended-growth processes ▪ Potential membrane fouling that affects the ability to treat design flows ▪ Waste sludge from the membrane process may be more difficult to dewater ▪ Aeration limitations

Sources: Artiga et al., 2005; Liang et al., 2007, You et al., 2007; Metcalf and Eddy, 2008; Gil et al., 2010

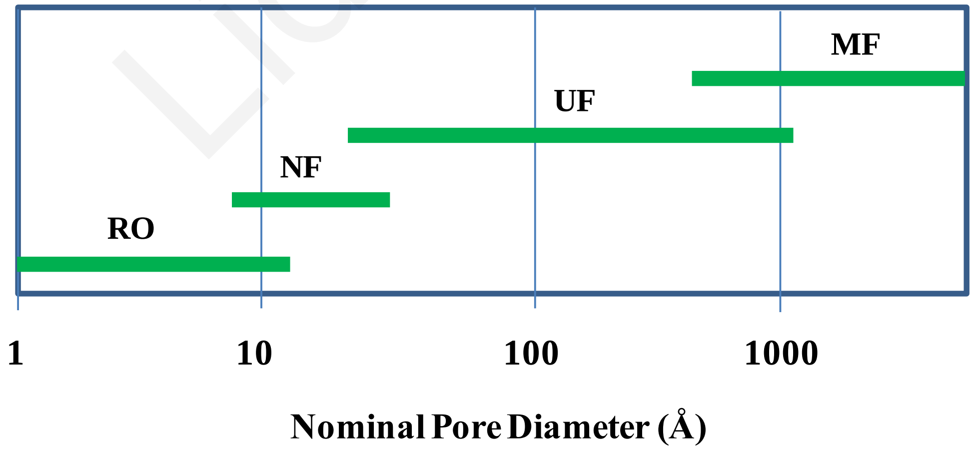
One of the main disadvantages of MBR is the necessity to clean the membranes routinely. Pretreatment functions are necessary, mainly to maintain membrane integrity and to prevent wastewater constituents from changing the hydraulics of the membranes. The term fouling is used to describe the potential deposition and accumulation of constituents of the feed stream on the membrane. In the activated sludge reactor, rejected components in the mixed liquor biomass coat the outer layer of the membranes during effluent withdrawal. Accumulated cake and finer particles adsorb onto the membrane, and cause an increase in pressure loss. The biomass not only contains biological flocs, formed by the conglomeration of microorganisms within floc matrices, but a whole range of soluble, insoluble, and colloidal compounds that are either contained in the incoming wastewater or result from bacterial metabolism (Metcalf and Eddy, 2008).

In practice, membrane fouling can be controlled by two types of approaches, i.e. (a) periodical air scouring, backwashing and chemical cleaning (Ang et al., 2006), and (b) the addition of adsorbents and pretreatment by coagulation (Kim et al., 2008). A recent study has shown that direct addition of a coagulant in the bioreactor is able to mitigate membrane fouling (Tian et al., 2008). The integration of coagulation into MBR is termed membrane coagulation bioreactor (MCBR). In fact, the most important trend in the development of membrane filtration for water treatment is the integration of different pretreatment strategies to improve the performance of low pressure membranes (Huang et al., 2009).

3.3 Membrane filtration and separation technologies for the treatment of winery and other agro-industrial wastewater streams

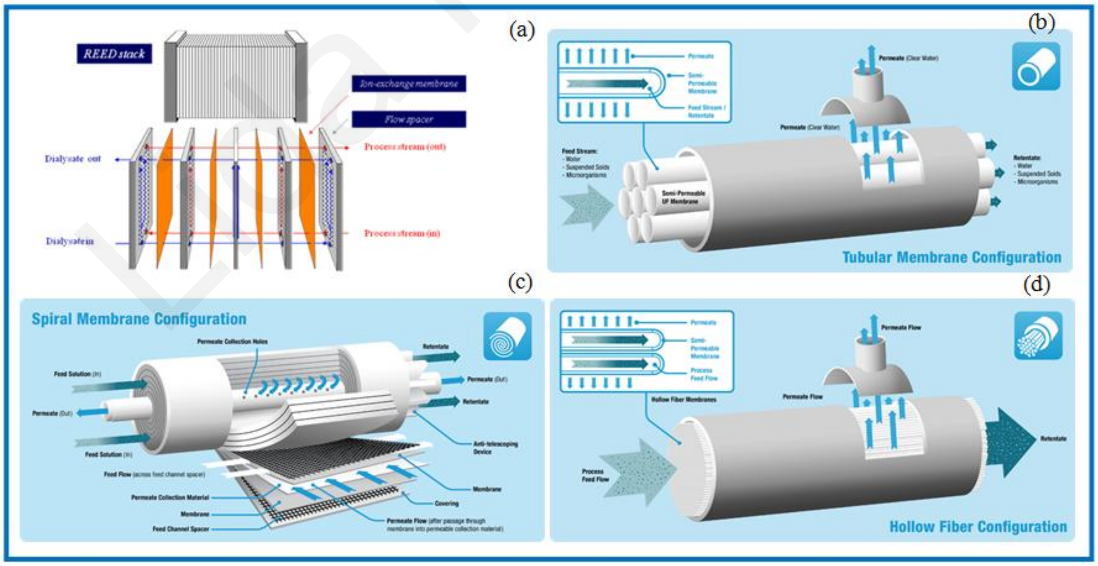
Membrane filtration and separation processes are becoming increasingly widespread in water treatment and wastewater reclamation/reuse applications, where a high product quality is desired. These processes are often chosen since these applications achieve high removals of constituents such as dissolved solids, organic carbon, inorganic ions, and regulated and unregulated organic compounds (Bellona et al., 2004).

In general, membrane filtration can be divided into four categories, depending on the effective pore size of the membrane, and hence the size of the impurities removed. In order of decreasing pore size, they are as follows: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) (Metcalf and Eddy, 2008). In Schematic 3.4, MF, UF, NF and RO range nominal membrane pore sizes are presented. RO membranes are effectively non-porous and, therefore, exclude particles, and even many low molar species such as salt ions, organics, etc. (Perry and Green, 2008).



Schematic 3.4: Range of nominal membrane pore sizes

Moreover, there are four main types of membrane modules: plate-and-frame, tubular, spiral wound, and hollow fiber, as shown in Schematic 3.5. The plate-and-frame module is the simplest configuration, consisting of two end plates, the flat sheet membrane and spacers (Schematic 3.5(a)). In tubular modules, the membrane is often on the inside of a tube, and the feed solution is pumped through the tube (Schematic 3.5(b)). The most popular module in industry for RO membranes is the spiral wound module (Schematic 3.5(c)). In the spiral wound membrane, a flexible permeate spacer is placed between two flat membrane sheets. The membranes are sealed on three sides. The open side is attached to a perforated pipe. A flexible feed spacer is added and the flat sheets are rolled into a tight circular configuration. In addition, the hollow fiber membrane module consists of a bundle of hundreds to thousands of hollow fibers. The entire assembly is inserted into a pressure vessel. The feed can be applied to the inside of the fiber (inside-out flow) or the outside of the fiber (outside-in flow) (Schematic 3.5(d)) (Metcalf and Eddy, 2008).



Schematic 3.5: (a) plate and frame, (b) tubular, (c) spiral wound and (d) hollow fibers modules.

Sources: http://www.kochmembrane.com; http://www.jurag.dk

Membranes can also be classified according to their material of construction, while most of membranes are synthetic organic polymers. RO membranes are typically either cellulose acetate (CA) (made from acetylated cellulose), or thin film composite (TFC) (based on polysulfone coated with aromatic polyamides) (Sourirajan, 1977).

The presence in winery wastewater, as well as in other agro-industrial wastewater of stable or non-biodegradable dissolved organic pollutants, asks for the adoption of physical treatments, such as membrane filtration processes (ultrafiltration (Canepa et al., 1998; Akdemir and Ozer, 2009), nanofiltration (Ferarrini et al., 2001), and reverse osmosis (Reimann, 1997; Sridhar et al., 2002; Madaeni an Mansourpanah, 2003; Nataraj et al., 2006; Vourch et al., 2008)), as shown in Table 3.7.

Table 3.7: Membrane filtration and separation technologies applied for the treatment of winery and other agro-industrial wastewater

Wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
pH= 7.0-12.9 COD= 100-150 g L^{-1} BOD_5= 30-60 g L^{-1} TS= 80-120 g L^{-1} TSS= 20-60 g L^{-1} TN= 12-14 g L^{-1} TPh= 10-15 g L^{-1} Total sugars= 20-40 g L^{-1}	Vegetation waters from olive oil industry (OMW) (Italy)	Ultrafiltration (UF) followed by two reverse osmosis (RO) units, working in series	COD removal, recovery of polyphenols and volume recovery	The pilot plant operated for about 6 months using polysulfone UF membranes, absorbing polymers and polypiperazine amide RO membranes. The process was optimized so that from wastewater with a COD content of about 100-150 g L^{-1} it was possible to obtain a COD reduction of about 99% with the recovery of polyphenols (99.7% in the concentrate) to use in alimentary industries and of a "concentrated paste", for oil extraction, phurphurale production or combustion for energy recovery. The volume recovery of this membrane process was about 70%, with fresh water with good characteristics both for recycling and for agricultural irrigation, as permeate.	Canepa et al., 1988
COD= 1000-43000 mg L^{-1}	Agricultural wastewater (pig and cattle slurry, milkhouse wastewater and vegetable wash water) (Germany)	UF followed by RO	Effect of organic matter concentration on flux and permeability of the membranes; COD removal	The test results showed that the permeate flux for both the UF and RO was considerably affected by the concentration of the organic matter in the wastewater. On the other hand, the permeability of the membranes decreases with increasing concentration of the organic matter in the wastewater, and that is independent of the kind of wastewater. The COD rejection rates were 87% for milking plant wastewater, 79% for pig slurry, 72% for cattle slurry, and 28% for potato wash-water filtration.	Reimann, 1997

pH= 3.2 Reducing sugars= 166 g L^{-1} TPh= 144 mg L^{-1} Conductivity= 190 mS cm^{-1} Turbidity= 89 NTU Temperature= 11-25 ^{o}C Working pressures= 32, 40 and 45 bar Feed flow rate= 1999 L h^{-1}	Synthetic winery wastewater - white grape juice	Two nanofiltration (NF) membranes	Effect of temperature and feed pressure on permeate flux and total polyphenols removal	The performance of two NF membranes (DS and DK) was compared and the effects of the working temperature and pressure on the selected NF-DS membrane were tested. The permeate flux of NF-DS membrane increased by 3% per degree of temperature rise, while a linear relationship between transmembrane pressure and permeate flux was established. By increasing the working pressure from 32 to 45 bar the membrane permeability doubled at 15 ^{o}C. The total polyphenols rejection of NF-DS membrane at the lower temperature tested (15 ^{o}C), and with constant pressure (32 bar) reached 74%.	Ferrarini et al., 2001
pH= 5.0 COD= 2550 mg L^{-1} BOD_5= 421 mg L^{-1} TDS= 10411 mg L^{-1} TSS= 400 mg L^{-1} Conductivity= 12.5 mS cm^{-1} Oil content < 50 mg L^{-1} Color= yellowish green Calcium= 300 mg L^{-1} Chlorides= 400 mg L^{-1} Sulphates= 130 mg L^{-1}	Wastewater from a vegetable oil industry (India)	Reverse osmosis	COD, TDS, color and BOD_5 removal. Effect of feed TDS concentration and feed pressure on permeate flux and percent rejection of pollutants	The effect of varying feed pressure (0-69 bar) and feed total dissolved solids (TDS) concentration (0.54-5.2% w/v) on separation performance of the TFC polyamide RO membrane was determined. At original effluent composition and feed pressure of 55.2 bar, high rejection of TDS (99.4%), and COD (98.2%) along with complete rejection of color and BOD_5 was achieved with a reasonably high flux of 52.5 L m^{-2} h^{-1}. The flux and percent rejection of pollutants improved significantly with increasing transmembrane pressure at constant feed concentration, but decreased with increasing feed TDS concentration.	Sridhar et al., 2002
COD= 29000-32000 mg L^{-1}	Wastewater from an alcohol manufacturing plant (Iran)	Reverse osmosis	Efficiency of eight RO membranes on COD removal	Altogether eight polymeric membranes (FT30, PVD, DSII, DS, BW30, 37100, 3750 and NF45) were tested for COD rejection. Neither of the membranes could reduce the COD to a desirable level (i.e. less than 200 mg L^{-1}) in a single step. The NF45 membrane decreased COD to a greater extent than the other membranes tested (52%). As a result, at least two consecutive membrane units were needed to reduce the COD to a desirable level (< 200 mg L^{-1}).	Madaeni and Mansourpanah, 2003

pH= 3.0 COD= 100000 mg L^{-1} TDS= 51500 mg L^{-1} Conductivity= 346 mS cm^{-1} Potassium= 2050 mg L^{-1} Chlorine= 4900 mg L^{-1} Color= dark brown	Molasses-based distillery spent wash water (India)	NF followed by RO	Color, TDS, Potassium and COD removal	The feasibility of the membranes for treating molasses-based distillery effluents by varying the feed pressure (0-70 bar) and feed concentration was tested on the separation performance of thin-film composite NF and RO membranes. Complete color removal by NF; and a high rejection of 99.80% TDS, 99.90% of COD, and 99.99% of potassium was achieved from the RO runs, by retaining a significant flux as compared to pure water flux, which shows that membranes were not affected by fouling during wastewater run.	Nataraj et al., 2006
pH= 5.8-7.2 COD= 380-38500 mg L^{-1} TOC= 96-13200 mg L^{-1} DOC= 24-9000 mg L^{-1} TSS= 10-796 mg L^{-1} TKN= 25-1890 mg L^{-1}	Dairy wastewater (mixtures of milk, whey and cream) (France)	Reverse osmosis	Permeate flux, water recovery, TOC, TKN and conductivity removal	RO treatment of the selected wastewater was carried out until 90-95% water recovery was achieved with an average permeate flux around 11 L h^{-1}m^{-2}. TOC of purified water was lower than 7 mg L^{-1} mainly due to the presence of lactose, reaching a reduction of TOC higher than 99.8%. Moreover, the RO process allowed a conductivity and TKN reduction of 97.8% and 96.3%, respectively.	Vourch et al., 2008
pH= 4.8 COD= 84000 mg L^{-1} TOC= 35542 mg L^{-1} TSS= 11200 mg L^{-1} Oil and grease= 25100 mg L^{-1}	Olive mill wastewater (OMW) from 3 phase olive oil mill (Turkey)	Two UF membranes	COD, TOC, TSS and oil and grease removal	Ultrafiltration membranes used were JW and MW membranes. The highest permeate flux (25.9 L m^{-2} h^{-1}) was obtained using MW membrane under operational conditions of Qf =200 L h^{-1} flow rate and pressure 4 bar, while the highest removal were obtained at Qf =100 L h^{-1} flow rate and pressure 1 bar. Although discharge effluent standards are not obtained, COD, TOC, TSS, oil and grease removal efficiencies were 92.3%, 92.7%, 97.1%, and 98.9%, respectively.	Akdemir and Ozer, 2009
pH= 4.6±0.2 COD= 40.3±1.0 g L^{-1} TOC= 12.9±0.5 g L^{-1} TS= 24.8±0.5 g L^{-1} TSS= 6.8±0.7 g L^{-1} TN= 0.24±0.05 g L^{-1} Oil and grease= 4.2±1 g L^{-1} Conductivity= 5.3±0.2 mS cm^{-1}	Olive mill wastewater (OMW) (Turkey)	OMW was centrifuged, and filtered via UF (UC010) membrane followed by NF (NP010, NP030, and NF270) and RO (XLE and BW30)	Membrane fluxes, COD and conductivity removal through the different membranes used	The membrane fluxes reached values of up to 21.2, 5.2, 28.3, 15.5, and 12.6 L m^{-2} h^{-1} for NP010, NP030, NF270, XLE, and BW30 membranes, respectively. The maximum COD removal efficiencies obtained at 10 bars were 60.1%, 59.4%, and 79.2% for NP010, NP030, and NF270 nanofiltration membranes, respectively; while they were 96.3% and 96.2% for XLE and BW30 reverse osmosis membranes, respectively. Besides, conductivity removal efficiencies obtained at 25 bars were 93.2% and 94.8% for XLE and BW30 membranes, respectively.	Coskun et al., 2010

3.3.1 Assessment of the performance of the membrane filtration and separation processes

Several membrane filtration and separation processes for the treatment of various agro-industrial wastewater streams have been applied, worldwide, as shown in Table 3.7. For winery wastewater, only one membrane filtration process (nanofiltration) has been reported so far. However, various types of membrane filtration and separation processes (ultrafiltration, nanofiltration and reverse osmosis) have been successfully applied for the treatment of other agro-industrial wastewater, such as dairy, molasses-based distillery, olive mill wastewater, etc.

Membrane filtration involves the passage of wastewater, through a thin membrane for the purpose of removing particulate material, pathogens, organic matter, nutrients, and dissolved substances not removed by biological treatment processes (Metcalf and Eddy, 2008). According to Ferrarini et al. (2001) a significant reduction of total polyphenols in the permeate (74%) of a synthetic winery wastewater (white grape juice) was achieved by nanofiltration. Moreover, it was noticed that the working pressure and temperature affected the selectivity of NF membranes. More specifically, the permeability of NF membranes doubled by increasing the working pressure from 32 to 45 bar, while the permeate flux of NF membrane increased by about 3% per degree of temperature rise.

Since, limited literature (Ferrarini et al., 2001) exists for the treatment of winery wastewater by membrane filtration and separation processes; the membrane applications of other agro-industrial wastewater were also reviewed. High COD removal efficiency for olive mill wastewater (OMW) was observed by using UF without pretreatment, which reached values higher than 90% (Akdemir and Ozer, 2009). Moreover TOC, TSS and oil and grease removal efficiencies were also high for OMW, reaching values of 92.3%, 92.7%, 97.1%, and 98.9%, respectively (Akdemir and Ozer, 2009).

On the other hand, RO has been found to be a very promising separation process for the treatment of olive oil industry effluent and water recovery; due to the high fluxes obtained (52.5 L m^{-2} h^{-1}), alongside significant rejection of TDS (99.4%), COD (98.2%), BOD (100%), and color (100%) (Sridhar et al., 2002). Moreover, RO achieved a sufficient COD reduction equal to 52% for a wastewater stream from an alcohol manufacturing plant (Madaeni and Mansourpanah, 2003). While, according to Vourch et al. (2008) the high quality of the RO permeate dairy effluent (removal of DOC 99.8%, conductivity 97.8%, and TKN 96.3%) allowed to be reused in the dairy plant for heating, cleaning and cooling purposes.

Ultrafiltration followed by reverse osmosis achieved to reduce the organic content, expressed as COD from 28-87% for several agricultural wastewater (pig and cattle slurry, milkhouse wastewater and vegetable wash water) (Reimann, 1997). In this work it was noticed that the permeability of the membranes decreases with increasing the concentration of the organic matter in the wastewater, and this is independent of the kind of wastewater (Reimann, 1997). Additionally, in the study of Canepa et al. (1988) the efficiency of the combined application of membranes and adsorption processes to the treatment of vegetation waters coming from olive oil factories was investigated. More specifically, the pilot plant operated using polysulfone UF membranes, absorbing polymers and polypiperazine amide RO membranes. This combined process was found able to achieve a COD reduction of about 99%, with the recovery of polyphenols (99.7%

in the concentrate) to use in alimentary industries and of a "concentrated paste" for oil extraction, phurphurale production or combustion.

A hybrid nanofiltration and reverse osmosis pilot plant was used to remove the color and contaminants of the molasses-based distillery spent wash water. Complete color removal by NF; and a high rejection of 99.8% TDS, 99.9% of COD, and 99.99% of potassium was achieved from the RO runs, by retaining a significant flux as compared to pure water flux, which shows that membranes were not affected by fouling during wastewater run (Nataraj et al., 2006).

A combination of centrifuging, ultrafiltration and reverse osmosis processes with XLE and BW30 membranes under 25 bars for the treatment of OMWs was able to reduce initial COD concentration of 40000 mg L^{-1} to 1000 mg L^{-1}. Therefore, the overall COD removal efficiency was 97.5%, while the conductivity removal efficiency was higher than 93.2% (Coskun et al., 2010).

From all the above, it can be noted that the membrane filtration and separation processes, seem to be promising technologies for the treatment of winery wastewater, since they are effective in the treatment of more complicated agro-industrial effluents, such as olive mill wastewater (OMW). The COD rejection rates that can be achieved are on average between 52 and 99.9%, while also significant solids removals can be obtained (even up to 99.8%). However, the appropriate management of the concentrate produced from these processes, and the fouling of the membranes, are the two major problems that must be handled, in order to be able to establish an integrated treatment.

Membrane fouling, is an important consideration in the design and operation of membrane systems as it affects pretreatment needs, cleaning requirements, operating conditions, cost, and performance. Membrane fouling occurs depending on the site-specific physical, chemical, and biological characteristics of the feedwater, the type of the membrane and the operating conditions (Fritzmann et al., 2007). In general, fouling occurs either on the surface of a membrane or within its pores, and it causes a decrease in flux (Kang and Cao, 2011). There are four major types of fouling: biofouling, scaling, organic and colloidal (Fritzmann et al., 2007).

Biofouling results by the microbial contamination of the feed water and produces a biofilm on the surface of the membrane, which increases the resistance to water permeation through the membrane (Li et al., 2008). Existing membrane biofouling, results in increased energy demand for salt separation and the deterioration of product water quality (Zhang et al., 2011). Scaling is caused by super-saturation of inorganic compounds concentrated on the feed side. Super-saturated salts can precipitate on the membrane surface building a thin layer, which hinders mass transfer through the membrane. Scaling always occurs at the membrane surface, because of the increased salt concentration near the membrane caused by concentration polarization. Some of the most important scaling substances are $CaCO_3$, $CaSO_4$, $BaSO_4$ and silica (Fritzmann et al., 2007). Organic fouling results from substances such as hydrocarbons, which coat the surface and/or plug pores in the porous support layer (Metcalf and Eddy, 2008). Colloidal fouling mainly stems from particles, such as clay or silica, accumulating on the surface of the membrane (Zhang et al., 2011).

Typically, three strategies are used to control membrane fouling: (a) pretreatment of feedwater, (b) hydraulic flushing, and (c) chemical cleaning of the membranes (Metcalf and Eddy, 2008; Kang and Cao, 2011).

Feedwater pretreatment is necessary to prolong membrane life and prevent its fouling, by reducing the TSS and bacterial content of the feedwater. Pretreatment usually consists of chemical coagulation, fine filtration, ultrafiltration or microfiltration, scaling control (e.g. softening), and acidification for pH regulation (Malaeb and Ayoub, 2011). Moreover, chemical precipitation can be used to remove the sparingly soluble salts, such as calcium carbonate ($CaCO_3$). The most commonly used method of limiting the accumulation of particulate matter on the membrane is to maintain a cross-flow velocity across the membrane, called hydraulic flushing. Some membrane systems are designed to be flushed periodically to limit the accumulation of material on the membrane surface (Metcalf and Eddy, 2008). Chemical cleaning is used to remove constituents that are not removed with conventional cross-flow hydraulic flushing. Some recently developed membranes can withstand cleaning solutions varying from a pH of 1 to 13. High pH solutions are useful for the removal of biofouling and organic fouling, while low pH solutions are useful for the removal of calcium carbonate ($CaCO_3$) deposits (Li et al., 2008; Kang and Cao, 2012).

Another significant drawback of the membrane processes, is the fact that these processes do not really destroy the pollutants, but merely concentrate them into smaller volumes of wastewater, i.e. concentrate. As a result, the concentrate generated in membrane separation processes contains high levels of refractory organic pollutants and inorganic salts (Wang et al., 2012); and need further treatment before its disposal into the environment (Dialynas et al., 2008). Traditionally, the membrane concentrate is discharged into the natural water body - surface waters, oceans, groundwaters - (with or without dilution, depending on the local discharge regulations to avoid degradation of the water body) or treated by evaporation (Zhang et al., 2011). The former methods are not environmentally friendly, and the latter process is very costly (when conventional energy is used for evaporation, potentially even less environmentally friendly) (Zhang et al., 2011).

Due to the fact that there are elevated concentrations of micropollutants in the concentrate, direct disposal into the environment could be hazardous to aquatic organisms. As a consequence, further treatment of the concentrate is necessary in order to minimize the environmental impacts upon disposal of this point source flow (Benner et al., 2008; Dialynas et al., 2008). Complete oxidation of all organic compounds, including biological organisms, to carbon dioxide would be the highest level of organic compounds control (Westerhoff et al., 2009).

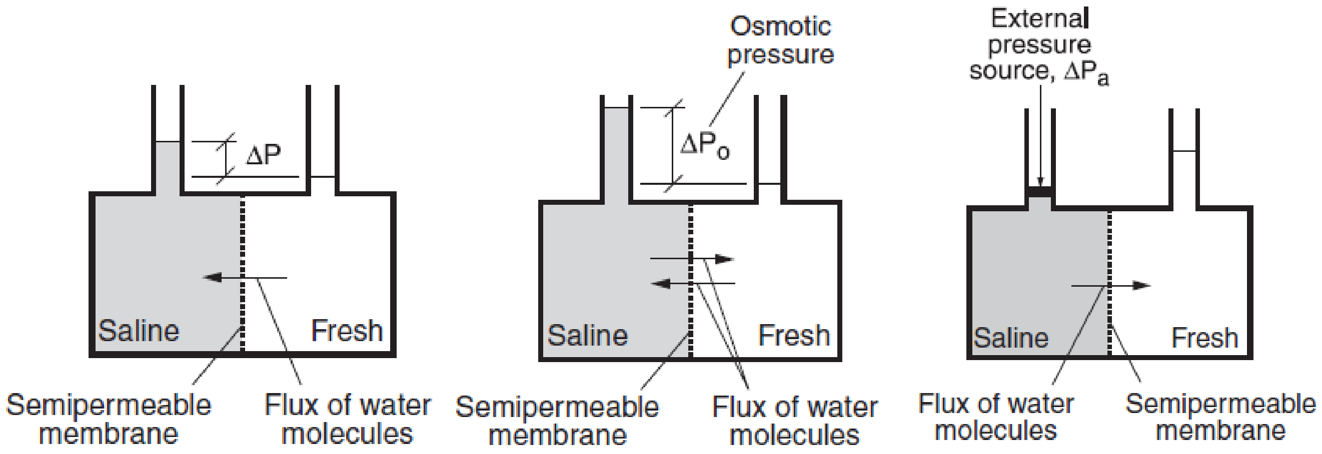
The organic pollutants in the membrane concentrate are difficult to be treated by conventional biological methods, and consequently it is vital to explore appropriate processes for the effective abatement of organic compounds under an acceptable level (Hermosilla et al., 2012; Zhou et al., 2012; Wang et al., 2012). The characteristics of the concentrate depend on the feedwater characteristics, the pretreatment, the membrane process used, the recovery, and the additional chemicals used (van der Bruggen, et al., 2003). Several advanced oxidation treatment options (e.g. Fenton and electro-Fenton, TiO_2 photocatalysis, ozonation, sonolysis and H_2O_2 oxidation) showed satisfactory performance in removing the organic matter from various membrane concentrate streams

(Dialynas et al., 2008; Westerhoff et al., 2009; Radjenovic et al., 2011; Zhou et al., 2011b). Since no literature on the treatment of winery or other agro-industrial membrane concentrate exists so far, a brief review on AOPs used for the purification of membrane concentrate of other industrial and municipal wastewater was conducted, as shown in Section 3.4 (Table 3.11).

3.3.2 Membrane separation treatment (reverse osmosis) applied in this thesis

In the framework of this thesis, reverse osmosis was used for the treatment of raw winery wastewater. The RO operating principles, the main characteristics, as well as the various advantages and disadvantages of the process are presented in detail in this section.

In order to better understand the reverse osmosis process, the phenomenon of osmosis should be firstly explained. As shown in Schematic 3.6(a), where two solutions having different solute concentrations are separated by a semi-permeable membrane, a difference in the chemical potential will exist across the membrane. Water will tend to diffuse through the membrane from the lower-concentration (higher-potential) side to the higher-concentration (lower-potential) side. In effect, the water passing through the membrane is trying to dilute the higher ionic concentration solution to equalize the concentrations on both sides of the membrane. This balancing pressure difference is termed “osmotic pressure” (Schematic 3.6(b)), and it is a function of the solute characteristics, concentration and temperature. If a pressure gradient opposite in direction and greater than the osmotic pressure is imposed across the membrane, flow from the more concentrated to the less concentrated region will occur, and this is termed “reverse osmosis” (Schematic 3.6(c)) (Metcalf and Eddy, 2008).



$\Delta P < \Delta P_o$ | **$\Delta P = \Delta P_o$** | **$\Delta P > \Delta P_o$**

Schematic 3.6: (a) Osmotic flow, (b) Osmotic equilibrium, and (c) Reverse osmosis

Source: Metcalf and Eddy, 2008

In other words, osmosis is the passive transport of water across a selectively permeable membrane in order to reduce a concentration difference of a solute between a concentrate and permeate solution separated by the membrane. Reverse osmosis accomplishes the opposite, where water of a solute solution is forced across a membrane that is ideally impermeable to a solute, resulting in a solute enriched concentrate. For semi-permeable membranes, a solute present in the feed stream will diffuse much slower (or not at all) than the water across a membrane and ideally result in a solute free permeate stream (Bellona et al., 2004).

Reverse osmosis membranes are typically thin film composite (TFC) membranes in a spiral wound configuration or hollow fiber with a pore size of approximately 0.5 nm. Spiral wound configurations have a low modular cost, and are economical to operate because of low power consumption. Pressure drops range from 100 to 140 kPa. Plugging of the spiral wound elements can be minimized by filtering the influent to 1 to 10 μm, depending on the spacer construction (Metcalf and Eddy, 2008).

In this thesis, a spiral-wounded polyamide membrane (TFC) (AG2521TF, Desal) was selected and used, for the RO experiments at a pilot scale, as described in detail in Chapter 5 (Section 5.2.4).

RO is currently considered to be the state-of-the-art in industrial wastewater treatment applications (Tam et al., 2007; Cséfalvay et al., 2008). The process efficiency depends on operational parameters and on membrane and feed water properties as well (Malaeb and Ayoub, 2011). The RO is the tightest possible membrane process in liquid/liquid separation, and therefore produces the highest water quality of any pressure driven membrane process (Simate et al., 2011). The development of low-pressure RO processes has made them an attractive alternative for the treatment of aqueous wastes since they offer high fluxes and solute separations, and can operate over wide temperature and pH ranges (Madaeni and Mansourpanah, 2003). Reverse osmosis general characteristics are presented in Table 3.8.

Table 3.8: General characteristics of reverse osmosis

Factor	**Reverse osmosis**
Membrane driving force	Hydrostatic pressure difference
Typical separation mechanism	Solution/diffusion and exclusion
Typical pore size	Dense (< 2 nm)
Typical operating range, μm	0.0001 - 0.001
Molecular weight cut-off	< 300
Permeate description	Water, very small molecules, ionic solutes
Typical constituents removed	Very small molecules, color, hardness, sulfates, nitrated, sodium, other ions
Operating pressure*	12 - 18 bar
Energy consumption*	1.5 - 2.5 kWh m^{-3}
Material	Cellulosic, aromatic polyamide, thin film composite**
Configuration	Spiral wound, hollow fiber

Source: Metcalf and Eddy, 2008
* when treating wastewater with a TDS concentration in the range from 1000 to 2500 mg L^{-1}.
** with surface layer formed from different types of polyamide compounds. Support structure usually made of polysulfone.

Reverse osmosis has been used in many applications including sea and brackish water desalination, as well as industrial process water for the removal of dissolved constituents (Metcalf and Eddy, 2008). More specifically, RO process has been demonstrated to be useful in the treatment of a wide variety of effluents including chemical, electrochemical, textile, pulp and paper, petroleum and petrochemical, food, tanning and metal finishing industries, as well as for the treatment of municipal and agro-industrial wastewater (e.g. molasses-based distillery wastewater, olive mills wastewater, pig and cattle slurry, milkhouse wastewater, vegetable wash water, etc.) (Canepa et al., 1988; Reimann, 1997; Ferrarini et al., 2001; Benito and Ruiz, 2002; Bodalo-Santoyo et al., 2003; Madaeni and Mansourpanah, 2003; Badani et al., 2005; Nataraj et al., 2006; Rektor et al., 2007; Csefalvay et al., 2008; Dialynas and Diamadopoulos, 2008; Radjenovic et al., 2008; Vourch et al., 2008; Zhang et al., 2009; Coskun et al., 2010). A brief review of the RO processes used for the treatment of various agro-industrial effluents has already been presented in Table 3.7. Reverse osmosis typically removes 95 to 99.5% of the total dissolved solids (TDS); 95 to 97% of dissolved organic matter (DOM), and 98 to 99% of monovalent ions (Metcalf and Eddy, 2008).

However, RO has very strict feedwater requirements regarding mainly the concentration of the suspended solids, fibres and oily constituents (Bodalo-Santoyo et al., 2003). As a consequence, pretreatment of the feedwater must be selected to allow successful performance of the specific membranes. In more details, the characteristics of the feedwater that must be considered include the following (Metcalf and Eddy, 2008):

- Suspended solids or turbidity to prevent clogging.
- Organics that promote membrane degradation and destruction.
- Iron, manganese, and other precipitates.
- Oil and grease, or floatables to prevent fouling.
- Erosive substances to minimize membrane wear.
- Temperature within membrane operating limits.
- pH to prevent membrane degradation.

The advantages offered by RO have been well documented in the literature, such as the excellent effluent quality (Tam et al., 2007). However, just like other membrane separation processes, RO suffers from various drawbacks, such as the concentrate generated that contains high levels of refractory organic pollutants and inorganic salts (Wang et al., 2012), the membrane fouling, etc. In Table 3.9 the advantages and disadvantages of the RO process are summarized in detail.

Table 3.9: Advantages and disadvantages of the RO process

Advantages	Disadvantages
▪ RO membranes can remove more than 90% of TDS and more than 95% of dissolved organic matter ▪ Easy operation ▪ Flexibility to provide higher quality water, if desired ▪ The low-pressure RO membranes can operate over wide temperature and pH ranges	▪ Requires high pressure to achieve high salt or organic compounds rejection ▪ Requires pretreatment processes to minimize scaling and fouling ▪ Requires chemical addition for RO fouling control ▪ More routine maintenance may be required to maintain performance ▪ The concentrate generated requires a further treatment before its disposal in the environment

Sources: Madaeni and Mansourpanah, 2003; Metcalf and Eddy, 2008

3.4 Advanced Oxidation Processes for the treatment of winery wastewater

The growing demand from society for disinfection and detoxification of polluted waters from different sources, materialized in very strict governmental regulations, has led, in the last few decades, to the development of new and more effective water purification non-biological technologies (Litter, 2005). It has been frequently observed that pollutants contained in winery wastewater like various recalcitrant high molecular weight compounds (e.g. polyphenols, tannins and lignins) not amenable to biological treatment, may also be characterised by high chemical stability and/or by difficulty to be completely mineralized. In these cases, it is necessary to adopt reactive systems, much more effective than those adopted in conventional treatment processes (Arienzo et al., 2009a; Lucas et al., 2010). A lot of researches have addressed this objective in the last decades pointing out the prominent role of a special class of oxidation techniques defined as advanced oxidation processes (AOPs), which usually operate at or near ambient temperature and pressure (Fernández-Alba et al., 2002).

All AOPs are characterized by a common chemical feature: the capability of exploiting the high reactivity of hydroxyl radicals (HO•) in driving oxidation processes, which are suitable for achieving the degradation to less reactive pollutants or complete mineralization (Andreozzi et al., 1999; Klavarioti et al., 2009). Second to fluorine (E^0 = 3.03 V) the HO• is the strongest known oxidant with a potential of 2.80 V. The relative strength of various oxidizing species compared to the hydroxyl radicals is shown in Table 3.10. Hydroxyl radicals are extraordinarily reactive species, and they attack most of the organic molecules quickly (Litter, 2005). Rate constants (k_{HO}, $r=k_{HO}\,[HO^{\bullet}]C$) for most reactions involving hydroxyl radicals in aqueous solution are usually on the order of

10^6-10^9 M^{-1} s^{-1} (Andreozzi et al., 1999; Malato et al., 2003; Klavarioti et al., 2009). They are also characterised as non-selective, which is a useful attribute for an oxidant used in wastewater treatment and for solving pollution problems. The versatility of AOPs is also enhanced by the fact that they offer different possible ways for HO• radicals production, thus allowing a better compliance with the specific treatment requirements (Andreozzi et al., 1999).

Table 3.10: Redox potential of common oxidizing species

Species	**E^0 (V, 25 °C)***
Fluorine	3.03
Hydroxyl radical	**2.80**
Atomic oxygen	2.42
Ozone	2.07
Hydrogen peroxide	1.78
Perhydroxyl radical	1.70
Permanganate	1.68
Chlorine dioxide	1.57
Hypochlorous acid	1.49
Chlorine	1.36

* Redox potentials referred to normal hydrogen electrode (NHE)

The most widely known AOPs include: H_2O_2/UV systems, treatment with ozone (often combined with H_2O_2, UVA, or both), heterogeneous photocatalysis (TiO_2), Fenton (Fe^{2+}/H_2O_2) and photo-Fenton type reactions ($Fe^{2+}/hv/H_2O_2$), and sololysis. A list of the most common methods of hydroxyl generation in AOPs is presented in Table 3.11.

Table 3.11: AOPs used for water and wastewater treatment and their key reactions

AOP	Key reactions	Description
UV	$R\text{-}R + h\nu \rightarrow R\text{-}R^* \rightarrow 2R^{\bullet}$ $R\text{-}R^* + O_2 \rightarrow R\text{-}R^{\bullet+} + O_2^{\bullet-}$ $^3DOM^* + {}^3O_2 \rightarrow DOM + {}^1O_2$	▪ UV photolysis is a process in which compounds absorb photons and the energy released drives oxidation processes induced by light. Direct irradiation leads to the promotion of a molecule from the fundamental state to an excited singlet state. The formed radicals initiate chain reactions to produce the final low-weight products. In the presence of oxygen, additional reactions generating the superoxide radicals ($O_2^{\bullet-}$) are possible. ▪ The success of direct photolysis is highly dependent on the photoreactivity of the specific organic compounds. ▪ Disadvantages: UV irradiation with lamps is expensive.
UV/H_2O_2	$H_2O_2 + h\nu \rightarrow HO^{\bullet} + HO^{\bullet}$ $HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O$ $HO_2^{\bullet} + H_2O_2 \rightarrow HO^{\bullet} + H_2O + O_2$	▪ The most direct method for generation of hydroxyl radicals is through the cleavage of H_2O_2. An optimal H_2O_2 concentration exists because overdosing of H_2O_2 would lead to reaction with HO• and formation of HO_2•. The major factors affecting this process are the initial concentration of the target compound, the amount of H_2O_2 used, wastewater pH, presence of bicarbonate and treatment time. ▪ Advantages: the oxidant is commercially accessible, thermally stable, and can be stored in the site of use. There is no mass transfer problems associated with gases, the capital investment is minimal, and the operation is simple. ▪ Disadvantages: unable to utilize solar light as the source of UV light; H_2O_2 has poor UV absorption characteristics.
O_3	$O_3 + R \rightarrow R_{ox}$ $2O_3 + 2H_2O \rightarrow 2HO^{\bullet} + O_2 + 2HO_2^{\bullet}$	▪ In the absence of light, O_3 can react directly with an organic substrate (R), through a slow and selective reaction, or through a fast and non-selective radical reaction that produces HO•. ▪ Advantages: The process is performed at ambient temperature and no harsh chemicals used. ▪ Disadvantages: expensive production of ozone; low solubility of O_3 in water; O_3 is selective; formation of by-products (bromates); need of temperature control, due to the risk of volatilization of initial or intermediate compounds.
H_2O_2/O_3	$O_3 + H_2O_2 \rightarrow HO^{\bullet} + O_2 + 2HO_2^{\bullet}$	▪ H_2O_2 initiates O_3 decomposition by electron transfer. ▪ Advantages: The process is fast, and can treat organic pollutants at very low concentrations (ppb). ▪ Disadvantages: Additional cost of H_2O_2.
UV/O_3	$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2$ $O_3 + h\nu \rightarrow O_2 + O\ ({}^1D)$ $O\ ({}^1D) + H_2O \rightarrow 2\ HO^{\bullet}$	▪ The generated H_2O_2 is photolyzed, generating HO• radicals, and also reacts with the excess of ozone. ▪ The rate of UV/O_3 increased with increasing light intensity, ozone concentration and pH, and decreased with increasing inorganic carbon concentration. ▪ Disadvantages: elevated costs.
UV/H_2O_2/O_3	$O_3 + H_2O_2 + h\nu \rightarrow O_2 + HO^{\bullet} + HO_2^{\bullet}$	▪ The addition of light to the H_2O_2/O_3 process produces a net increase in the efficiency, through the extra generation of HO•. ▪ Disadvantages: high operational costs.
UV/TiO_2	$TiO_2 + h\nu \rightarrow TiO_2\ (e_{CB}^- + h_{VB}^+)$ $HO^- + h_{VB}^+ \rightarrow HO^{\bullet}$ $O_2 + e_{CB}^- \rightarrow O_2^{\bullet-}$	▪ Takes place in aquatic suspensions of semiconductors (TiO_2) in the presence of irradiation (ultraviolet or visible). The process starts, when a particle of semiconductor excited by light of energy higher than that of the band gap and then electron-hole (e-/h+) pairs are formed. ▪ Advantages: operation at ambient conditions and without overpressure; lack of mass transfer limitations when nanoparticles are used as photocatalysts; possible use of solar irradiation; complete oxidation of the substances into CO_2 and other inorganic species; the oxygen needed for the reaction could be obtained directly from atmosphere; TiO_2 is a cheap, innocuous, commercially available in various crystalline forms and particle characteristics, non-toxic, photochemically stable and can be reused. ▪ Disadvantages: the low quantum yield; the catalyst removal and regeneration.

Sonolysis	$H_2O \rightarrow H^\bullet + HO^\bullet$	▪ Sonolysis generated by ultrasound waves in liquid media, results in a remarkably suitable medium for high-energy chemistry. ▪ The sonochemical degradation in aqueous phase involves several reaction pathways and zones such as pyrolysis inside the bubble and/or at the bubble-liquid interface and hydroxyl radical-mediated reactions at the bubble-liquid interface and/or in the liquid bulk. ▪ Several factors may affect the sonolysis efficiency in a complex way. The most important ones are the frequency and intensity of ultrasound, the reactor geometry, the physicochemical properties of the liquid medium (vapour pressure, surface tension, viscosity, etc.), the temperature, the presence of gases (oxygen, ozone) and the type and the nature of contaminant. ▪ Advantages: The use of mild conditions (i.e., room temperature, normal pressure) for sample pretreatment, low reagent consumption, reduced contamination or volatilization risks, and diminished production of hazardous laboratory wastes. ▪ Disadvantages: high operational cost.
Fenton (H_2O_2/Fe^{2+})	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$	▪ Fenton process (dark Fenton) is a reaction between Fe^{2+} and H_2O_2 in acidic aqueous solution that forms HO•. ▪ Advantages: Fenton reagent appears to be a very powerful oxidizing agent. Fe^{2+} is abundant and nontoxic at low concentrations and there are no mass transfer limitations because all of the reagents are in the liquid phase. The process is simple taking place at low temperatures and at atmospheric pressure. ▪ Disadvantages: low pH (2.8-3.0); iron removal requirement.
Photo-Fenton ($UV/H_2O_2/Fe^{2+}$)	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$ $Fe^{3+} + H_2O \rightarrow Fe^{2+} + H^+ + HO^\bullet$	▪ The photo-Fenton process involves irradiation with sunlight or an artificial light source, which increases the efficiency of the process, by photoreducing the Fe^{3+} to Fe^{2+}, and by the generation of additional HO•. ▪ Advantages: In the presence of Fenton reagent, photochemical reactions can be driven with photons of low energy, belonging to the visible part of the spectrum (λ<580 nm). ▪ Disadvantages: low pH (2.8-3.0); iron removal requirement.
Electro-Fenton	$Fe^{3+} + e^- \rightarrow Fe^{2+}$ $O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	▪ There are two main types of Fenton process involving the use of electrochemically produced reagents. ▪ In cathodic Fenton process, the source of Fe^{2+} may be direct Fe^{2+} addition or may be produced by reduction of Fe^{3+} at the cathode. Additionally, the source of H_2O_2 may be either by direct H_2O_2 addition or it may be produced by reduction of dioxygen at the cathode. ▪ In anodic Fenton process, an iron electrode is used as the anode and becomes the source of Fe^{2+}. ▪ Advantages: In this process there is no need for addition of other chemical reagents except a catalytic quantity of ferrous ions and there is no pollution displacement to another medium. ▪ Disadvantages: high iron concentration requirements; elevated costs.

Sources: Roche et al., 1994; Roche and Prados, 1995; Stefan et al., 1996; Andreozzi et al., 1999; Cater et al., 2000; Chiron et al., 2000; Capelo et al., 2001; Guivarch et al., 2003; Malato et al., 2003; Bahnemann, 2004; Malato, 2004; Parsons, 2004; Litter, 2005; Eren, 2006; Stasinakis, 2008; Klavarioti et al., 2009; Malato et al., 2009; Mahamuni et al., 2009.

A more detailed description of the aforementioned AOPs is beyond the scope of this thesis. The investigative focus lies on the photo-Fenton oxidation, which can be conducted under solar irradiation; process fundamentals and mechanisms are described thoroughly in the next Section 3.4.2. The successful application of several AOPs for treating winery wastewater, as well as a brief review on AOPs applied for the purification of membrane concentrate for various industrial and municipal wastewater are presented in Table 3.12.

Table 3.12: Advanced Oxidation Processes (AOPs) applied for the treatment of winery wastewater and membrane concentrates

Wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
I. Winery wastewater					
pH= 3.76 COD= 15000-16500 mg L^{-1} TS= 9100 mg L^{-1} TPh= 400-700 mg L^{-1} Turbidity= 115 NTU Aromaticity= 11.7 u.a. (254nm)	Wine distillery wastewater (vinasses) (Spain)	Fenton oxidation (H_2O_2/Fe^{2+}) followed by coagulation / flocculation (using $Ca(OH)_2$ as base-precipitant)	Effect of [Fe^{2+}], [H_2O_2] and effluent dilution on COD, total phenolic compounds removal	There was a positive influence of the ferrous dose and the initial concentration of H_2O_2 on the degree of COD removal. The COD removal efficiency by Fenton oxidation was influenced by effluent dilution. The biodegradability of effluent (BOD_5/COD) was increased by increasing the dilution of effluent. COD removal of approx. 70% were noted for the maximum dilution (3/16); while the later stage of coagulation/flocculation has only a moderate influence on the global process (~15% additional COD removal).	de Heredia et al., 2005a
pH= 4.0 COD= 4000 mg L^{-1} TOC= 1723 mg L^{-1}	Synthetic winery wastewater - diluted red wine in ultra pure water	Photocatalytic systems using hydrogen peroxide: (H_2O_2/UV), (H_2O_2/UV/TiO_2) and (H_2O_2/UV/clay) - photo-Fenton	COD removal, comparison between artificial and natural (solar) irradiation, mineralization	H_2O_2/TiO_2 treatment produced the highest efficiency, reaching 52-58% of COD removal in winery wastewater samples. Although the H_2O_2/clays system led to lower COD removal (34-45%), it required a H_2O_2 dosage between three and six times lower than the H_2O_2/TiO_2 treatment. On the other hand, the degradation of organic matter was not efficient when no H_2O_2 was added to the photocatalytic systems. However, direct TiO_2 photo-activation acted as an oxidation system, since the addition of TiO_2 alone (1.0 g L^{-1}) led to a COD removal of 26% in winery samples. Artificial radiation provided better results than natural light for all the cases. Complete mineralization of organic matter was not achieved, since the TOC removal was always lower than the COD removal. Moreover, the iron that the clays contain was partially dissolved in the samples, which could contribute to enhancing the photo-Fenton reaction. The iron can operate as a homogeneous catalyst as	Navarro et al., 2005

				well as a heterogeneous one.	
pH= 3.5 COD= 5000-10000 mg L^{-1} TOC= 1500-3000 mg L^{-1} Particle size of clay= 80-500 µm	Synthetic winery wastewater - diluted red wine (WV) or grape juice (WG) in ultra pure water	Heterogeneous photo-Fenton oxidation	Effect of initial concentration, H_2O_2 and clay concentration, and reaction time on TOC removal	Applying photo-Fenton treatment in heterogeneous phase under energetic conditions for synthetic samples simulating winery effluents resulted in purification levels of up to 50% (measured as TOC). Increasing the initial concentration of the organic matter (measured as COD) to 10000 mg L^{-1} and the particle size of the clays (to 500 µm) resulted in a decrease in the degree of degradation. However, an increase in the H_2O_2 concentration (up to 0.3 M), the clay concentration (up to 2 g L^{-1}), or the reaction time (up to 60 min), produced a higher degree of degradation (up to 55%, measured as COD).	Mosteo et al., 2006
pH= 3.5 COD= 5000-10000 mg L^{-1} TOC= 1500-3000 mg L^{-1}	Synthetic winery wastewater - diluted red wine (WV) or grape juice (WG) in water	Homogeneous photo-Fenton oxidation	TOC removal	At the optimum conditions (5 mg L^{-1} Fe^{2+} and 0.03 M H_2O_2) the photo-Fenton treatment reached purification levels of up to 95% (measured as TOC) for synthetic samples prepared by diluting commercial grape juice in ultrapure water (WG), and of 50% for samples prepared from commercial wine (WV). Differences in the degree of degradation were attributed mainly to the generation of intermediate compounds, which inhibit the photo-Fenton process (acetic acid among others).	Ormad et al., 2006
pH= 7.1±0.4 COD= 9250±380 mg L^{-1} BOD_5= 7100±654 mg L^{-1} TPh= 268±33 mg L^{-1}	Winery wastewater - diluted with tap water (1:1) (Spain)	Photocatalytic ozonation ($UV/O_3/TiO_2$)	COD and TOC removal and effect of pH	The combination of UV-A/Vis radiation and ozone, in the presence of titanium dioxide, had been investigated as a potential destructive technology for the treatment of winery effluents. Adsorption onto the TiO_2 surface accounted for a 15-20% removal of the initial COD. The rest of the subsystems attained COD conversion values in the range of 37-43%. The photocatalytic ozonation of the effluent, under the optimum operating conditions, increased the COD depletion to a value close to 80%. Moreover, the system UV-A/Vis/O_3/TiO_2 led to the total mineralization of the effluent (100% TOC removal). The working pH was studied in the range of 3-11, and the optimum value being located in the acidic region (pH=3).	Gimeno et al., 2007

Commercial grape juice: COD= 4500 mg L^{-1} TOC= 1185 mg L^{-1} **Commercial red wine:** COD= 4440 mg L^{-1} TOC= 1155 mg L^{-1} **Mixture of wine and grape juice:** COD= 4474 mg L^{-1} TOC= 1165 mg L^{-1}	Synthetic winery wastewater - diluted commercial grape juice, commercial red wine and mixture of both in Milli-Q water	Heterogeneous photocatalysis (TiO_2, TiO_2/H_2O_2, $TiO_2/Na_2S_2O_8$) and homogeneous photo-Fenton oxidation (pilot-scale plant)	Effect of winery wastewater composition and Fe^{2+} concentration on TOC and total polyphenols removal and on toxicity towards *Vibrio fischeri*	This study showed that heterogeneous photocatalysis of simulated winery wastewater is inefficient in removing TOC, with best performance of only 25% TOC degradation. Homogeneous photocatalysis with solar Fenton in the CPC reactor showed a TOC removal efficiency of 46% with simulated wine wastewater, and 93% with grape juice wastewater, in 400 min of treatment. However, TOC removal was 90% in a 50:50 simulated wine wastewater and diluted grape juice mixture after 150 min. No significant increase in the efficiency of the treatment was observed by increasing ferrous concentration. Furthermore, toxicity (*Vibrio fischeri*) decreases sharply during the photo-Fenton reaction from 48 to 28%. At the same time, total polyphenols decrease 92%, improving thus wastewater biodegradability.	Lucas et al., 2009a
pH= 4.7-5.3 COD= 4047-8827 mg L^{-1} TOC= 1255-2506 mg L^{-1} TSS= 1200-1300 mg L^{-1} TPh= 28-134 mg L^{-1}	Winery wastewater (Portugal)	Flocculation, Ozonation and Fenton oxidation	COD, TSS and TPh removal	In this work the best integration strategy involving flocculation, ozonation and Fenton process to depurate winery wastewater was assessed in order to provide industry with useful data which can help in the choice of a proper treatment scheme. Thus, each individual process was studied and optimized, and 10%, 30% and 54% of COD removal was obtained by flocculation (using 4350SH flocculant), Fenton (using 4.0 g L^{-1} of iron and 9 g L^{-1} of H_2O_2), and ozonation (using 3 g O_3 h^{-1}), respectively. Comparatively to these separated methodologies, the winery effluent can be much more effectively treated by coupling the ozonation treatment with the Fenton process, reaching 73% of COD reduction. Complete degradation of TPh, and high TSS removal (94%) was also obtained.	Martins et al., 2009

pH= 4.0 COD= 4650 mg L^{-1} TOC= 1255 mg L^{-1} TPh= 103 mg L^{-1}	Winery wastewater (Portugal)	Ozone-based advanced oxidation processes (O_3, O_3/UV and $O_3/UV/H_2O_2$) on a pilot-scale bubble column reactor	COD and TOC removal, pH, ozone consumption and reaction kinetics	At the natural pH of the wastewater (pH=4) the effectiveness of each AOP followed the sequence: $O_3/UV/H_2O_2 > O_3/UV > O_3$. The rate of COD and TOC removal was enhanced by operation at neutral (pH=7) and at alkaline pH (pH=10). The rate of ozone consumption in the reactor with the O_3/UV and $O_3/UV/H_2O_2$ processes was in the range of 70-95% during the experiments, suggesting an effective use of the ozone supplied to the system. The highest TOC removal was achieved by $O_3/UV/H_2O_2$ at pH=10 after 300 min, and was equal to 64%. In all the experiments the disappearance of the winery wastewater organic load was described by pseudo-first order apparent reaction kinetics.	Lucas et al., 2010
COD= 970±78 mg L^{-1} TOC= 370±7.4 mg L^{-1} BOD_5= 291±58 mg L^{-1} BOD_5/COD= 0.3 TPh= 350±35 mg L^{-1}	Synthetic winery wastewater - mixing of six phenolic acids	Fenton oxidation	Effect of ferrous and peroxide concentration on COD, TOC, BOD_5 and phenolic acids removal. Toxicity towards *Vibrio fisheri* and biodegradability	A TOC removal in the range of 15.0-58.8% was attained within the operational conditions used. On the hydrogen peroxide injection methodology, the results showed that the continuous introduction of small volumes is advantageous when compared with one single addition of the overall volume at the zero reaction time with a mineralization improvement of 11%. The use of $FeSO_4 \cdot 7H_2O$ correspondent to a Fe^{2+} load of 271 mg; $[H_2O_2]$= 488 mM, injected in twelve aliquots each 30 min during 6 h of reaction reached optimal efficiencies with the parent compounds (phenolic acids, quantified by HPLC and the Folin-Ciocalteau method) quickly totally removed (100%); and TOC, COD and BOD_5 removal of 67%, 83% and 58%, respectively. Toxicity assessment by *Vibrio fischeri* light inhibition revealed that Fenton's process reduces the effluent ecological impact related with the decomposition of the toxic phenolic acids. Indeed, EC_{50} changed from 32.2% dilution to no-dilution needed. The analysis of BOD_5/COD ratio pointed out a high improvement of the treated wastewater biodegradability from 0.30 to 0.80 meaning that the application of Fenton's oxidation as a pretreatment enables a further application of an efficient post-biological technology, which	Martins et al., 2010

				was also confirmed by respirometry.	
pH= 3.0 COD= 19833±160 mg L^{-1} TOC= 9320±20 mg L^{-1} BOD_5= 8745±1750 mg L^{-1} TSS= 2200±110 mg L^{-1} VSS= 800±80 mg L^{-1} TPh= 90±18 mg L^{-1} Temperature= 25 ^{o}C Biodegradability= 27±8% Toxicity= 100±8% EC_{50}= 4.5±2%	Wine-distillery wastewater (Portugal)	Flocculation, Ozonation and Fenton oxidation	TSS, COD and BOD_5 removal	The commercial flocculant 923PWG led to up to 84% of TSS removal even if a very low COD abatement was observed (<2%). The optimum Fenton conditions were $[H_2O_2]$= 0.5 M and $[H_2O_2]/[Fe^{2+}]$= 10 mol mol^{-1}, and were able to achieve 45% COD and 20% BOD_5 removal. Ozonation showed poor results regarding organic matter removal (~10% expressed as COD); however, it seems to be an interesting methodology raising the effluent's biochemical oxygen demand BOD_5/COD ratio even up to 0.81 especially when pH=3. According to these results, a highly biodegradable stream is reached using Fenton's peroxidation followed by ozonation at pH=3 (BOD_5/COD=0.98) that can be further biologically treated.	Martins et al., 2011
pH= 12.40 TOC= 2674 mg L^{-1} TS= 12.06 mg L^{-1} Sedimentable Solids= 0.80 mL L^{-1}	Winery wastewater (Spain)	Coagulation-flocculation and solar Fenton oxidation at a pilot scale	Effect of pH, H_2O_2 and oxalic acid concentration and temperature on TOC removal	The temperature and the initial concentrations of H_2O_2 and oxalic acid were the most significant factors affecting the winery wastewater mineralization. Under optimal conditions (pH 3.5, $[H_2O_2]$= 250 mg L^{-1}, $[Fe^{2+}]$= 10 mg L^{-1}, $[H_2C_2O_4]$= 30 mg L^{-1} and T= 28 ^{o}C), 61% TOC removal from the treated wastewater was achieved in 360 min. The correlation between consumed hydrogen peroxide and removed TOC was found to remain constant. Thus the addition of H_2O_2 can be used to control the degree of mineralization for this type of wastewater. Photocatalytically degraded wastewater, containing significant residual organic content, can be treated at a later stage by a biological process, thus reducing the cost of its total mineralization using just photochemical oxidation methods.	Monteagudo et al., 2012

II. Membrane concentrate streams					
DOC= 10-10.2 mg L^{-1}	RO concentrate of municipal wastewater (Greece)	Electrolytic oxidation, heterogeneous photocatalysis (UVA/TiO_2) and sonolysis	DOC removal	The three oxidation methods employed (electrolytic oxidation over a boron-doped diamond electrode, UVA/TiO_2 photocatalysis and sonolysis at 80 kHz), showed similar behavior: during the first few minutes of treatment there was a moderate removal of DOC followed by further oxidation at a very slow rate. Electrolytic oxidation was capable of removing up to 36% of DOC at 17.8 A after 30 min of treatment, sonolysis removed up to 34% of DOC at 135 W after 60 min, while photocatalysis was capable of removing up to 50% of DOC at 60 min of treatment.	Dialynas et al., 2008
pH= 7.0 COD= 138 mg L^{-1} DOC= 40 mg L^{-1} TDS= 5560 mg L^{-1} Conductivity= 10 mS cm^{-1}	RO concentrate from secondary treated effluent (USA)	Heterogeneous photocatalysis (UV/TiO_2) followed by a simple biological system (sand filter)	DOC removal	UV/TiO_2 can remove a high percentage of organic matter (80%) from this RO concentrate. The combination of AOPs and a simple biological system (e.g. sand filter) can remove higher levels of organic matter at lower UV dosages; because AOPs produce biologically degradable material (e.g. organic acids) that have low HO• rate constants, meaning that their oxidation, rather than that of the primary organic matter in the RO concentrate, dictates the required UV energy inputs. At the highest applied UV dose (10 kWh m^{-3}), the DOC in the RO concentrate decreased from 40 to 8 mg L^{-1} (80% removal) of which approx. 6 mg L^{-1} were readily biologically degradable. Therefore, after combined UV treatment and biodegradation, the final DOC concentration was 2 mg L^{-1}, representing 91% DOC removal.	Westerhoff et al., 2009
pH= 7 COD= 470 mg L^{-1} BOD= 80 mg L^{-1} DOC= 95 mg L^{-1} Chloride= 440 mg L^{-1} BOD/COD= 0.17	RO concentrate from a wastewater treatment plant of a photo processing unit (Belgium)	Homogeneous Fenton oxidation and ozonation (UV/O_3)	COD and TOC removal, enhancement of biodegradation	Comparing all the investigated dosages (1.4-28 mM) of Fenton reagent, the hydrogen peroxide dosage of 2.4 mM has the most positive impact on both the BOD/COD ratio (0.14), and the COD removal (80%). Ozone treatment was proved to be rather inefficient for the reduction of COD and TOC, usually not exceeding 50% and 40% removal, respectively. On the other hand, the oxidation by ozone and UV achieves a more positive effect on the enhancement of the biodegradability, and more specifically the	van Aken et al., 2010

				biodegradation rate is increased with 10% by Fenton oxidation and with 38% by ozone/UV.	
pH= 8.2-8.4 DOC= 2.5-27.7 mg L^{-1} TDS= 346-1392 mg L^{-1} Turbidity= 0.3-0.9 NTU Conductivity=525-2109 μS cm^{-1} Alkalinity= 172-282 mg L^{-1} Hardness= 186-260 mg L^{-1}	Membrane concentrate of natural waters containing pesticides (bromoxynil and trifluralin) (Canada)	UV/H_2O_2 and ozonation (O_3/H_2O_2)	Pesticide degradation, toxicity (*Vibrio fisheri*) and electrical energy per order	The results of this study indicated that high pesticide degradation was achieved using O_3/H_2O_2 for all concentrate matrices (75-87%); while significant lower degradation achieved using UV/H_2O_2 (51-80%). However, the toxicity (*Vibrio fisheri*) obtained during the O_3/H_2O_2 process was higher than that obtained during UV/H_2O_2 treatment. Low levels of pesticide oxidation were observed in experiments using mixture of pesticides during all treatment options. In terms of electrical energy per order, the O_3/H_2O_2 treatment was found to be more efficient for bromoxynil concentrates, and UV/H_2O_2 for concentrate containing trifluralin (<0.5 kWh m^{-3} $order^{-1}$).	Chelme-Ayala et al., 2010
pH= 7-9 COD= 220-260 mg L^{-1} BOD_5= 10-20 mg L^{-1} Cu^{2+}= 4-6 mg L^{-1} Ni^{2+}= 4-6 mg L^{-1}	RO concentrate from a metal plating wastewater (China)	Homogeneous Fenton oxidation followed by a biological aerated filter	Effect of pH, H_2O_2 and ferrous dosage, hydraulic retention time on COD, Cu^{2+} and Ni^{2+} removal	A combination process including Fenton oxidation and a biological aerated filter was used to treat RO concentrate containing complex Cu and Ni from metal plating. During the Fenton treatment, Cu and Ni ions were released due to the degradation of organic compounds, and then removed by pH adjustment and coagulation. The concentrate was further treated using a biological aerated filter. Optimum conditions were as follows: initial pH of influent of 4.0; dosage of H_2O_2 of 5.0 mmol L^{-1}; ratio of (Fe^{2+})/(H_2O_2) of 0.8; precipitation pH of Cu and Ni ions of 8.0; and a hydraulic retention time of the biological aerated filter of 2.5 h. The results showed that concentrations of effluent COD, Cu and Ni ions were less than 40 mg L^{-1}, 0.5 mg L^{-1} and 0.3 mg L^{-1}, respectively; indicating removal up to 85% for COD, 92% for Cu^{2+}, and 95% for Ni^{2+}. While only Fenton oxidation achieved removal of 51.7% for COD, and more than 85% for Cu and Ni ions as well.	Huang et al., 2011

pH= 6.9±0.2 COD= 60±0.5 mg L^{-1} TOC= 18±2 mg L^{-1} TDS= 1129±40 mg L^{-1} TKN= 10±3 mg L^{-1} Turbidity= 3.2±0.4 NTU Conductivity= 1.7 mS cm^{-1}	RO concentrate of municipal wastewater (Singapore)	Heterogeneous photocatalysis (UVA/TiO_2), sonolysis, ozonation (O_3) and H_2O_2 oxidation, as well as their combinations	DOC and COD removal and ecotoxicity towards *Vibrio fisheri*	Simple ozone treatment could only remove a small fraction (22%) of DOC with marginal improvements at higher energy input. Nevertheless, O_3 treatment provided better performances that UV irradiation or H_2O_2 addition. The highest efficiency was obtained by the combination of ozonation with photocatalysis (UVA/TiO_2/O_3), which reach a DOC removal of 44%. Coupling pretreatment with coagulation, the various AOPs could efficiently remove the organics, resulting in an overall DOC removal of 34-68%, a significant improvement in biodegradability (7-20 times), and a decrease of ecotoxicity towards *V. fisheri* (~40%). The results also demonstrated that a simple integrated method, $FeCl_3$ coagulation + photocatalysis (UVC/TiO_2), could finally achieve 95% of the organics removal from the RO concentrate within 6 h.	Zhou et al., 2011b
pH= 7.5±0.5 COD= 530±74 mg L^{-1} BOD_5= 21±4 mg L^{-1} TOC= 111±13 mg L^{-1} TSS= 21±7 mg L^{-1} TP= 12±5 mg L^{-1} TN= 105±22 mg L^{-1} Conductivity= 3.7 mS cm^{-1} Temperature= 23±2 ^{o}C	RO concentrate from a paper mill (Spain)	Conventional Fenton and photo-Fenton oxidation and heterogeneous photocatalysis (UV/TiO_2) combined with a biological treatment	COD, TOC removal and effect of pH	Photo-Fenton process was able to totally remove the COD (100%) of the RO concentrate from a paper mill, while conventional Fenton achieved an 80% reduction of the COD at best. In addition, although these optimal results were produced at pH 2.8, it was also proved that Fenton processes are able to achieve good COD reduction efficiencies (>60%) without adjusting the initial pH value, provided the natural pH of this wastewater was close to neutral. Finally, although TiO_2-photocatalysis showed the least efficient (40% COD and 35% TOC removal), it improved the biodegradability of the concentrate, so its combination with a final biological step almost achieved the total removal of the COD.	Hermosilla et al., 2012

pH= 7.7 COD= 3896 mg L^{-1} BOD_5= 2.6 mg L^{-1} TOC= 1347 mg L^{-1} TDS= 18910 mg L^{-1} TN= 234 mg L^{-1}	Nanofiltration (NF) concentrate of landfill leachate (China)	Electro-Fenton (E-Fenton)	Effect of initial pH, density, $FeSO_4$ dosage and cathode surface on TOC and TN removal	Under optimal reaction conditions that included a current density of 30 mA cm^{-2}, $FeSO_4$ dosage of 10 mM, initial pH of 3, and cathode area of 20 cm^2, the TOC and TN removal efficiencies were 82% and 51%, respectively, after 6 h of the electro-Fenton treatment.	Wang et al., 2012
pH= 7.2-7.6 COD= 120-150 mg L^{-1} TOC= 15-18 mg L^{-1} TSS< 25 mg L^{-1} Conductivity= 22.3 mS cm^{-1}	RO concentrate from a steel plant (China)	Electro-Fenton (E-Fenton)	Effect of pH, Fe^{3+} concentration and cathodic potential on COD removal	The COD removal achieved the best performance under the conditions of cathodic potential -0.72 V, Fe^{3+} concentration 0.2 mM, initial pH 3, in which more than 62% of COD could be removed, and could meet the local wastewater discharge requirement (COD< 50 mg L^{-1}).	Zhou et al., 2012

3.4.1 Assessment of the performance of advanced oxidation processes

The use of a strong oxidizing agent can result in a high degree of wastewater treatment, including the breakdown of recalcitrant and toxic compounds. In theory, depending on the oxidation potential of the agent and the contact time, complete mineralization can occur.

i) AOPs applied for the treatment of winery wastewater

In recent years there has been growing interest in advanced oxidation processes (AOPs) for the treatment of industrial effluents, and therefore for the treatment of winery wastewater as well, as shown in Table 3.12. In the study of Navarro et al. (2005) the application of H_2O_2 as oxidant combined with light (artificial or natural) was investigated, in order to reduce the organic matter in effluents from wine industry. Moreover, the combination of H_2O_2 with heterogeneous catalysts: TiO_2 and clays containing iron minerals was studied. The addition of photocatalysts to the system reduces the required H_2O_2 concentration. The highest efficiency after 80 min (58% of COD removal) was reached by combining H_2O_2 and TiO_2. The H_2O_2/clays system produces lower COD removal (45%); however, it requires a H_2O_2 dosage between three and six times lower than the H_2O_2/TiO_2 (Navarro et al., 2005). Additionally, applying photo-Fenton oxidation in heterogeneous phase for the treatment of winery wastewater (synthetic samples) results in purification levels of up to 50% (measured as TOC) (Mosteo et al., 2006). On the other hand, the use of homogeneous photo-Fenton oxidation for the treatment of winery wastewater, proved to be highly effective, reaching TOC removal between 50-95% (Ormad et al., 2006) and COD removal up to 70% (de Heredia et al., 2005a).

Gimeno et al. (2007) investigated the combination of UV-A/Vis radiation and ozone, in the presence of titanium dioxide, as a potential destructive technology for the treatment of winery effluents. Adsorption onto the TiO_2 surface accounts for a 15-20% removal of the initial COD. The rest of the subsystems attained COD conversion values in the range of 37-43%. The photocatalytic ozonation of the effluent, under the operating conditions investigated, increases the COD depletion to a value close to 80%.

Flocculation, Fenton oxidation and ozonation processes were investigated in order to define the best integration strategy that may conduce to optimal efficiency of degradation of winery wastewater (Martins et al., 2009). A COD removal of 10% was obtained by flocculation (using 4350SH flocculant), 30% by Fenton (using 4.0 g L^{-1} of iron and 9 g L^{-1} of hydrogen peroxide) and 54% by ozonation (using 3 g O_3 h^{-1}). The higher COD and TSS removal (73% and 94%, respectively) were achieved combining the ozonation treatment with the Fenton process. Complete degradation of total phenolic compounds was obtained, together with a significant decrease on TOC and color (Martins et al., 2009).

A mixture of six phenolic acids, corresponding to an initial TOC of 370 mg L^{-1}, was studied by Fenton oxidation aiming to improve the biodegradability of synthetic winery wastewater (Martins et al., 2010). TOC, COD and BOD_5 removal of 67%, 83% and 58%, respectively were attained within the optimum operational conditions used. Toxicity assessment by *Vibrio fischeri* light inhibition revealed that Fenton’s process reduces the effluent ecological impact related with the decomposition of the toxic phenolic acids. Indeed, EC_{50} changed from 32.2% dilution to no-dilution needed. The analysis of BOD_5/COD ratio pointed out a high improvement of the treated wastewater biodegradability from 0.30 to 0.80 meaning that the application of Fenton oxidation as a pretreatment enables a further application of an efficient post-biological technology which was also confirmed by respirometry. Furthermore, according to Martins et al. (2011) a moderate COD removal of 45% was observed after Fenton oxidation of wine-distillery wastewater; while single ozonation does not improve the COD abatement (<10%), causing on the other hand a high increase on biodegradability (BOD_5/COD= 0.8).

Research is continually being conducted into larger scales, and as presented below, the use of pilot-scale reactors for the advanced treatment of winery wastewater has been proved to be effective. Lucas et al. (2009a) studied the degradation of simulated winery wastewater at a pilot-scale compound parabolic collector (CPC) solar reactor. The effluents used were reproduced with synthetic samples prepared by diluting commercial grape juice, commercial red wine, and a mixture of both (wine and grape juice) in Milli-Q water. This study showed that heterogeneous photocatalysis (TiO_2, TiO_2/H_2O_2, $TiO_2/Na_2S_2O_8$) of simulated winery wastewater is inefficient in removing TOC, with best performance of only 25% TOC degradation. On the other hand, homogeneous photocatalysis with solar Fenton in the CPC reactor showed a TOC removal efficiency of 46% with simulated wine wastewater and 93% with grape juice. However, TOC removal was 90% in a 50:50 simulated wine wastewater and diluted grape juice mixture. In the study of Lucas et al. (2010) the effectiveness of different ozone-based AOPs (O_3, O_3/UV and $O_3/UV/H_2O_2$) for the treatment of winery wastewater, at a pilot-scale reactor was investigated. The highest TOC removal was achieved by $O_3/UV/H_2O_2$ at pH=10 after 300 min, and was equal to 64%. In a recent study of Monteagudo et al. (2012), the mineralization of wastewater from an actual winery using a ferrioxalate-induced solar photo-Fenton process was analyzed. First, a physicochemical pretreatment of the raw winery wastewater was conducted, using either coagulation/flocculation or precipitation methods. Next, a photochemical reaction was carried out at a pilot plant consisting of a compound parabolic collector (CPC) solar reactor to remove the TOC content of the wastewater. Temperature and the initial concentrations of H_2O_2 and oxalic acid were the most significant factors affecting the wastewater mineralization; and under optimal conditions, 61% TOC removal from the treated winery wastewater was achieved in 360 min.

From all the above, it can be noted that the homogenous photo-Fenton oxidation seems to be the most effective advanced oxidation process for the treatment of winery wastewater, both at bench and pilot scale, achieving COD and TOC removals up to 70% and 95%, respectively. Moreover, ozonation seems also to be a promising process for the treatment of winery wastewater, reaching a COD removal up to 64% at a pilot scale.

ii) AOPs applied for the treatment of membrane concentrate streams

Since no literature on the treatment of winery or other agro-industrial membrane concentrate exists so far, a brief review on various AOPs used for the treatment of membrane concentrate of other industrial and municipal wastewater was conducted, as shown in Table 3.12.

Heterogeneous photocatalysis (UV/TiO_2) proved to be an effective process for the treatment of RO concentrate from secondary treated effluent, reaching a DOC removal of 80% (Westerhoff et al., 2009), and a COD removal of 50% (Dialynas et al., 2008).

Electrolytic oxidation, as well as sonolysis, was capable of removing only up to 34-36% of COD, of the RO concentrate of municipal wastewater (Dialynas et al., 2008). On the other hand, the combination of ozonation with photocatalysis (UV/TiO_2/O_3) has moderate efficiency in the treatment of RO concentrate of municipal wastewater (44% DOC removal); while the combination of $FeCl_3$ coagulation and photocatalysis (UVC/TiO_2), could finally achieve 95% of the organics removal from the RO concentrate within 6 h (Zhou et al., 2011b).

The results of the study of Chelme-Ayala et al. (2010) indicated that high pesticide degradation was achieved using O_3/H_2O_2 (up to 87%) for membrane concentrate of natural waters containing pesticides; while lower degradation achieved using UV/H_2O_2 (<80%). Additionally, Fenton oxidation reached 80% COD removal of an RO concentrate from a wastewater treatment plant of a photo processing company; while ozone treatment was proved to be rather inefficient for the reduction of COD (50%) (van Aken et al., 2010). A combination process including Fenton oxidation and a biological aerated filter was used to treat a RO concentrate containing complex Cu and Ni from metal plating; reaching removal up to 85% for COD, 92% for Cu^{2+}, and up to 95% for Ni^{2+} (Huang et al., 2011). Moreover, according to the study of Hermosilla et al. (2012), the photo-Fenton oxidation was able to totally remove the COD of the RO concentrate from a paper mill; the conventional Fenton achieved an 80% reduction of the COD, while TiO_2-photocatalysis showed to be the least efficient (40% COD removal). Finally, the organic matter removal efficiency of electro-Fenton was high both for the concentrate of landfill leachate (82% DOC removal) (Wang et al., 2012), as well as for the RO concentrate from a steel plant (62% COD removal) (Zhou et al., 2012).

In summary, the most efficient advanced process for the treatment of the various membrane concentrate streams, seems to be the photo-Fenton oxidation, being able to achieve a COD removal of the order of 80%; the electro-Fenton, reaching a satisfactory COD removal of the order of 62%; while the combination of the Fenton oxidation with a biological process seems to be very promising yielding a slightly higher COD removal up to 85%.

3.4.2 Solar photo-Fenton oxidation applied in this thesis

An advanced oxidation process and specifically the photo-Fenton oxidation using solar irradiation was used in this thesis as a post-treatment of the two biologically pretreated winery effluents (SBR and MBR) (Section 3.2.2). The Fenton process fundamentals and mechanisms, the advantages and disadvantages of this process, as well as the successful applications of Fenton and photo-Fenton processes for the treatment of various wastewater streams are presented in detail in the following sections.

Although Fenton's reagent was discovered by H.J.H. Fenton in the 1890s, its application as an oxidizing agent to destroy toxic organics was not introduced until the late 1960s. Fenton's reagent refers to a mixture of hydrogen peroxide and ferrous salts, which is an effective oxidant of a large variety of organic substrates (Goldstein et al., 1993). The ferrous ion initiates and catalyses the decomposition of H_2O_2, resulting in the generation of a range of free radicals, including the highly reactive and non-selective hydroxyl radicals (HO•), which are capable to react with a wide range of organic compounds in water matrices and cause their chemical decomposition (Lucas and Peres, 2006).

The Fenton's reagent oxidation has proved very effective in the removal from water and wastewater of many hazardous organic pollutants, including persistent organic pollutants (POPs) that are degraded to harmless compounds, namely CO_2, water and inorganic salts (Buyukkamaci, 2004; Gravotto et al., 2005).

Furthermore, process efficiency may be enhanced in the presence of light irradiation (hv) (i.e., photo-Fenton), which increases the rate of contaminant degradation by stimulating the reduction of Fe^{3+} to Fe^{2+} (Pérez et al., 2002; Parsons, 2004; García-Montaño et al., 2006; Anastasiou et al., 2009). The photo-Fenton is an advantageous process because: Fe^{2+} is abundant and nontoxic at low concentrations, hydrogen peroxide can be easily handled and it is an environmentally friendly compound. Furthermore, there are no mass transfer limitations because all of the reagents are in the liquid phase. The design of reactors for technological application is rather simple (Litter, 2005). Fenton reagent appears to be a very powerful oxidizing agent. Moreover, the process is simple taking place at low temperatures and at atmospheric pressure. The chemicals (ferrous ion and hydrogen peroxide) are readily available at moderate cost and there is no need for special equipment (Lucas et al., 2006). In the presence of Fenton reagent, photochemical reactions can be driven by photons of low energy, belonging to the visible part of the spectrum ($\lambda<580$ nm).

Many researchers, according to the literature, take into account the possibility of driving the Fenton process with solar radiation (solar photo-Fenton). This is because photo-Fenton process seems to be the most apt of all AOPs to be driven by sunlight, because soluble iron-hydroxy, and especially iron-organic acid complexes absorb even part of the visible light spectrum, not only ultraviolet radiation (Malato et al., 2009). The illumination leads not only to the formation of additional hydroxyl radicals, but also to recycling of ferrous (Fe^{2+}) catalyst by reduction of Fe^{3+}. In this way, the concentration of Fe^{2+} is increased and the overall reaction is accelerated (Tamimi et al., 2008). Thus, the photo-Fenton process is a potential low cost AOP that can be operated under solar irradiation (Perez et al., 2002; García-Montaño et al., 2006; Malato et al., 2009).

However, the application of Fenton and photo-Fenton processes based on homogeneous ferrous or ferric salts usually suffers a major drawback associated with the narrow pH range of operation, typically between 2.5 and 3.5, and more usually around 2.8, to avoid the formation and subsequent precipitation of iron oxyhydroxides (Pignatello, 1992; Pariente et al., 2008). This means not only additional cost through the consumption of reagents for acidification and subsequent neutralization, but also an increase of the treated water's salt loads (Malato et al., 2009). In this respect, the immobilization of Fenton's catalyst on a heterogeneous matrix would enable its use under non-controlled pH conditions. The recovery of the catalyst is easy and precipitation of the ferric hydroxide is not expected (Santos et al., 2007; Pariente et al., 2008; Malato et al., 2009).

After more than 110 years after the Fenton reaction was discovered, it is now well known that this oxidation system is based on the formation of reactive oxidizing species able to efficiently degrade the pollutants of the wastewater stream. The nature of these species, the mechanism and the key intermediates are still under discussion, and it has been a subject of controversy in the past and recent Fenton oxidation related literature (Fentona and Chemizmu, 2009). There is something intriguing and at the same time fascinating that a simple reaction (of Fe^{2+} ions with H_2O_2), proves to be very difficult to describe and understand.

3.4.2.1 Fenton chemistry

Behind oxygen, silicon and aluminium, iron is the fourth most abundant element in the earth's crust. It occurs in oxidation numbers from -II to +VI. In aqueous solution the most abundant iron species are ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) (Malato et al., 2009). Dissolved ferrous and ferric iron species are present in octahedral complexes with six ligands in water. Iron is complexed by water and hydroxyl ligands provided that no other complexing substances are present. How many of these ligands are hydroxyl ions, depends on the solution's pH, which influences directly the acid/base equilibrium of the aquo complex. Ferric iron is the more critical iron species in the photo-Fenton process, because its hydroxides precipitate at lower pH than those of ferrous iron. It is crucial to understand the hydrolytic speciation of Fe^{3+} species in order to accurately interpret their absorption spectra and photochemical behavior.

Consequently, the pH-dependent hydrolytic speciation of Fe^{3+} can be represented by the following iron aquo complexes, (Eqs. (3.1)-(3.4)).

$$[Fe(H_2O)_6]^{3+} \leftrightarrow [Fe(H_2O)_5(OH)]^{2+} + H^+ \qquad (3.1)$$

$$[Fe(H_2O)_5(OH)]^{2+} \leftrightarrow [Fe(H_2O)_4(OH)_2]^{+} + H^+ \qquad (3.2)$$

$$[Fe(H_2O)_4\,(OH)_2]^{3+} \leftrightarrow [Fe(H_2O)_3(OH)_3] + H^+ \qquad (3.3)$$

$$2Fe_{aq}^{3+} \leftrightarrow [Fe_2(OH)_2]^{4+} + 2H^+ \qquad (3.4)$$

Figure 3.1 shows the equilibrium concentrations of the most important ferric iron aquo complexes in the absence of other complexing substances, at different pH for a ferric iron concentration of 20 mg L^{-1} (Malato et al., 2009).

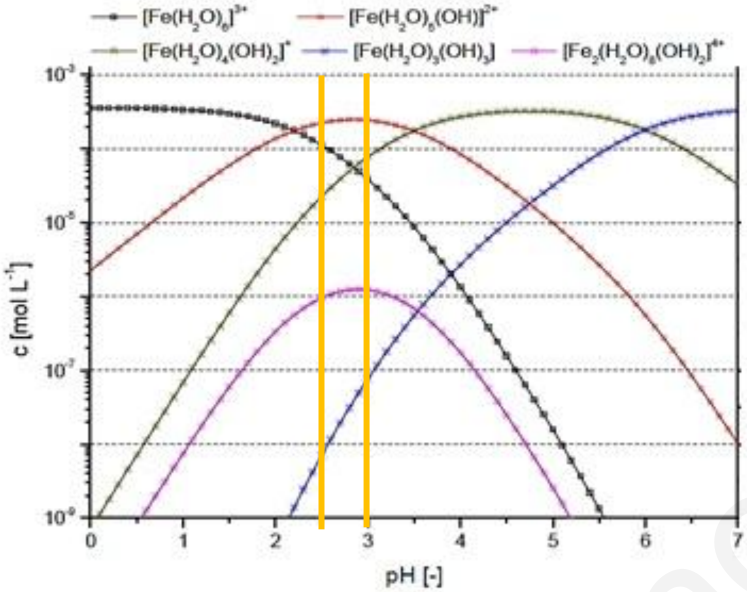


Figure 3.1: Ferric (Fe^{3+}) iron species present in aqueous solution at different pH at a concentration of 20 mg L^{-1}, calculated with equilibrium constants provided by Flynn (1984) at T=20 ^{o}C.

Source: Malato et al., 2009

As shown in Figure 3.1, $[Fe(H_2O)_5(OH)]^{2+}$ is the dominant monomeric Fe^{3+}-hydroxy complex species between pH 2.5 and 3.0 (yellow lines). The maximum catalytic activity of the Fe^{2+}/Fe^{3+}-H_2O_2 system is at a pH of about 2.8-3.0. At pH > 5.0, ferric hydroxide $[Fe(H_2O)_3(OH)_3]$ is generated, while at pH < 2.0 the ferric ions form $[Fe(H_2O)_6]^{3+}$ which at higher pH this complex undergoes hydrolysis and subsequent precipitation. The precipitation process starts at pH=2.5-3.5 with the formation of dimers and oligomeres, which gradually polymerise further and lose water until forming finally insoluble iron hydroxides (Malato et al., 2009).

Reaction between dissolved Fe^{2+} and H_2O_2 in acidic aqueous solution leads to oxidation of Fe^{2+} to Fe^{3+} and is thought to form the highly reactive hydroxyl radicals (HO•) (Eq. 3.5). The reaction is spontaneous and can occur without the influence of light:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \quad (3.5)$$

This reaction is often known as the Fenton reaction, after Fenton (1894) who first described the process, but is actually the Haber-Weiss reaction, which is a specific example of the Fenton reaction. It was proposed by Haber and Weiss (1934), and further developed by Barb et al. (1951a, b) and Walling (1975).

There were two suggestions for the Fenton mechanism, a radical, which considers a hydroxyl radicals ($HO^{\bullet}$) production, and a non-radical, considering ferryl ion (Fe^{4+}) production (Fentona and Chemizmu, 2009). The radical mechanism has been broadly accepted for reactions in acidic milieu. Yet, it should be mentioned that discussion is still on-going and the occurrence of ferrate and ferryl iron (+IV and +V), at least in intermediate complexes, has been proposed (Pignatello, 1992; Bossmann et al., 1998; Malato et al., 2009). The free radical mechanism proposed by Barb et al. (1951b) consists of the following steps Eqs. (3.6)-(3.9) (Kremer, 1999):

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \quad (3.6)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + H^{+} + O_2 \quad (3.7)$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{3+} + HO_2^{-} \quad (3.8)$$

$$Fe^{2+} HO^{\bullet} \rightarrow Fe^{3+} HO^{-} \quad (3.9)$$

Fenton is a chain reaction including a step (Eq. (3.5)) serving as chain initiation, steps Eqs. (3.8) and (3.9) as termination, and the cycle (Eqs. (3.5)-(3.6)-(3.7)) forms the chain which is the site of O_2 evolution (Kremer, 1999).

Kremer, (1999) concluded that it is difficult to accept the existence of free radical mechanism because this mechanism either in the formulation of Haber and Weiss (1934) or in that of Barb et al. (1951b) recognizes only Fe^{2+} and Fe^{3+} as the forms of iron in the system. In the free radical mechanism, reaction (Eq. (3.6)) becomes insignificant at low H_2O_2 as a mode of reaction of HO• (Fentona and Chemizmu, 2009). Hydroxyl radicals could then react with Fe^{2+} and produce Fe^{3+} (Eq. (3.10)). As an alternative, HO• could react with Fe^{3+} (Eq. (3.11)):

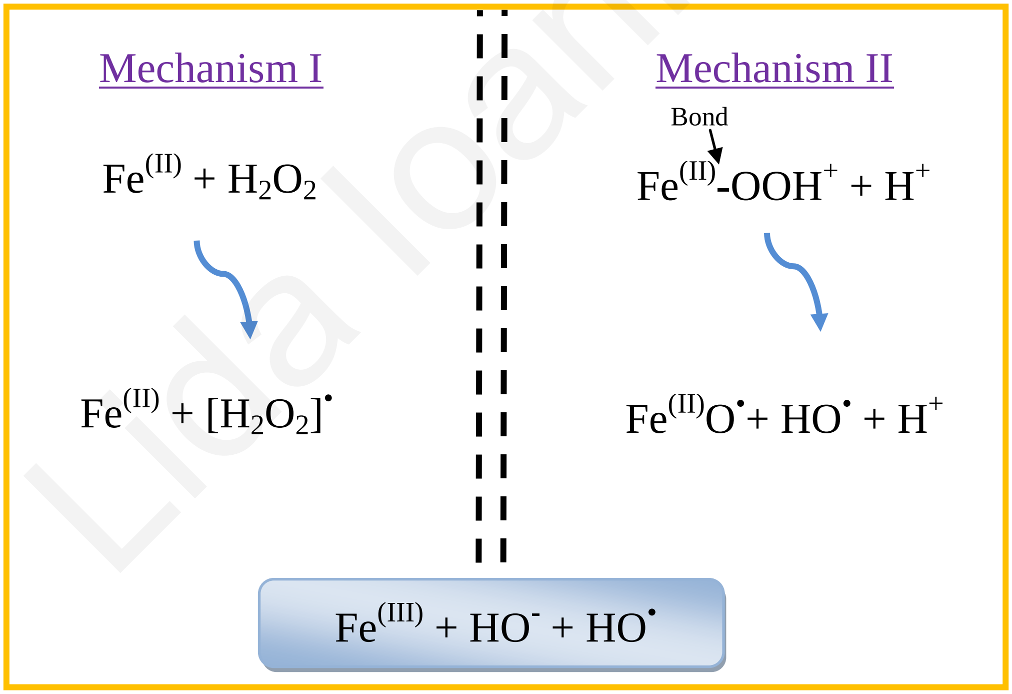
$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \tag{3.10}$$

$$Fe^{3+} + HO^{\bullet} \rightarrow FeOH^{3+} \tag{3.11}$$

If this reaction (Eq. (3.11)) occurred, it would be even more plausible to assume that the pair Fe^{3+} + HO• (as products of Fenton reaction (Eq. (3.5)) would not become separated at all, and the species $FeOH^{3+}$ would appear instead. It can be stated that the species $FeOH^{3+}$ is merely the protonated form of FeO^{2+} $(FeO^{2+} + H^{+} \rightarrow FeOH^{3+})$ (Kremer, 1999).

Many studies examining the nature of reactive oxidizing species in the Fenton reaction have been conducted, and many possible mechanisms of reaction were presented. Some of these studies suggested that the classical Fenton reaction occurs using only Fe^{2+} as an electron donor to H_2O_2. Such would be an outer sphere electron transfer reaction with no direct bonding interactions between the electron donor and the acceptor, (Mechanism I, Schematic 3.7). However, the outer sphere mechanism is thermodynamically unfavorable and unlikely to occur (Parsons, 2004; Fentona and Chemizmu, 2009).

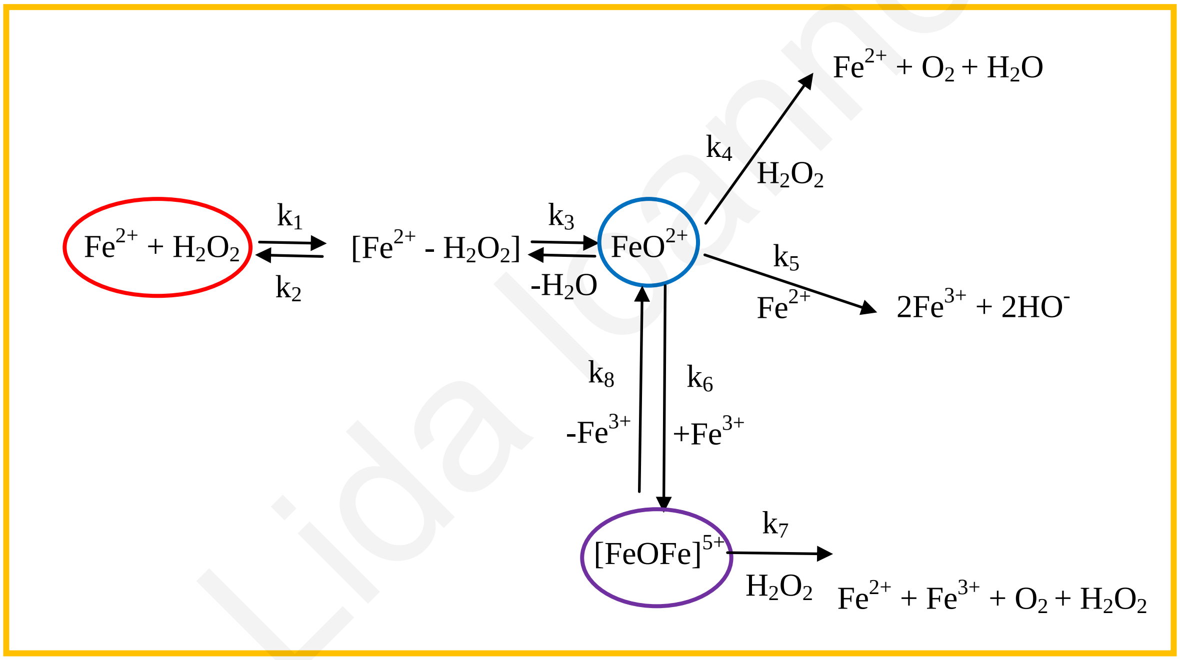
On the other hand, recent studies have shown and favored the inner sphere electron transfer mechanism, which involves direct bonding between the iron and the hydrogen peroxide. This interaction can produce an iron-peroxo complex, Fe-OOH$^+$ which may react further to generate hydroxyl radicals (one-electron oxidant) (Mechanism II, Schematic 3.7) (Fentona and Chemizmu, 2009). The key question therefore is which of these species is the major oxidant in these reactions (Mwebi, 2005).



Schematic 3.7: Basic reactions and intermediates involved in the classic Fenton process

Source: Fentona and Chemizmu, 2009

Kremer, (1999) proposed a non-radical mechanism for the Fenton reaction (Schematic 3.8). The reaction starts with the reversible formation of a primary intermediate $[Fe^{2+}\cdot H_2O_2]$ from Fe^{2+} and H_2O_2 (exchange of a H_2O molecule in the hydration shell of Fe^{2+} ions by H_2O_2). From the primary complex a secondary intermediate FeO^{2+} is formed by the loss of H_2O. This species is the key intermediate in the reaction. It can react either with Fe^{2+} ions to produce Fe^{3+} (k_5), or with H_2O_2 to produce O_2 (k_4). FeO^{2+} can further react with Fe^{3+} and form a binuclear species $[FeOFe]^{5+}$ (k_6). This species can react with H_2O_2 to produce O_2 (k_7) or to decompose back to FeO^{2+} and Fe^{3+} (k_8).



Schematic 3.8: Mechanism of Fenton reaction as proposed by Kremer (1999)

The mechanism of Fenton reactions is still poorly understood, and there is much disagreement in the literature on the exact intermediates that form including whether or not the HO• itself forms (Kremer, 1999).

Considering the fact that Fenton reaction is common in chemical, biological, and environmental systems where conditions may be very diverse, it is highly probable that there is more than one universal Fenton mechanism. It is possible that both hydroxyl radicals and ferryl ions can coexist in Fenton chemistry (Fenton and Fenton-like reactions), and depending on the environmental conditions or operating parameters, one of them will predominate. Given the above doubts, it is desirable to carry out further in-depth research either to prove the above hypothesis or to prove that there is only one mechanism (radical or non-radical) of the Fenton reaction (Fentona and Chemizmu, 2009).

Mixtures of ferrous iron and hydrogen peroxide, as already mentioned, are called Fenton reagent. If ferrous is replaced by ferric iron it is called Fenton-like reagent. Eqs. (3.12)-(3.18) show the reactions of ferrous iron, ferric iron and hydrogen peroxide in the absence of other interfering ions and organic substances. The regeneration of ferrous iron from ferric iron by Eqs. (3.16)-(3.18), is the rate limiting step in the catalytic iron cycle, if iron is added in small amounts. The listed rate and equilibrium constants for Eqs. (3.12)-(3.18) were reported by Sychev and Isak (1995).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{-} + HO^{\bullet} \qquad k=53 - 76\ M^{-1}s^{-1} \tag{3.12}$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \qquad k=2.6 - 5.8\times10^{8}\ M^{-1}s^{-1} \tag{3.13}$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{3+} + HO_2^{-} \qquad k=0.75 - 1.5\times10^{6}\ M^{-1}s^{-1} \tag{3.14}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^{+} + HO_2^{\bullet} \qquad k=1 - 2\times10^{-2}\ M^{-1}s^{-1} \tag{3.15}$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^{+} \qquad k=0.33 - 2.1\times10^{6}\ M^{-1}s^{-1} \tag{3.16}$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \qquad k=0.05 - 1.9\times10^{9}\ M^{-1}s^{-1} \qquad (3.17)$$

$$H_2O_2 + HO^{\bullet} \rightarrow H_2O + HO_2^{\bullet} \qquad k=1.7 - 4.5\times10^{7}\ M^{-1}s^{-1} \qquad (3.18)$$

If organic substances (quenchers, scavengers or in the case of wastewater treatment pollutants) are present in the system $Fe^{2+}/Fe^{3+}/H_2O_2$, they react in many ways with the generated hydroxyl radicals. Yet, in all cases the oxidative attack is electrophilic, and the rate constants are close to the diffusion-controlled limit. The following reactions with organic substrates have been reported (Legrini et al., 2003):

Hydrogen abstraction from aliphatic carbon atoms, Eq. (3.19).

$$HO^{\bullet} + RH \rightarrow R^{\bullet} + H_2O \qquad (3.19)$$

Electrophilic addition to double bonds or aromatic rings, Eq. (3.20).

$$R-CH=CH_2 + HO^{\bullet} \rightarrow R-C^{\bullet}H-CH_2OH \qquad (3.20)$$

Electron transfer reaction, Eq. (3.21).

$$HO^{\bullet} + RX \rightarrow RX^{\bullet +} + HO^{-} \qquad (3.21)$$

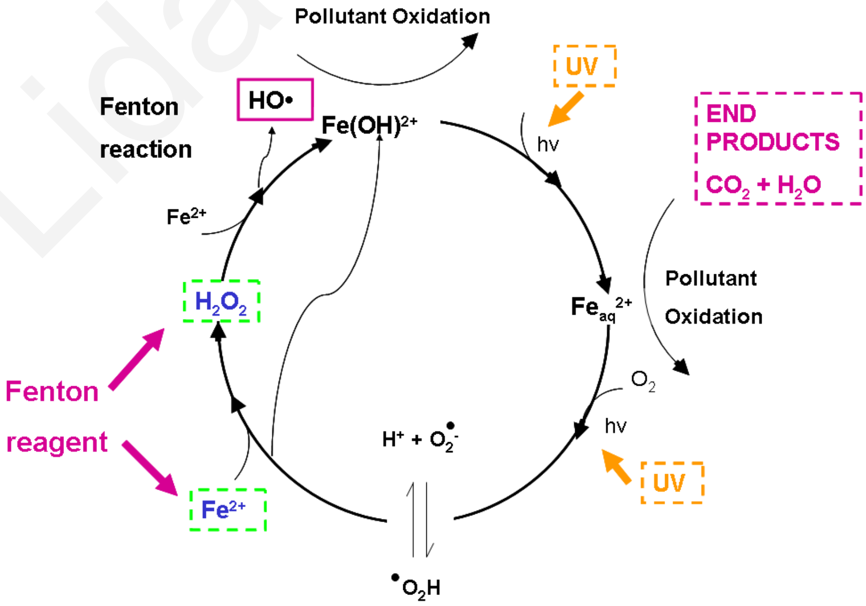
Due to the high oxidation potential of the HO•, it can also react with inorganic ions present in the solution. Several authors have described the strong negative effect of the presence of carbonate and phosphate in the Fenton reaction, while the effect of other ions, such as chloride or sulphate is not as strong (Pignatello, 1992; De Laat et al., 2004; Kavitha and Palanivelu, 2004). Phosphate has a double detrimental effect; first, it precipitates iron and second, it scavenges hydroxyl radicals.

It is known that the efficiency of the Fenton reaction for the degradation of a great variety of toxic and non-biodegradable organic pollutants depends mainly on several operating parameters, such as H_2O_2 concentration, Fe^{2+}/H_2O_2 ratio, pH and reaction time. The initial concentration of the pollutant and its character, as well as temperature and solution salinity, also have a substantial influence on the final efficiency. The effect of these parameters is discussed, in the next Chapters (Chapter 7).

The Fenton process efficiency, as mentioned before, may be enhanced in the presence of light irradiation (hv) (i.e. photo-Fenton), by photoreducing the ferric iron to ferrous iron, and the generation of additional hydroxyl radicals (Eq. (3.22)) (Pérez et al., 2002).

$$Fe^{3+} + H_2O + hv \rightarrow Fe^{2+} + H^{+} + HO^{\bullet} \qquad (3.22)$$

A schematic representation of the photo-Fenton process, where it is evidence that the light increases the catalytic iron cycle and the formation of hydroxyl radicals, is illustrated in Schematic 3.9.



Schematic 3.9: Sequence of the main reactions occurring in the photo-Fenton system

It has been demonstrated that Fenton and photo-Fenton processes are used to treat a variety of agro-industrial effluents such as livestock (Lee and Shoda, 2008), olive mill (Dogruel et al., 2009; Kallel et al., 2009), winery wastewater (Mosteo et al., 2006; Ormad et al., 2006; Mosteo et al., 2008; Lucas et al., 2009a; Martins et al., 2011; Monteagudo et al., 2012); as well as other industrial and municipal wastewater (Yang and Long, 1999; Maciel et al., 2004; Shemer et al., 2006; Tekin et al., 2006; Yavuz et al., 2007; Kajitvichyanukul, et al., 2008; Trovó et al., 2008). A brief review of the successful applications of Fenton and photo-Fenton oxidation for the treatment of other agro-industrial wastewater, except winery wastewater (that was previously presented in Table 3.12), is presented in Table 3.13.

Table 3.13: Applications of Fenton and photo-Fenton processes for the treatment of agro-industrial wastewater streams

Wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
COD= 110 mg L^{-1} TOC= 75 mg L^{-1}	Water containing natural phenolic pollutants	Photo-Fenton oxidation	Phenolic and COD removal	In this work, six model phenolic compounds (vanillin, protocatechuic acid, syringic acid, p-coumaric acid, gallic acid and l-tyrosine) were chosen for a demonstration of degradation by photo-Fenton reaction, under artificial light at bench-scale experiments and under sunlight at pilot-scale experiments. All compounds were completely mineralized (100%). No non-degradable intermediates were produced, either in experiments with single substances or in a more complex matrix of a mixture of phenolic compounds. The maximum COD removal achieved was 87% after 90 min of treatment.	Gernjak et al., 2003
pH= 8.4-8.7 COD= 5000-5700 mg L^{-1} Color= dark grey	Livestock wastewater (Japan)	Fenton oxidation	Effect of H_2O_2 and ferrous dosage, pH and reaction time on COD and color removal	The optimum ratio of H_2O_2 to the initial COD was 1.05 mg L^{-1} and the optimum molar ratio of H_2O_2/Fe^{2+} was 2; while the optimum pH and the optimum reaction time were 3.5 and 30 min, respectively. Under optimal experimental conditions, the removal ratios of COD and color of the supernatant after static precipitation of the produced sludge were 88% and 95.4%, respectively. Addition of Fenton's reagents in several aliquots did not affect the efficiencies of COD and color removal.	Lee and Shoda, 2008
pH= 3 and 4.6 COD= 39240 mg L^{-1} TOC= 13430 mg L^{-1} BOD_5= 15030 mg L^{-1} TSS= 5310 mg L^{-1} VSS= 5050 mg L^{-1} TKN= 6.7 mg L^{-1}	Olive mill wastewater (OMW) (Turkey)	Fenton oxidation	COD, TPh and antioxidant activity abatements obtained for Fenton's oxidation of OMW at pH 3.0 and pH 4.6	COD removal achieved via Fenton's oxidation both at pH=3.0 and pH=4.6 (the original pH of the OMW) remained in the range of 40-50%. As anticipated, the effect of Fenton's treatment was more pronounced in the soluble size range. Fenton's oxidation at pH=3.0 resulted in 46% and 63% removal for total phenols and antioxidant activity, respectively.	Dogruel et al., 2009

pH= 5.2 COD= 19000 mg L^{-1} TPh= 672 mg L^{-1}	Diluted olive mill wastewater (OMW) (Tunisia)	Fenton oxidation with zero-valent iron	COD and phenolic compounds removal and enhancement of biodegradability	The application of zero-valent Fe/H_2O_2 procedure allows high removal efficiency of pollutants from OMW. The optimal experimental conditions were found to be continuous presence of iron metal, acidic pH (2-4) and 1M hydrogen peroxide solution. At a pH value within 2 and 4 the maximum COD removal of 92% was reached. After 24 h of Fenton reaction, the BOD_5/COD ratio reaches the value of 0.53, which indicates that the wastewater is now already biodegradable. This improvement in the biodegradability is obtained by the complete removal (100%) of phenolic compounds from the OMW.	Kallel et al., 2009
pH= 5.2 COD= 115000 mg L^{-1} TSS= 32000 mg L^{-1} TN= 420 mg L^{-1} TP= 120 mg L^{-1} TPh= 5580 mg L^{-1} Oil and grease=23000 mg L^{-1}	Olive mill wastewater (OMW) (Turkey)	Physicochemical pretreatment followed by Fenton and Fenton-like oxidation processes	Effect of pH, ferrous and H_2O_2 dosage on COD and TPh removal	Raw OMW has toxic and inhibitory effects on activated sludge mixture and these effluents almost cannot be treated by means of classical biological processes. In this study, OMW were treated using a physicochemical pretreatment followed by Fenton and Fenton-like processes. Chemical pretreatment (acid cracking and coagulation-flocculation) positively affected the biodegradability and inhibition on activated sludge was considerably removed (>67% COD and >72% total-phenol removal). Fenton and Fenton-like processes showed high COD (>80%) and total phenolic compounds (>85%) removal performance on evaluated effluents. Inhibitory effect of Fenton-like reagents applied samples on activated sludge mixture was considerably removed.	Mert et al., 2010
COD= 2000-7000 mg L^{-1} TOC= 180-300 mg L^{-1}	Olive mill wastewater - pretreated by sedimentation and sand filtration (Tunisia)	Photo-Fenton oxidation	Color, aromaticity and COD removal	Almost complete color and aromaticity removal were achieved and at the same time more than 90% of COD and 80% of TOC were reduced during the treatment under the following optimized conditions: 3 mg L^{-1} H_2O_2, 30 mg L^{-1} Fe^{2+}, pH 3 and T= 26 ^oC.	Ahmed et al., 2011

pH= 5.1-5.5 COD= 32.2-58.5 g L^{-1} DOC= 9.9-24.1 g L^{-1} BOD= 9.2-15.6 g L^{-1} TS= 43.3-198.0 g L^{-1} TSS= 17.6-56.7 g L^{-1} TP= 100-220 mg L^{-1} TN= 160-290 mg L^{-1} TPh= 1.5-2.0 g L^{-1}	Olive mill wastewater (OMW) (Cyprus)	Coagulation-flocculation followed by solar photo-Fenton oxidation	COD, TSS and TPh removal, toxicity towards *Daphnia magna* and phytotoxicity assays (*Sinapis alba*, *Lepidium sativum* and *Sorghum saccharatum*)	Pre-conditioning by coagulation-flocculation using $FeSO_4·7H_2O$ as the coagulant, and an anionic polyelectrolyte (FLOCAN 23) as the flocculant was performed to remove the solid content of the effluent. The addition of 6.67 g L^{-1} of $FeSO_4·7H_2O$ and 0.287 g L^{-1} of FLOCAN 23 led to the optimal removal of TSS (97%), of COD (72%), and of TPh (40%). Finally, solar photo-Fenton was applied as a post-treatment method; oxidation for 240 min at 0.2 g L^{-1} Fe^{2+}, 5 g L^{-1} H_2O_2, and pH=3 reduced the remaining COD and TPh by 73% and 87%, respectively. Toxicity assays to *Daphnia magna*, as well as phytotoxicity tests to three plant species (*Sinapis alba*, *Lepidium sativum* and *Sorghum saccharatum*) to untreated OMW, as well as to oxidized samples were also performed; indicating the evolution of more biologically potent products during the oxidation.	Papaphilippou et al., 2012

3.5 Combined processes for the treatment of winery wastewater

Advanced oxidation processes (AOPs) can reduce pollutant concentrations, while some processes produce more oxidized compounds, which are in most cases more easily biodegradable than the former ones. Although AOPs are expensive to install and operate, they may be unavoidable for the tertiary treatment of refractory organics present in industrial effluents to allow safe discharge of industrial contaminants (Tabrizi and Mehrvar, 2004). Effective treatment of various industrial effluents, as well as winery wastewater, may require a combination of AOPs and biological processes, in order to exploit their individual quantities and, thus, reach the desired quality within reasonable economical limits. On one hand, AOPs have shown their worthiness for toxic compounds elimination in water and wastewater treatment, however, the total mineralization through these processes is very expensive. On the other hand, biological treatment is relatively cheap and reliable process, but there are substances, which are unable to deal with. A combination of both processes would mean a cheaper option for total organic degradation from a toxic wastewater or a wastewater containing refractory organics (Comninelli et al., 2008; Oller et al., 2011; Cassano et al., 2011). The successful applications of the combined biological and AOPs processes used for the treatment of winery wastewater are presented in Table 3.14.

Table 3.14: Combined processes (biological + AOPs) applied for the treatment of winery wastewater

Winery wastewater initial quality characteristics	Matrix	Technology characteristics	Measure of treatment efficiency	Main findings	References
			I. AOPs as pretreatment		
pH= 3.7-4.1 COD= 24500 mg L^{-1} BOD_5= 11150 mg L^{-1} TS= 21510 mg L^{-1} MLSS= 12510 mg L^{-1} Alkalinity= 450 mg $CaCO_3$ L^{-1}	Wine-distillery wastewater (vinasses) after centrifugation and filtration in order to remove TSS (Spain)	Combined actions of ozonation ($UV/H_2O_2/O_3$) and aerobic activated sludge	COD and total aromatic compounds removal	Over the range of variables studied, 5-25.2% removal of COD, and 16.8-51.4% removal of the total aromatic compounds were attained in the single ozonation process of the wine vinasses, while the consumed ozone yield was around 50% in the single ozonation. Additionally, the aerobic degradation of these effluents by an activated sludge system led to COD removal between 31 and 85% for a hydraulic retention time between 24 and 72 h. The combined process of an ozonation step followed by an activated sludge step provided an enhancement (~12%) in the substrate removal obtained in relation to that obtained in the single aerobic treatment of wastewater without ozone pretreatment.	Benitez et al., 2003
pH= 3.5±0.3 COD= 21715±1236 mg L^{-1} BOD_5= 13440±1578 mg L^{-1} TOC= 7363±697 mg L^{-1} TS= 748±132 mg L^{-1} TSS= 485±74 mg L^{-1} TKN= 1354±432 mg L^{-1} TPh= 735±127 mg L^{-1} Conductivity= 3.12±0.4 mS cm^{-1}	Diluted wine-distillery wastewater with municipal wastewater (1:10) (Spain)	Combined ozonation-activated sludge and activated sludge-ozonation systems	Effect of ozone dose on COD, BOD_5, TOC, TKN and TPh removal	The percentage removal of each parameter investigated (COD, TKN and TPh), with the exception of BOD_5, increased linearly with the increasing ozone dose. Ozonation pretreatment of wine-distillery wastewater enhanced the performance of a subsequent activated sludge system, due to the increase of wastewater biodegradability induced by ozonation. After ozonation, biological treatment seemed to be an interesting solution, especially for BOD_5 reduction, where almost complete elimination was achieved. Considering the second sequence of treatments, namely activated sludge followed by ozonation, the former revealed, once more, a great potential because it could be applied in diluted wine-distillery wastewater, with significant	Beltran et al., 2004

				reductions in COD (60%), BOD_5 (97%), TOC (42%), TKN (78%), and TPh (40%).	
pH= 3.5 COD= 3300 mg L^{-1} TOC= 969 mg L^{-1} Tartaric acid= 1210 mg L^{-1} Malic acid= 1.0 mg L^{-1} TPh= 99 mg L^{-1} Ethanol= 1200 mg L^{-1} Glucose + fructose= 100 mg L^{-1}	Winery wastewater (Spain)	Combined actions of heterogeneous solar Fenton oxidation and activated sludge	COD and TOC removal	After 24 h of heterogeneous solar Fenton treatment, the organic matter (measured as TOC) was reduced to about 50%. The post-treatment (activated sludge process), allowed the elimination of 90% of the initial TOC present, without producing non-desired side effects, such as the bulking phenomenon, which is usually detected when this treatment is used alone. The final effluent had a COD value of 128 mg L^{-1} while the influent's COD concentration was 3300 mg L^{-1}, (96% overall COD removal). The coupled system proved to be an efficient process for the treatment of winery wastewater.	Mosteo et al., 2008
pH= 4.1±0.2 DOC= 882±81 mg L^{-1} COD= 2958±67 mg L^{-1} BOD_5= 1500±100 mg L^{-1} TPh= 0.51±0.05 mg L^{-1}	Winery wastewater (Portugal)	Combined actions of solar Fenton oxidation and immobilized biological reactor (IBR)	COD removal	The pre-oxidation step enhanced the biodegradability of the winery wastewater, increasing the microbial degradation rate, which consequently decreased the aeration demands and the retention time in the aeration tank. Considering a target COD value of 150 mg L^{-1} (higher than 83% COD removal) in agreement with Portuguese legislation for discharge into water bodies, 290 mM of H_2O_2 and an UV solar dose of 100 kJ L^{-1} were necessary, while with the combination of the IBR process, approximately 10 and 6 days (time necessary for the biological oxidation) were required.	Souza et al., 2013
II. AOPs as post-treatment					
pH= 3.8 COD= 18500 mg L^{-1} TS= 13000 mg L^{-1} TPh= 630 mg L^{-1} Volatile acidity= 980 mg L^{-1}	Wine distillery wastewater (vinasses) (Spain)	Combined actions of aerobic biological treatment followed by Fenton oxidation	COD, total phenolic compounds and aromatic compounds removal	Using a biological treatment followed by an AOP had been shown to be highly effective, as the aerobic biological treatment removed the majority of the organic compounds present. The subsequent Fenton process 'polished' this effluent and removed the majority of the remnant phenolic compounds. More specifically, aerobic biological degradation reduced the COD between 75 and 94%, and the total	de Heredia et al., 2005b

Aromatic compounds= 4300 mg phenol L^{-1}				phenolic compounds in the range 54 to 79%. Fenton oxidation degraded the pretreated biologically wastewater with high efficiency; COD removal was in the range 50 to 80%. Aromatic and total phenolic compounds removal was always higher than 90%.	
pH= 10.6 COD= 16250 mg L^{-1} TS= 15086 mg L^{-1} TSS= 1259 mg L^{-1} BOD_5= 3250 mg L^{-1} Conductivity= 3.3 mS cm^{-1} Cu= 0.5 mg L^{-1} Ni= 0.1 mg L^{-1} Cr= 0.12 mg L^{-1} Cd= bdl Zn= 1 mg L^{-1}	Winery wastewater (Cyprus)	Combined actions of Sequential Batch reactor (SBR) and photo-Fenton oxidation	COD and BOD_5 removal	The SBR process was significantly reduced the organic load of winery wastewater (about 93% COD and BOD_5 removal); reaching an effluent with COD and BOD_5 values of 1060 and 210 mg L^{-1}, respectively. This effluent was subjected to photo-Fenton oxidation, under continuous UV-A irradiation provided by a 125 W lamp. In general, organic matter degradation increased with increasing photo-Fenton treatment time, reaching values of COD and BOD_5 removal as high as 80% after 4 h of reaction. Hence, the combined biological + photo-Fenton oxidation resulted in 95% COD removal.	Anastasiou et al., 2009
pH= 3.9 COD= 20000 mg L^{-1} BOD_5= 11000 mg L^{-1} TSS= 8600 mg L^{-1} VSS= 6200 mg L^{-1} TN= 208 mg L^{-1} TP= 280 mg L^{-1} TPh= 680 mg L^{-1}	Winery wastewater (Portugal)	Long-term aerated storage followed by Fenton oxidation	COD removal	The long-term hydraulic retention time, 11 weeks, contributed remarkably to the reduction of COD (about 90%), and the combination with the Fenton's reagent led to a high overall COD reduction that reached 99.5% when the mass ratio (R= H_2O_2/COD) used was equal to 2.5, maintaining constant the molar ratio H_2O_2/Fe^{2+}=15. The final winery effluents (COD residual= 100 mg L^{-1}) could be reused, rejected in the water streams or on soil, according to existing Portuguese law.	Lucas et al., 2009b

3.5.1 Assessment of the performance of the combined processes

Depending on the nature of the pollutants and the level of contaminants, detoxification might be difficult and/or even impossible to achieve by single conventional biological methods. In such cases, biological processes alone are not able to reach effluent standards for the discharge into the environment (i.e. surface water); therefore, a pretreatment or post-treatment is required. The choice of the correct combination system should be carried out considering several aspects, both technical (treatment efficiency, plant simplicity, flexibility, etc.) and economical (capital and operating costs including reagent and energy consumption, sludge and gas disposal, maintenance, etc.) (Tabrizi and Mehrvar, 2004).

It has been shown that the combination of biological and advanced oxidation treatment processes has the following advantages: (a) chemical pretreatment can protect the microorganisms from inhibitory or toxic compounds; (b) the cost of chemical treatment can be decreased by using a cost-effective biological pre or post-treatment; (c) total residence time is flexible as a result of different choices that are possible for chemical and biological reactor residence times; and (d) total mineralization can be achieved for the organics, while the total cost can be minimized (Lee et al., 2001; Comninellis et al., 2008).

i) AOPs as pretreatment

Coupling chemical pre-oxidation with biological post-treatment is conceptually beneficial, as it can lead to increased overall treatment efficiencies compared with the efficiency of each individual stage (Mantzavinos and Psillakis, 2004). The main role of the chemical pretreatment is partial oxidation of the biologically persistent part to produce biodegradable reaction intermediates (Comninellis et al., 2008). The percentage of mineralization should be minimal during the pretreatment stage, in order to avoid unnecessary expenditure of chemicals and energy, thereby lowering the operating cost (Oller et al., 2006). This is important because electricity represents a major part of the total operating cost of photocatalytic reactors (Bandara et al., 1997). However, if the pretreatment time is too short, the reaction intermediates generated could still be structurally very similar to the original non-biodegradable and/or toxic components (Oller et al., 2006).

The main drawback of the AOPs is their high cost in comparison to the biological treatments. This cost difference is caused not only by the type of treatment itself, but also by the nature of the effluent and the treatment volume, which is usually on a smaller scale for AOPs. The higher costs of AOPs have caused many authors to study the possibility of a combined process with a first, more costly, photocatalytic stage followed by less expensive biological treatment (Oller et al., 2011). Regardless of the industry type, this strategy is based on the knowledge that some substances reduce their toxicity when they are partially oxidized (Ballesteros Martin et al., 2010). Thus, an ecotoxic effluent can be treated to some extent with an AOP until toxicity is minimal and the pollutants in water are biodegradable. Then, a biological process can be applied until the effluent is totally decontaminated and so, reducing the costs that would be incurred if only an AOP were used instead of a combined process (Pérez et al., 2013).

In the case of the winery wastewater, ozonation and heterogeneous photo-Fenton have been used as pretreatment of activated sludge systems, so far. The oxidation of the organic substrate present in effluents generated in wine distilleries (vinasses) was studied by both an ozonation process and by an aerobic activated sludge system (Benitez et al., 2003). Only 5-25.2% removal of COD and 16.8-51.4% removal of the total aromatic compounds are attained in the single ozonation process of the wine distillery wastewater. On the other hand, the aerobic degradation of these effluents by an activated sludge system leads to COD removal between 31 and 85% for a hydraulic retention time between 24 and 72 h. The combined process of an ozonation step followed by an activated sludge step provides an enhancement in the substrate removal (~12%) obtained in relation to that obtained in the single aerobic treatment of wastewater without ozone pretreatment (Benitez et al., 2003).

Moreover, according to Beltran et al. (2004) single ozonation was inefficient for the treatment of wine-distillery effluents, reaching only 4% of COD removal; while single activated sludge system was achieved to reach a higher COD removal equal to 29.5%. Only a small increase on the COD reduction (32%) was achieved with the use of ozonation as post-treatment of biological process. Best results were attained with the integrated ozonation-activated sludge system, with significant reductions in COD (65%), BOD_5 (97%), TOC (42%), TKN (78%) and total phenolic content (40%). Additionally, Mosteo et al. (2008) studied the degradation of winery wastewater by a heterogeneous solar assisted photo-Fenton process and activated sludge treatment combined system. After 24 h of chemical treatment, the organic matter was reduced about 50% (expressed as TOC). The latter biological treatment (activated sludge process), allowed the elimination of 90% of the initial TOC present. As a consequence, the coupled system proved to be an efficient process for the treatment of winery wastewater.

In the study of Souza et al. (2013) the pre-oxidation step enhances the biodegradability of the winery wastewater, increasing the microbial degradation rate, which consequently decreases the aeration demands and the retention time in the aeration tank. Considering a target COD value of 150 mg L^{-1} (higher than 83% COD removal) in agreement with Portuguese legislation for discharge into water bodies, 290 mM of H_2O_2 and an UV solar dose of 100 kJ L^{-1} are necessary, while with the combination of the IBR process, approximately 10 and 6 days (time necessary for the biological oxidation) are required.

ii) AOPs as post-treatment

According to the literature (Scott and Ollis, 1995; Oller et al., 2006) there are two types of wastewater as potentially treatable by a biological process followed by AOPs: (a) wastewater containing biorecalcitrant compounds such as large macromolecules like soluble polymers that are not easily biodegradable due to their large size and lack of active centers and (b) the highly biodegradable industrial wastewater, which still requires chemical post-treatment, as it contains a large amount of biodegradable organic compounds in addition to small concentrations of recalcitrant compounds.

As a consequence, several studies have been performed in which firstly the highly biodegradable part of the winery wastewater is eliminated biologically and then the recalcitrant contaminants are degraded by post-treatment via the application of an advanced chemical oxidation process.

According to de Heredia et al. (2005b), who evaluated the degradation of wine distillery wastewater by applying combined biological and Fenton oxidation treatment, the biological degradation reduced the COD between 75 and 94% and the phenolic content in the range of 54 to 79%, depending on the initial organic content. Fenton oxidation as post-treatment further degraded the biologically pretreated wastewater, reaching a further reduction of COD (80%); while the aromatic and phenolic compounds removal was higher than 90%. Moreover, the work of Anastasiou et al. (2009) assessed the efficiency of coupling physical and biological treatment with photo-Fenton oxidation, with the latter serving as the final polishing step. A partially treated winery wastewater effluent by sequential batch reactor (SBR) was subjected to photo-Fenton oxidation, under continuous UV-A irradiation. The maximum COD and BOD removal was 80% after 4 h of photo-Fenton reaction. Hence, the combined biological + photo-Fenton oxidation resulted in 95% total COD removal.

Furthermore, the combined process of aerobic degradation followed by Fenton's reagent oxidation was also employed to treat winery wastewater by Lucas et al. (2009b). The results indicated that aerobic biological treatment reached a very high COD removal, corresponding to the biodegradable fraction of COD, followed by Fenton oxidation as the final polishing step. The main conclusions were: aerobic biological degradation rates were between 76% and 96% for the COD removal at bench scale, and between 64% and 96% at pilot scale. The combined process (long-term aeration/Fenton reagent) led to a COD removal higher than 99%, with final effluents (COD residual=100 mg L^{-1}) able to be safely reused or disposed of in water streams or on soil, according to the existing Portuguese law.

It can be concluded that a combination of advanced oxidation and biological treatment (as pre or post-treatment) can lead to a higher level of COD reduction than any single-stage treatment under the same operating conditions.

CHAPTER 4: OBJECTIVES OF THE STUDY

The first objective of this thesis was the identification of the environmental impacts related to the wine production process, using Cyprus wineries as a case study, and the various actions that could be implemented by wineries, in order to minimize or eliminate those impacts.

Furthermore, the research work aimed at the optimization of an advanced oxidation process (AOP), and specifically the solar photo-Fenton process (hv/Fe^{2+}/H_2O_2), as post-treatment, for the removal and the possible mineralization of the organic content of winery effluents which have been pretreated by a biological process (a) a Sequencing Batch Reactor (SBR), and (b) a Membrane Bioreactor (MBR). The study includes bench- and pilot-scale experiments for both biologically pretreated flows.

In addition, the thesis investigated a membrane separation process, the reverse osmosis (RO), for the purification of raw winery effluents. As well known, RO process does not really destroy the pollutants, but merely concentrates them into smaller volumes of wastewater, the concentrate. As a result, the concentrate generated during the RO process contains high levels of refractory organic pollutants and inorganic salts, and needs further treatment before its disposal in the environment. For this reason, solar photo-Fenton oxidation was applied for the treatment of the produced RO concentrate, in order to reduce its organic content, as well as its toxicity.

Moreover, a high performance liquid chromatography (HPLC) method has been also developed for studying the phenolic compounds concentration during the RO process to the concentrate stream, in raw winery wastewater. Finally, the overall cost of construction, as well as the cost for a five-year operation and maintenance of the complete system including the biological system (MBR), the solar Fenton system and the membrane-based RO system, for a typical medium-size winery that produces an average of 50 m^3 d^{-1} of wastewater, was estimated.

Neither the use of solar photo-Fenton oxidation as post-treatment for biologically treated effluents by SBR and MBR processes, as well as its upscaling to industrial application, nor the integrated study of the treatment of winery wastewater using RO at a pilot scale and solar photo-Fenton oxidation for the purification of the produced concentrate, have been reported in the literature so far. It should be also noted that the solar irradiation is a green technology and along with its low environmental footprint can contribute to the sustainable management of winery wastewater. Moreover, neither an integrated cost analysis (cost of investment, operation and maintenance for five years) of a biological, a reverse osmosis and a solar Fenton system for the treatment of winery wastewater has yet been published in the literature.

The specific objectives of this thesis are presented in more detail below.

4.1 Environmental problems related to the wine production process in Cyprus

The first objective included the identification of the exact environmental impacts related to the wine production process in Cyprus, through a review, which was prepared based on the relevant available literature, a questionnaire designed specifically for collective relevant information from the operators (Annex), site visits to wineries in Cyprus and Italy, and personal communication with the owners of numerous wineries in Cyprus.

4.2 General guidelines for adopting environmental friendly practices for wineries

The work related to the first objective included the identification of the various actions that could be implemented by the wineries, in order to minimize or even eliminate their environmental impacts, concerning the management of their wastewater and solid waste, the conservation of water and energy, as well as the reduction of chemical use; through the development of a practical, simple and comprehensible tool, for the implementation of Best Practices concerning environmental management in the wine manufacturing industry.

4.3 Bench-scale solar photo-Fenton experiments

Initially, bench-scale experiments using a solar simulator in combination with a photochemical batch reactor were carried out, in order to evaluate the influence of various operational parameters on the efficiency of solar photo-Fenton oxidation, as post-treatment of the two biological processes examined in this thesis (SBR and MBR). In more detail the following have been studied:

A. For the optimization of the process

- The effect of ferrous (Fe^{2+}) concentration
- The effect of hydrogen peroxide concentration
- The effect of pH
- The effect of solar irradiation
- The effect of temperature

B. For the monitoring and evaluation of the process

- The color removal
- The total phenolic compounds removal
- The mineralization of the effluents organic load
- The DOC removal kinetics
- The evaluation of the toxicity and phytotoxicity (*Daphnia magna*, *Sinapis alba*, *Lepidium Sativum, Sorghumm Saccharatum*)

4.4 Pilot-scale solar Fenton experiments

The solar Fenton oxidation was further investigated at a pilot-scale setup. The pilot-scale experiments were carried out for both biologically pretreated flows (SBR and MBR), in order to determine the most efficient combined treatment that could be further investigated for an industrial-scale application.

The aforementioned experiments were carried out in a compound parabolic collector (CPC) solar prototype photocatalytic reactor, located at the premises of the University of Cyprus (UCY), with treated volume of 60 L and irradiated volume of 21.4 L, as described in more detail in Section 5.2.3.

The main objectives of the pilot-scale study were:

- The optimization of the solar Fenton process for the determination of the optimum operating conditions for the two biologically pretreated flows (SBR and MBR)
- The color removal
- The mineralization of the effluent organic load (DOC)

- The study of the DOC removal kinetics

Additionally, various toxicity and phytotoxicity assays (*Daphnia magna, Sinapis alba, Lepidium Sativum and Sorghumm Saccharatum*) were performed to evaluate the potential toxicity of the two biologically pretreated flows, and their oxidation products during the various stages of the solar Fenton process. Considering the fact that the treated effluent samples are intended mainly for irrigation purposes (e.g. vineyards), the toxicity measurements were considered as an indispensable task.

4.5 Industrial-scale solar Fenton operation

The objective was the monitoring of the efficiency of the solar Fenton as post-treatment of the most efficient biological treatment at an industrial scale. For this reason a solar driven industrial-scale plant was placed side by side with the MBR, which resulted to be the most efficient biological process, in order to complete the winery wastewater treatment plant (WWWTP). Due to the fact, that winery wastewater can be discharged into the environment mainly for irrigation purposes (i.e. vineyards), it is important to comply with the national environmental limits. As a consequence, solar Fenton oxidation was used as a polishing step of the winery wastewater, in order to comply with these limits and reduce its ecotoxicity as well.

The industrial-scale study focused on the COD, DOC and color removal of the winery effluent. Moreover, the evaluation of the toxicity and phytotoxicity (*Daphnia magna, Sinapis alba, Lepidium sativum,* and *Sorghumm saccharatum*) was studied, in order to evaluate the potential toxicity of the effluent treated by MBR followed by solar Fenton oxidation.

4.6 Reverse osmosis - pilot-scale experiments

Another objective of this thesis was the investigation of the efficiency of a membrane separation process, and specifically reverse osmosis, for the treatment of raw winery wastewater. RO experiments were performed at a pilot-scale RO unit, which was equipped with a spiral-wounded polyamide membrane, at the Laboratoty of Environmental Technology and Management of the Department of Environmental Engineering, at the Technical University of Crete (TUC). The RO study included a detailed evaluation of a number of process parameters for the RO streams (i.e. feed, concentrate and permeate). Since RO does not really destroy the pollutants, but merely concentrates them into smaller volumes of wastewater, i.e. RO concentrate, solar photo-Fenton oxidation was applied for the purification of the produced concentrate, in order to achieve an integrated treatment of winery wastewater.

In more detail the following have been studied:

- The efficiency of a pilot-scale RO process to remove the organic compounds (expressed as COD) from winery wastewater.
- The optimum experimental conditions (with regard to pressure and temperature) of the RO process, during which the maximum volume recovery and organic content removal could be achieved.
- The evaluation of the toxicity of the permeate resulting from the RO process, against one aquatic microorganism (*D. magna*) and three plant species (*Sinapis alba, Lepidium sativum, Sorghum saccharatum*), as the treated winery wastewater can be disposed of into surface waters or used for irrigation.

- The investigation of the potential enhancement on the efficiency of the RO treatment of winery wastewater by using a recirculation mode (with regard to volume recovery and organic load removal).
- The concentration of the phenolic compounds that are present in the raw winery wastewater in the concentrate stream, with a view to future recovery, due to being high-value natural compounds. A high performance liquid chromatography (HPLC) method has been also developed for studying the phenolic compounds concentration during the RO process, in raw winery wastewater. The development of the analytical method was achieved in collaboration with the laboratory of Environmental Organic Chemistry - Micropollution of the Department of Environmental Engineering, at the Technical University of Crete.
- The assessment of the efficiency of the solar photo-Fenton oxidation for the treatment of the concentrate resulted from the RO process, in order to achieve an integrated treatment of winery wastewater.

4.7 Cost estimation of an integrated winery wastewater treatment

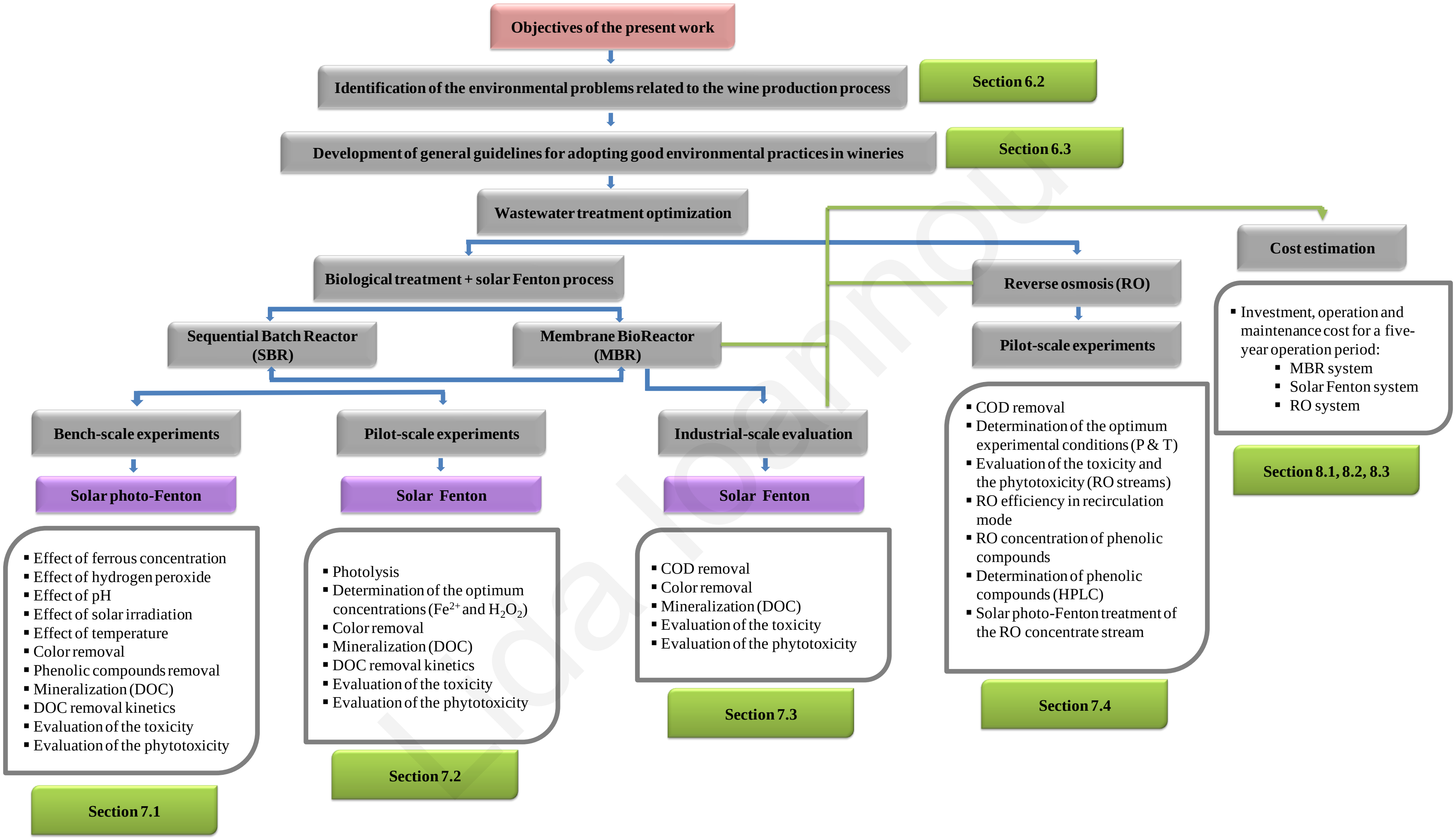
The aim of this task was to establish the overall ownership cost of an MBR, a solar Fenton system, and an RO system for a five-year period. For this reason, cost estimation was based on the initial investment cost, as well as the operation and maintenance cost for five-year time. Both of these costs establish the total ownership cost. It should be mentioned that for the calculation of the operating costs the seasonal variations in flow have been taken into consideration. The cost benefit analysis performed in this thesis was based on a typical medium-size winery that produces an average of 50 m^3 of wastewater per day.

In more detail the following were studied:

- Total investment, operation and maintenance cost for a five-year operation period for the MBR system
- Total investment, operation and maintenance cost for a five-year operation period for the solar Fenton system
- Total investment, operation and maintenance cost for a five-year operation period for the RO system

Part of the work was carried out in the framework of the **WINEC** project (Project title: Advanced systems for the enhancement of the environmental performance of WINEries in Cyprus). This project was co-funded by the LIFE financial instrument of the European Commission through grant LIFE08/ENV/000455. Also, part of the work has been supported by the activities of the International Research Center “NIREAS” (Strategic Infrastructure Project ΝΕΑ ΥΠΟΔΟΜΗ/ΣΤΡΑΤΗ ΙΙ/0308/09), which is co-funded by the Republic of Cyprus and the European Regional Development Fund.

The main tasks of this thesis are summarized and shown in Schematic 4.1.



Schematic 4.1: The main tasks included in this thesis

CHAPTER 5: EXPERIMENTAL

5.1 Materials and methods

5.1.1 Reagents

The reagents used for the solar Fenton experiments at bench, pilot and industrial scale were iron sulfate heptahydrate ($FeSO_4.7H_2O$, Sigma Aldrich), hydrogen peroxide (H_2O_2, 30% w/w, Merck), and sulphuric acid for pH adjustment (H_2SO_4, 95-97%, Merck). The Fenton reaction was terminated at specific time intervals by adding manganese dioxide (MnO_2, particle size 10 μm, reagent grade ≥ 90%, Sigma Aldrich) for the COD and DOC determination.

For the toxicity and phytotoxicity analyses, the treated solutions were neutralized by 2N sodium hydroxide (NaOH, Panreac). The residual hydrogen peroxide was removed from the treated samples by adding catalase (Micrococcus lysodeikticus 17000 U mL^{-1}, Fluka Biochemika).

For the measurement of total dissolved iron, 1,10-phenanthroline ($C_{12}H_8N_2 \cdot H_2O$), ascorbic acid ($C_6H_8O_6$), ammonium acetate ($CH_3CO_2NH_4$, ≥97%) and acetic acid (CH_3COOH) were used, all supplied by Sigma Aldrich. The residual hydrogen peroxide (H_2O_2) during the Fenton process was measured using ammonium metavanadate (NH_4VO_3) supplied by Fluka.

For the assessment of the qualitative characteristics of the winery wastewater used for all the experiments, the following reagents were used. Potassium hydrogen phthalate (KHP, Sigma Aldrich) was used as standard solution for the calibration of COD and DOC, and glycose-glutamic acid solution (Sigma Aldrich) was used as standard solution for the calibration BOD_5. For the measurement of BOD_5, allylthiourea (ATH, Sigma Aldrich) and potassium hydroxide (KOH, ACS reagent, ≥85%, pellets) were used.

For the measurement of oil and grease, hydrochloric acid (HCl, 37%, AR grade, Sigma Aldrich) was used for the pH adjustment, n-hexane (96%, analytical grade, Scharlau) was used as the extracting solvent, and sodium sulfate anhydrous (Na_2SO_4, 99%, Riedel-deHaen) was used to drain the solvent layer.

Gallic acid (≥99%, Sigma Aldrich) was used as standard solution for the measurement of total phenolic compounds, while Folin-Denis' Reagent and sodium carbonate solution (Na_2CO_3, purum anhydrous, ≥98%) were also used, both supplied by Fluka Analytical.

For the measurement of metals, the samples were acidified with concentrated nitric acid (HNO_3, reagent grade, >90%) to pH <2, which was supplied by Sigma Aldrich, and buffer solutions for each of the metals measured (Perkin Elmer Pure) were used as standards.

Hydrochloric acid (HCl 37%, AR grade, Sigma Aldrich) and/or sodium hydroxide (NaOH, ACS reagent, >97%, pellets) were used for the pH adjustment.

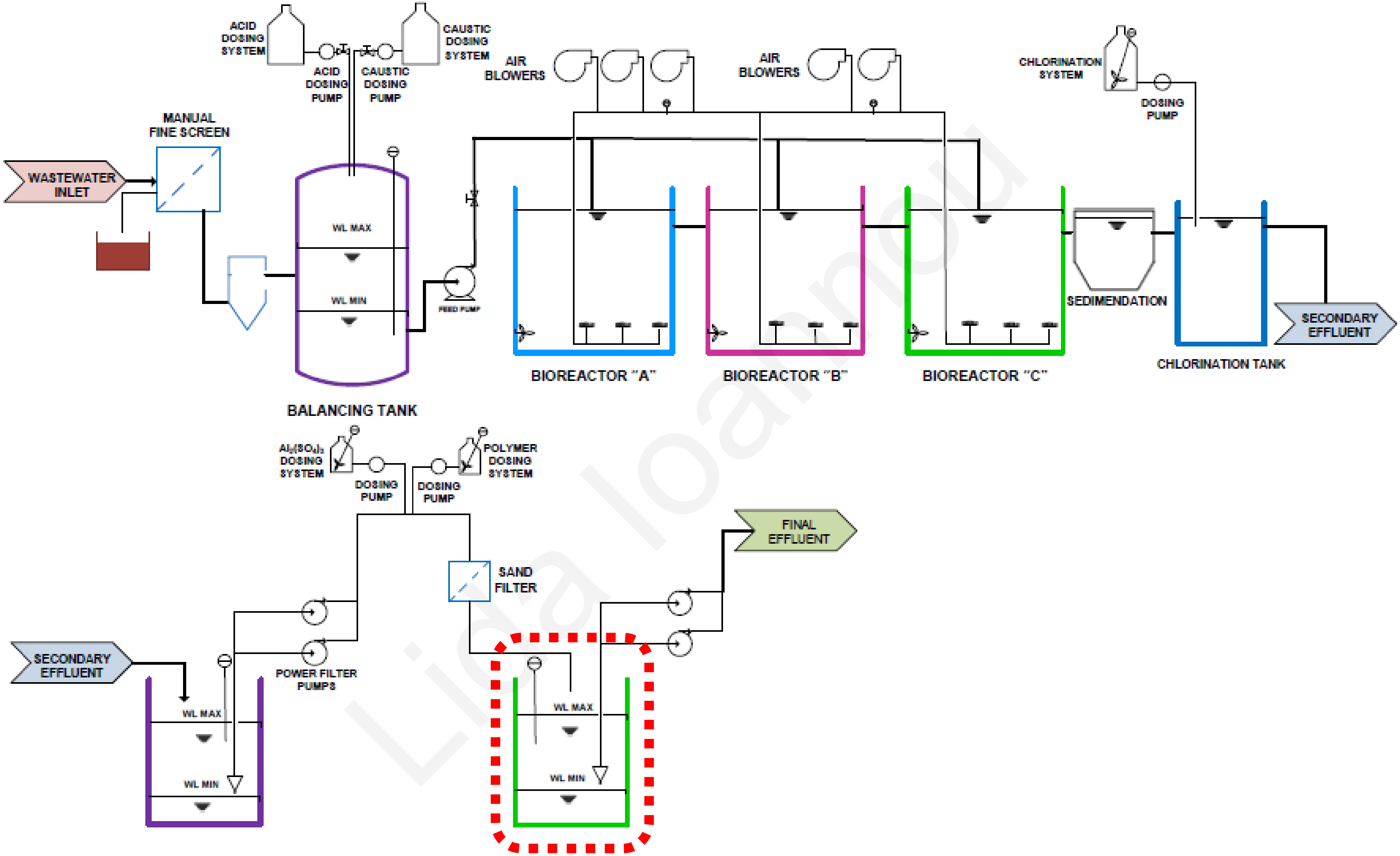
Ultrapure water (milli-Q PURELAB Ultra ELGA Water Dispenser; Millipore) was used throughout the experimental procedure.

For the chromatographic analysis of phenolic compounds the following were used: gallic acid (≥99%, Sigma Aldrich), vanillic acid (97%, Sigma Aldrich), epicatechin (≥98%, HPLC, Sigma Aldrich), p-coumaric acid (≥98%, HPLC, Sigma Aldrich), ferulic acid (≥99%, HPLC, Fluka Analytical), HPLC grade methanol (MeOH, >99.9%, Sigma Aldrich), acetic acid (CH_3COOH, ReagentPlus®, ≥ 99%, Sigma Aldrich), and ultrapure water (UPW) (EASYpureRF - Barnstead/Thermolyne, USA).

5.1.2 Winery wastewater used for the advanced oxidation experiments

The biologically treated winery wastewater samples used for the solar photo-Fenton and the solar Fenton experiments (bench, pilot and industrial scale) were collected after the SBR and MBR stage of two winery wastewater treatment plants (WWWTPs).

SBR is applied at a winery with annual wastewater production of about 10000 m^3. The complete treatment system consists of (a) the preliminary treatment (screening, balancing tank and pH adjustment), and (b) the biological treatment (three sequential batch reactors, sedimentation tank, chlorination tank and storage tank) as shown in Schematic 5.1. The SBR is an activated sludge process designed to operate under non-steady state conditions. Screening and large solids removal take place in the pretreatment, via a static basket screen with an opening of 2 mm. The winery effluent is then collected in a balancing tank, where the pH is corrected by the addition of caustic soda solution to a neutral pH; and afterwards it is pumped to the first bioreactor. The biological treatment consists of three bioreactors as shown in Schematic 5.1, and the secondary treated effluent after sedimentation and chlorination, is passed through a sand filter and then stored in a final effluent tank. The sampling point from which the winery effluent pretreated by SBR was collected is depicted in Schematic 5.1 (red dashed lines).



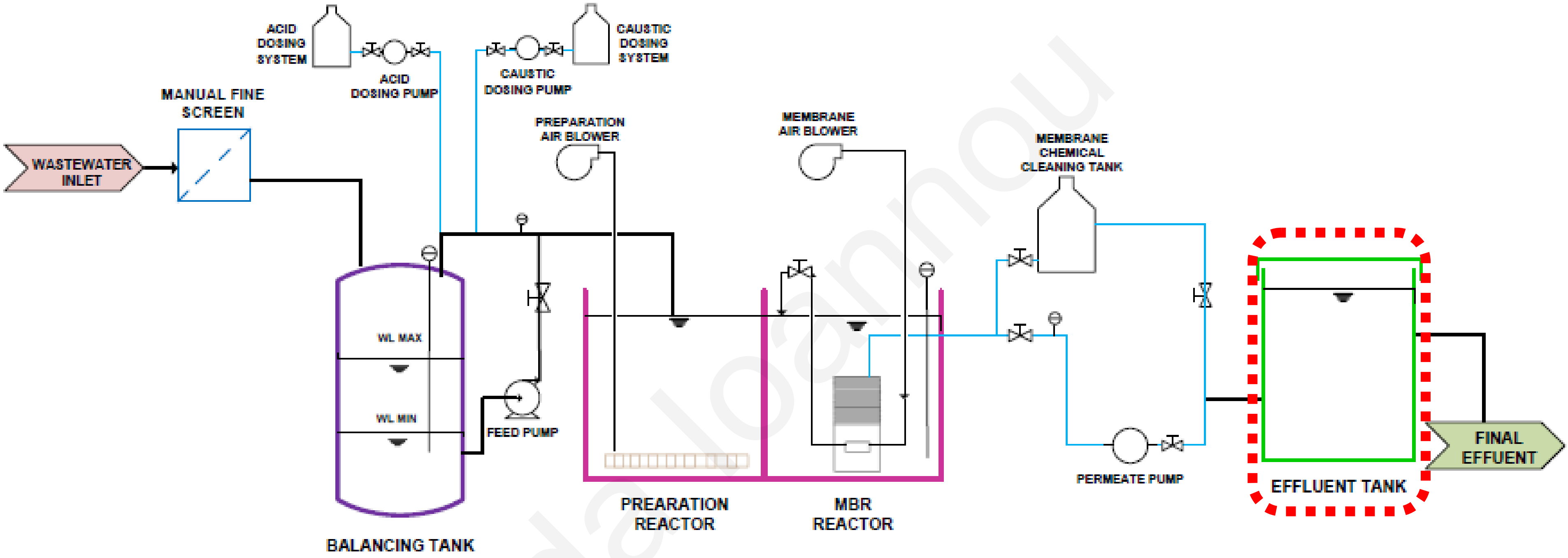
Schematic 5.1: Winery wastewater treatment plant I (which includes SBR treatment)

The SBR was manufactured to operate under specific seasonal distribution of hydraulic and pollution loads, as shown in Table 5.1.

Table 5.1: SBR hydraulic and pollution loads

	January to July	August to October	November	December
Flow (m^3 d^{-1})	5	50	30	25
BOD_5 (kg m^{-3})	4	2.5	2	2
COD (kg m^{-3})	7.2	4.4	3.6	3.6

MBR is applied at a winery with annual wastewater production of about 411 m^3. The complete treatment system consists of (a) the preliminary treatment (screening, equalization/balancing tank, pH adjustment), and (b) the biological treatment (pre-aeration/nitrification, membrane reactor, storage/irrigation tank), as shown in Schematic 5.2. A static basket screen with an opening of 2 mm is used to remove large solids, in order to avoid abrasion problems with grape seeds circulating between the membranes. The balancing tank is used to balance the variations in organic loads, pH and flow resulting from batch operation of the winemaking process. The winery effluents are acidic (pH<5), and thus caustic soda is also added to adjust the influent pH to neutral. The wastewater emanating from the balancing tank is then fed into the first compartment of the reactor. The biological treatment consists of two compartments, the aeration tank, where nitrogen is removed biologically through nitrification, and the membrane bioreactor, where the treated effluent permeates through a membrane to a common manifold, and is stored in a final effluent tank. The sampling point from which the winery effluent pretreated by MBR was collected is depicted in Schematic 5.2 (red dashed lines). The MBR was fabricated to operate with a range of daily flow of 4-8 m^3 day^{-1}, with an average influent BOD_5 of about 2500 mg L^{-1} and COD of about 6000 mg L^{-1}.



Schematic 5.2: Winery wastewater treatment plant II (which includes MBR treatment)

5.1.2.1 Quality of the winery effluents used for the solar photo-Fenton bench-scale experiments

Samples for the bench-scale experiments were collected between October - November 2010 (treated by SBR), and between November - December 2011 (treated by MBR). All wastewater samples analyzed in this work were grab samples collected in amber glass bottles (1 L each), placed in a cool box, and vacuum filtered through 1 μm glass fibre filters (Whatman, USA) immediately after their arrival at the laboratory. Samples were then stored at -18 ^{o}C until analysis, again in amber glass bottles. The samples were analyzed before use for a number of qualitative characteristics, as summarized in Table 5.2. All parameters were measured according to Standard Methods (APHA, 1998) described in detail in the following sections.

Table 5.2: Main qualitative parameters of the winery effluents treated by SBR and MBR, used for the bench-scale experiments

Parameter	SBR effluent	MBR effluent
pH (20 ^{o}C)	8.20 - 8.30	8.10 - 8.30
Total Solids (mg L^{-1})	3672 - 3740	1980 - 2040
Total Volatile Solids (mg L^{-1})	2430 - 2612	950 - 842
Suspended Solids (mg L^{-1})	225 - 245	8 - 11
Suspended Volatile Solids (mg L^{-1})	140 - 170	2 - 4
Total phenolic compounds (mg L^{-1})	3.8 - 4.7	bdl
COD (mg L^{-1})	264 - 270	100 - 120
DOC (mg L^{-1})	60 - 80	25 - 35
Soluble BOD$_5$ (mg L^{-1})	111 - 113	<5
Total Nitrogen (mg L^{-1})	6.7 - 6.8	2 - 5.7
Total Phosphorous (mg L^{-1})	32.00 - 46.80	0.17 - 0.40
Oil and grease (mg L^{-1})	4.0 - 6.0	0.2 - 0.3
Cu (mg L^{-1})	0.18 - 0.20	0.09 - 0.15
Cd (mg L^{-1})	0.17 - 0.19	0.05 - 0.09
Fe (mg L^{-1})	0.05 - 0.07	0.02 - 0.04
Sodium (mg L^{-1})	1.42 - 1.50	1.30 - 1.50
Potassium (mg L^{-1})	4.50 - 4.90	4.00 - 4.45

bdl: below detection limit

5.1.2.2 Quality of the winery effluents used for the solar Fenton pilot-scale experiments

Samples treated by MBR were collected between March - June 2012, while samples treated by SBR were collected in June 2012. The experiments in the solar prototype photocatalytic reactor were performed as described in detail in Section 5.2.2. The qualitative characteristics of the winery effluents used are shown in Table 5.3.

Table 5.3: Main qualitative parameters of the winery effluents treated by SBR and MBR, used for the pilot-scale experiments

Parameter	SBR effluent	MBR effluent
pH (20 ^{o}C)	8.20	7.80 - 8.50
Total Solids (mg L^{-1})	2120	1800 - 2130
Total Volatile Solids (mg L^{-1})	1750	1300 - 1720
Suspended Solids (mg L^{-1})	245	7 - 12
Volatile Suspended Solids (mg L^{-1})	132	2 - 3
COD (mg L^{-1})	270	130 - 190
DOC (mg L^{-1})	74	30 - 45
Soluble BOD$_5$ (mg L^{-1})	97	<5
Total Nitrogen (mg L^{-1})	70.0	1.0 - 1.6
Total Phosphorous (mg L^{-1})	4.30	0.20 - 0.38
Oil and grease (mg L^{-1})	3.0	0.1 - 0.2
Cu (mg L^{-1})	0.15	0.08 - 0.10
Cd (mg L^{-1})	0.22	0.06 - 0.07
Fe (mg L^{-1})	0.02	0.01 - 0.02
Sodium (mg L^{-1})	2.10	1.40 - 1.60
Potassium (mg L^{-1})	6.70	4.10 - 4.70

5.1.2.3 Quality of the winery effluents used for the solar Fenton industrial-scale process

Winery effluents treated by MBR were collected and used for the industrial-scale operation in September 2012 (vintage period), and the corresponding qualitative characteristics are shown in Table 5.4. The industrial-scale treatment was carried out as described in detail in Section 5.2.3.

Table 5.4: Main qualitative parameters of the winery effluent treated by MBR, used for the industrial-scale process

Parameter	MBR effluent
pH (20 ^{o}C)	7.80
Total Solids (mg L^{-1})	2000
Volatile Total Solids (mg L^{-1})	1420
Suspended Solids (mg L^{-1})	9
Volatile Suspended Solids (mg L^{-1})	2
COD (mg L^{-1})	180
DOC (mg L^{-1})	47
Soluble BOD_5 (mg L^{-1})	<5
Total Nitrogen (mg L^{-1})	3.6
Total Phosphorous (mg L^{-1})	0.30
Oil and grease (mg L^{-1})	0.2
Cu (mg L^{-1})	0.06
Cd (mg L^{-1})	0.04
Fe (mg L^{-1})	0.01
Sodium (mg L^{-1})	1.20
Potassium (mg L^{-1})	5.00

5.1.3 Winery wastewater used for the reverse osmosis experiments

5.1.3.1 Quality of the winery wastewater used for the RO pilot-scale experiments

The raw winery wastewater samples used for the reverse osmosis experiments were collected in October 2012 (vintage period), from a winery located in Chania, Crete, Creece; since the specific experiments were carried out on a pilot plant installed at the Technical University of Crete (Laboratory of Environmental Technology and Management). Due to the high suspended solids content and in order to avoid fouling of the membrane, the winery wastewater was first centrifuged (Sigma 4-16K, 14000 rpm, 5 min), and then filtered through glass microfilter (Whatman, 1 μm) before fed to the RO unit. The main qualitative characteristics of the filtered winery wastewater are illustrated in Table 5.5.

Table 5.5: Main qualitative parameters of the winery effluent treated by filtration, used for the reverse osmosis experiments

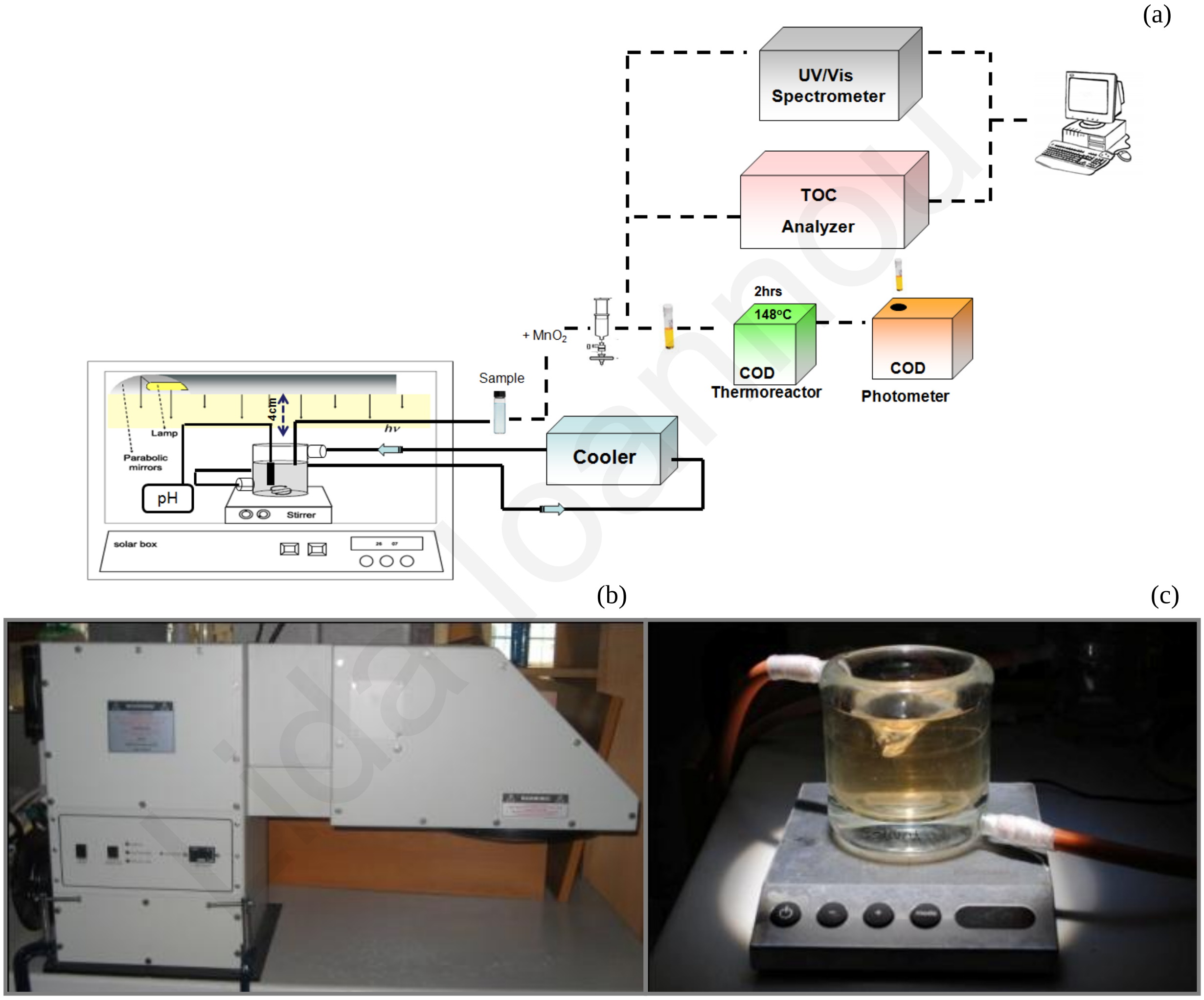
Parameter	Winery effluent
pH (20 ^oC)	6.00
Conductivity (mS cm^{-1})	3.34
Total Solids (mg L^{-1})	5040
Suspended Solids (mg L^{-1})	66
Total phenolic compounds (mg L^{-1})	20.0
COD (mg L^{-1})	5353
BOD_5 (mg L^{-1})	424
Total Nitrogen (mg L^{-1})	10.0
Total Phosphorous (mg L^{-1})	2.10

5.2 Experimental procedure

5.2.1 Experimental setup and description of the bench-scale solar photo-Fenton treatment process

For the solar photo-Fenton experiments, irradiation was provided by a 1 kW lamp (Xe-OP) in a solar simulator (Newport model 91193). The irradiation intensity of the simulator was 272.3 W m^{-2} determined by a radiometer (Newport type 70260). Solar photo-Fenton experiments were conducted in a photochemical batch reactor constructed by Pyrex (inner diameter 7 cm; height 9 cm) with a maximum capacity of 350 mL. The vessel content was continuously stirred (550 rpm), while the reaction temperature was kept at 25±0.1 ^{o}C through a water cooling system. Photocatalytic experiments were performed in triplicate and, when necessary in quadruplicate, and mean values are quoted as results. The relative standard deviation of the experiments never exceeded 5%.

Three hundred (300) mL of winery wastewater after filtration through Whatman glass microfiber filter - 1μm, was fed into the reactor and the pH was adjusted to 3.0 by adding the required amount of 2M H_2SO_4 aqueous solution. Then, the appropriate amount of Fe^{2+} was added using an aqueous solution of $FeSO_4.7H_2O$. The mixture was mixed well before the addition of the appropriate volume of H_2O_2 (30 % w/w). Irradiation was allowed just after the addition of the H_2O_2, and this was considered as time zero. Samples were withdrawn at specified intervals from the reactor and were transferred in vials. The residual hydrogen peroxide was removed from the treated samples with MnO_2 (for the measurements of COD and DOC) and with catalase (for the measurements of toxicity and phytotoxicity). Catalase is a protein containing carbon and therefore, cannot be used to remove the residual H_2O_2 in the experiments for COD and DOC measurements (Ghaly et al., 2001). The samples, when MnO_2 was used, were filtered after the removal of H_2O_2 through 0.22 µm filter (Milli-pore) to remove the MnO_2 particles. The bench-scale experimental setup, the photochemical reactor, and the solar simulator used in this study, are presented in Schematic 5.3.



Schematic 5.3: (a) Bench-scale experimental setup of the solar photo-Fenton treatment, (b) solar simulator (Newport model 91193), and (c) photochemical batch reactor.

5.2.2 Experimental setup and description of the pilot-scale solar Fenton treatment process

The solar Fenton experiments were carried out in a compound parabolic collector (CPC) pilot plant installed at the premises of the University of Cyprus (UCY), as shown in Schematic 5.4.

Schematic 5.4: Pilot-scale setup of solar Fenton treatment

The solar prototype photocatalytic reactor (pilot-scale plant) consists of glass tubes and is mounted on a fixed platform tilted at the local latitude (35^o), operated in batch mode. The reflecting surface is constructed by resistant and highly reflecting polished aluminium. The contaminated water flows directly from one tube to the other and finally to a reservoir tank (meander flow). A centrifugal pump returns the water to the collectors in a closed circuit. The overall capacity of the reactor V_T consists of the total irradiated volume Vi (tubes volume) and the dead reactor volume (tank, piping and valves). Storage tank, flow meters, pH sensor, air blower, control panel, pipes, and fittings complete the installation, as shown in Table 5.6. Furthermore, three reagent tanks along with their dosing pumps are installed in the solar driven pilot-scale plant, which can automatically dose reagents (H_2SO_4, H_2O_2, $FeSO_4.7H_2O$) directly to the storage tank. The automated dosing system was not used for the thesis experiments, as the pilot-scale plant was operated in batch mode. The maximum capacity of the pilot-scale plant is 100 L and the minimum operating volume is 60 L, while the irradiated volume inside the tubes is ca. 21.4 L. For the various experiments carried out, the solar driven pilot-scale plant was operated with the minimum operating volume of 60 L.

Table 5.6: Mechanical parts of the solar driven pilot-scale plant

Equipment	**Material and Capacity**
Storage tank	100 L Polyethylene container
Air blower (increase the dissolved oxygen)	Flow rate: 26 L min^{-1}
Feed pump (effluent transfer)	Centrifugal: 0.37 kW Capacity: 40-150 L h^{-1}
Acid dosing pump and solution tank (pH adjustment)	Electronic pump (0.74 L h^{-1}) 20 L Polyethylene container
Peroxide dosing pump and solution tank (Fenton process)	Electronic pump (0.74 L h^{-1}) 20 L Polyethylene container
$FeSO_4$ dosing pump and solution tank (Fenton process)	Electronic pump (0.74 L h^{-1}) 20 L Polyethylene container
Complete system for measurement and control of pH	Electronic monitoring of pH
Complete system for measurement and control of H_2O_2	Electronic monitoring of H_2O_2
Compound Parabolic Collectors	6 Borosilicate glass tubes (55mm x 1.5m) Wall thickness 1.8 mm
Piping	UPVC PN10

It should be mentioned that the pilot-scale experiments were performed with winery wastewater pretreated by SBR and MBR (for quality characteristics see Table 5.3).

The UV solar radiation was continuously recorded, with a UV radiometer (UV_air_ABC), which was mounted on the platform of the CPCs. During the loading of the reactor with the chemicals, the collectors were covered with a thick plastic sheet to avoid any photoreaction during preparation. At the beginning, the reactor was filled with the biologically pretreated wastewater (by SBR or MBR). Then a sample was taken, representing the initial concentration of the effluent. The pH was then adjusted with dilute H_2SO_4 2M and the appropriate volume of ferrous iron solution was added. Mixing was following for 10 min. Hydrogen peroxide was then added, and after 10 min of dark Fenton oxidation a sample was taken representing the zero-illumination time. Just after that the collectors were uncovered. This was the time when solar Fenton process began. Samples were withdrawn during the process, at periodic intervals and were further analyzed. The main characteristics of the solar driven pilot-scale plant are shown in Table 5.7.

Table 5.7: Main characteristics for the solar driven pilot-scale plant

Platform latitude (^{o})	35
Coordinates	Latitude: 35° N Longitude: 33.25 E
Modules	1
Total volume V_T (L)	100
Irradiated volume V_i (L)	21.4
Number of tubes	6
Tube material	Borosilicate
Tube length (mm)	750
Diameter (mm)	50
Storage tank material	Polyethylene
Flow (L h^{-1})	150
Cover material	Thick beige plastic sheet

5.2.3 Description of the industrial-scale solar Fenton treatment process

The solar Fenton treatment was carried out in a compound parabolic collector (industrial-scale plant) installed at the premises of the winery wastewater treatment plant II (which includes MBR treatment), as shown in Schematic 5.5. It should be noted that the industrial-scale solar plant has been placed side by side with the membrane bioreactor (MBR), in order to complete the winery wastewater treatment plant II (Schematic 5.2).

Schematic 5.5: Industrial-scale setup of solar Fenton treatment

The major machinery equipment comprising the industrial-scale plant is the same but in larger sizes than those of the pilot-scale plant and is shown in Table 5.8. The maximum capacity of the industrial-scale plant is 250 L (used in this study as well), and the irradiated volume inside the tubes is 85.4 L.

Table 5.8: Mechanical parts of the solar driven industrial-scale plant

Equipment	**Material and Capacity**
Storage tank	250 L AISI 304 container
Air blower (increase the dissolved oxygen)	Flow rate: 120 L min^{-1}
Feed pump (effluent transfer)	Centrifugal: 0.37 kW Capacity: 60 L min^{-1}
Compound Parabolic Collectors	24 Borosilicate glass tubes (55 mm x 1.5 m) Wall thickness 1.8 mm
Piping	UPVC PE

The main characteristics of the solar driven industrial-scale plant are shown in Table 5.9.

Table 5.9: Main characteristics for the solar driven industrial-scale plant

Platform latitude (^{o})	35
Coordinates	Latitude: 35° N Longitude: 33.25 E
Modules	2
Total volume V_T (L)	250
Irradiated volume V_i (L)	85.4
Number of tubes	24
Tube material	Borosilicate
Tube length (mm)	1500
Diameter (mm)	50
Storage tank material	Stainless Steel
Flow (L h^{-1})	600
Cover material	Thick grey plastic sheet

All runs at bench, pilot and industrial-scale treatment were performed at least in triplicate and mean values are quoted as results.

5.2.4 Experimental setup and description of the pilot-scale reverse osmosis treatment process

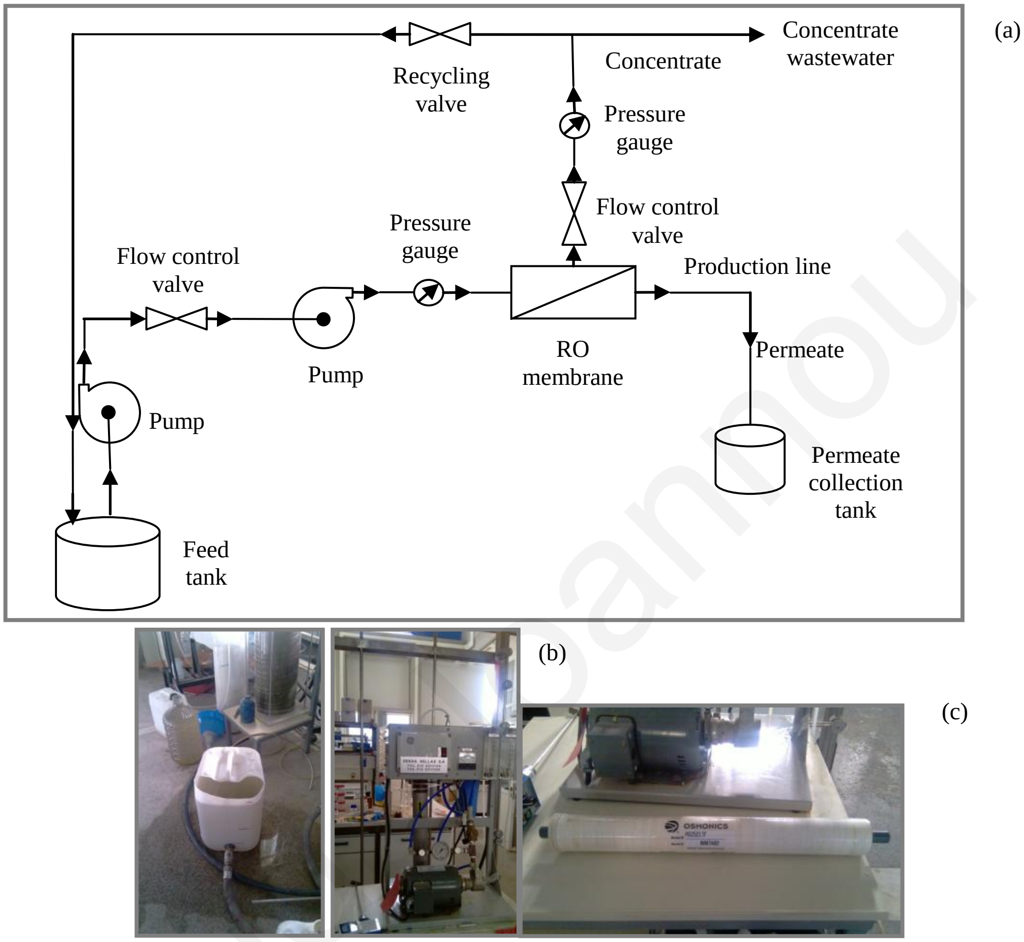
The experiments were carried out at a pilot-scale reverse osmosis unit, installed at the Laboratory of Environmental Technology and Management of the Department of Environmental Engineering, at the Technical University of Crete (TUC), Greece. The RO pilot unit manufactured by GE-Osmonics (model E2/375-2535), is equipped with an AG2521TF spiral-wounded polyamide membrane (Desal). The technical characteristics of the unit are presented in Table 5.10.

Table 5.10: Technical characteristics of the reverse osmosis unit

Process parameters	Value
Membrane type	Desal, AG2521TF
Nominal membrane surface area, m^2	1.2
Operating temperature range, ^oC	12 - 29
Pump power, kW	0.37
pH range	5.5 - 8.5

Experiments in single-pass, as well as in recirculation mode were performed, in order to investigate the optimum experimental conditions (with regard to pressure and temperature), during which the maximum volume recovery and organic compound removal could be achieved.

A plastic feed tank of 30 L capacity was provided for storage and supply of the effluent to the system, as well as for the collection of the recycled concentrate, when the system was working in recirculation mode. A pressure pump (Wausau Wisconsin 54401 - Model APB48517D1306AP, 0.37 kW) was used for transporting the feed liquid through the system. Permeate and concentrate flow rates were measured by two glass rotameters containing metal floats. Two pressure gauges were used to indicate the pressure of the inlet and the outlet of the membrane. A flow diagram of the RO unit, the RO system and the membrane used, are shown in Schematic 5.6.



Schematic 5.6: (a) Flow diagram of the RO process, (b) the RO pilot unit, and (c) the RO membrane

Prior to its use, the RO membrane was washed with distilled water for 4 h and then conditioned with citric acid according to the manufacturer’s instructions. In addition, cleaning with distilled water was also used for cleaning between filtration experiments. More intense cleaning was not necessary due to the short duration of the experimental runs, while no reduction in clean water flux was observed.

5.3 Analytical methods

5.3.1 Analysis of Dissolved Organic Carbon (DOC)

The dissolved organic carbon (DOC) was determined in order to evaluate the degree of mineralization of the contaminants during solar photo-Fenton and solar Fenton process (at bench, pilot and industrial scale). DOC was monitored by direct injection of the filtered winery wastewater samples into a Shimadzu TOC-VCPH/CPN, TOC analyzer.

The basic principle for the quantitation of total organic carbon (TOC) relies on the oxidation of the organic carbon presented in wastewater to carbon dioxide (CO_2) by dry combustion at 720 °C. The final determination of CO_2 is carried out by infrared spectrometry (ISO 8245:2000).

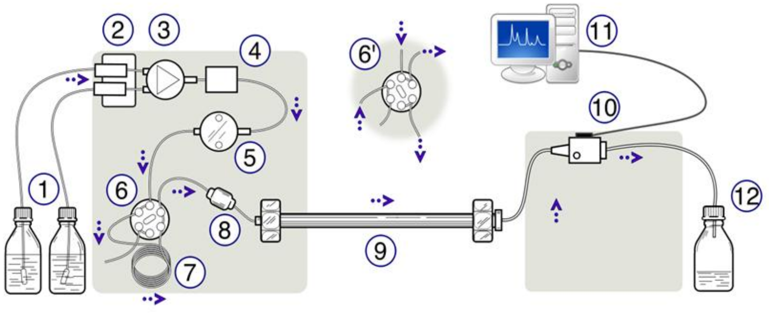
Prior to the determination of TOC, a filtered and acidified water sample is sparged with oxygen to remove total inorganic carbon (TIC). The acidification is usually performed using HCl solution. The water is then injected onto a combustion column packed with platinum-coated alumina beads held at 680 °C. Non-purgeable organic carbon compounds are combusted and converted to CO_2, and then a CO_2 free carrier gas delivers the sample combustion products to the cell of a non-dispersive infrared detector (NDIR) gas analyzer, where CO_2 is detected. The absorbance of CO_2 generates a signal which is evaluated by the equipment’s software, and which is proportional to the quantity of carbon in the sample.

The TOC analyzer used in this study was provided by Shimadzu (Shimadzu TOC-VCPH/CPN) equipped with an autosampler. Due to the narrow capillary tubes in analyzer, any suspended solids in the sample have to be removed prior the injection in the system. Therefore, the analyzer can only be used to measure dissolve organic carbon (DOC).

In this work sample filtration through 0.22 μm pore size syringe driven filters (Millipore) was applied. After filtration the sample was injected and automatically analyzed for DOC by the TOC analyzer as described above. Each measurement was based on two injections with a maximum coefficient of variance of less than 2% (otherwise automatic sample re-injection takes place). The calibration curve was made from a standard aqueous solution of potassium hydrogen phthalate (KHP) over the range 0 - 10 mg L^{-1}. Calibration and quality maintenance were performed regularly to ensure the correct operation of the equipment.

5.3.2. Analysis of the phenolic compounds with High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a dynamic physicochemical method of separation technique in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (mobile phase) moves relatively to the stationary phase (Dong, 2006). The stationary phase may be a solid or a liquid that is immobilized or adsorbed on solid particles. Mobile phase can be run as a single or mixed solvent running at a constant composition (isocratic elution) or two (or more) separate mobile phases can be pumped simultaneously at varying concentrations (gradient elution). An HPLC schematic layout and the HPLC used in this study are shown in Schematic 5.7.



(1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in "inject position", (6') Switching valve in "load position", (7) Sample injection loop, (8) Pre-column or guard column, (9) Analytical column, (10) Detector (i.e. UV, FL), (11) Data acquisition, (12) Waste or fraction collector.

Schematic 5.7: HPLC schematic diagram and HPLC (Alliance 2690, Waters) used for the analytical determination of phenolic compounds in winery wastewater, at the Technical University of Crete (TUC)

The sample solution is injected into the mobile phase through the injector port. As the sample solution flows with the mobile phase through the stationary phase, the components of that solution will migrate according to the non-covalent interactions of the compounds with the two phases. These interactions (hydrophobic or van der Waals interactions) determine the degree of migration and separation of the components contained in the sample. For example, solutes which have stronger interactions with the mobile phase than with the stationary phase will elute from the column faster, and thus have a shorter retention time (t_R). The composition of the mobile phase can be modified in order to manipulate the interactions of the sample and the stationary phase.

Additionally, in order to improve the separation and peak resolution, the pH of the mobile phase can be adjusted accordingly.

The type of liquid chromatography (LC) used for the analytical determinations in this thesis was a reversed-phase LC. The separation mechanism in reversed-phase LC depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the hydrophobic stationary phase. Reverse phase operates by using a stationary phase that consists of silica-based packings with n-alkyl chains (e.g. C_8, C_{18}) covalently bound to them. C_8 signifies an octyl chain and C_{18} an octadecyl ligand in the silica medium. The distribution of the solute between the two phases depends on the binding properties of the medium, the hydrophobicity of the solute and the composition of the mobile phase. The more hydrophobic the medium on each ligand, the greater is the tendency of the column to retain hydrophobic compounds.

A UV-diode array (UV-DAD) and a Fluorescence detector (FL) were used as detectors in the HPLC system of this study. A diode array consists of a number of photosensitive diodes placed side by side and insulated from one another in the form of a multi-layer sandwich. The light source is usually polychromatic (e.g. light from a deuterium lamp) and after passing through the cell, the light is dispersed by a quartz prism or a diffraction grating onto the surface of the diode array. Many organic compounds have characteristic spectra in the UV, which can be used to help identify the substance passing though the sensor cell. The analytes eluted through the sensor cell and all the outputs from the array can be acquired and subsequently processed by a computer device. The result is used to construct absorption spectra that can be compared with standard spectra for identification purposes.

A UV/VIS detector monitors the absorption of light with a specified wavelength. However, some substances absorb light at one wavelength, and then emit light called fluorescence at another wavelength. This is a phenomenon in which substance absorbs light to reach a high-energy level when emits light to return to its original level. Such, a substance has specific wavelengths of light that it absorbs (excitation wavelength, λ_{ex}) and emits (emission wavelengths, λ_{em}). Fluorescence (FL) detectors are probably the most sensitive among the existing modern HPLC detectors. Typically, fluorescence sensitivity is 10 - 1000 times higher than that of the UV detector for strong UV absorbing materials. Fluorescence detectors are very specific and selective among the others optical detectors. This is normally used as an advantage in the measurement of specific fluorescent species in samples (Kazakevich and McNair, 2000).

High performance liquid chromatography (HPLC: Alliance 2690, Waters) was employed in this study. Separation was achieved on a Luna C-18 (2) column (5 μm, 250 mm × 4.6 mm) and a security guard column (4 mm × 3 mm), both purchased from Phenomenex. The HPLC used for the chromatographic analysis, was placed in the Laboratory of the Environmental Organic Chemistry - Micropollution of the Department of Environmental Engineering, at the Technical University of Crete (TUC).

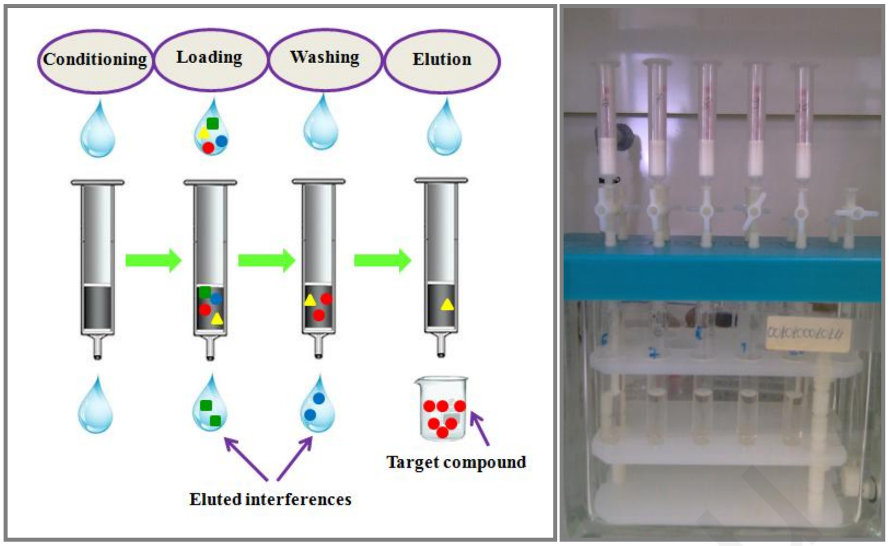
The HPLC method used is a modified version of the method developed by Rodriguez-Delgado et al. (2001). The gradient eluted consisted of solvent A (methanol-acetic acid-water (10:2:88, v/v)) and solvent B (methanol-acetic acid-water (90:2:8, v/v)), with a total flow-rate set of 1.0 mL min^{-1} and 25 ^{o}C, while the injection volume was 50 μL. Run time was set at 70 min with a constant flow rate at 1.0 mL min^{-1}, in accordance with the following gradient time table: at zero time, 100% A; after 30 min, the pumps were adjusted to 85% A and 15% B; at 50 min, 50% A and 50% B; at 51 min, 100% B and at 61 min 100% A. Detection was achieved through two detectors connected in series, a diode array detector (Waters 996) and a fluorescence detector (Waters 474 Scanning Fluorescence Detector), in which the excitation and emission wavelengths were set at 280 nm and 360 nm, respectively.

The detection of gallic acid was done at 271 nm (t_R=5.578 min), vanillic acid at 260 nm (t_R=23.001 min), epicatechin at 278 nm (t_R=26.942 min), p-coumaric acid at 309 nm (t_R=36.277 min), and ferulic acid at 322 nm (t_R=40.134 min).

5.3.2.1 Solid Phase Extraction (SPE)

Pretreatment before HPLC analysis is often required for samples of low concentration or samples containing analytical contaminants. It improves reproducibility and sensitivity in analysis, and protects HPLC columns. A wide variety of sample pretreatment methods have been used in combination with LC chromatography.

As a consequence, prior to HPLC analysis the RO permeate samples were subjected to solid phase extraction (SPE) using a Sep-Pak® Vac 3 mL C18 cartridge (Waters, Milford, MA, USA) previously activated with methanol (HPLC grade, >99.9%, Sigma Aldrich) (3 mL). For the conditioning of the cartridges, 6 mL of acidified water with acetic acid at pH 2.5, were used. Afterwards, 10 mL of sample was percolated through the cartridge and then washed using 3 mL of acidified water-methanol (9:1, v/v). Finally, the phenolic compounds were eluted using 3 mL of acidified water-methanol (3:7, v/v) at a rate of 1-2 mL min^{-1}, and 50 μL were injected in the HPLC. Schematic 5.8 shows the solid phase extraction (SPE) process and the SPE system used in this study.



Schematic 5.8: Solid phase extraction (SPE) process and system used for the extraction of phenolic compounds from winery wastewater, at TUC

5.3.3 Determination of heavy metals with Atomic Absorption Spectroscopy (AAS)

Metals present in the wastewater samples were determined by atomic absorption spectrometry (AAS). Atomic Absorption Spectroscopy is designed to determine the amount (concentration) of an object element in a sample, utilizing the phenomenon that the atoms in the ground state absorb the light of characteristic wavelength passing through an atomic vapor layer of the element.

Usually the apparatus consists of a light source, a sample-atomizer, a spectroscope, a photometer, and a recording system. For the light source, a hollow cathode lamp and a discharge lamp are mainly used. To the sample-atomizer, the flame type, electrothermal type, and the cold-vapor type are applied.

In this study, a flame type AAS was used. In flame atomic absorption spectrometry, a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the flame. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used. The amount of energy absorbed in the flame at the characteristic wavelength is proportional to the concentration of the element in the sample. A standard curve is obtained by plotting the absorbance of standards against the concentration. The sample concentration is computed directly from the standard curve (APHA 3111C). Metals were determined using the Atomic Absorption Spectrometer (Perkin Elmer - Aanalyst 200), as shown in Schematic 5.9.

Schematic 5.9: Atomic Absorbance Spectrometer (Perkin Elmer - Aanalyst 200) used for the measurement of heavy metals

5.3.4 Other analytical measurements

5.3.4.1 pH and conductivity

Laboratory pH measurements were carried out using a PL-600 lab pH meter (EZDO pH/mV/Temp meter) equipped with a standard glass electrode. A calibration of the pH-meter was performed using two standard buffer solutions at pH 4.00 and pH 7.00 (obtained from Panreac). At the solar driven pilot- and industrial-scale plants, pH measurements were performed with a portable pH-meter (Gondo EZDO). At the pilot-scale reverse osmosis unit at TUC, the pH of the samples was recorded with a MicropH2002 (Crison) and their conductivity with a MicroCM222 (Crison).

5.3.4.2 Total Solids (TS)

To determine the total solids (TS), a well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103-105 ^{o}C. The increase in weight over that of the empty dish represents the total solids (APHA 2540B), as shown in Eq. (5.1).

$$mg_{TS}/L = \frac{(A-B)\times 1000}{Sample\ Volume\ (mL)} \tag{5.1}$$

where A, is the weight of dried residue + dish, (mg) and B is the weight of dish, (mg).

The evaporation of the total solids on a steam bath is shown in Schematic 5.10.

Schematic 5.10: Total solids evaporation on a steam bath

5.3.4.3 Total Suspended Solids (TSS)

Total suspended solids (TSS) refer to matter suspended in water or wastewater. A well-mixed sample is filtered through a weighed standard glass-fiber filter (0.45 μm, Sartorius Stedim Biotech) and the residue retained on the filter is dried to a constant weight at 103 ^{o}C to 105 ^{o}C, as shown in Schematic 5.11. The increase in weight of the filter represents the TSS (APHA 2540D), as shown in Eq. (5.2).

$$mg_{TSS}/L = \frac{(A-B)\times 1000}{Sample\ Volume\ (mL)} \tag{5.2}$$

where A, is the weight of filter with the dried residue (mg) and B is the weight of filter (mg).

Schematic 5.11: Filters with the dried residue of total suspended solids

5.3.4.4 Total Volatile Solids (TVS) and Volatile Suspended Solids (VSS)

To determine the total volatile solids (TVS) and volatile suspended solids (VSS), the residue after drying at 103-105 ^{o}C taken from the implementation of the methods for TS and TSS is ignited to constant weight at 550 ^{o}C. The remaining solids represent the fixed total or suspended solids, while the weight lost on ignition is the volatile solids (APHA 2540E), as shown in Eq. (5.3).

$$mg_{VTS\ \&\ VSS}/L = \frac{(A-B)\times 1000}{Sample\ Volume\ (mL)} \tag{5.3}$$

where A, is the weight of residue + dish or filter before ignition, (mg) and the B is the weight of residue + dish or filter after ignition, (mg).

5.3.4.5 Color

The color measurement was carried out according to the Tristimulus Filter Method, using a UV/Vis Jacso V-530 spectrophotometer (APHA 2120D). Prior to color measurements, the wastewater samples were filtered using 0.22 μm Millipore filters to remove any turbidity. To determine the color, a light scan from 350 to 700 nm was performed (Kang et al., 2000; Kang et al., 2002; Pala and Tokat, 2002). Color content was determined by measuring the absorbance at three wavelengths (390, 400 and 410 nm), and taking the sum of these absorbances (Pala and Tokat, 2002). The percentage of color removal was calculated from the difference in absorbance values before and after treatment (Selcuk, 2005; Gao et al., 2007; Streethawong and Chavadej, 2008).

5.3.4.6 Hydrogen peroxide concentration

The residual peroxide concentration during photo-Fenton process is a very important parameter to evaluate. When H_2O_2 is completely consumed, which can happen in a very short time depending on the organic matter concentration, the degradation during the photo-Fenton reaction practically stops making new additions of the oxidant necessary.

Hydrogen peroxide consumption was determined by a fast, simple spectrophotometric method using ammonium metavanadate in acidic medium, based on the red-orange color of peroxovanadium cation (VO_2^{3+}) formed during the reaction of H_2O_2 with metavanadate, at λ_{max}=450 nm (Nogueira et al., 2005). The presence of H_2O_2 in the treated samples was also monitored using hydrogen peroxide test strips (Quantofix peroxide, 1-100 mg L^{-1}, Sigma Aldrich).

The procedure for measuring H_2O_2 is as follows: 4 mL of sample is mixed with 4 mL of ammonium metavanadate solution and 2 mL of deionized water. The reaction generates a red-orange color which does not change within 2 hours. The absorbance of the samples is measured at 450 nm using a spectrophotometer, and the H_2O_2 concentration is obtained by a calibration curve.

5.3.4.7 Total iron concentration

Total iron concentration was monitored by colorimetric determination with 1,10-phenanthroline, according to ISO 6332:1996, using spectrophotometer (UV-Vis Jasco V-530). Dissolved iron forms a stable red-orange colored complex with 1,10-phenanthroline, which is strongly absorbed at 510 nm. A series of iron standards is measured at this wavelength and a calibration plot of absorbance vs. concentration is prepared. The absorbance of the unknown sample is measured and the calibration curve is used to calculate the concentration of iron in the sample.

The procedure for measuring the concentration of total iron is as follows: 4 mL of sample is mixed with 1 mL of 1,10-phenanthroline (0.1% w/v in deionised water), 1 mL of acetate buffer solution (250 g L^{-1} ammonium acetate and 700 mL acetic acid in deionised water), and a spatula tip of ascorbic acid. After some minutes of reaction the absorbance is measured at 510 nm in a spectrophotometer against a blank (1,10-phenantroline is replaced with the same volume of distilled water). Moreover, it should be noted that when total iron is to be determined, Fe^{3+} must first be reduced to Fe^{2+} with the addition of ascorbic acid, which is an effective reductant in acidic solution (Mortatti et al., 1982).

5.3.4.8 Biological Oxygen Demand (BOD)

The BOD test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation (in the presence of microorganisms) of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material.

The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature (20 ^{o}C) for 5 days. Dissolved oxygen (DO) is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement. BOD measurements in a 5-day test period are known as BOD_5 (APHA 5210B). BOD was determined using the 444406 OxiDirect meter.

5.3.4.9 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is defined as the quantity of oxygen which is required to oxidize the organic matter present in a sample under controlled conditions (temperature, time, oxidizing agent).

The organic matter in a sample is oxidized in an acidic solution of potassium dichromate ($K_2Cr_2O_7$) in the presence of silver sulfate (Ag_2SO_4) which acts as a catalyst. Under these conditions and when the sample is digested, dichromate ion ($Cr_2O_7^{2-}$) is reduced to the chromic ion (Cr^{3+}). The produced Cr^{3+} ions, which generate a green color, are measured photometrically (APHA 5220D).

Merck®Spectroquant kits were used for the COD determination. The measurement range for the samples analysed was between 25 and 1500 mg O_2 L^{-1}. Three (3) mL of a sample, filtered with 0.22 μm filters (Millipore), was mixed with the reaction solution in the test tube and heated for two hours at 148 ^{o}C in a thermo block (WTW CR 3200). After cooling down to room temperature the test tube was introduced into the photometer (Photolab S6 and Merck, Pharo 100 Spectroquant®) and measured at 445 nm. The calibration was made from a standard aqueous solution of potassium hydrogen phthalate (KHP) over the range 25-1500 mg L^{-1}.

5.3.4.10 Total Nitrogen (TN)

Total nitrogen can be determined through oxidative digestion of all nitrogenous compounds to nitrate according to the persulfate method. Alkaline oxidation at 100 ^{o}C to 110 ^{o}C converts organic and inorganic nitrogen to nitrate. Total nitrogen is determined by analyzing the nitrate in the digestate. A standard curve is prepared by plotting the absorbance of the standard nitrate solutions carried through the digestion procedure against their concentrations. The total N of the sample is computed by the standard curve (APHA 4500-NC).

The total N values obtained from the method described above were confirmed using Koroleff's method as well. For this method, Merck®Spectroquant kits were used. Organic and inorganic nitrogen compounds are transformed into nitrate (NO_3^-) according to Koroleff's method by treatment with an oxidizing agent in a thermoreactor. In concentrated sulfuric acid, this nitrate reacts with a benzoic acid derivative to form a red nitro compound that is determined photometrically.

5.3.4.11 Total Phosphorous (TP)

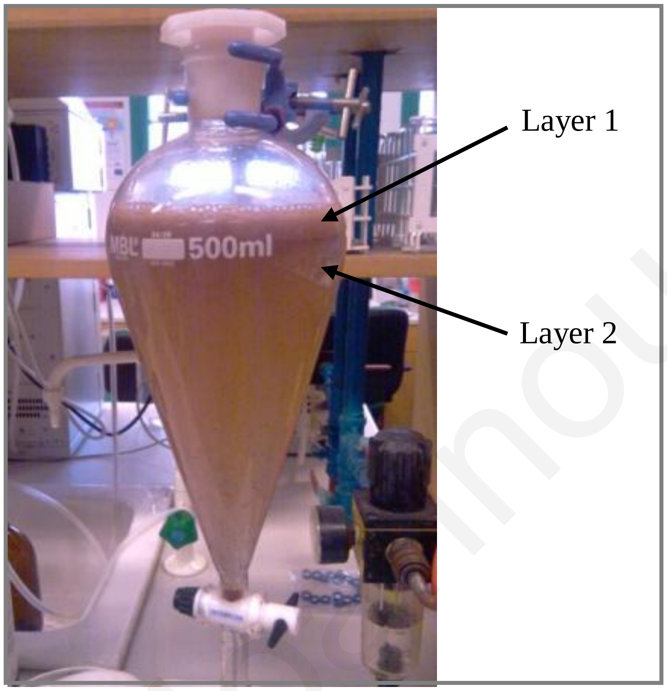
Total phosphorous is measured by the persulfate digestion procedure (APHA 4500-PB). The sample is digested with a sulfuric acid solution containing ammonium persulfate. Ammonium molybdate and potassium antimonyl tartrate react in acidic medium with orthophosphate ions to form a phosphomolybdic acid. Ascorbic acid reduces this acid to phosphomolybdenum blue (PMB) that is determined photometrically. A standard curve is prepared by plotting the absorbance of the standard phosphorous solutions carried through the digestion procedure against their concentrations. The TP of the sample is computed by the standard curve. For this method, Merck®Spectroquant kits were also used.

5.3.4.12 Oil and grease

“Oil and grease” is defined as any material recovered as a substance soluble in the solvent. It includes other material extracted by the solvent from an acidified sample (such as sulfur compounds, certain organic dyes, etc.) and not volatilized during the test (APHA 5220B).

Dissolved or emulsified oil and grease is extracted from water by intimate contact with an extracting solvent (e.g. n-hexane). Some extractable, especially unsaturated fats and fatty acids, oxidize readily; hence, special precautions regarding temperature and solvent vapor displacement are included to minimize this effect. Organic solvents shaken with some samples may form an emulsion that is very difficult to break, but this method includes a means for handling such emulsions (e.g with centrifugation).

The procedure for measuring oil and grease is as follows: First the sample is acidified with 1:1 HCl to pH lower than 2 and then is transferred to a separatory funnel. Afterwards, 30 mL extracting solvent (100% n-hexane) is added to separatory funnel, which is shaked vigorously for 2 min, until the separation of the layers is achieved as shown in Schematic 5.12. The aqueous layer is drained and a small amount of organic layer is transferred into the original sample container. Then the solvent layer is drained through a funnel containing a filter paper and 10 g sodium sulfate anhydrous, both of which have been solvent-rinsed, into a clean, tared distilling flask. The extraction is repeated twice with 30 mL solvent each time. The solvent layer in the tared flask is evaporated in a water bath at 85 °C, and after that it is cooled in desiccator for 30 min and weighted.



Schematic 5.12: Separation of the layers in oil and grease extraction

If the organic solvent is free of residue, the gain in weight of the tared distilling flask is due to oil and grease. Total gain in weight, A, of tared flask, less calculated residue from solvent blank, B, is the amount of oil and grease in the sample (Eq. (5.4)):

$$mg/L_{oil\ \&\ grease} = \frac{A-B\times 1000}{Volume\ sample(mL)} \tag{5.4}$$

5.3.4.13 Total phenolic compounds (TPh)

The concentration of total phenolic compounds was measured with the Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965). Folin-Ciocalteu colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenolic compounds. A calibration curve was made by observing the absorbencies of standards of various concentrations of gallic acid. The samples were tested and measured against this curve.

Analyses of the Folin-Ciocalteu type are convenient, simple, require only common equipment, and have produced a large body of comparable data. Under proper conditions, the assay is inclusive of monophenols and gives predictable (but variable by reactive groups per molecule) reactions with the types of phenols found in nature. Because different phenols react to different degrees, expression of the results as a single number such as milligrams per liter gallic acid equivalence is necessarily arbitrary. Because the reaction is independent, quantitative, and predictable, analysis of a mixture of phenols can be recalculated in terms of any other standard (Singleton et al., 1999).

The assay in fact measures all compounds readily oxidizable under the reaction conditions and its inclusiveness allows certain substances to also react that are either not phenols or seldom thought of as phenols (e.g. proteins) (Singleton et al., 1999). According to Medina-Remón et al. (2009) the Folin-Ciocalteu assay is affected by several interfering substances such as sulfur dioxide, ascorbic acid, sugar, aromatic amines, organic acid, Fe(II) and nonphenolic organic substances that react with the FC reagent. Judicious use, with consideration of potential interferences in particular samples and prior study if necessary, can lead to very informative results (Singleton et al., 1999).

The FC method has been adopted as the official procedure for total phenolic compounds levels in wine; the Office International de la Vigne et du Vin (OIV), the international body that certifies specific procedures for wine analysis, accepts the FC method as the standard procedure for total phenolic compounds analysis (OIV, 1990). Furthermore, FC assay is a worldwide indicative method widely accepted and used for the determination of total phenolic compounds (Chiselli et al., 1998; Asami et al., 2003; Lopez-Velez et al., 2003; Medina-Remón et al., 2009).

The procedure for measuring the total phenolic compounds is as follows: 1 mL of sample is mixed with 70 mL of ultra pure water and 5 mL Folin-Denis' reagent (Fluka Analytical) and is pipetted into a 100 mL volumetric flask. After 1 to 8 min at room temperature 15 mL of sodium carbonate solution (Fluka Analytical) is added, which is then filled to the mark with ultra pure water. After 2 h of incubation the absorbance is measured at 765 nm in a spectrophotometer against a blank.

5.4 Toxicity assays

The quality control of wastewater based on global chemical measures of total organic pollution load such as total organic carbon (TOC) or the detection of specific pollutants, is not efficient to assess the environmental risk. Therefore, effective tools for the evaluation of the potential negative effects on living organism are needed (Farré and Barceló, 2003).

Toxicity bioassays are applied worldwide as tools for wastewater quality control (Fernández-Alba et al., 2002; Hernando et al., 2005). The use of single organisms to evaluate wastewater toxicity cannot provide a real assessment of the risk. Therefore, an appropriate way to assess the risk of wastewater is the use of a variety of toxicity tests with representative organisms of different biological organization (Hernando et al., 2005).

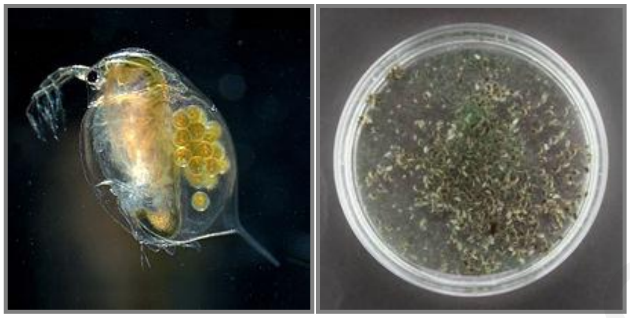
Toxicity measurements were carried out in winery samples at various times of solar Fenton treatment at bench, pilot and industrial scale; and at the three streams of the RO process (feed, permeate and concentrate). The toxicity assays used in the present study are: (a) Daphtoxkit F^{TM} toxicity test and (b) Phytotestkit microbiotest toxicity test. The toxicity tests were conducted according to the standard testing protocols for *Daphnia magna* and the phytotoxicity with 3 species of plant seeds: (a) monocotyl Sorgho (*Sorghum saccharatum*), (b) dicotyls garden cress (*Lepidium sativum*), and (c) mustard (*Sinapis alba*).

It is important to mention that the H_2O_2 present in all solar Fenton treated samples (bench, pilot and industrial scale) can affect toxicity measurements, and therefore had to be eliminated before any bioassay. The residual H_2O_2 was removed from the treated samples with catalase solution (*Micrococcus lysodeikticus*, Fluka), which is non toxic to microorganisms (Kaludjerski and Gurol, 2004). Catalase is an enzyme which catalyzes the decomposition of H_2O_2 to water and molecular oxygen. One unit of catalase corresponds to the amount of enzyme which decomposes 1 μmol H_2O_2 per minute at pH 7 and 25 °C.

5.4.1 Daphtoxkit F^{TM} toxicity test

This test, which is currently widely used for toxicity monitoring purposes, is based on the observation of the freshwater species *Daphnia magna* (Schematic 5.13) immobilization after 24 and 48 h of exposure in the samples.

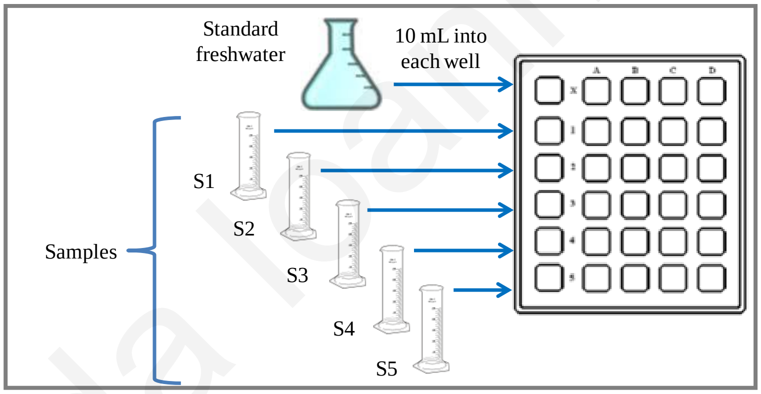
The freshwater zooplankter *Daphnia* (Arthropoda: Crustacea) is known to be of pivotal ecological importance in lakes and ponds, where it is a highly efficient grazer on phytoplankton and a preferred prey item for planktivorous fish and other predators (Miner et al., 2012).



Schematic 5.13: *Daphnia magna* species

Sources: http://en.wikipedia.org/wiki/File:Daphnia_pulex.png;
http://www.google.com.cy/imgres?q=Daphnia+magna+petri+dish&um

The toxicity of winery wastewater was assessed using the commercially available Toxkit Daphtoxkit (Creasel, Belgium). The toxicity studies were performed in accordance to the testing conditions prescribed by ISO 6341: 2010. The water used to activate and hatch the organisms (72-90 h) was synthetic freshwater containing $NaHCO_3$, $CaCl_2$, $MgSO_4$ and KCl (dilution solution). Sufficient amount of dissolved oxygen (~5 mg L^{-1}) was achieved by aeration. The water used for the dilutions was prepared a day prior to its use, in order to provide oxygen saturation and ensure complete salts dissolution and homogenization. Cultures were grown under continuous illumination of 6000 lux at a constant temperature of 20-22 ºC. Appropriate adjustment of the pH value of the samples in the range of 7±0.5 was carried out with 1M solution of NaOH or HCl. Two hours before testing, the neonates were fed using a dilution of *Spirulina* microalgae in order to preclude mortality by starvation, thus avoiding biased test results. Analysis was carried out on specific test plates, which were filled with the examined samples, as shown in Schematic 5.14. For statistically acceptable evaluation of the effects, each test sample as well as the control, was tested in quadruplicate. After the transfer of five *Daphnia* neonates into the cells, the test plates were covered and incubated at 20 ºC in the dark. Observations of test populations were made at 24 and 48 h of exposure and any dead or immobilized neonates were recorded. The neonates were considered immobile, if after 24 or 48 h of incubation with the toxicant they remained settled at the bottom or did not resume swimming within the observation period. It should be noted that tests in which the control survival was less than 90% were considered invalid and were repeated.

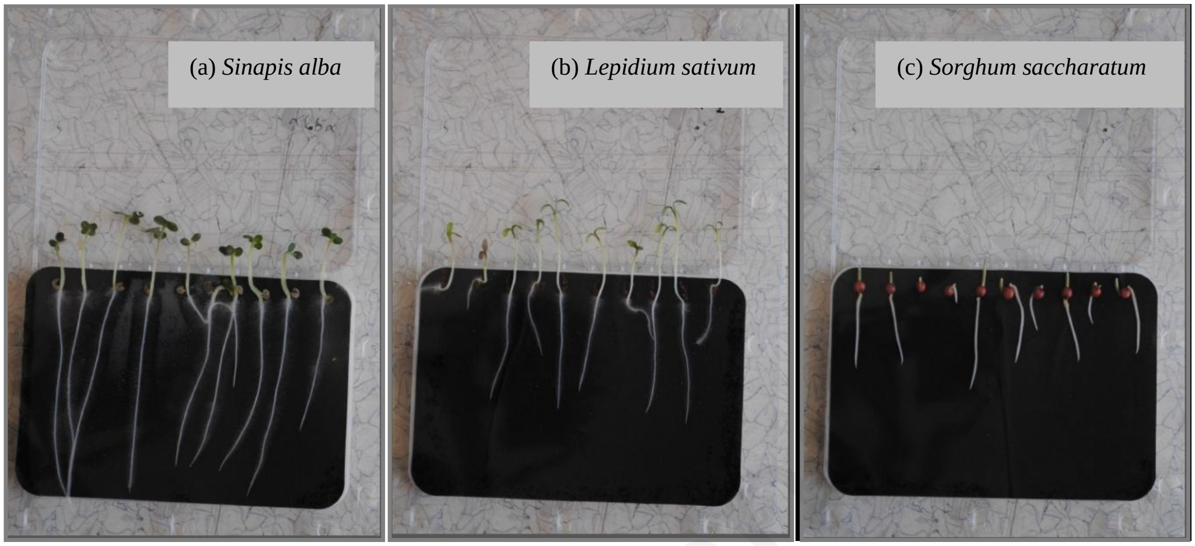


Schematic 5.14: Filling of the test plate of Daphtoxkit F^{TM} toxicity test

5.4.2 Phytotestkit microbiotest toxicity test

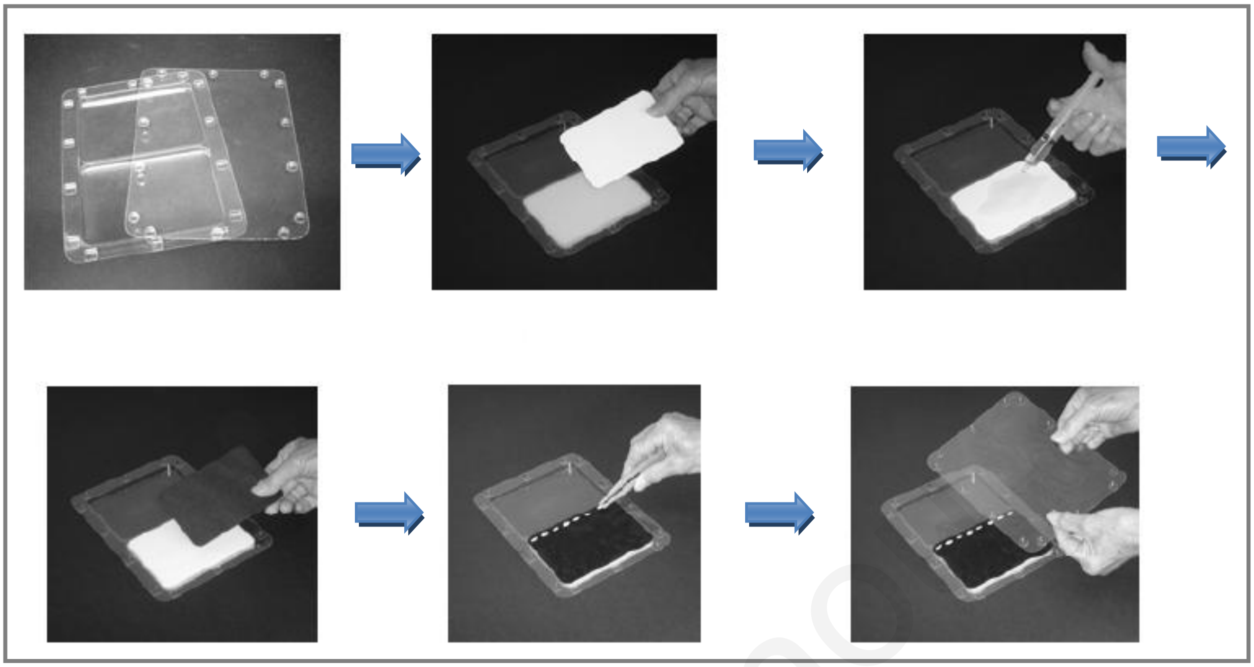
The Phytotestkit microbiotest (MicroBioTests Inc.) is measuring the decrease (or the absence) of germination and early growth of plants which are exposed directly to the samples spiked onto a thick filter paper. A control test was performed using tap water. The plants used for the Phytotestkit microbiotest were: the monocotyl Sorgho (*Sorghum saccharatum*), the dicotyl garden cress (*Lepidium sativum*), and the dicotyl mustard

(*Sinapis alba*) (Schematic 5.15). These species are frequently used in phytotoxicity analyses due to their rapid germination and growth of roots and shoots and their sensitivity to low concentrations of phytotoxic substances (MicroBioTests Inc.).



Schematic 5.15: Phytotest kit plant types: (a) *Sinapis alba*, (b) *Lepidium sativum*, and (c) *Sorghum saccharatum*. The above photos were taken after the exposure of these plants in the solar photo-Fenton treated samples for 3 days

Seeds of the selected test plant(s) are positioned at equal distance near the middle ridge of the test plate, on a black filter paper placed on top of the spiked filter paper (20 mL sample) as shown in Schematic 5.16. After closing the test plates with their transparent cover, the test plates are placed vertically in a holder and incubated at 25 ^{o}C and for 3 days. At the end of the incubation period a digital picture was taken of the test plates. The pictures were stored in a computer file and the length measurements of the roots and the shoots were performed using the Image Tool 3.0 for Windows. The bioassays were carried out in three replicates for each sample and for each type of plant.



Schematic 5.16: Phytotestkit procedure

The test results were evaluated comparing the mean number of germinated seeds and the mean root and shoot length for the three replicates in the control and in each examined sample. The inhibition percentage of the chemical compounds on seed germination (GI), shoot growth (SI), and root growth (RI) was calculated by applying the following formula (Eq. (5.5)):

$$\%GI, SI \,\&\, RI = \frac{A-B}{A} \times 100 \tag{5.5}$$

where A, is the average number of germinated seeds or the average shoot length or the average root length in the control water, and B the same parameter for the test wastewater.

CHAPTER 6: IDENTIFICATION OF THE ENVIRONMENTAL IMPACTS RELATED TO THE WINE PRODUCTION IN CYPRUS

6.1 Introduction

The identification of the exact environmental impacts related to the wine production process in Cyprus were investigated through a review, which was prepared based on the relevant available literature, a questionnaire designed specifically for collecting relevant information from the operators (Annex), site visits to wineries in Cyprus and Italy, and personal communication with the owners of numerous wineries.

In order to achieve best results, a questionnaire was designed to collect the relevant data and information through a telephone survey targeting all 54 wineries in Cyprus. The most updated list and the contact details of the wineries were obtained through the website of the Cypriot Wine Products Council. The survey was conducted between July and August 2010.

In specific, the overall research included the following fields:

- ***Winery details:*** Annual quantity of grapes received (tonnes per year).
- ***Environmental measures:*** General measures implemented by the wineries in the fields of wastewater, water consumption, energy, solid waste, chemicals use, air emissions and odors prevention.
- ***Winery wastewater:*** Monitoring of the annual winery wastewater volume produced, wastewater drainage/collection system, how and where wastewater is disposed off,

how the wastewater is treated before being disposed in the environment, awareness on the national legislation on the treatment and disposal of winery wastewater, and location of the main wastewater collection tank.

- ***Water consumption:*** Practices applied for monitoring the water consumption, identification of possible leaks and water saving measures.
- ***Energy consumption:*** Practices applied for monitoring the energy consumption, identification of possible excessive energy consumption values and energy saving measures applied.
- ***Solid waste:*** Practices applied for the management of organic and packaging waste, management of wine sludge and monitoring of the annual quantity of solid waste produced.
- ***Chemicals:*** Identification of chemicals used for cleaning/disinfection, pesticides and fertilizers, monitoring of the chemicals' quantities, measures to limit chemicals' use, use of environmentally friendly cleaning products, frequency of vineyard spraying, monitoring of the fertilizers quantity used, use of organic fertilizers, and maintenance of an archive with the specifications of chemical products, fertilizers, etc.
- ***Air emissions and odors:*** Specification of air emissions and of the way of monitoring, if any.
- ***Noise:*** Identification of possible noise sources, noise measurements and measures for noise minimization/elimination.

- ***Environmental awareness training:*** Areas covered by the environmental training and staff awareness on environmental legislation related to the operation of wineries, if any.

From the 54 wineries that were included in the list of the Wine Products Council in the summer 2010, 46 responded. It was not made feasible to collect information from eight wineries, since it was impossible to communicate via telephone with six wineries, while two winery owners refused to participate in this research.

For the processing of the results obtained, the wineries were divided into two categories based on the national legislation for wineries; (a) the small wineries, that receive less than 1000 tonnes of grapes per year, and (b) the large wineries, that receive more than 1000 tonnes of grapes per year. Decree 38/2007 requires that small wineries, receiving less than 1000 tonnes of grapes per year should obtain permission prior to the disposal of their effluents. In order to obtain a permit, the volume of their wastewater should not exceed 500 m^3 per year, during the peak months (August up to November). The wastewater should be transferred to a septic tank to remove the solids and then stored in a second tank prior to their final use for irrigation. During the disposal of wastewater, surface stagnant waters or flows to the nearby areas that could endanger public health should be avoided. On the other hand the large wineries (receiving more than 1000 tonnes of grapes per year) are obliged to treat their effluents in their privately owned wastewater treatment plants.

Solid waste from the activities of a winery includes organic waste and grape pomace (also called marc), as well as packaging waste, old equipment, etc. As far as organic waste is concerned (e.g. stems and skins), according to Decree 38/2007, these have to be reused as food for animals, as an organic fertilizer or rejected in landfills. Grape pomace, should be suitably stored in a way that would deter liquids leakages. It could be then utilised as livestock feed, composted or, as a final solution, disposed in approved landfills. Solid waste, such as paper, plastic, old equipment (e.g. office items, instruments, etc.), should be collected and given for recycling or recovery, or returned to the suppliers, or delivered in approved public areas, according to the Packaging and Packing waste Laws (2002 until 2006), (No 32(I)/2002, 133(I)/2003, 159(I)/2005, 48(I)/2006).

6.2 Environmental problems related to the wine production process in Cyprus

In this section, the final results and conclusions obtained through the survey on the environmental problems related to the wine production process in Cyprus are presented. As mentioned earlier, from the 54 wineries that were included in the list of the Wine Products Council in summer 2010, 46 have participated in the survey. Out of the 46 wineries surveyed, 42 are considered as small wineries and 4 as large wineries receiving more than 1000 tonnes grapes per year. The major results of the review can be summarized as follows:

Winery wastewater:

- The annual volume of wastewater produced in each winery ranged from 1 to 1200 tonnes per year for the small wineries (based mainly on estimations), and between 867 and 17000 tonnes per year for the large wineries.
- Most of the small wineries (71% ≈ 30 wineries) have a single wastewater collection system, only 9 (21%) have more than one wastewater collection systems, while 4

(10%) have no wastewater collection system. All 4 large wineries have a single wastewater collection system for all wet-working areas.

- Two wineries mix the collected rainwater in their premises with their wastewater.
- All large wineries utilise/dispose their wastewater after treatment, while only 13 small wineries (28%) do the same.
- Almost all the small wineries (88% ≈ 37 wineries) are owners of the area where their wastewater is disposed, while only the 50% (2 wineries) of the large wineries are also owners of the area.
- Only 2 wineries out of the 46 dispose their wastewater in an environmentally sensitive area.
- Almost all wineries (91% ≈ 42 wineries) have their main wastewater collection tank at the lowest point of the site, so they do not need any pumping system.

Water consumption:

- The majority of wineries (76% ≈ 36 wineries) monitor their monthly water consumption via water meters and through the local Water Board accounts.
- Only a 13% (6 wineries) of the wineries have noticed water spills/leakages and all fixed the problem immediately.

Energy consumption:

- Almost all wineries (45 out of 46) monitor their monthly energy consumption through the Electricity Authority of Cyprus.

- The 29 out of the 46 wineries (63%) implement specific measures for energy saving. These include insulation systems, use of double-glazing windows, pumping of cold air for cellar cooling, utilization of energy released by the chiller for heating and use of renewable energy sources.

Solid wastes:

- The majority of the wineries (74% ≈ 34 wineries) use the solid organic waste as land fertilizer, 6 wineries (13%) as animal feed, and the remaining 6 (13%) use it in both the above ways.
- More than the half of wineries (65% ≈ 30 wineries) recycle their packaging waste, especially glass and paper.
- All 4 large wineries are members of Green Dot Cyprus, while only 15 out of the 42 small wineries (36%) are also members.
- The majority of small wineries (79% ≈ 33 wineries) use their produced wine sludge as a fertilizer, and only 2 wineries dispose it to their drain field or transfer it to a disposal area. Two of the large wineries use it as a fertilizer, one provides it to a cement factory for further use, and the last one transfers it to a disposal area.
- The small wineries, (with one exception), do not monitor the annual quantity of the organic solid waste produced, while the majority of the large wineries (75%) monitor it. Based on estimations, the annual quantity of solid organic waste produced ranges between 0.5 and 400 tonnes for the small wineries, and between 30 and 700 tonnes for large wineries.

Chemicals used:

- The chemicals mostly used are for disinfection (e.g. sulphurous) and cleaning (e.g. caustic soda, citric acid and oxonium). Five wineries (11%) use only water (e.g. hot water or steam) for cleaning and disinfection purposes.
- The pesticide mostly used is sulphur; however, 12 wineries (26%) surprisingly, were not aware of the pesticides used in their vineyards.
- Regarding the fertilisers, only 10 wineries (22%) use biological fertilisers, while 3 wineries do not use any fertilisers at all. Most wineries use synthetic fertilisers, while 12 wineries (26%) were not aware about the fertilisers used.
- More than half of the wineries (72% ≈ 33 wineries) monitor the quantities of the chemicals used and keep records.
- The majority of wineries (78% ≈ 36 wineries) make efforts to limit the use of chemicals through the use of chemicals at a minimum quantity and the use of only water and steam.
- The results show that the frequency of vineyards spraying (application of insecticides in vineyards) ranges between 1 to 7 times per year, with an average of 2 to 5 times per year.
- More than half of the wineries (26 out of 46 ≈ 57%) examine whether spraying is or is not necessary, in order to reduce it to the minimum required.
- From the 46 wineries, 16 (35%) monitor the quantity of fertilizers they use for vine cultivation, while 4 wineries (9%) monitor only the organic fertilizers they use.
- Only a 24% (11 wineries) of the wineries use organic fertilizers.

- Only 8 of the wineries (17%) are registered in an organization for the certification of their organic cultivation, and the area of their organic vineyards ranges between 8 and 32 decares.
- Half of the small wineries (21 wineries), and 3 out of the 4 large wineries (75%) keep records with the specifications of the chemical products and fertilizers used.

Air emissions:

- None of the 46 wineries monitor the air emissions produced by the wine production process. They all mentioned that only CO_2 is emitted in very small quantities and that it is practically difficult to measure it.

Noise:

- Almost all wineries (42 out of the 46 ≈ 91%) considered that no intense noise is observed during their operation. Only 4 small wineries (9% of all wineries) measure their noise levels in order to check whether they generate excessive noise. While 16 wineries (35%) implement specific measures for noise elimination/minimization (e.g. insulation of area where the equipment kept, use of noise traps, use of modern equipment that does not produce significant noise, etc.).

Environmental awareness:

- 17 out of the 46 wineries (37%) train their staff on environmental awareness and safety issues, either as part of a formal training related to food safety and environmental management or through the organisation of internal meetings with their staff. During these meetings, they discuss various issues including wastewater and solid waste management, reduction of energy and water consumption, the prudent use of pesticides and fertilisers, and additional issues.

- Only few of the wineries (9% ≈ 4 wineries) also train their temporary employees through seminars on environmental management issues, while only one winery maintains up to date records for staff training.
- 34 out of the 46 wineries (74%) state that their personnel is aware of the environmental legislation that is related to the operation of wineries.

With regard especially to winery wastewater, which is one of the most important management problem that most wineries face, as mentioned before, almost all small wineries (40 out of 42) as Figure 6.1 shows, do not monitor their produced wastewater. They can, however, provide an estimation of the wastewater produced. On the other hand, 75% (3 out of 4) of the large wineries do monitor their produced wastewater. After those estimations, the annual volume of wastewater produced ranged from 1 to 1200 tonnes per year for the small wineries, and from 867 to 17000 tonnes per year for the large wineries. These extraordinary in some cases produced volumes of wastewater, show the size of the problem, regarding the management and the treatment of winery wastewater, as well as their safe disposal in the environment.

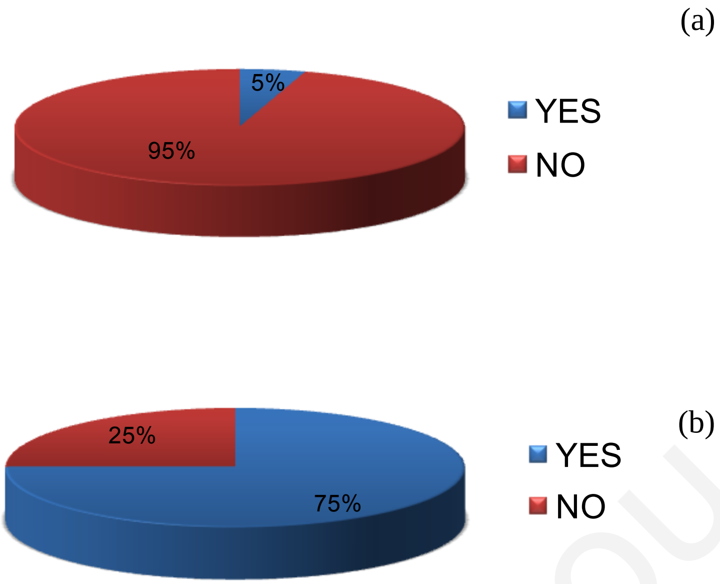


Figure 6.1: Percentages *on whether wastewater volume is monitored* for (a) small wineries (receiving less than 1000 tn yr^{-1} grapes), and (b) large wineries (receiving more than 1000 tn yr^{-1} grapes).

In terms of the winery wastewater treatment applied by the small wineries, 17 of them use a sealed tank (sometimes combined with a septic tank) for the collection of wastewater. In some wineries the wastewater collection is made in a sealed tank combined also with pH neutralization (1 winery), while wine sludge is also isolated for composting. In eighteen wineries a septic tank/drain field is used for the treatment of winery wastewater, one winery is connected to the local community sewerage system, while 5 do not treat their winery wastewater at all (Figure 6.2a). Figure 6.2b shows that three (75%) of the large wineries operate a private wastewater treatment plant, while one uses a sealed tank combined with filtration. The above wastewater treatment methods used, especially from small wineries, show that there is a lack of awareness on the serious impacts that winery wastewater can cause to the environment. In addition, many of the winery owners seem not to be aware and as a consequence, not to comply with the existing legislation regarding the management of their wastewater (Decree 38/2007).

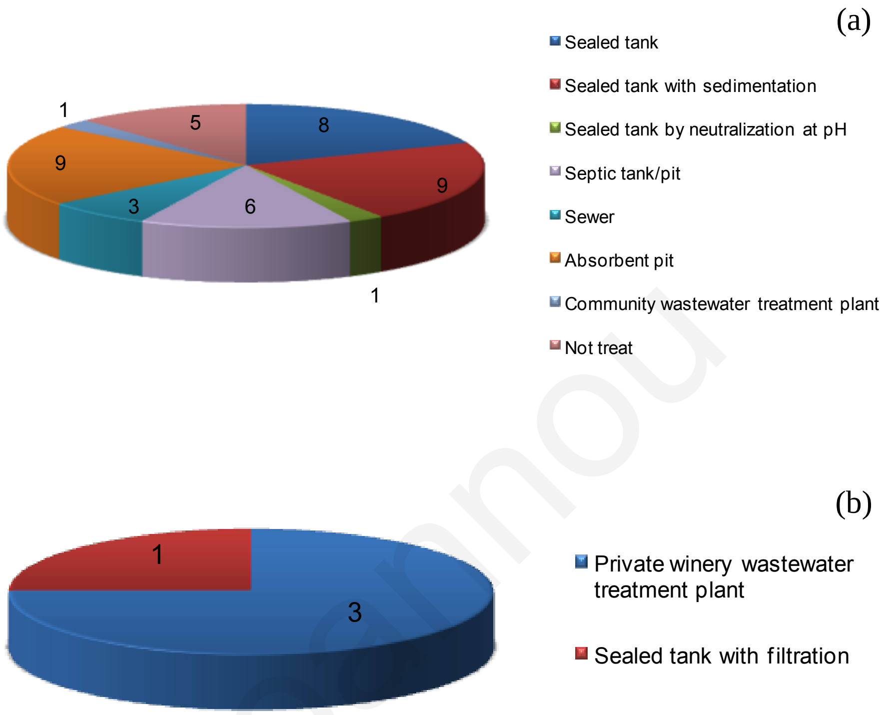


Figure 6.2: Classification of (a) small wineries and (b) large wineries regarding their wastewater treatment methods.

Regarding the ways that small wineries dispose their wastewater to the environment, 13 wineries use wastewater for irrigation (vineyard or tree irrigation), 8 dispose it in fields, wastewater from 1 winery ends up in a water receiver, 2 transfer it to a disposal site, in 1 wastewater is left to evaporate, while for the remaining 18, wastewater either remains in their septic tank, drain field or sealed tank or ends up in the community sewerage system (Figure 6.3a). On the other hand, 2 out of the 4 large wineries use their treated wastewater for vineyard irrigation, 1 sends them in a cement factory for further use, while the last one is connected with a local sewerage system (Figure 6.3b). The fact that a lot of small wineries used their untreated or not appropriate treated wastewater for irrigation of their own vineyards, shows once again their unawareness about the noticeable environmental impacts of winery wastewater (e.g. its high acidity that affects crop growth; its nutrients may be toxic to crops in large amounts; its metal contamination is toxic to plants; etc.) (Arienzo et al., 2009b; Mosse et al., 2010; EPA, 2011).

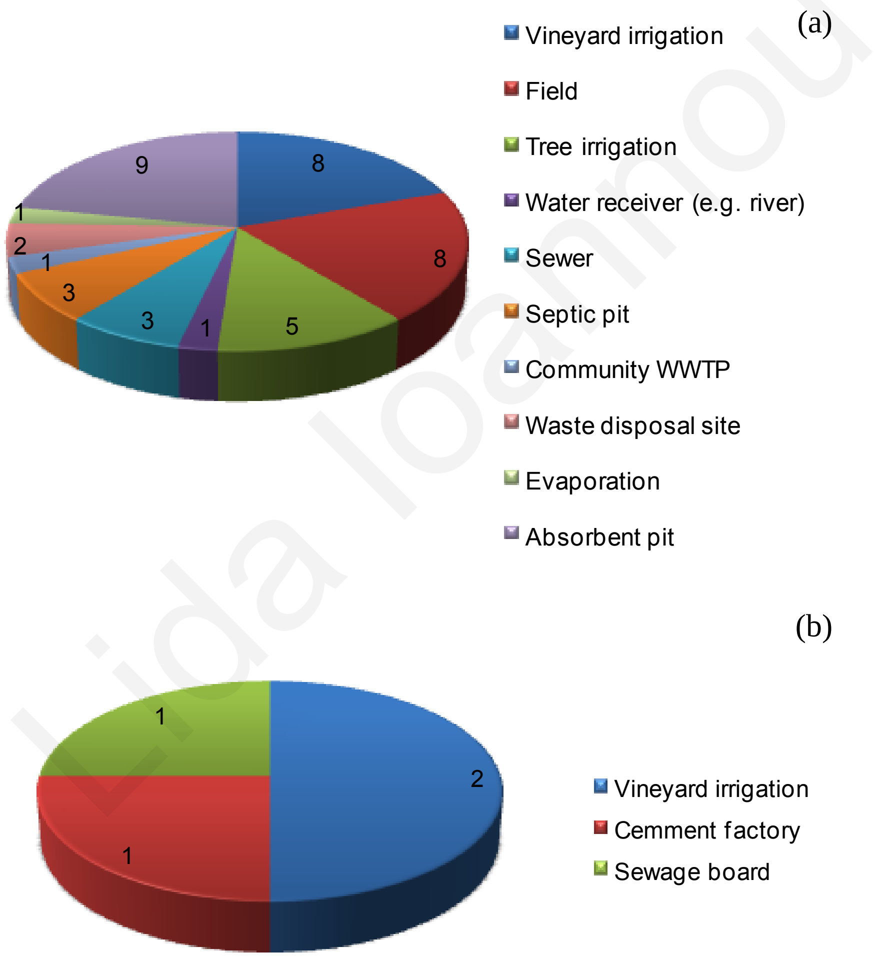


Figure 6.3: Classification of (a) small wineries and (b) large wineries regarding their wastewater disposal methods.

All large wineries (100%) utilize wastewater after treatment as mentioned before. On the other hand, only 13 out of the 42 small wineries do the same, as shown in Figure 6.4. It is also worth noticing that a number of wineries consider sedimentation in the septic or sealed tank as a treatment, while others do not. The other 28 wineries utilize wastewater without any treatment, except for the one that evaporates it, and cannot include its winery to any of the two categories.

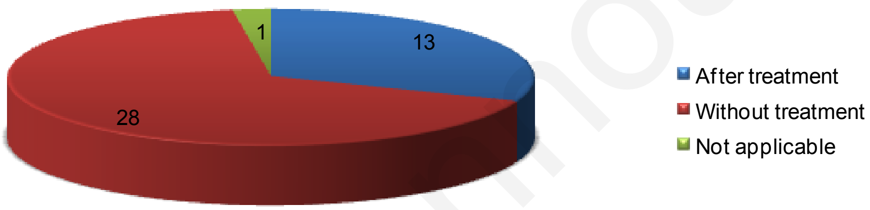


Figure 6.4: Classification of small wineries regarding their utilization of wastewater before or after treatment.

With respect to the 42 small wineries, 15 of them (36%) implement water saving measures. The importance of the reduction in the amount of water consumed in a winery, falls in the several environmental and economic benefits, including conservation of water resources and consequently, lower wastewater discharge volumes. The water saving measures that have been reported are the following: use of untreated wastewater for irrigation - 7 wineries, use of treated wastewater for irrigation - 2 wineries, reuse of water with the caustic soda for tanks cleaning - 2 wineries, use of drop systems for vineyard irrigation - 2 wineries, collection of rain water from the roof for the irrigation of vineyards - 1 winery, uses economic toilet flushers - 1 winery (Figure 6.5a). Most of the large wineries (75% = 3 wineries) implement water saving measures. Specifically, 2 wineries mentioned that they use treated wastewater for irrigation; 2 of them reuse also water with caustic soda in order to wash the tanks, and 1 of them uses water through a borehole for the cooling system (Figure 6.5b).

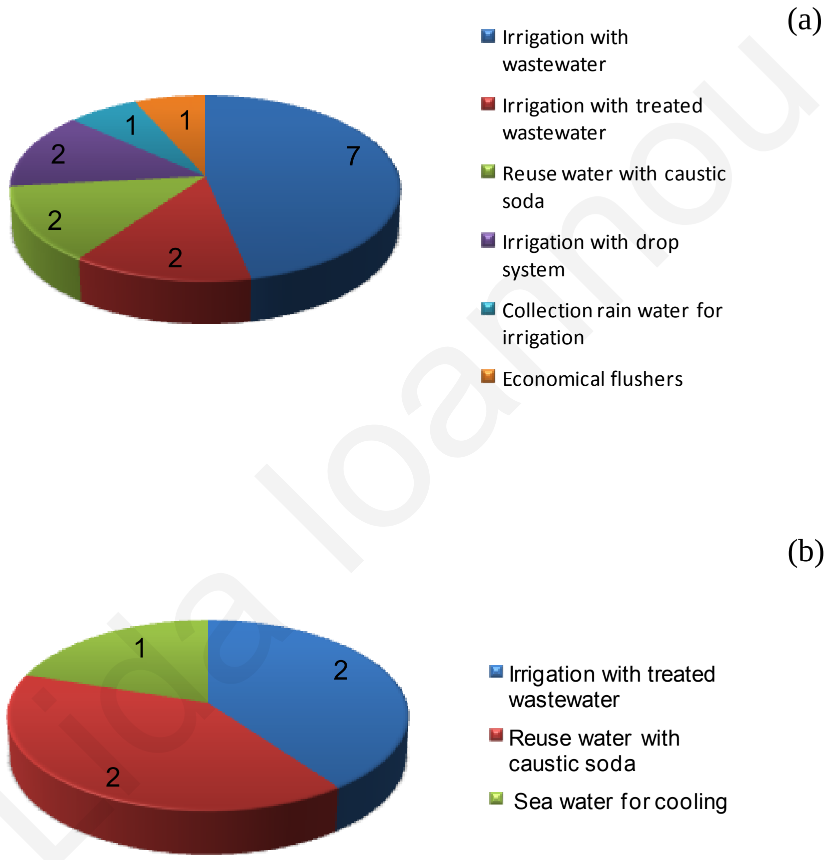


Figure 6.5: Classification of (a) small wineries and (b) large wineries as regards the water saving measures implemented.

Finally, all large wineries are aware of the national legislation on the treatment and disposal of their winery wastewater. With respect to the small wineries, 25 wineries (60%) declared they are fully or partially aware of the national legislation, while 17 (40%) are not aware at all.

According to all the above results obtained through this research, it is apparent that the treatment and management of winery wastewater, is one of the biggest problem that wineries and especially small ones, face. The fact that they do not monitor the annual wastewater produced, they are not all aware of the national legislation on the treatment and disposal of winery wastewater, many of them, dispose their wastewater into the environment without any treatment, and not all of them implement water saving measures, demonstrates clearly the significance of the problem.

The environmental impact of wastewater from the wine industry is noticeable, as already mentioned in previous Section 2.1, due to the high organic load, the large volumes and the seasonal variability (Chatzilazarou et al., 2010). Winery wastewater can cause eutrophication of water resources (e.g. natural streams, rivers, dams and wetlands) (van Schoor, 2005). Furthermore, winery effluents can cause salinity, contamination with a wide range of chemicals, loss of soil structure and increased susceptibility to erosion (Mosse et al., 2011).

The importance of water is definitely worldwide. In Cyprus, water is the most important resource and a prerequisite for societal and economical progress. Its scarcity has acted as a limiting constraint for the development of agriculture and for other economic activities, such as tourism. The drastic reduction of the water supply coupled with the concurrent increase of the demand for water have brought about the full utilization and even overuse of the available traditional water sources, i.e. groundwater and surface water.

Approximately half of the European countries are facing water stress issues today. It is estimated that 20-40% of Europe's available water is being wasted (leakages in the supply system, no water saving technologies installed, too much unnecessary irrigation, dripping taps, etc.) (European Union, 2010). In an effort to combat the problem of water stress, a number of governmental authorities have turned their attention to the utilization of treated domestic and industrial effluents, in order to alleviate water scarcity (Bixio et al., 2006). Treated domestic and industrial wastewater recycling for industrial, agricultural and non-potable municipal uses is an increasingly important component of water resources management practices worldwide.

Figure 6.6 shows the water situation in different European countries, according to statistic data of the European Environmental Agency, (2009). The water stress index (WSI) - the ratio of a country's total water withdrawal to its total renewable freshwater resources - serves as a rough indicator for the pressure exerted on water resources (note, however, that not all water uses are causing comparable stress). With values of less than 10%, water stress is considered low. A ratio in the range of 10-20% indicates that water availability is becoming a constraint on development, and that significant investments are needed to provide adequate supplies. A water stress index above 20% is supposed to necessitate comprehensive management efforts to balance supply and demand, and actions to resolve conflicts among competing uses (Bixio et al., 2006). Moreover, WSI values above 40% indicate severe water stress and clearly unsustainable use of the water resource.

Cyprus, Bulgaria, Belgium, Spain, FYR of Macedonia, Italy and Malta are currently using up to 20% or more of their long-term supplies every year. Cyprus, which has been suffering severe drought, consumed much more than 40% of its renewable supplies, in 2009, according to the data of the European Environment Agency. As a consequence, Cyprus is recognized as the first country in European Union facing water stress issues. To this effect, except of the use of treated domestic wastewater, the use of treated industrial and agro-industrial wastewater for irrigation of agriculture areas and recharging groundwater aquifers is of a great importance. As a consequence, regarding the wineries, the use of high quality treated winery effluents as an additional source of water can be an effective solution for the water requirements at the winery (i.e. vineyards' irrigation, cleaning activities, etc.).

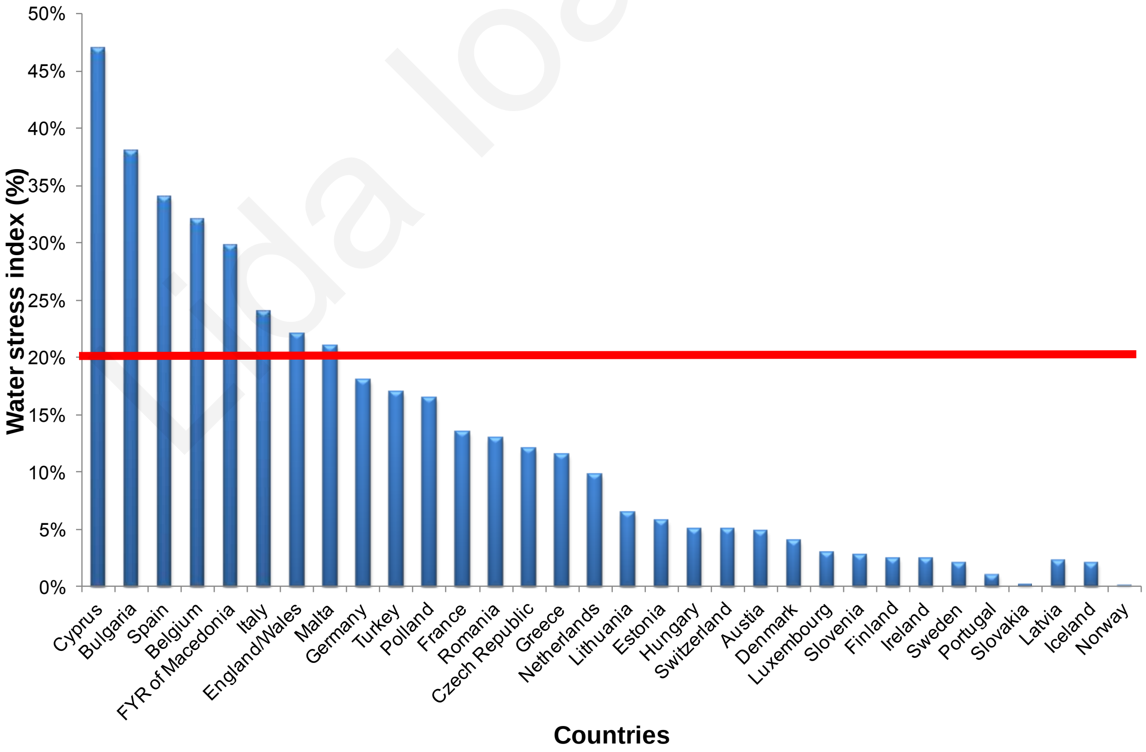


Figure 6.6: Water stress index for the European countries for the year 2009

Source: European Environmental Agency (2009)

6.3 Best Practices concerning environmental management in the wine manufacturing industry

In the increasing globalization of the world economy, business must reduce all unnecessary costs in order to become more competitive. Such savings could come from economizing renewable resources, and wineries should adopt such efforts and become more sustainable, enhancing also their environmental performance. For example, various treatment methods have been successfully documented for solid and liquid waste produced by the wine industry (Chapter 2 and 3). Certain solid and liquid wastes have by-products with an economic value in other industries, such as pharmaceutical and food industries, and can be recycled into other useful products (Brown and Pollard, 2009). The management and the disposal of the generated waste, as well as the overall environmental management of wineries is therefore, a growing interest to the wine operators.

The environmental performance of the wine industry does not receive as much attention and media coverage as some other industries. Nevertheless, environmental issues relating to wine production have gained prominence in recent years. The most serious of these environmental issues include conserving water supply and water quality, reducing organic waste and packaging materials, reducing the use of chemical, pesticides and herbicides, reducing the loss of natural habitats and species, as well as reducing greenhouse gas (GHG) emissions (Marshall et al., 2005; Musee et al., 2007; Broome and Warner, 2008; Ruggieri et al., 2009).

In order to promote active and voluntary environmental efforts, it is necessary to precisely measure and evaluate the impacts or burden of organization’s activities on the environment, and the outcomes of environmental actions (environmental performance). Based on data gathered from the survey on the environmental impacts of wineries and through relevant literature review, a practical tool, for the implementation of *Best Practices* concerning environmental management in the wine manufacturing industry was developed. This is presented below:

6.3.1 Best practices for winery wastewater management

The **best management practices** of winery wastewater should include at least the following (Artiga et al., 2005; Galitsky et al., 2005; Walsdorff et al., 2005; EPA, 2006; EPA, 2011):

- reduction of winery wastewater by cleaning activities
  - by using a closed system of cleaning operations, and
  - by using compressed air instead of water whenever possible,
- measuring the volumes of cleaning water used, wastewater produced and wastewater irrigated, so as to improve the overall management of the wastewater storage, treatment and reuse system,
- avoiding acid and chlorine washing as much as possible, as it makes effluent treatment and disposal more difficult. The use of hot water washing (with pressure) as an alternative, must be considered,
- storage of wastewater for at least 24 h before discharge to treatment systems to avoid variability throughout the day,
- separation and collection of sludge from the wastewater, before the main wastewater treatment procedure,

- biological treatment of winery wastewater, through different methods (i.e. comprising oxidation and activated sludge tanks, or membrane bioreactor (MBR), etc.),
- sampling and analysis of the treated effluent from the tanks, in order to ensure that sufficient quality treatment has been achieved, and that all parameters are below the legal limits before this is conveyed to the public sewer or discharged in the environment.

6.3.2 Best management practices for water conservation

The **best management practices** for water conservation should include at least the following (Vancouver, 1997; EPA, 2006; Walsdorff et al., 2007; Beddow, 2011):

- installation, monitoring and control of water meters at various sections of the winery,
- stopping water flow during breaks, with the exception of water used for cleanup,
- installation of flow control valves and an automatic valve to interrupt the water supply when there is a production stoppage,
- use of a closed system for cleaning operations,
- use of low-volume/high-pressure washers,
- use of compressed air instead of water, whenever possible,
- sweeping floors before water washing,
- ozone tank cleaning of barrels, (ozone cleaning will generally eliminate the need for hot water in barrel cleaning),
- replacement of old equipment (e.g. bottle washers),

- recycling of alkali cleaning solutions (caustic soda) (pH~13.5) until the solution becomes frothy (pH ~10.0), for the cleaning of tanks,
- using stormwater to operate cooling towers in preference to bore water, as this will substantially reduce the amount of water removal (bleeding) required to stop salts accumulating,
- collection and reuse for irrigation of rainwater and/or treated wastewater effluents of good quality, and
- training of employees in order to consume as much less water as possible.

6.3.3 Best management practices for energy conservation

The **best management practice**s for energy conservation are the following (Vancouver, 1997; Galitsky et al., 2005; Walsdorff et al., 2005; EPA, 2006; EPA, 2011):

Electrical energy:

- implementation of good housekeeping measures, such as turning off equipment and lights when not in use, or use of automatic switch off of lamps after employees or visitors leave working area,
- use of fluorescent lights and/or lower wattage lamps (energy efficient lamps),
- use of more efficient equipment when replacing old equipment (such as motors and heating units),
- installation of computerized controllers to better regulate motor output,
- installation of timers and thermostats to control heating and cooling, and,
- preventative maintenance of operational processes and pipes so as to improve efficiency and minimize losses.

Thermal energy:

- improvement or enhancement of the insulation on heating or cooling lines, pipes, valves or flanges, refrigeration systems and bottle washers. Insulation represents a cheap and effective way to reduce energy consumption,
- installation of roof insulation of winery premises,
- institution of preventative maintenance to reduce leakages and avoid steam trap bypass,
- using of more efficient equipment, the adjustment of burners for optimal air/fuel ratios, the insulation of steam pipes, and the systematic maintenance of process operations to ensure their efficiency,
- hot water tank to be of appropriate size so as to optimize hot water production,
- performance of a hot water balance of the entire facility to determine when, where and how hot water is being utilized, and identify areas where reductions in consumption can be made,
- use of renewable - solar energy for water heating (solar panels) and photovoltaic panels,
- use of natural gas instead of diesel, and
- enhancement of employees’ awareness of energy conservation.

6.3.4 Best practices for solid waste management

The **best management practices** for both categories of winery solid waste are (Galitsky et al., 2005; Walsdorff et al., 2005; EPA, 2006; Bustamante et al., 2007):

Organic solid wastes:

- utilization of the solid organic waste materials produced in winemaking process (e.g. stalk, marc, lees, etc.) as compost on the vineyards, in lieu of synthetic fertilizers or as soil mulch under the vines,
- use of worm farms or distillation of the solid remains of grapes,
- collection of screening, sludge's and other solids removed from liquid wastes that will not and/or cannot be used agronomically, and disposed of at a legal point of disposal, and finally
- cultivated lands that receive solid wastes from wine making process shall be managed to prevent runoff and erosion.

Non organic solid wastes:

- minimization of waste produced,
- separately collection of the different types of waste (e.g. glass, paper, plastic, etc.) and monitoring of their quantities,
- reuse of packaging waste like wooden pallets and carton boxes,
- preferable use of environmentally friendly raw materials,
- use of certified corks and non toxic glue for the labels,
- achievement membership to relevant recovery schemes and forums, and
- use of electronic systems allowing for the continuous monitoring of particular types of waste, as well as ensuring that proper handling of waste is achieved.

6.3.5 Best management practices for chemicals

The **best management practices** for chemicals used in the winery are the following (Boland and Tee, 2005; Galitsky et al., 2005; EPA, 2006):

- reduction of chemical use within the winery,
- recirculation of cleaning materials, wherever possible,
- minimizing the use of inorganic fertilizers, herbicides and insecticides,
- staff chemical handling training,
- Material Safety Data Sheets (MSDS) to be kept for all pesticides, herbicides and chemicals,
- wherever possible using less pesticide or choosing a product with a lesser environmental impact,
- checking labels for information on toxicity, and choosing the least toxic, where possible,
- using pesticides that will have the least impact on non-target organisms, and
- avoiding spraying in adverse weather conditions.

6.4 Potential business benefits of adopting good practices

In order to effectively establish environmental management practices and provide for emergency action, winery managers need to be aware of their potential environmental impacts, the generation of potential pollutants, as well as alternative solutions in order to minimize their impacts.

There are a number of incentives for minimizing/eliminating waste, both for wine industry and society at large (Vancouver, 1997). Firstly, the potential economic benefits for a winery from pollution prevention include reduced costs for waste management. In addition, savings may be realized through a reduction in costs for insurance rates, environmental clean-up, legal liability, health and safety costs (Yapijakis, 1992). As a result, pollution prevention can be seen as an internal source of company funds, which can be used to improve its overall competitive strength (Dickens, 1993). Secondly, the pollution prevention reduces the amount of pollutants being released to the environment. This minimizes the potential adverse impacts associated with environmental degradation and results in a cleaner environment (EPA, 2006). Furthermore, there is a positive effect with regard to the public relations of the company, due to the fact that the public interest in pollution prevention has been continuously increasing (Marshall et al., 2010). Consequently, a company can improve its public image in the marketplace by adopting pollution prevention as a part of its management policy (EPA, 2011). Moreover, by adopting a pollution prevention program, the overall work environment improves, due to the increasing employee interest and their participation in pollution prevention. This results in a corresponding improvement in employee morale, teamwork and productivity. However, pollution prevention reduces both long- and short-term liabilities and improves employee safety (Yapijakis, 1992). Furthermore, by adopting pollution prevention as a business strategy, companies may ease future legal liabilities and real estate transactions. Pollution prevention helps also, the facility to meet the current environmental legislation, and to avoid extra waste management costs (Dickens, 1993; EPA, 2011). And finally, pollution prevention focuses research and development in areas with the greatest potential for economic and environmental benefits. This could result in the development and implementation of new technologies or processes which may provide the company with new assets and a competitive edge (Yapijakis, 1992; Marshall et al., 2010).

Summarizing, all the above mentioned results, show the real size of the problem that the Cypriot wineries faced, especially, concerning the management of their wastewater. Moreover, the literature review on the environmental problems related to the wine sector (Chapter 2), and the visits of four wineries in other European countries (e.g. Sicily and Italy), that have been made in the framework of this thesis; prove that the management of wastewater is one of the major problems that the wineries faced all over the world. The appropriate treatment of winery wastewater is an imperative task, in order to be reused for irrigation or cleaning purposes; especially for Cyprus which is facing considerably water stress issues. As a consequence, there is a need to develop a comprehensive treatment for these effluents in order to reduce their organic content, as well as their toxicity and phytotoxicity. Thus, the idea and the objective of this thesis was born, which was the establishment of an integrated scheme for the optimized wastewater treatment of winery effluents, as presented in detail in the subsequent Chapter.

CHAPTER 7: TREATMENT OF WINERY WASTEWATER

7.1 Bench-scale solar photo-Fenton treatment using winery effluents biologically pretreated by (a) a sequential batch reactor (SBR) and (b) a membrane bioreactor (MBR)

7.1.1 Effect of ferrous dosage

The amount of ferrous ion is one of the main parameters that influences the Fenton and photo-Fenton process, and relates to the type of the reactor used and the type of pollutants (Tamimi et al., 2008; Malato et al., 2009; Navarro et al., 2011). It is well known that when Fe^{2+} concentration is increased, the catalytic effect is enhanced, since more hydroxyl radicals (HO•) are produced (Muruganandham and Swaminathan, 2004; Tamimi et al., 2008). The chain reactions that take place during these processes produce additional HO•, which are capable to react with a wide range of organic compounds in water matrices and cause their chemical decomposition.

According to Zapata et al. (2009), when the iron concentration is very low, H_2O_2 is consumed by less desirable reactions, while part of H_2O_2 is decomposed into molecular oxygen and water, without the generation of HO•. In the presence of higher iron concentrations, the process is accelerated due to the regeneration of Fe^{2+} from ferric (Fe^{3+}) iron, that results in the rapid generation of additional HO• (Zapata et al., 2009; Abdessalem et al., 2010). On the other hand, too high concentrations of iron can generate dark zones in the photoreactor, because the incident ray is attenuated too strongly along the optical pathlength, reducing therefore the process efficiency (Malato et al., 2009).

Moreover, according to Xu et al. (2009), although more HO• can be produced at higher Fe^{2+} concentrations through Eq. (7.1), these HO• can be scavenged by reacting with other ferrous ions (Eq. (7.2)), leading to a reduced amount of HO•.

$$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + HO^{-} + Fe^{3+} \tag{7.1}$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \tag{7.2}$$

In this study, in order to obtain the optimal initial Fe^{2+} concentration, the investigation was carried out in the range of 3-20 mg L^{-1} at pH=3.0 for the winery effluent pretreated by SBR, and in the range of 1-5 mg L^{-1} at pH=3.0 for the winery effluent pretreated by MBR, while the initial concentration of H_2O_2 was kept constant at 500 mg L^{-1} for both biologically pretreated flows. The hydrogen peroxide concentration of 500 mg L^{-1} was used based on similar experiments performed in our laboratory on the treatment of other agro-industrial wastewater, as well as based on other studies on the treatment of wastewater streams with similar range of COD (e.g. for a wastewater containing pesticides with initial COD=200 mg L^{-1} a dosage of H_2O_2=1132 mg L^{-1} was used by Martín et al. (2009); for a petrochemical wastewater with initial COD=94 mg L^{-1} a dosage of H_2O_2=380 mg L^{-1} was used by Gao et al. (2004)). The results are shown in Figure 7.1(a,b). As seen, the increase of ferrous concentration had a beneficial effect on the reduction of COD.

The reduction of COD in both biologically pretreated flows was found to be always faster in the early stages of the reaction than in the later stages. At very low Fe^{2+} dosage (1 and 2 mg L^{-1}) a longer treatment time was required for the same % COD removal compared to higher Fe^{2+} dosage (3, 5 and 10 mg L^{-1}), as shown in Figure 7.1(b). More specifically, the

1 mg L^{-1} Fe^{2+} dose required 90 min in order to achieve a COD removal of 49±2.0%, while on the other hand, for the 3 mg L^{-1} Fe^{2+}, 30 min was sufficient time for almost the same COD removal (47±2.3%). Therefore, the extra 60 min of the treatment time required for the 1 mg L^{-1}, it was considered as a quite significant difference. The same behavior was also observed for the effluent pretreated by SBR (Figure 7.1(a)). Yang et al. (1998) reported that ferrous ion catalyses H_2O_2 to form hydroxyl radicals quickly in the first stages of the reaction, and for this reason higher reduction of COD occurs. After 120 min of reaction, the reduction of COD ranged slightly between 65±3.0 to 71±2.7% for the effluent pretreated by SBR, and 52±3.1 to 58±2.5% for the effluent pretreated by MBR, for all the Fe^{2+} concentrations tested.

Moreover, it should be noted that the initial COD value of the winery effluent pretreated by SBR was on average equal to 267 mg L^{-1}, while the residual COD after the solar photo-Fenton treatment at the optimum conditions presented in Figure 7.1(a) (i.e. 5 mg L^{-1} Fe^{2+} and 500 mg L^{-1} H_2O_2), was 82.7±8.2 mg L^{-1}. On the other hand the initial COD of the winery effluent pretreated by MBR was on average equal to 110 mg L^{-1}, and the residual COD after the solar photo-Fenton treatment at the optimum conditions presented in Figure 7.1(b) (i.e. 3 mg L^{-1} Fe^{2+} and 500 mg L^{-1} H_2O_2) was 46.2±3 mg L^{-1}.

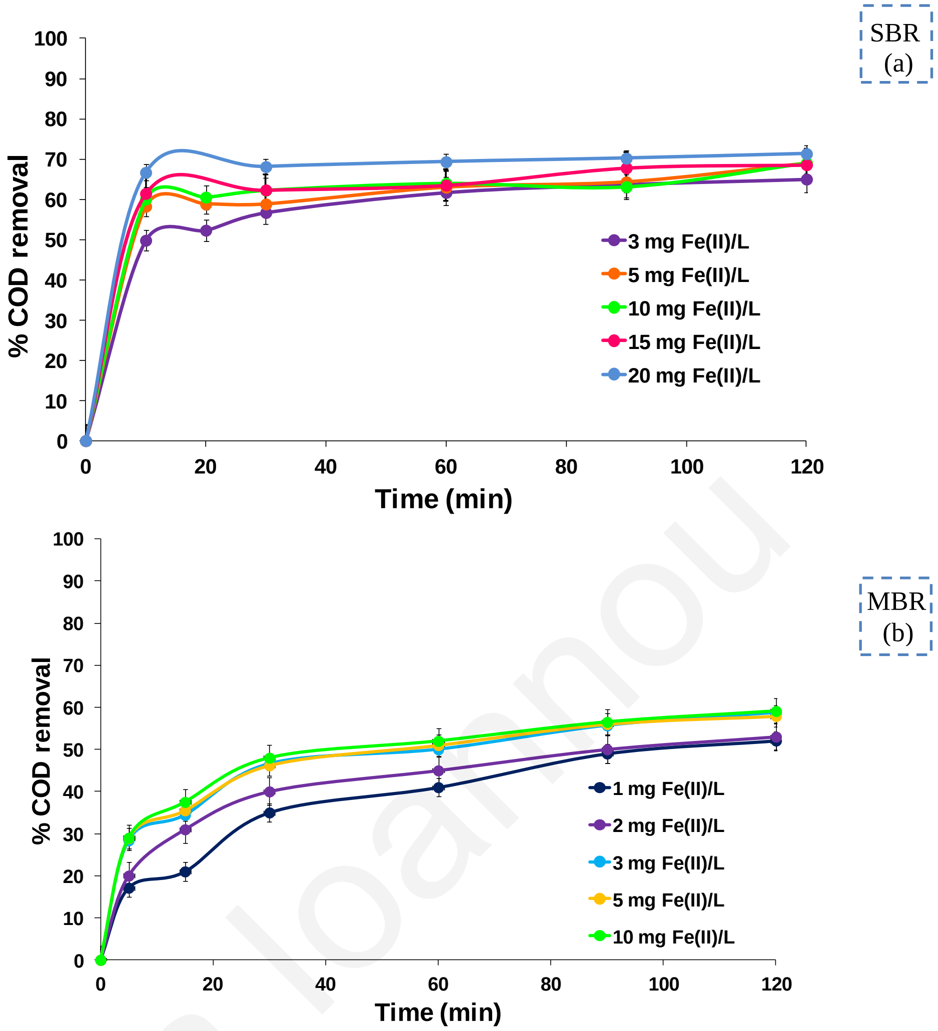


Figure 7.1: Effect of the initial ferrous concentration on the COD removal of the winery effluents pretreated by (a) SBR and (b) MBR; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** COD_0=267 mg L^{-1}, $[Fe^{2+}]_0$=3-20 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** COD_0=110 mg L^{-1}, $[Fe^{2+}]_0$=1-10 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}.

The ferrous dosage is especially important for large-scale wastewater treatment plants since it can influence not only the capital costs as it determines the size of the photoreactor, but also the operating costs as shorter reaction times are required. However, if the iron concentration is high, the problem of the resulting iron-separation step arises at the end of the photocatalytic process. Consequently, it is preferable to select a lower ferrous concentration by which it would be possible to achieve as short reaction time as possible, without the need of further treatment for iron removal, in order to comply with the existing regulations (Gernjak et al., 2003; Evgenidou et al., 2007b). It should be noted that the regulations in Cyprus (Cyprus Law: 106(I)/2002) set the value of 5 mg L^{-1} as the maximum iron concentration in treated effluents that can be discharged in water dams and other water bodies.

The inner filter effects present in the treated solution are another important issue regarding the influence of the iron concentration on the reaction rate during the solar photo-Fenton process. Inner filter effects refer to the competitive absorption of photons by other light absorbing species, usually the organic contaminants present in the wastewater. The photons absorbed by the organic contaminants instead of the catalyst (especially at low concentrations of iron) may be considered lost in terms of efficient photon use. The competitive light absorbance may lead also to some direct photolysis reactions, which normally have a low quantum yield and a low efficiency in the degradation (Malato et al., 2009).

For all the above mentioned reasons, the optimum ferrous concentrations were considered to be 5 and 3 mg L^{-1} for the winery effluents pretreated by SBR and MBR, respectively.

7.1.2 Effect of H_2O_2 concentration

To render the solar photo-Fenton process competitive with other processes, it is essential that its application represents a low cost operation, which basically implies a better control of H_2O_2 dosage. The objective of this evaluation was to select the optimum operational dosage of H_2O_2 for the solar photo-Fenton process.

In order to examine the effect of hydrogen peroxide concentration on the COD removal for the biologically pretreated flows, experiments were carried out at different initial concentrations of the oxidant (H_2O_2).

Figure 7.2(a,b) shows the effect of the initial concentration of H_2O_2 which was studied in the range of 50-1000 mg L^{-1} at pH=3.0, and at a constant Fe^{2+} concentration of 5 mg L^{-1} for the effluent pretreated by SBR; and in the range of 25-500 mg L^{-1} at pH=3.0, and at a constant ferrous concentration of 3 mg L^{-1} for the effluent pretreated by MBR.

The influence of oxidant concentration on the kinetics was investigated by several previous studies (Ghaly et al., 2001; Lucas and Peres, 2007; Abdessalem et al., 2010; Navarro et al., 2011), and the main findings show that neither too low hydrogen peroxide concentration (leading to a rate reduction of the Fenton reaction), nor too high concentration (H_2O_2 act as scavenger of the hydroxyl radicals produced) may be applied. Usually however, there is a rather broad concentration interval between both extremes, where none of these two phenomena occurs (Malato et al., 2009).

As shown in Figure 7.2(a), by increasing the concentration of H_2O_2 from 50 to 500 mg L^{-1} the reduction of COD increased from 58±4.0 to 69±3.1% for the effluent pretreated by SBR, respectively. This can be explained by the effect of the additionally produced hydroxyl radicals (Tamimi et al., 2008). Further increase of the concentration of H_2O_2 from 500 to 1000 mg L^{-1} did not cause any significant change in the COD reduction. On the other hand in the case of the effluent pretreated by MBR, as shown in Figure 7.2(b), by increasing the concentration of H_2O_2 from 25 to 250 mg L^{-1} the reduction of COD increased from 52±3.1 to 70±3.3%. Further increase of the concentration of H_2O_2 from

250 to 500 mg L^{-1} caused a reduction in the COD removal from 70±3.3% to 58±2.5%. The explanation is that a higher increase in the oxidant concentration can slow the degradation process (adverse effect) because of the enhancement of the competition reactions in the case of excess of H_2O_2; mainly the recombination of hydroxyl radicals produced, as well as the HO• with H_2O_2, contributing to the HO• scavenging capacity (Eq. (7.3)-(7.5)) (Ghaly et al., 2001; Tamimi et al., 2008; Abdessalem et al., 2010; Navvaro et al., 2011).

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \tag{7.3}$$

$$H_2O_2 + HO^{\bullet} \rightarrow H_2O + HO_2^{\bullet} \tag{7.4}$$

$$HO_2^{\bullet} + HO^{\bullet} \rightarrow H_2O + O_2 \tag{7.5}$$

Therefore, the concentrations of 500 and 250 mg L^{-1} of H_2O_2 were considered as the optimal dosages for the solar photo-Fenton treatment of the winery effluents pretreated by SBR and MBR, respectively. It should be mentioned, that the effluent pretreated by SBR required a higher oxidant concentration (500 mg L^{-1}) for the removal of the same percentage of COD (~70%), compared to that needed for the effluent pretreated by MBR (250 mg L^{-1} H_2O_2). This was actually expected due to the higher initial COD content (267 mg L^{-1}) of the effluent pretreated by SBR compared to that of the effluent pretreated by MBR (110 mg L^{-1}), as shown in Table 5.2 (Chapter 5).

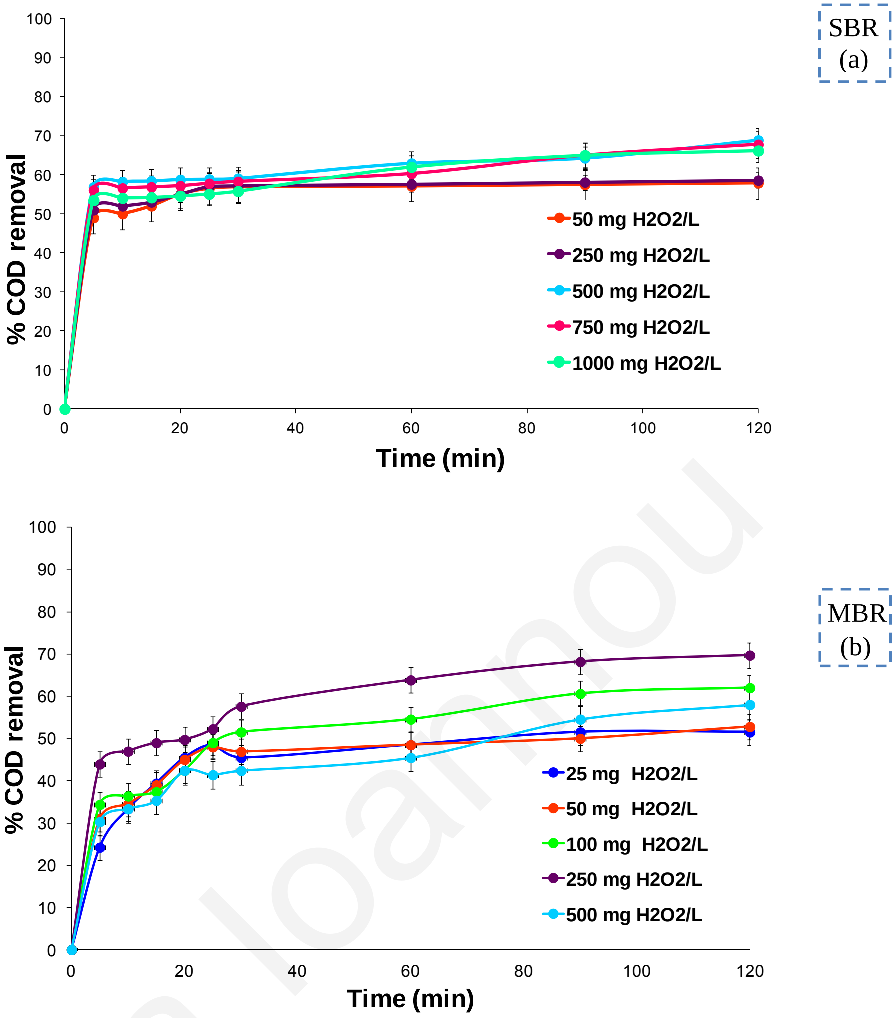


Figure 7.2: Effect of the initial hydrogen peroxide concentration on the COD removal of the winery effluents pretreated by (a) SBR and (b) MBR; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** COD_0=267 mg L^{-1}, $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=50-1000 mg L^{-1}; **(b)** COD_0=110 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=25-500 mg L^{-1}.

Moreover, it should be noted that the major part of COD was removed during the first 30 min of treatment for both flows, as shown in Figure 7.2. In specific, at the optimum concentrations of H_2O_2 (500 mg L^{-1} and 250 mg L^{-1}), 59±2.7% and 58±3.2% of the COD was removed during the first 30 min of solar photo-Fenton treatment, for the effluent pretreated by SBR and MBR, respectively. An extra 6 and 10% of COD was removed by increasing the treatment time at 90 min (65±1.7 and 68±2.5%); after which a plateau was reached. At the end of the treatment, a COD removal of 69±3.1 and 70±3.3% was achieved (120 min) for the effluent pretreated by SBR and MBR, respectively. In addition, interesting was the fact that the residual COD after the end of the solar treatment of the effluent pretreated by SBR was 82.7±8.2 mg L^{-1}, while for the effluent pretreated by MBR was much lower and equal to 33±3.6 mg L^{-1}. This was also expected due to the difference in the initial COD of the two winery effluents as already mentioned (Table 5.2, Chapter 5).

7.1.3 Effect of pH

The pH affects the oxidation of organic substances both directly and indirectly. As known, the photo-Fenton reaction is strongly affected by the pH (Ghaly et al., 2001; Navarro et al., 2011). During the photo-Fenton process, the pH of the solution controls both the generation of hydroxyl radicals and the concentration of Fe^{2+}, and thus influences the oxidation efficiency (Muruganantham and Swaminathan, 2004; Lucas and Peres, 2006; Tamimi et al., 2008; Malato et al., 2009). The effect of pH on the COD reduction of the winery effluents pretreated by SBR and MBR is depicted in Figure 7.3(a,b).

The experiments were carried out at pH 2.0, 3.0 and 4.0, by adding the appropriate amount of 2M H_2SO_4 solution. At pH=2.0 and after 120 min of solar irradiation, the COD removal was 45±1.5% for the effluent pretreated by SBR, and 50±3.1% for the effluent pretreated by MBR. On the other hand, at pH=4.0, the COD removal after 120 min of treatment was reduced to 39±2.7% and 42±3.4% for the effluents pretreated by SBR and MBR, respectively. At pH=3.0 the highest COD removal was obtained, which was approximately 70% for both biologically pretreated flows.

As known, for pH values below 3.0, the reaction of hydrogen peroxide with Fe^{2+} is seriously affected causing a reduction of the hydroxyl radicals production; for pH=1.0 hydroxyl radicals are not produced (Peres et al., 2004). The low COD removal at pH=2.0 is also due to the hydroxyl radicals scavenging by H^+ ions (Eq. (7.6)) (Lucas and Peres, 2007; Tamimi et al., 2008).

$$HO^{\bullet} + H^{+} + e^{-} \rightarrow H_2O \qquad (7.6)$$

Inhibition of the HO• radicals formation at pH below 3 seems also to be due to the small amount of soluble iron (Fe^{3+}), responsible for the continuity of the oxidation process, inducing the formation of $Fe(OH)^{2+}$ and $Fe(OH)_2^{+}$ (Peres et al., 2004; Lucas and Peres, 2006). Moreover, the lower efficiency at pH=2.0 is due to the formation of the complex species $[Fe(H_2O)_6]^{2+}$, which react more slowly with H_2O_2 and therefore, produce less HO• (Xu et al., 2009). In addition, at pH < 3.0, the reaction system is autocatalytic, because Fe^{3+} decomposes H_2O_2 in O_2 and H_2O through a chain mechanism (Eqs. (7.7)-(7.11)) (Litter, 2005).

$$Fe^{3+} + H_2O_2 \Leftrightarrow Fe - OOH^{2+} + H^{+} \qquad (7.7)$$

$$Fe - OOH^{2+} \rightarrow HO_2^{\bullet} + Fe^{2+} \qquad (7.8)$$

$$HO_2^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + HO_2^{-} \qquad (7.9)$$

$$HO_2^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + O_2 + H^{+} \tag{7.10}$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \tag{7.11}$$

On the other hand, the COD removal was found to decrease at pH values equal or higher than 4, possibly because iron precipitates as hydroxide, resulting in the inactivation of Fe^{2+} and in the reduction of the transmission of the solar radiation (Tamimi et al., 2008). Moreover, for pH values above 4 the degradation decreases probably due to the fact that the dissolved fraction of iron species decreases because it precipitates as ferric hydroxide $Fe(OH)_3$, hindering the regeneration of the active species (Fe^{2+}) (Peres et al., 2004). Additionally, the oxidation potential of hydroxyl radicals is known to decrease with increasing pH (Tamimi et al., 2008). Another reason for the inefficient degradation at pH > 3 is the dissociation and auto-decomposition of H_2O_2 (Badawy and Ali, 2006).

Thus, pH=2.8-3.0 is frequently used as an optimum pH for the solar photo-Fenton treatment, as at this pH, precipitation does not take place and the dominant iron species in solution is $Fe(OH)^{2+}$ (Eq. (7.12)), which is the most photoactive ferric iron-water complex (Section 3.3.2.1, Chapter 3) (Malato et al., 2009). This is due to the fact that apart from efficiently generating Fe^{2+}, it generates further hydroxyl radicals (Navarro et al., 2011).

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + HO^{\bullet} \tag{7.12}$$

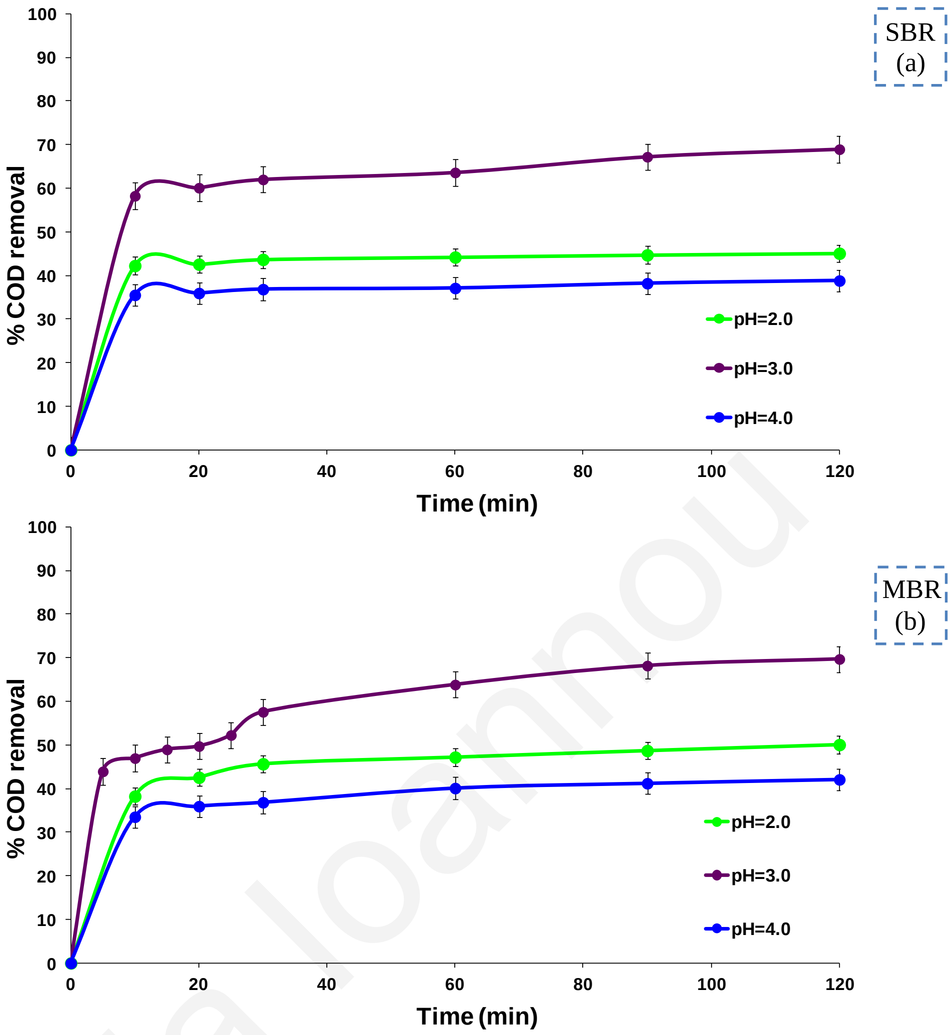


Figure 7.3: Effect of the initial solution pH on the COD removal of the winery effluents pretreated by (a) SBR and (b) MBR; (pH=2.0-4.0 and T=25±0.1 ^{o}C) **(a)** COD_0=267 mg L^{-1}, $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** COD_0=110 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=250 mg L^{-1},.

Moreover, it should be noted that at the optimum pH of 3 the COD removal for the effluent pretreated by SBR was 59±2.7% at the first 30 min of the reaction, while a small increase up to 69±3.1% was observed by increasing the treatment time at 120 min. The same trend was also observed for the effluent pretreated by MBR, where a COD reduction of 46±3.0% was observed during the first 10 min, and then increased to 58±3.2% at 30 min, until it reached a plateau after 90 min of reaction, yielding a reduction of 70±3.3% at the end of the treatment.

7.1.4 Effect of solar irradiation

In order to evaluate the efficiency of the Fenton reagent and the beneficial effect of solar irradiation on the COD reduction of both biologically pretreated effluent streams (SBR or MBR), the following experiments were performed: (i) Fe^{2+} + H_2O_2 (dark Fenton), (ii) photolysis (only solar irradiation), (iii) solar irradiation + Fe^{2+}, (iv) solar irradiation + H_2O_2 (photo-bleaching), and (v) solar irradiation + Fe^{2+} + H_2O_2 (solar photo-Fenton). For both effluents the optimum experimental conditions determined in Sections 7.1.1 and 7.1.2 were used. More specifically, in the case of the effluent pretreated by SBR, the concentrations of Fe^{2+} and H_2O_2 were 5 and 500 mg L^{-1}, respectively. In the case of the effluent pretreated by MBR the optimum concentrations of Fe^{2+} and H_2O_2 were 3 and 250 mg L^{-1}, respectively. Figure 7.4(a,b) shows the COD removal of the abovementioned processes for the two biologically pretreated flows.

Dark Fenton experiments were conducted to assess the oxidation power of the Fenton reagent in the absence of solar light. Dark Fenton experiments achieved a COD reduction of the order of 45±2.0 and 49±1.8% for the effluents pretreated by SBR and MBR, respectively. It is widely known that the active oxidants in dark Fenton systems are also the hydroxyl radicals. The generally accepted origin of HO• in dark conditions is the free radical chain mechanism (Pignatello et al., 1999), presented in an abbreviated form in Eqs. (7.13)-(7.16):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet \qquad (7.13)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\bullet + H^+ \qquad (7.14)$$

$$Fe^{3+} + HO_2^\bullet \rightarrow Fe^{2+} + O_2 + H^+ \qquad (7.15)$$

$$HO^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O \qquad (7.16)$$

The solar irradiation alone (photolysis), for both biologically pretreated flows, showed no significant COD reduction (6.0±0.9% and 6.5±1.1%), after 120 min of irradiation. Photolysis involves the interaction of artificial or natural light with the target molecule and the induction of photochemical reactions which can lead to its direct degradation to by-products whose further decomposition eventually yields mineral end-products (Klavarioti et al., 2009). Generally, photolysis is ineffective in treating winery wastewater matrices, as it was also reported by Mosteo et al. (2008). Moreover, it should be noted that UV photolysis can selectively reduce some organic compounds, but alone is not sufficient for removing pollutants (Jing and Cao, 2012).

The combination of solar irradiation and catalyst (Fe^{2+}) alone at acidic conditions (pH=3.0) led also to a very small reduction of COD (7.6±2.4% and 8.6±3.1%) after 120 min of treatment for both effluents, while 6.7±2.0% and 5.8±1.0% of COD was removed in the first 30 min of treatment for the effluents pretreated by SBR and MBR, respectively. It must be noted, that even in the absence of the oxidant, but in the presence of catalyst, photodegradation still occurs due to charge transfer photolysis of Fe^{3+}(Cieśla et al., 2004). Fe^{3+}- hydroxocomplexes undergo photochemical reduction to Fe^{2+} under UV irradiation, and Fe^{2+} is reoxidized by oxidants like dissolved oxygen (DO), giving rise to the basic Fe^{3+}/Fe^{2+} redox cycle. $Fe(OH)^{2+}$ is the dominant complex at pH 2.5 to 5 and it absorbs light in the UV range with higher absorption coefficients than that of aqueous Fe^{3+}. Its photolysis leads to Fe^{2+} and HO•, as shown in Eq. (7.17). The quantum yield of this reaction is low and depends on the irradiation wavelength (Larson et al., 1991; Litter, 2005). As a result, the reduction observed in the presence of iron alone is relatively low and more specifically, in the case of the biologically treated winery effluent streams studied in this thesis, is more or less the same as in the photolysis, as shown in Figure 7.4.

$$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + HO^{\bullet} \tag{7.17}$$

Additional experiments were also performed to assess the contribution of photo-bleaching in the presence of the aforementioned optimum H_2O_2 concentrations for each winery effluent (Section 7.1.2). The COD removal due to photo-bleaching (solar irradiation + H_2O_2) was 25±2.0% and 29±3.3% for the effluents pretreated by SBR and MBR, respectively, after 120 min of treatment. It should be noted that 22±1.7% and 25±2.1% of COD removal was observed in the first 30 min of solar treatment, for the effluents pretreated by SBR and MBR, respectively. The increased COD removal compared to that obtained by solar irradiation alone (photolysis) can be attributed to the formation of extra hydroxyl radicals (HO•) due to the irradiation of H_2O_2 (Litter, 2005).

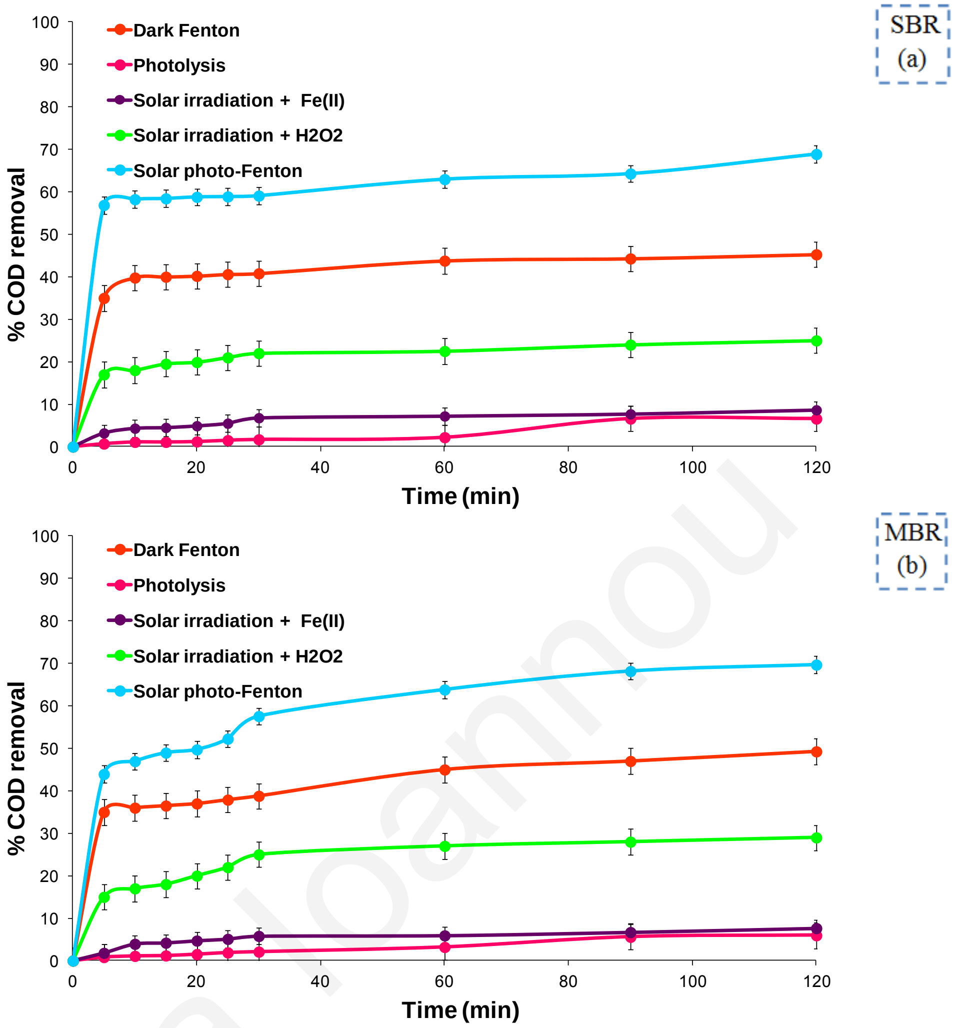


Figure 7.4: COD removal for winery effluents pretreated by (a) SBR and (b) MBR, under different processes; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** COD_0=267 mg L^{-1}, $[Fe^{2+}]_0$=0 and 5 mg L^{-1}, $[H_2O_2]_0$=0 and 500 mg L^{-1}; **(b)** COD_0=110 mg L^{-1}, $[Fe^{2+}]_0$=0 and 3 mg L^{-1}, $[H_2O_2]_0$=0 and 250 mg L^{-1}.

The COD reduction was strongly accelerated by the solar photo-Fenton process, and after 120 min of treatment was almost 70% for both effluents, from which the ~59% was removed during the first 30 min of solar treatment (Figure 7.4). The high efficiency of solar photo-Fenton process is due to the formation of more hydroxyl radicals than during the other processes (Litter, 2005), as well as the regeneration of Fe^{2+} from the photolysis of the complex $Fe(OH)^{2+}$ in solution (Eq. (7.17)) (Kavitha and Palanivelu, 2004). In a study of Pignatello et al. (1999) under combined thermal process and solar light, the oxidation power of Fenton reagent was significantly increased due mainly to the photo-reduction of Fe^{3+} to Fe^{2+}, which could react with H_2O_2 establishing a cycle mechanism of generating additional hydroxyl radicals (Eq. (7.18)).

$$Fe^{3+} + H_2O + h\nu \rightarrow HO^{\bullet} + Fe^{2+} + H^{+} \tag{7.18}$$

Interestingly however, was the fact that for all the abovementioned processes as shown in Figure 7.4, after the first 30 min of solar treatment, the reduction of COD reached a plateau, with a small increase with further increasing the treatment time, for both biologically pretreated flows. The fact that 30 min of treatment is capable of removing the major part of COD of both winery effluents, is crucial for a large-scale application, since it can influence the operating costs, as shorter reaction times are required.

Hence, the relative efficiencies of the processes investigated can be given in the following order: solar photo-Fenton (solar irradiation + Fe^{2+} + H_2O_2) > dark Fenton (Fe^{2+} + H_2O_2) > photo-bleaching (solar irradiation + H_2O_2) > solar irradiation + Fe^{2+} ≈ photolysis (solar irradiation alone).

7.1.5 Effect of temperature

It has been reported that an elevated temperature can significantly increase the activity of the photo-Fenton system (Solozhenko et al., 1995; Lunar et al., 2000; Göb et al., 2001; Sagawe et al., 2001; Lee et al., 2003; Gernjak et al., 2006; Malato et al., 2009; Zapata et al., 2010). According to Lee and Yoon, (2004) using solar energy not only as the photon source, but also as the heat source, the system activity can be greatly increased. To investigate the effect of temperature on the reduction of COD of the winery wastewater, experiments were conducted at reaction temperatures varying from 15 to 45 ^{o}C. Temperatures over 45 ^{o}C were not examined, as solar driven reactors cannot operate at higher temperatures under real conditions (Zapata et al., 2009).

Figure 7.5(a,b) shows the COD reduction of the winery effluents pretreated by SBR and MBR, and the rate constant obtained at each temperature (inset graphs). As expected, the process efficiency was found to increase with temperature, due to the fact that the regeneration of ferric to ferrous ions is faster at higher temperatures (Malato et al., 2009). In the case of the effluent pretreated by SBR, at 15 ^{o}C the reduction of COD was 46±2.7%, at 25 ^{o}C the reduction was increased to 69±3.1%, while with further increase of temperature to 35 ^{o}C and 45 ^{o}C a plateau was reached, and the COD removal was 71±0.9% and 72±1.5% after 120 min of treatment, respectively. In addition, in the case of the effluent pretreated by MBR, a similar behavior was observed; more specifically, the COD reduction was found to increase with increasing temperature from 40±2.2% at 15 ^{o}C to 70±3.3% at 25 ^{o}C, while only a small increase was observed with further increase of the temperature to 73±3.1% at 45 ^{o}C.

On the other hand, it should be mentioned that with increasing the temperature to 45 ^{o}C a faster COD reduction was observed, since in the case of the effluent pretreated by MBR, 65±2.8% of COD was removed in the first 30 min of treatment, compared to 58±3.0% of COD that was removed at the same treatment time at the lower temperature of 25 ^{o}C. The same trend was also observed for the winery effluent pretreated by SBR, during the first

30 min of treatment (62±2.1% at 25 ^{o}C to 68.5±1.8% at 45 ^{o}C). Consequently, the highest treatment efficiency (i.e. faster COD reduction and shorter illumination time required) was obtained at 45 ^{o}C.

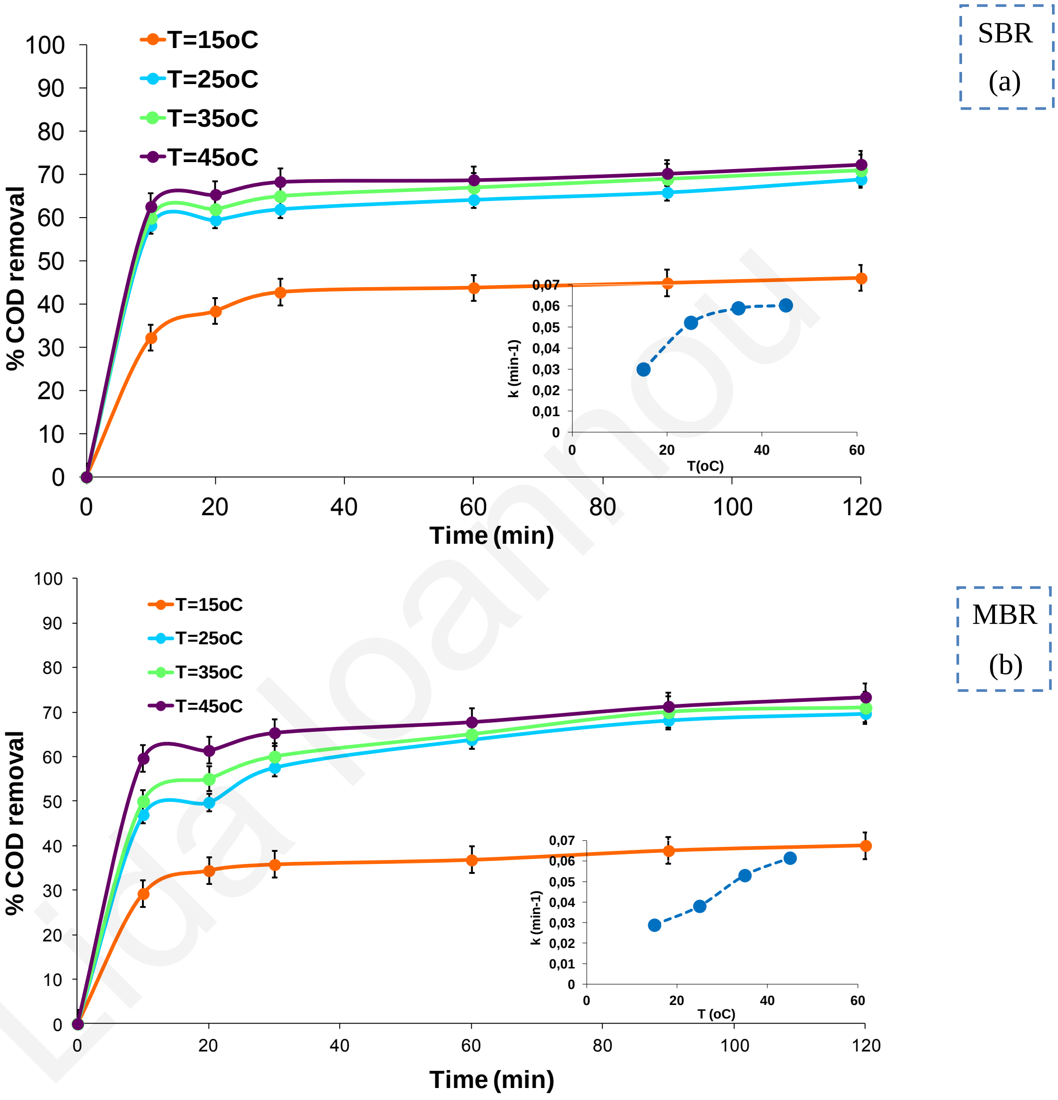


Figure 7.5: Effect of temperature on the COD removal of the winery effluents pretreated by (a) SBR and (b) MBR; Inset graphs show the effect of temperature on the rate constant, k; (pH=3.0 and T=15-45±0.1 ^{o}C) **(a)** COD_0=267 mg L^{-1}, $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** COD_0=110 mg L^{-1} $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=250 mg L^{-1}.

The abovementioned enhancement of COD reduction with increasing temperature can be also attributed to the increase of the $Fe(OH)^{2+}$ concentration and to the temperature dependence of the quantum yield of the photochemical reduction of Fe^{3+} (Lee and Yoon, 2004).

Furthermore, it must be noted that the stability of the hydrogen peroxide is greatly influenced by temperature. According to Malato et al. (2009), the temperature also affects the hydrogen peroxide consumption efficiency, because at higher temperatures more H_2O_2 is consumed to reach the same level of mineralization. This can be explained by looking at the thermal reactions involved in the reduction of ferric iron, particularly Eqs. (7.19)-(7.21), as opposed to the photoreduction of ferric iron.

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \tag{7.19}$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \tag{7.20}$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \tag{7.21}$$

Whereas, in the thermal pathway, H_2O_2 is consumed for the reduction of ferric iron without producing HO•, in the photoreduction pathway H_2O_2 is not consumed (Malato et al., 2009). This seems an obvious and easy explanation, but normally the issue becomes more complicated when other reactions are involved (Santos et al., 2007). Moreover, a further increase of the temperature at values higher than 50 ^{o}C, according to Zapata et al., (2009) can cause a decrease on the photo-Fenton efficiency, due to the significant loss of iron by precipitation.

7.1.6 Total phenolic compounds removal

Numerous phenolic compounds are present in wines as a result of their extraction from the skin, flesh, and seeds of grapes (Arienzo et al., 2009b; Mosse et al., 2012). Many of the compounds present in wine have the potential to occur in the wastewater generated. Phenolic compounds constitute a relatively small portion of the wastewater COD, but can have great negative effects and environmental damages if released untreated. These compounds have also been implicated in the inhibition of biological treatment systems for winery effluents (Strong and Burgess, 2008).

Additionally, some phenolic compounds may be resistant to biological degradation, and are known to be toxic to microorganisms and phytotoxic in many instances (DellaGreca et al., 2001; Wang et al., 2002; Saadi et al., 2007; Strong and Burgess, 2008). The persistence of phenolic compounds in the treated effluents is of concern from a reuse perspective. According to Strong and Burgess, (2008) phenols can even inhibit microorganisms that are capable of using aromatic compounds as their sole source of carbon and energy. Phenols can be toxic or even lethal to fish at relatively low concentrations of 5 to 25 mg L^{-1}. Moreover, various phenolic compounds are responsible for strong inhibitory effects on microbial activity, and therefore should be removed during wastewater treatment, to avoid environmental risks after their disposal (Melamane et al., 2007). As a consequence, for sustainable reuse to occur there is a need for a treatment process that can effectively remove phenolic compounds from winery wastewater (Mosse et al., 2012).

The reduction of the total phenolic compounds after the two biological processes (SBR and MBR) used in this study, as well as during the solar photo-Fenton oxidation was investigated, by the Folin-Ciocalteau method (as described in Section 5.3.4, Chapter 5).

In the case of the SBR treatment, the total phenolic compounds in the raw winery wastewater were 52 mg L^{-1} (evaluated from grap samples taken from the influent of the winery wastewater treatment plant I (Schematic 5.1, Chapter 5)); while after the SBR treatment were reduced to 4.3 mg L^{-1}. The effluent concentration of the total phenolic compounds was considerably reduced after the SBR treatment, although a significant concentration of total phenolic compounds was still detected in the effluent, proving that some of those compounds are not readily biodegradable.

Figure 7.6 shows the reduction of the total phenolic compounds originally present in the winery effluent pretreated by SBR during the solar photo-Fenton process. After 150 min of solar photo-Fenton oxidation, a high reduction of the total phenolic compounds was observed (71±5.0%), which corresponds to a residual total phenolic compounds concentration of 1.2±0.2 mg L^{-1}, and to an overall total phenolic compounds reduction of 97.7±5.0% (biological + solar photo-Fenton). Moreover, it should be noted that the total phenolic compounds removal for the effluent pretreated by SBR was 64±3.5% at the first 60 min of the solar treatment, while a small increase up to 71±5.0% was observed by increasing the treatment time at 150 min.

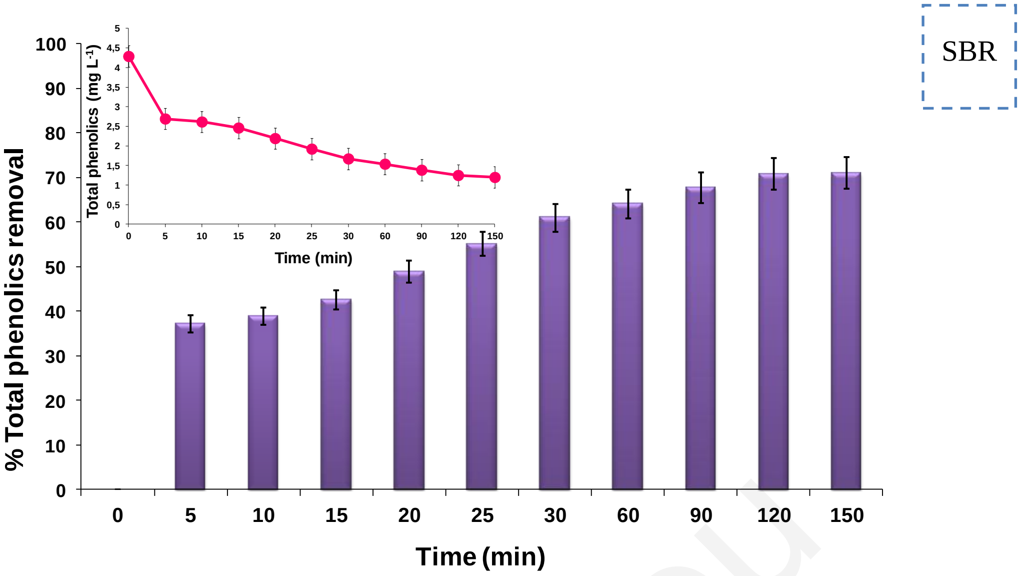


Figure 7.6: % Total phenolic compounds' removal for the effluent pretreated by SBR during the solar photo-Fenton process. Inset graph shows the concentration of total phenolic compounds vs. treatment time; TPh_0=4.3 mg L^{-1} $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}, pH=3.0 and T=25±0.1 ^{o}C.

In addition, in the case of the MBR treatment, the total phenolic compounds in the raw winery wastewater (evaluated from grap samples taken from the influent of the winery wastewater treatment plant II (Schematic 5.2, Chapter 5)), were 40 mg L^{-1}. In the case of this biologically treated winery effluent, the total phenolic compounds measured, were below the limit of detection of the method (Folin-Ciocalteau), and therefore it was not further investigated. The limit of detection (LOD) was calculated from the parameters obtained from calibration curves, using the formula LOD = 3 sa/b (LOD=0.4 mg L^{-1}), where sa is the standard deviation of the y-intercept of the regression line and b is the slope of the calibration curve (Seruga et al., 2011). As a consequence, MBR treatment seems to be an efficient process able to completely remove the phenolic compounds from winery wastewater.

7.1.7 Color removal

The initial color of red wine is mainly due to anthocyanins extracted from grape skin during crushing, pressing and fermentation (Eiro and Heinomen, 2002; Monagas et al., 2005; García-Falcón et al., 2007). As a consequence, raw winery effluents are also considerably colored (dark red) mostly due to the natural colors of grapes (Schematic 7.1(a)). In general, color in water solutions is divided into true color and apparent color (Bennett and Drikas, 1993). The apparent color is determined using the original sample without the recovery of suspended solids or turbidity. However, for the true color measurement, suspended solids and turbidity removal are required (Kao et al., 2001). According to Satyawali and Balakrishnan, (2008b) winery wastewater dark red color hinders photosynthesis by blocking sunlight, and is therefore deleterious to aquatic life.

Commonly applied treatment methods for color removal from colored industrial wastewater, consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods (Robinson et al., 2001; Azbar et al., 2004).

There is a limited literature dealing with the color removal for raw winery wastewater (Shepherd et al., 2001). However, according to our knowledge, the treatment of winery wastewater by conventional biological processes can be considered as a good solution for their color removal, but not for complete removal, since both after the SBR and MBR treatment the effluents were still colored (yellowish). This indicates that after the biological treatment, some of the colored compounds are still present in the winery effluents, because of their resistance to biodegradation (Sreethawong and Chavadej, 2008).

Figure 7.7(a,b) depicts the removal for the color present in the winery effluents pretreated by SBR and MBR, during solar photo-Fenton oxidation.

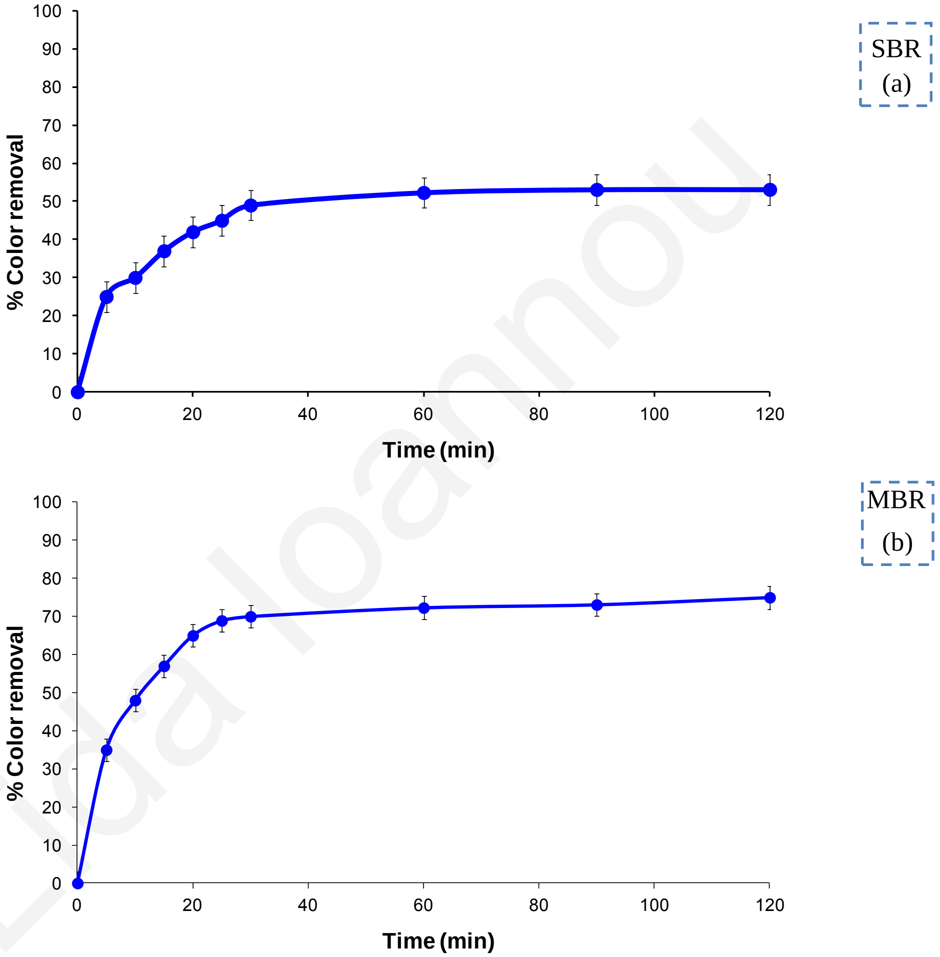
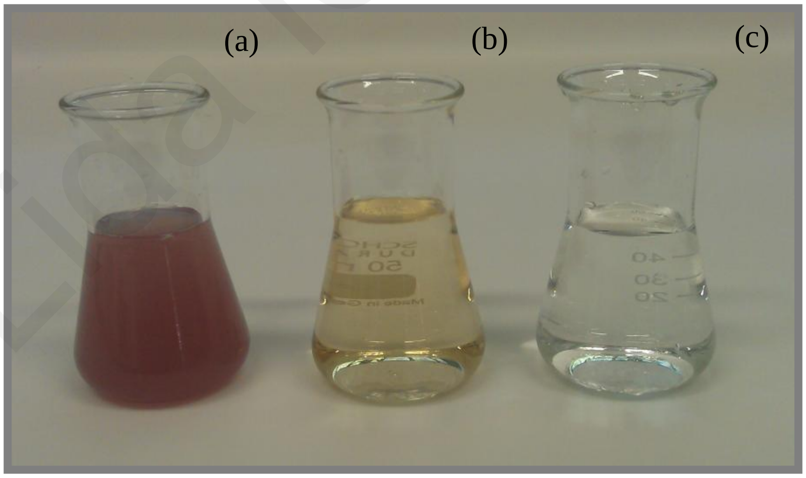


Figure 7.7: % Color removal for the winery effluents pretreated by (a) SBR and (b) MBR during solar photo-Fenton treatment; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=250 mg L^{-1}.

A maximum removal of 53±2.4% (at the same time the COD removal was 69±3.1%) was reached after 120 min of treatment for the effluent pretreated by SBR. It should be noted that almost half of the color removal (25±3.1%) was observed in the first 5 min of solar photo-Fenton process. The maximum color removal for the effluent pretreated by MBR was 75±2.2% (at the same time the COD removal was 70±3.3%) after 120 min of solar photo-Fenton oxidation, while 48±4.3% of the color removal was observed in the first 10 min of solar treatment.

As mentioned before, a number of organic compounds were not easily biodegraded causing a color problem after the biological treatment (SBR and MBR), as shown in Schematic 7.1(b). Thus, solar photo-Fenton oxidation was suitable for effectively oxidizing these refractory organics and it significantly removed the color from winery wastewater, as shown in Schematic 7.1(c).



Schematic 7.1: Winery wastewater (a) before treatment, (b) after biological treatment (SBR and MBR), and (c) after further treatment with solar photo-Fenton oxidation.

7.1.8 Mineralization (DOC)

The determination of the total organic carbon (TOC) removal during the solar photo-Fenton oxidation was generally used to assess the degree of mineralization in the treated wastewater samples (e.g. winery effluents pretreated by SBR and MBR). In the present study, TOC measurements represent the dissolved organic carbon (DOC) since all samples were filtered before tested (Millipore, 0.22 μm).

DOC concentrations in the solar photo-Fenton treated winery effluents were measured at constant Fe^{2+} concentration, at varying H_2O_2 concentrations and treatment time. Figure 7.8(a,b) shows the DOC removal as a function of the treatment time, which was studied in the range of $[H_2O_2]$=50-1000 mg L^{-1} and at a constant Fe^{2+} concentration of 5 mg L^{-1} for the effluent pretreated by SBR; and in the range of $[H_2O_2]$=25-500 mg L^{-1} and at a constant catalyst concentration of 3 mg L^{-1} for the effluent pretreated by MBR. The consumed hydrogen peroxide for the solar photo-Fenton process vs. treatment time (at the optimum concentrations: $[Fe^{2+}(SBR)]_0$=5 mg L^{-1}, $[H_2O_2(SBR)]_0$=500 mg L^{-1} and $[Fe^{2+}(MBR)]_0$=3 mg L^{-1}, $[H_2O_2(MBR)]_0$=250 mg L^{-1}) is presented in the inset graphs of Figure 7.8(a,b).

In the case of the winery effluent pretreated by SBR, the DOC removal increased gradually by increasing the oxidant concentration from 50 to 500 mg L^{-1}, due to the formation of additional hydroxyl radicals (HO•). However, as clearly seen in Figure 7.8(a), the use of excessive concentrations of peroxide (750 and 1000 mg L^{-1}) may have an adverse effect on the mineralization of the effluent pretreated by SBR, which can be attributed to the HO• scavenging effect of H_2O_2 (Muruganandham and Swaminathan, 2004). A similar DOC removal behavior was observed for the winery effluent pretreated by MBR. The DOC removal was found to increase by increasing the oxidant concentration from 25 to 250 mg L^{-1}, but further increase to 500 mg L^{-1} caused a reduction of the mineralization. The highest DOC removal was 48±3.3% (~424 mg L^{-1} of H_2O_2 consumed) for the effluent pretreated by SBR and 53±3.7% (~222 mg L^{-1} of H_2O_2 consumed) for the effluent pretreated by MBR, after 120 min of treatment; while more than the half of DOC (43±2.0% and 38±2.7%) was removed in the first 30 min of solar photo-Fenton process.

Moreover, it should be noted that the initial DOC value of the winery effluent pretreated by SBR was on average equal to 70 mg L^{-1}, while the residual DOC after the solar photo-Fenton treatment at the optimum conditions presented in Figure 7.8 (a) (i.e. 5 mg L^{-1} Fe^{2+} and 500 mg L^{-1} H_2O_2), reached 36.4±2.3 mg L^{-1}. On the other hand, the initial DOC of the winery effluent pretreated by MBR was on average equal to 30 mg L^{-1}, and the residual DOC after the solar photo-Fenton treatment at the optimum conditions presented in Figure 7.1(b) (i.e. 3 mg L^{-1} Fe^{2+} and 250 mg L^{-1} H_2O_2) was 14.1±1.1 mg L^{-1}.

In the various operating conditions studied, the extent of mineralization (as assessed by DOC) for both biologically treated flows was lower than the COD decrease; thus implying that partial oxidation reactions of the organic content prevail over total oxidation to carbon dioxide and water.

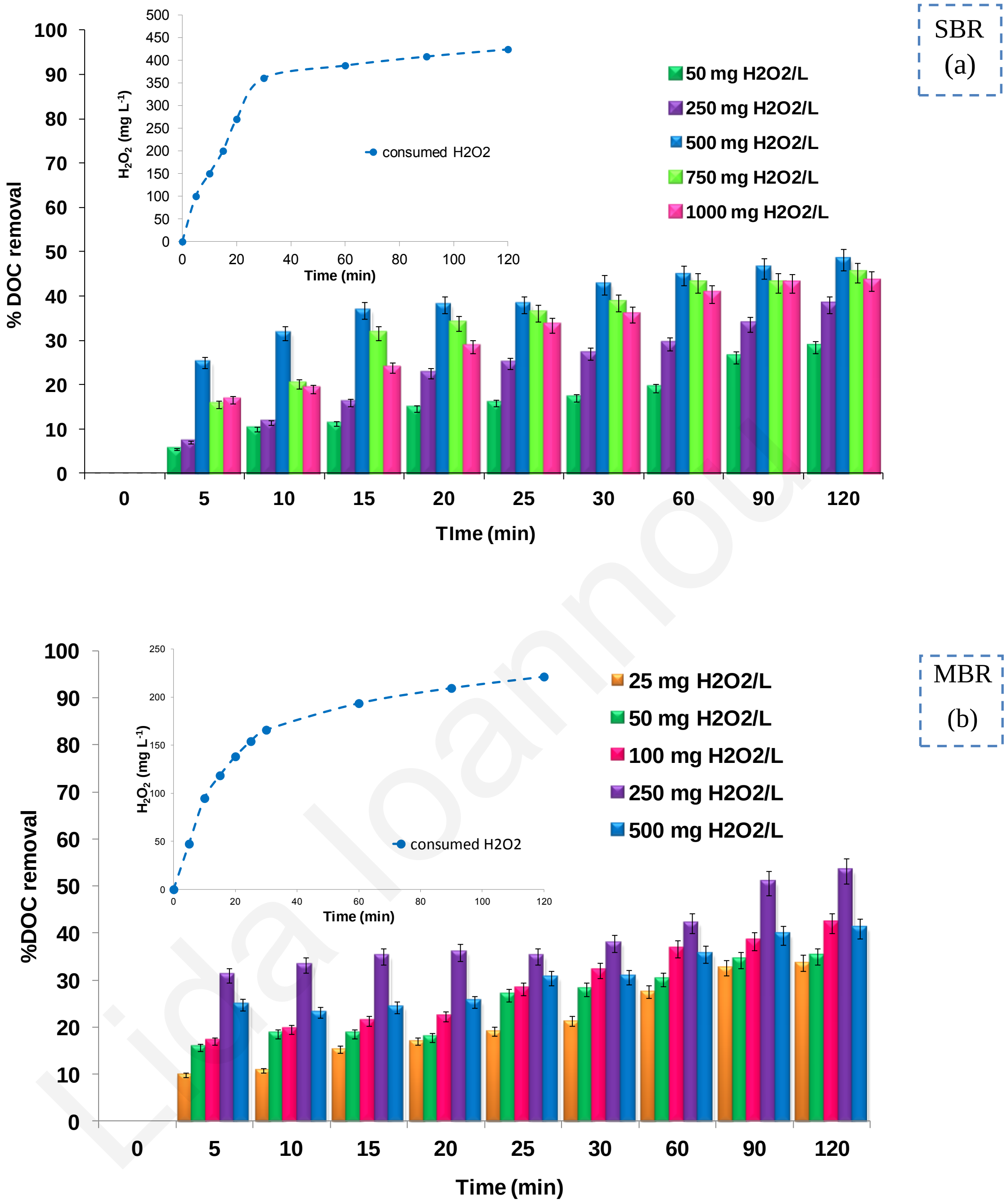


Figure 7.8: Effect of the initial H_2O_2 concentration on the DOC removal of the winery effluents pretreated by (a) SBR and (b) MBR; The inset graphs show the consumed hydrogen peroxide with the time at the optimum conditions of both effluents; (pH=3.0 and T=25±0.1 ^oC) **(a)** DOC_0=70 mg L^{-1} $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=50-1000 mg L^{-1}; **(b)** DOC_0=30 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=25-500 mg L^{-1}.

7.1.9 DOC removal kinetics

Due to the presence of a complex matrix and unknown multiple reactions, dissolved organic carbon (DOC) was used to describe the global kinetics of organic matter removal and to derive the essential kinetic parameters needed for reactor design and optimisation, as also reported by Lucas et al. (2010).

A kinetic study of the solar photo-Fenton process can be performed assuming that the reaction between hydroxyl radicals and the pollutant is the rate determining step as has been reported also by other authors (Malato et al., 2001; Chacón et al., 2006; Evgenidou et al., 2007b; Pariente et al., 2008). Generally, the behavior of the reaction rate (r) can be explained by the Eq. (7.22):

$$r = -\frac{dC}{dt} = k \times f(C) \tag{7.22}$$

where k is the kinetic constant and f(C) is a function of the organic matter concentration (Pignatello et al., 1999).

Although the kinetics of Fenton reaction can be quite complex because of the large number of steps and reactive species involved, HO• is usually regarded as the sole or the most important reactive species (Navarro et al., 2011), and the rate equation can be given by (Eq. (7.23)):

$$r = -\frac{dC}{dt} = k \;\; \times C_{HO^{\bullet}} \times C \tag{7.23}$$

where k is the reaction rate constant, $C_{HO\bullet}$ is the concentration of hydroxyl radicals, and C is the concentration of the organic matter.

Under the assumption that HO• radicals rapidly achieve a constant steady-state concentration in the solution, the winery wastewater DOC removal may be described by a pseudo first-order kinetic expression (Eq. (7.24)) (Evgenidou et al., 2007b; Sirtori et al., 2009; Navarro et al., 2011):

$$r = -\frac{dC}{dt} = k \times C_{HO\bullet} \times C = k_{app} \times C \tag{7.24}$$

where k_{app} is the apparent pseudo first-order constant.

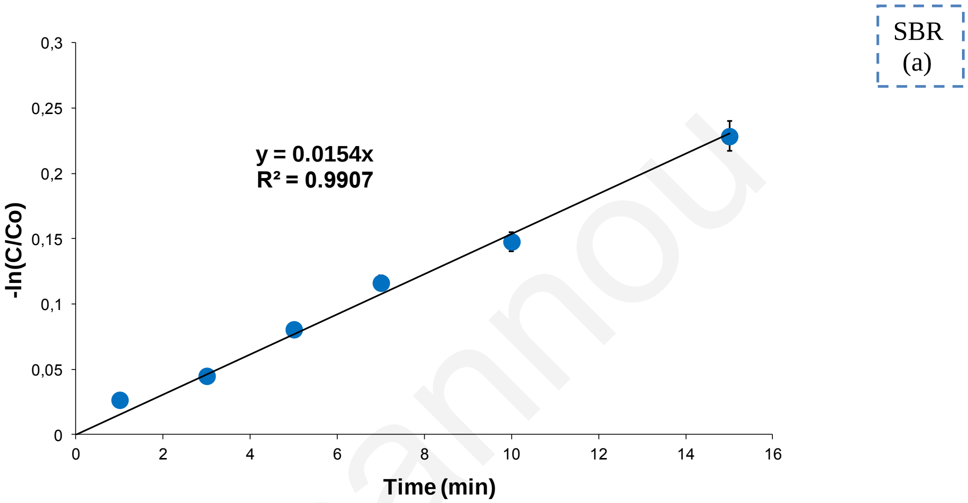
Therefore the concentration of the organic load of the winery wastewater can be given by the following kinetic expressions (Eqs. (7.25) and (7.26)):

$$C = C_o \times e^{-k_{app}t} \tag{7.25}$$

$$-\ln\frac{C}{C_o} = k_{app} \times t \tag{7.26}$$

where, C is the concentration of the DOC in the winery wastewater.

The experimental data fit well the linear kinetic equation (Eq. (7.26)), and consequently the removal of the DOC for both biologically pretreated flows, follows the pseudo first-order law, (Figure 7.9). The degradation rate constant, k (min^{-1}), is determined from the slop of the $-\ln(C/C_o)=f(t)$ plot, where C_o and C are the concentrations of DOC at times zero and t (min), respectively. The values of k were found to be 0.0154 min^{-1} and 0.0274 min^{-1} for the DOC removal for the effluents pretreated by SBR and MBR, respectively, at the optimum experimental conditions, as shown in Figure 7.9(a,b). It should be noted that linear fitting is valid for reaction times up to 15 min. This criterion was set assuming a steady state production of HO• from hydrogen peroxide decomposition, which is likely to occur mainly during the early stages of the reaction (Pariente et al., 2008).



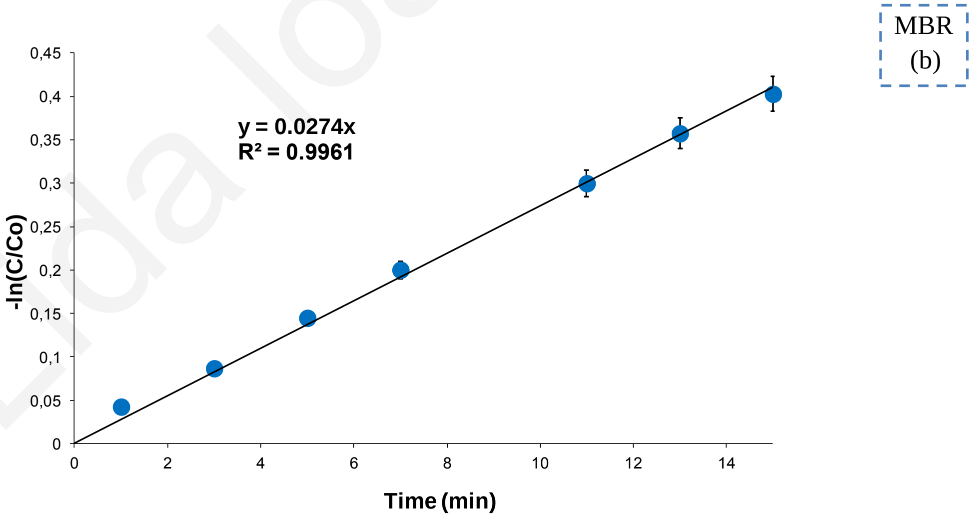


Figure 7.9: Average DOC removal kinetics for the winery effluents pretreated by (a) SBR and (b) MBR after solar photo-Fenton oxidation, at the optimum experimental conditions; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=250 mg L^{-1}.

Moreover, the degradation kinetic parameters provided above were estimated under different conditions of oxidant concentration (25-1000 mg L^{-1}), as described in Section 7.1.8. Each mineralization experiment described therein was used to calculate the rate constant by the linear fit, using only the experimental data from the first 15 min of reaction in order to avoid variations as a result of competitive effects of by-products, pH changes, etc (Evgenidou et al., 2007a; Michael et al., 2010). The experimental data under different concentrations of H_2O_2 fit also well the linear kinetic equation as depicted in Figure 7.10(a,b).

As shown in Figure 7.10 (inset graphs), an increase of the amount of hydrogen peroxide causes an increase of the reaction rate since more hydroxyl radicals are produced (Muruganandham and Swaminathan, 2004). This increase of the reaction rate continues up to a level which corresponds to an optimum concentration (Evgenidou et al., 2007b). At high dosages of the oxidant, the observed decrease of the reaction rate is due to the HO• scavenging effect of H_2O_2 and the recombination of hydroxyl radicals as well, according to the Eqs. (7.3)-(7.5) (Muruganandham and Swaminathan, 2004).

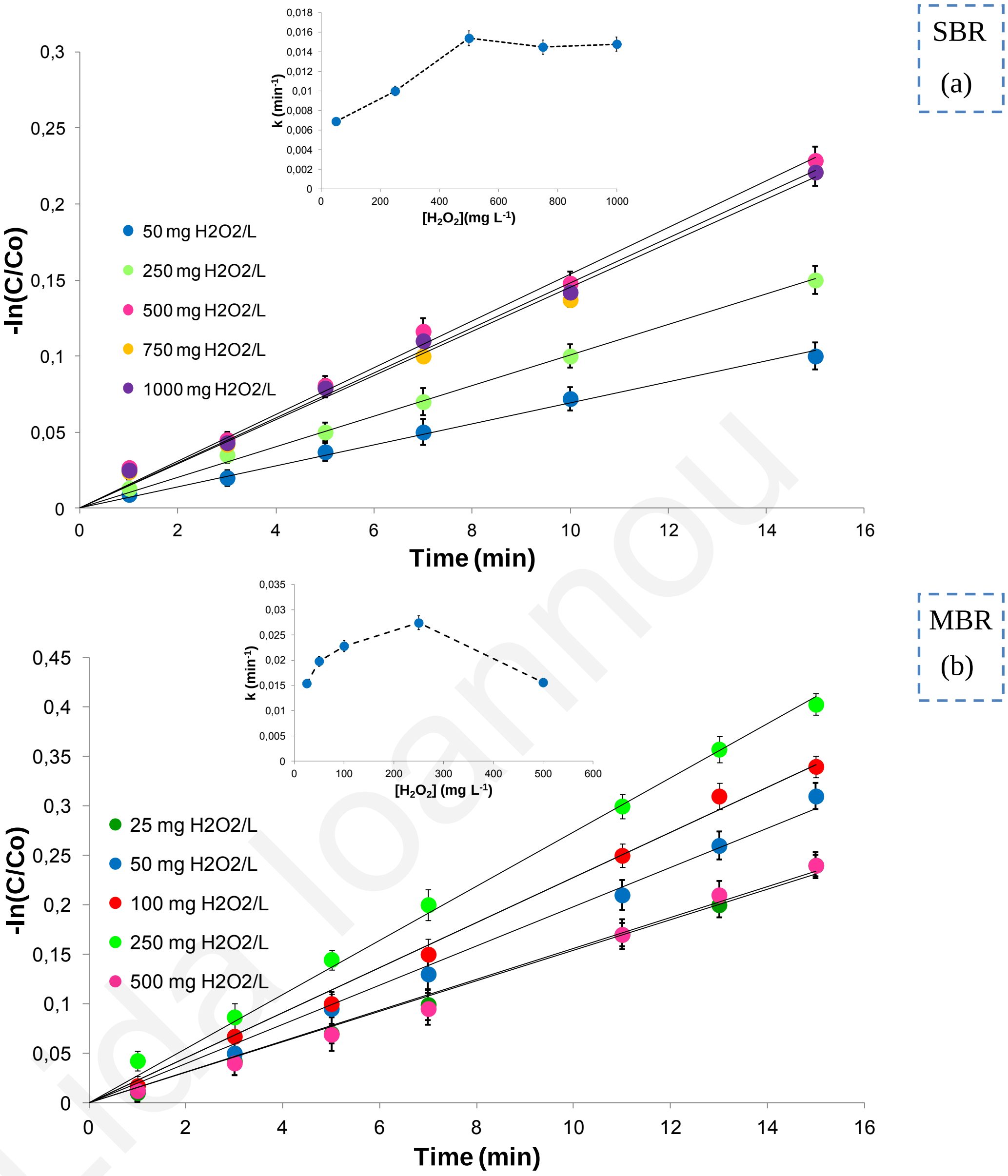


Figure 7.10: Fitting of the experimental data to the linear kinetic model for solar photo-Fenton process for the winery effluents pretreated by (a) SBR and (b) MBR; Inset graphs: effect of hydrogen peroxide concentration on the rate constant, k; (pH=3.0 and T=25±0.1 ^{o}C) **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=50-1000 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=25-500 mg L^{-1}.

7.1.10 Evaluation of the toxicity

The investigation of the toxicity of the two biologically pretreated flows, as well as the possible toxicity of the oxidation products formed during the solar photo-Fenton process was an imperative task of this study, since this would enable a more complete evaluation of the efficiency and environmental safety of the applied technology.

Winery wastewater can be characterized as an extremely complex mixture containing numerous organic, as well as inorganic compounds whose individual and combined contribution to plant phytotoxicity and toxicity generally is not known (Nielsen and Rank, 1994; Arienzo et al., 2009b; Mosse et al., 2010). Moreover, winery wastewater is characterized by high organic load, generally low pH and high salinity (Mosse et al., 2010). Land disposal of winery wastewater onto field crops, tree lots, pastures and vineyards is a widely used means of wastewater management (Crites et al., 2000). However, the aforementioned qualitative characteristics of the winery wastewater indicate that these effluents can pose an environmental risk (Mosse et al., 2010).

The quality control of wastewater based on global chemical measures of total organic pollution load such as DOC, COD or the detection of specific pollutants, is not sufficient to assess the environmental risk. Information on the potential biological effects of such wastewater stream is very important. Therefore, effective tools for the evaluation of the negative effects on living organisms are needed. The use of biological assays can provide a direct and appropriate measure of toxicity to complement the physicochemical measures of quality of wastewater (Nielsen and Rank, 1994; Farré and Barceló, 2003; Hernando et al., 2005). Toxicity testing has grown steadily in recent years, being a useful tool in environmental risk assessment (Farré and Barceló, 2003).

The possible toxicity of the two biologically pretreated flows was examined by two types of bioassays considering the final use of the treated effluents. Toxicity tests using *Daphnia magna* and three plant species (*Sinapis alba, Lepidium sativum, Sorghum saccharatum*) were considered suitable to evaluate the toxicity of the treated wastewater before its disposal in the environment (i.e. water bodies) and use for agricultural irrigation, respectively.

7.1.10.1 Daphnia magna

Toxicity measurements were performed in samples collected at various times of the solar photo-Fenton treatment and at various dilutions, using the Daphtoxkit F^{TM} magna toxicity test. Young daphnids, aged less than 24 h at the start of the test, were exposed to the test samples for a period of 24 and 48 h. Immobilization was recorded at 24 and 48 h and compared to control values (as described in more details in Chapter 5).

Firstly, it was considered necessary to investigate the potential reduction of the examined winery wastewater toxicity through dilutions. For this reason the winery effluents pretreated by SBR and MBR were subjected to various dilutions (concentration of winery wastewater 100% (undiluted), 75%, 50% and 25%) (Figure 7.11) and their toxicity was tested.

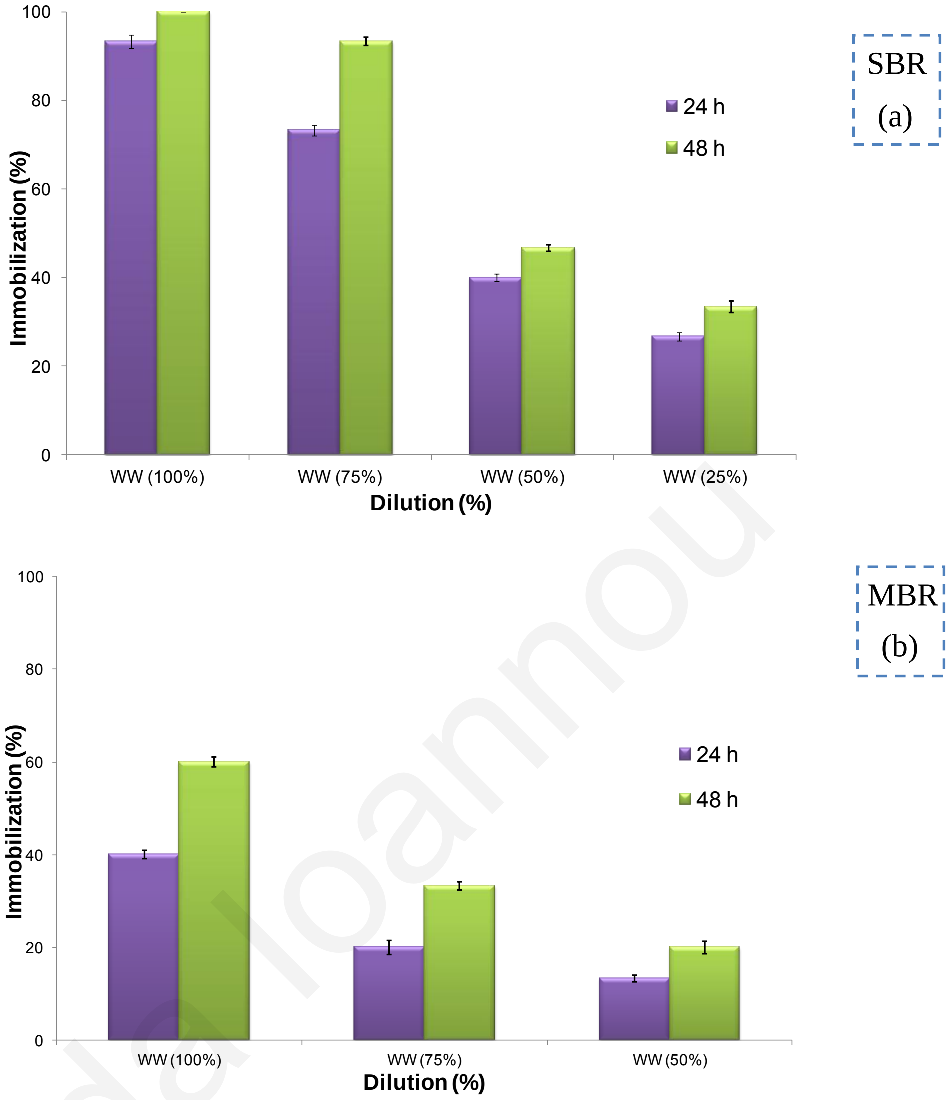


Figure 7.11: Effect of the initial concentration of the winery effluents pretreated by (a) SBR and (b) MBR on toxicity to *D. magna* for 24 and 48 h exposure times.

Figure 7.11(a) shows the effect of the initial concentration of the effluent pretreated by SBR on toxicity to *D. magna* for 24 and 48 h exposure times. As seen the effluent pretreated by SBR was found to be very toxic to daphnids for the two highest concentrations studied (100 and 75%) and the immobilization was higher than 73.3±2.0%, for both exposure times; while only at the lower concentration of 25% did toxicity decrease rapidly reaching 26.7±0.9% and 40±1.3% for 24 and 48 h of exposure, respectively.

As seen in Figure 7.11(b), the effluent pretreated by MBR was found to be relatively non-toxic to daphnids for the whole range of concentrations studied; only at the higher concentration of 100% and prolonged exposure of 48 h, toxicity reached 60±1.1%. For this reason only two dilutions were made in order to investigate the toxicity of the winery effluent pretreated by MBR. Thus, at the lower concentration of 50%, the toxicity was reduced to 13.3±0.7 and 20±1.4% after 24 and 48 h of exposure, respectively.

In Figure 7.12(a), the variation of the toxicity during the photocatalytic treatment of the winery effluent pretreated by SBR is presented. As shown, the raw effluent was found to be very toxic to *D. magna* (93.3±1.58 and 100±0.0% immobilization after 24 and 48 h of exposure, respectively). A high toxicity towards *D. magna* for 24 h of exposure was also observed for the solar photo-Fenton treated samples at 30 and 60 min with 100±0.0% immobilization. This high toxicity is attributed to the products formed by the oxidation of the winery wastewater, causing toxicity effects on daphnids at longer time of exposure. After 48 h of exposure to samples taken at 30, 60 and 90 min, immobilization was found to be 100±0.0, 100±0.0, 86.7±1.75%, respectively. Finally, after 120 min of treatment, the toxicity was reduced to 53.3±1.19% after 24 h of exposure, while after 48 h of exposure it remained constant to 86.7±1.76%. Since no significant decrease of the toxicity after 120 min of solar treatment was achieved, additional experiments were carried out to investigate whether the residual toxicity could be reduced after a longer treatment time. The results showed a significant decrease in daphnids immobilization (33.3±1.85%) after 240 min of treatment and 48 h of exposure.

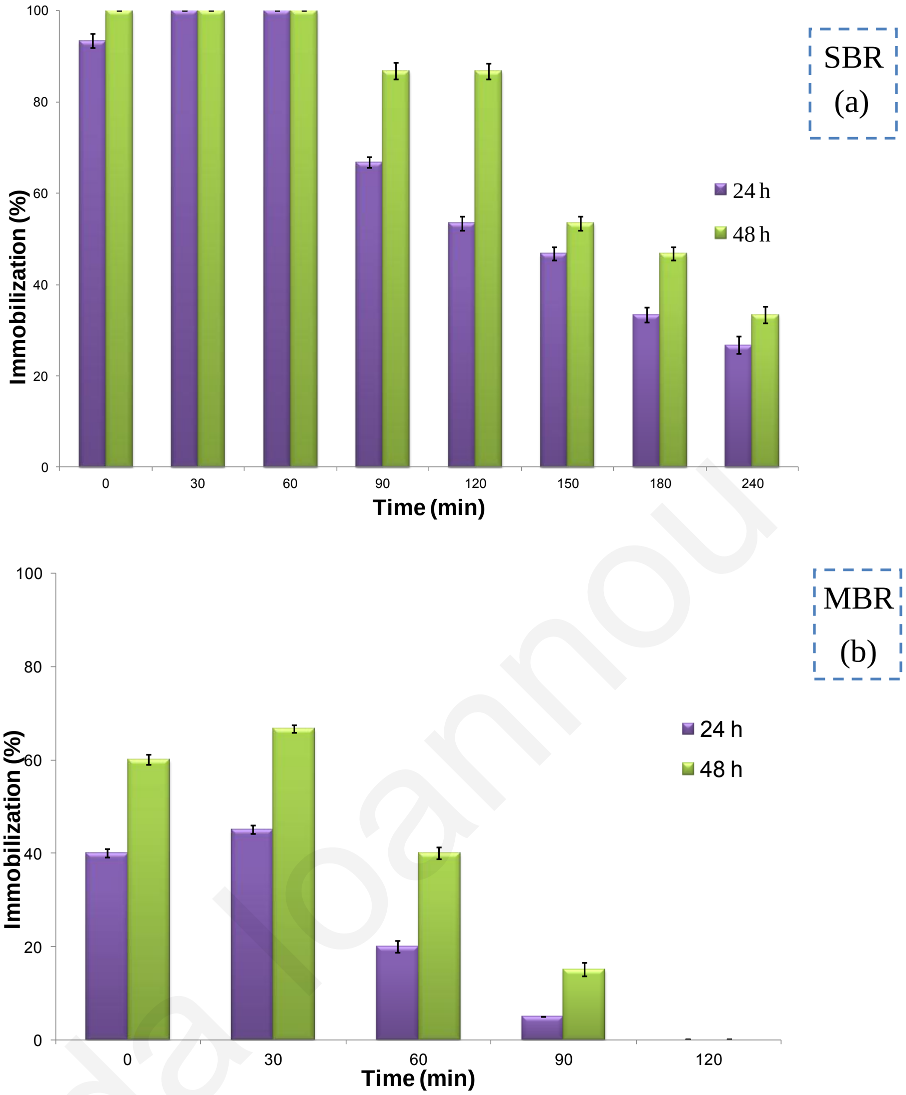


Figure 7.12: Evolution of toxicity to *D. magna* during the solar photo-Fenton oxidation of the winery effluents pretreated by (a) SBR and (b) MBR; **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1} and $[H_2O_2]_0$=250 mg L^{-1}.

On the other hand, as shown in Figure 7.12(b) the effluent pretreated by MBR was found moderately toxic to *D. magna* (40±0.9 and 60±1.1% after 24 and 48 h of exposure, respectively). However, the toxicity of the treated effluent at 30 min of solar oxidation increased up to 45±0.87 and 67±0.78% after 24 and 48 h of exposure, respectively, due to the potential formation of toxic oxidation products. After 48 h of exposure to samples taken at 60 and 90 min immobilization was reduced to 40±1.3 and 15±1.45%, respectively. Finally, prolonged solar photo-Fenton treatment of the effluent for 120 min led to a complete reduction of the toxicity, for both exposure times.

The winery effluent pretreated by MBR was found to be less toxic to *D. magna* than that pretreated by SBR, as was expected, due to the higher efficiency of MBR treatment and the lower remaining organic load (described in Section 5.1.2, Chapter 5). The same behavior was also observed for the effluent pretreated by MBR after 120 min of solar oxidation, in relation to that pretreated by SBR which was found to be more toxic. In the case of the effluent pretreated by SBR, 240 min were needed in order to reduce the immobilization of *D. magna* to 26±1.9 and 33±1.85% after 24 h and 48 h of exposure, respectively. On the other hand, in the case of the effluent pretreated by MBR, 120 min of solar photo-Fenton oxidation was sufficient for complete reduction of the toxicity.

Thus, in the case of the effluent pretreated by SBR, which was more toxic to *D. magna*, the efficiency of the combined dilution of the sample and solar photo-Fenton oxidation was also investigated. The evaluation of the toxicity to *D. magna* during the solar photo-Fenton oxidation, for 50% dilution of the untreated effluent is shown in Figure 7.13. The raw effluent after a 50% dilution was found to be moderately toxic to *D. magna* (40±0.7 and 45±0.8% immobilization after 24 and 48 h of exposure, respectively). An increased toxicity to *D. magna* was observed for the solar photo-Fenton treated samples at 30 min with 55±1.2 and 75±0.98% immobilization for 24 and 48 h of exposure, respectively, due to the potential formation of toxic oxidation products. After 48 h of exposure to samples taken at 60 and 90 min the toxicity was reduced to 75±2.3 and 40±3.1%, respectively. Finally after 120 min of solar oxidation the toxicity was almost eliminated, yielding 0 and

5±1% immobilization after 24 and 48 h of exposure, respectively. As a consequence, the combination of dilution and solar photo-Fenton oxidation was able to completely reduce the toxicity of the winery effluent pretreated by SBR.

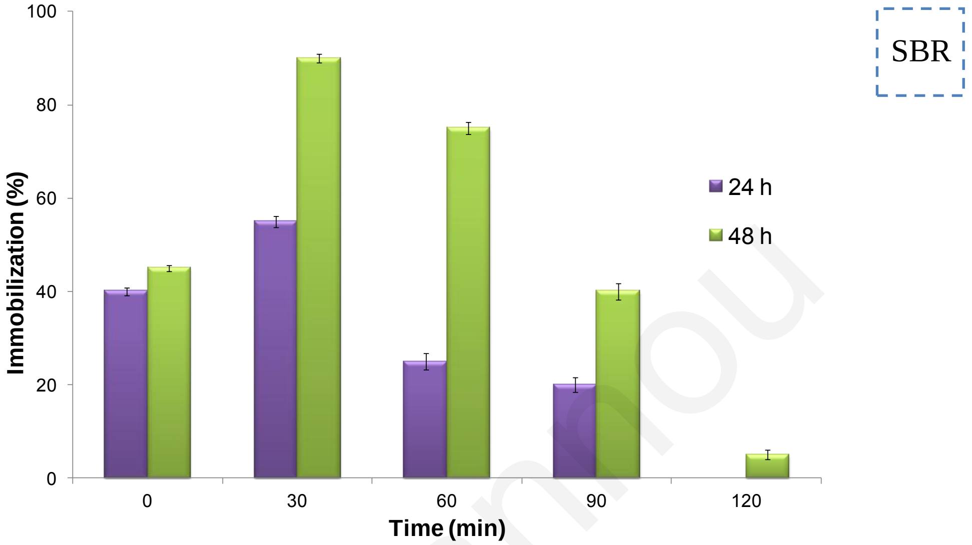


Figure 7.13: Evaluation of the toxicity of 50% diluted winery effluent pretreated by SBR to *D. magna* during the solar photo-Fenton oxidation; $[Fe^{2+}]_0$=5 mg L^{-1} and $[H_2O_2]_0$=500 mg L^{-1}.

7.1.10.2 Phytotestkit toxicity test

Phytotoxicity testing was conducted according to the standard testing protocols using 3 types of plant seeds: (i) mustard (*Sinapis alba*), (ii) dicotyls garden cress (*Lepidium sativum*), and (iii) monocotyl Sorgho (*Sorghum saccharatum*). The test results were evaluated comparing the mean number of germinated seeds and the mean root and shoot length for the three replicates in the control and in each sample examined. The inhibition percentage of the chemical compounds on seed germination (GI), shoot growth (SI) and root growth (RI) was calculated by applying the following formula (Eq. (7.27)):

$$\%GI, SI, RI = \frac{A-B}{A} \times 100 \qquad (7.27)$$

where A is the average number of germinated seeds or the average shoot length or the average root length in the control water, and B the same parameters for the test wastewater.

The phytotoxicity test was conducted on samples taken from the solar photo-Fenton process (at the optimum reagents concentrations, as determined in the Sections 7.1.1 and 7.1.2) at various times of treatment (0-240 min) for both biologically pretreated flows. The treated samples displayed a varying toxicity profile to each type of plant by means of GI, SI and RI, and the results are depicted in Table 7.1.

Table 7.1: Seed germination inhibition (GI), shoot inhibition (SI) and root inhibition (RI) during solar photo-Fenton oxidation (bench scale)

Plant species	Treatment time (min)	Germination inhibition (%GI)*		Shoot inhibition (%SI)		Root inhibition (%RI)	
		SBR	MBR	SBR	MBR	SBR	MBR
Sinapis alba	0	15.0±2.1	3.3±0.5	44.0±6.7	47.2±9.2	45.7±4.5	45.7±5.7
	30	5.0±0.8	10.0±1.8	65.4±5.2	67.4±5.7	86.1±7.2	86.1±6.2
	60	ni	5.0±1.1	58.0±3.4	60.4±6.9	79.3±6.9	79.3±7.9
	90	10.0±1.4	20.0±2.5	50.1±5.6	52.9±7.3	76.2±5.8	76.2±9.2
	120	ni	3.3±0.7	47.0±4.7	50.1±8.1	73.3±7.6	73.3±10.1
	150	3.3±0.5	3.3±0.3	44.9±5.1	42.7±5.4	58.8±9.2	61.8±6.3
	180	ni	ni	32.0±3.7	32.0±3.8	45.0±5.3	43.5±5.7
	240	ni	ni	20.3±4.2	24.8±4.2	35.8±4.1	28.2±4.2
Lepidium sativum	0	15.0±1.8	6.7±1.2	37.6±10.1	8.8±2.1	19.0±3.2	16.7±4.3
	30	15.0±2.2	25.0±2.8	38.9±5.9	63.2±10.2	68.7±7.9	86.4±5.9
	60	10.0±1.7	10.0±1.3	20.8±4.2	32.6±3.4	61.9±5.6	73.7±6.8
	90	10.0±1.5	15.0±1.7	32.1±7.3	47.0±5.8	48.9±3.7	61.1±7.2
	120	6.7±1.1	3.3±0.9	9.8±2.7	24.9±4.3	31.6±11.2	66.5±8.4
	150	3.3±0.6	ni	6.6±1.5	18.7±3.8	18.2±8.2	51.9±6.5
	180	3.3±0.5	3.3±0.5	4.5±1.2	7.1±2.7	23.0±6.1	35.8±3.7
	240	ni	ni	2.3±0.6	5.2±1.1	11.0±3.7	15.8±2.8
Sorghum saccharatum	0	10.0±2.3	ni	91.0±3.2	14±0.7	60.7±5.4	12.1±5.2
	30	10.0±1.7	5.0±0.7	77.4±11.4	94.0±5.1	96.1±3.4	93.4±2.9
	60	15.0±2.4	6.7±1.1	76.9±8.6	88.8±7.6	46.9±10.1	82.4±7.2
	90	10.0±1.6	5.0±1.0	83.5±5.4	83.4±9.7	46.4±6.8	72.2±6.7
	120	ni	ni	80.5±9.2	74.8±5.4	43.4±5.7	70.2±9.4
	150	3.3±0.5	3.3±0.8	59.9±4.7	52.9±9.2	37.9±4.9	39.4±6.1
	180	ni	ni	32.8±5.1	27.9±4.8	31.9±7.2	25.5±5.3
	240	ni	ni	20.8±2.9	1.9±0.5	21.8±2.7	9.0±2.6

*ni: no inhibition

Experimental conditions: $[Fe^{2+}(SBR)]_0$=5 mg L^{-1}, $[H_2O_2(SBR)]_0$=500 mg L^{-1}, $[Fe^{2+}(MBR)]_0$=3 mg L^{-1} and $[H_2O_2(MBR)]_0$=250 mg L^{-1}.

7.1.10.2.1 Seed germination inhibition

In general, the solar photo-Fenton process did not exert any significant influence on seed germination, and the inhibition level was altered from 0 to 25±2.8%, for both biologically pretreated flows. As can be seen in Table 7.1 the effluent pretreated by SBR, caused an inhibition on seed germination of the order of 10±2.3% for *Sorghum saccharatum* to 15±2.1% for *Sinapis alba* and *Lepidium sativum*. On the other hand, the effluent pretreated by MBR caused a low inhibition on seed germination for *Lepidium sativum* (6.7±1.2%), *Sinapis alba* (3.3±0.5%), and no inhibition for *Sorghum saccharatum*. According to Williams and Hoagland, (1982) the seed germination inhibition can differ between species and this is likely due to the variation in seed size, seed coat permeability, differential uptake of nutrients and toxins, and metabolism.

When the solar photo-Fenton oxidation was applied, the phytotoxic effects on seed germination varied among the plant species and the treatment time. In the case of the effluent pretreated by SBR, after 30 min of solar photo-Fenton treatment, the *Lepidium sativum* GI was the same as in the raw effluent (15±2.2%), which was the highest value. After 60 min of treatment, the *Sorghum saccharatum* GI was higher than that of the raw effluent (15±2.4%), due to the potential subsequent formation of oxidation products which were toxic to these seeds. After 120 min of photocatalytic treatment the GI decreased to zero for *Sinapis alba* and *Sorghumm sacharratum*, and to 6.7±1.1% for *Lepidium sativum*. Finally, at the end of the treatment (240 min) the GI was completely eliminated for all the plant species.

In the case of the effluent pretreated by MBR, after 30 min of solar photo-Fenton treatment, the highest GI was observed. More specifically, the GI at 30 min of treatment was 10±1.8% for *Sinapis alba*, 25±2.8% for *Lepidium sativum*, and 5±0.7% for *Sorghum saccharatum*, due to the subsequent formation of toxic oxidation products. After 60 min of treatment the GI decreased to values lower than 10% for all the three species, while after 90 min the GI increased again, for *Sinapis alba* (20±2.5%) and *Lepidium sativum* (15±1.7%), indicating that more toxic oxidation products were formed. The GI of all plant species was decreased by further increasing the treatment time from 90 to 150 min, while no inhibition was detected after 240 min of treatment, indicating that the toxic oxidation products were further oxidized to less toxic substances.

In summary, the GI of the effluent pretreated by SBR (10±2.3 to 15±2.1%) was higher than that of the effluent pretreated by MBR (0 to 6.7±1.2%). For both biologically pretreated flows, the GI increased during the first minutes of the solar photo-Fenton treatment, due to the subsequent formation of oxidation products, which were apparently toxic to plant species. Finally, the inhibition on the seed germination was eliminated at the end of the treatment (240 min) for each type of plant, and for both biologically pretreated flows.

7.1.10.2.2 Shoot growth inhibition

The phytotoxicity in the treated samples expressed as shoot growth inhibition (SI), varied differently compared to the inhibition of seed germination (GI). The growth of the shoots was affected by the treated samples, potentially reflecting their own specific mechanisms for growth and adaption to the oxidation products. Interestingly, however was the fact that the shoot inhibition of the treated samples was higher compared to the seed germination inhibition. As can be seen in Table 7.1 the effluent pretreated by SBR, caused significant SI of the order of 44±6.7% for *Sinapis alba*, 37.6±10.1% for *Lepidium sativum*, and 91±3.2% for *Sorghum saccharatum*. On the other hand, the effluent pretreated by MBR caused moderate SI for *Sinapis alba* (47.2±9.2%), and low inhibition for *Lepidium sativum* (8.8±2.1%) and for *Sorghum saccharatum* (14±0.7%).

When the solar photo-Fenton was applied, the phytotoxic effects on SI varied among the plant species and the treatment time. In the case of the effluent pretreated by SBR, the SI of *Sinapis alba* was increased at 30 min of solar photo-Fenton treatment yielding 65.4±5.2% inhibition, due to the subsequence formation of toxic oxidation products. On the other hand, the SI of *Lepidium sativum* remained almost constant at 30 min, and then it was reduced at 60 min, while at 90 min an increase of the SI was observed, due to the potential formation of oxidation products. Moreover, the SI behavior of *Sorghum saccharatum* showed a quite different trend; at 30 min of treatment was decreased, and then remained constant at 60 min, while at 90 min a small increase was observed, which continued until 120 min, due to the formation of phytotoxic oxidation products. The SI of all plant species, was decreased by increasing the treatment time from 120 to 240 min, which at the end of the treatment (240 min) was 20.3±4.2% for *Sinapis alba*, 2.3±0.6% for *Lepidium sativum*, and 20.8±2.9% for *Sorghum saccharatum*. The SI of all plant species at the end of the solar treatment was significantly lower than that of the effluent pretreated by SBR alone, indicating that less toxic oxidation products were formed at the end of the process.

In the case of the effluent pretreated by MBR, the SI of all three plant species increased rapidly during the first 30 min of solar photo-Fenton treatment. For *Sinapis alba* it increased from 47.2±9.2 to 67.4±5.7%, for *Lepidium sativum* from 8.8±2.1 to 63.2±10.2%, and for *Sorghum saccharatum* from 14±0.7 to 94±5.1%. After 60 min of treatment the SI of *Sinapis alba* and *Sorghum saccharatum* was continuously decreasing to reach 24.8±4.2 and 1.9±0.5% at the end of the treatment (240 min), indicating the removal or transformation of toxic oxidation products to less toxic substances. For *Lepidium sativum* the SI showed approximately the same trend, with an additional increase of SI at 90 min of treatment, and a further decrease until the end of the treatment, yielding 5.2±1.1% final inhibition. The SI of all plant species at the end of the solar photo-Fenton treatment was lower than that of the raw effluent.

7.1.10.2.3 Root growth inhibition

The growth of roots was affected by the solar photo-Fenton treated samples, reflecting again their own specific mechanisms for growth and adaption to the oxidation products. As can be seen in Table 7.1, the root growth inhibition (RI) of the effluent pretreated by SBR, was relatively low for *Lepidium sativum* (19±3.2%), while for *Sinapis alba* (45.7±4.5%) and *Sorghum sacharratum* (60.7±5.4%) was higher. In addition, the RI of the effluent pretreated by MBR, was low for *Lepidium sativum* (16.7±4.3%) and *Sorghum saccharatum* (12.1±5.2%), while it was higher for *Sinapis alba* (45.7±5.7%).

In the case of the effluent pretreated by SBR, after 30 min of solar photo-Fenton treatment the RI increased rapidly for all three plants, obtaining an RI value equal to 86.1±7.2% for *Sinapis alba*, 68.7±7.9% for *Lepidium sativum*, and 96.1±3.4% for

Sorghum saccharatum. This high phytotoxicity is attributed to the products formed by the oxidation of the winery wastewater, causing inhibitory effects on root growth of plant species. After 60 min of treatment the RI decreased gradually for all plant species. Finally at the end of the treatment (240 min) RI was lower than that of the raw effluent and equal to 35.8±4.1% for *Sinapis alba*, 11±3.7% for *Lepidium sativum*, and 21.8±2.7% for *Sorghum saccharatum*.

Interestingly however, was the fact that in the case of the effluent pretreated by MBR, the raw effluent induced a lower RI for all the three plant species, than after the first 120 min of solar photo-Fenton oxidation. After the first minutes of solar treatment, the RI was excessively increased. This indicates that the growth of roots seems to be the most sensitive to the potential formation of toxic oxidation products, compared to seed germination and shoot growth, for the effluent pretreated by MBR. At the end of the solar photo-Fenton treatment (240 min) the RI was lower than that of the raw effluent for all three plants, indicating the removal or transformation of the toxic oxidation products throughout the process. More specifically, the RI at 240 min of treatment was 28.2±4.2% for *Sinapis alba*, 15.8±2.8% for *Lepidium sativum*, and 9±2.6% for *Sorghum saccharatum*.

The above results describe the potential of the solar photo-Fenton process to finally reduce the phytotoxicity towards the examined plant species. The three plant species displayed an interesting profile concerning the seed germination, shoot and root growth inhibition stimulated by the remaining organic load of the two biologically pretreated flows but mainly the varying response to the oxidation products. It is apparent that the effluent pretreated by MBR is generally less phytotoxic to plant species compared to the effluent pretreated by SBR, and additionally less toxic to *D. magna*. However, it should be noted, that in the case of *Sinapis alba*, its shoot and root growth inhibition in the effluent pretreated by MBR was almost the same with that induced by the effluent pretreated by SBR.

According to our knowledge, although winery wastewater is well known to restrict plant growth, there are only limited studies that have been performed to date. In the following studies the phytotoxicity of real or semi-synthetic winery effluents, which have not been subjected to any treatment (biological or chemical) was investigated. Arienzo et al. (2009b) investigated the phytotoxicity of winery wastewater in order to design a winery wastewater treatment system involving constructed wetlands. The tolerance of three aquatic macrophytes species (*Phragmites australis*, S*choenoplectus validus* and *Juncus ingens*) to winery wastewater was studied through biomass production, total chlorophyll and nitrogen, phosphorous and potassium tissue concentrations. Moreover, the performance of two laboratory phytotoxicity assays was also evaluated; (i) garden cress (*Lepidium sativum*), and (ii) onion (*Allium coepa*). The results of these tests revealed that the winery effluent was highly toxic to plant species, due to the high concentrations of organic and phenolic compounds, which are usually considered responsible for the reduction in plant growth. A comparison between the cress and onion bioassays with the wetland microcosm results indicated that the thresholds for toxicity were of the same order of magnitude. Thus, the onion (*Allium coepa*) and garden cress (*Lepidium sativum*) tests could be effectively used in the wine industry for rapid assessment of the toxicity of the wastewater at different stages of treatment. In another study of Mosse et al. (2010) the seed germination of four common crop species (*Hordeum vulgare*, *Pennisetum glaucum*, *Medicago sativa* and *Phalaris aquatica*), in semi-synthetic winery wastewater, was investigated. It was found that winery wastewater had a phytotoxic effect on all species studied, with all species showing a similar trend of decreasing biomass (root and shoot) with increasing winery wastewater concentrations. Although overall germination was not affected, winery wastewater was shown to increase the time to germination, and restrict early growth. Whilst phenolic compounds are commonly present in winery wastewater, the synthetic sample, which was used in the study of Mosse et al. (2010), had relatively low phenolic compounds concentrations (<0.04 mg L^{-1}), thereby suggesting that phenolic compounds are also not solely responsible for the observed phytotoxicity. Hence, it is highly likely that the phytotoxicity arises from the complex combination of organic matter in the wastewater, which has also been demonstrated in the case of olive mill wastewater (OMW) (Capasso et al., 1992; Saadi et al., 2007).

In summary solar photo-Fenton oxidation was proved to be very efficient for the treatment of both biologically pretreated flows, reaching a COD removal of approximately 70%; while the residual COD values were 80 mg L^{-1} and 33 mg L^{-1} for the effluents pretreated by SBR and MBR, respectively. The parameters that influenced the kinetic parameters the most, were the operating conditions employed, such as ferrous and hydrogen peroxide concentration, the initial pH of the solution and the temperature. Solar photo-Fenton oxidation reduced the total phenolic compounds up to 70% for the effluent pretreated by SBR; while MBR treatment was able to completely remove these compounds. Both biologically pretreated flows were still colored (yellowish), indicating that after the biological treatment, some of the colored compounds were still present in the winery effluent, due to their bioresistance. However, solar photo-Fenton oxidation *effectively oxidized these refractory organics and significantly removed the color from both winery effluents. The extent of mineralization was lower than the COD removal, implying that partial oxidation reactions of the organic content prevail over total oxidation to* CO_2 *and water. The DOC removal followed during the solar photo-Fenton oxidation the pseudo first-order kinetic law in the first 15 min. It should be noted that linear fitting was considered valid for reaction times up to 15 min. This criterion was set assuming a steady state production of HO• from hydrogen peroxide decomposition, which is likely to occur mainly during the early stages of the reaction. Toxicity and phytotoxicity effects varied among the two biologically pretreated effluent streams, the three plant species and the treatment time, a fact that can be attributed to the potentially different oxidation products produced during the solar photo-Fenton treatment. Finally, prolonged solar photo-Fenton treatment of the winery effluents led to a complete reduction of the toxicity to D. magna, and to a significant reduction of the phytotoxicity for the three plant species examined.*

7.2 Pilot-scale solar Fenton treatment using winery effluents biologically pretreated by (a) a sequential batch reactor (SBR) and (b) a membrane bioreactor (MBR)

The next objective of this study was the investigation of the efficiency of solar Fenton oxidation for the removal of the organic load as well as the toxicity of the biologically treated winery streams, at a pilot-scale setup. The solar Fenton experiments were carried out in a compound parabolic collector (CPC) pilot plant installed at the premises of the University of Cyprus (UCY), as described in detail in Section 5.2.2. The solar driven pilot-scale plant consists of glass tubes, mounted on a fixed platform tilted at the local latitude ($35\ ^{o}$), and was operated in batch mode. The pilot-scale experiments were being carried out for both biologically pretreated flows by SBR and MBR, in order to determine the most efficient combined treatment that could be selected for industrial-scale application.

The comparison of the data derived from different days, at different times of the day and under different solar illumination conditions was performed based on Eq. (7.28). With this equation, combination of the data from several days' experiments and their comparison with other photocatalytic experiments is possible (Malato et al., 2003; Agüera et al., 2004; Pérez-Estrada et al., 2005; Gernjak et al., 2006; Oller et al., 2006; García-Montaño et al., 2008; Radjenovic et al., 2009; Zapata et al., 2010; Klamerth et al., 2011).

$$t_{30W,n} = t_{30W,n-1} + \Delta t_n \frac{UV}{30}\frac{V_i}{V_T}; \quad \Delta t_n = t_n - t_{n-1} \quad (n=1, t_n = 0\,\mathrm{min}) \tag{7.28}$$

where t_n is the experimental time for each sample, UV is the average solar ultraviolet radiation (λ<400 nm) measured between t_{n-1} and t_n (during Δt_n), and $t_{30W,n}$ is the "normalized illumination time". In this case, the time refers to a constant solar UV power of 30 W m^{-2} (typical solar UV power on a perfectly sunny day around noon) (Klamerth et al., 2010). V_T is the total volume of the wastewater loaded in the pilot-scale plant (60 L) and V_i is the total irradiated volume (21.4 L) (Chapter 5).

7.2.1 Photolysis

Preliminary photolytic experiments were carried out, in order to determine the contribution of this effect in the overall photocatalytic process in the solar driven pilot-scale plant. Photolysis experiments were performed at acidic pH=3.0 (same pH used for the solar Fenton process that was later on applied), and for a period of 480 min (t_{exp}).

Results obtained from photolysis (solar irradiation alone) indicated that the photolytic process was not responsible for the observed significant COD reduction of winery effluents, when irradiated in the presence of Fenton reagents. The COD reduction observed was 6.5±3.9% ($t_{30W,n}$=131.0 min) for the winery effluent pretreated by SBR, and 10±2.8% ($t_{30W,n}$=127.1 min) for the winery effluent pretreated by MBR, as shown in Figure 7.14. Moreover, it should be noted that the photolytic degradation was accompanied by a very low, almost insignificant reduction in DOC (<4%), for both biologically pretreated flows.

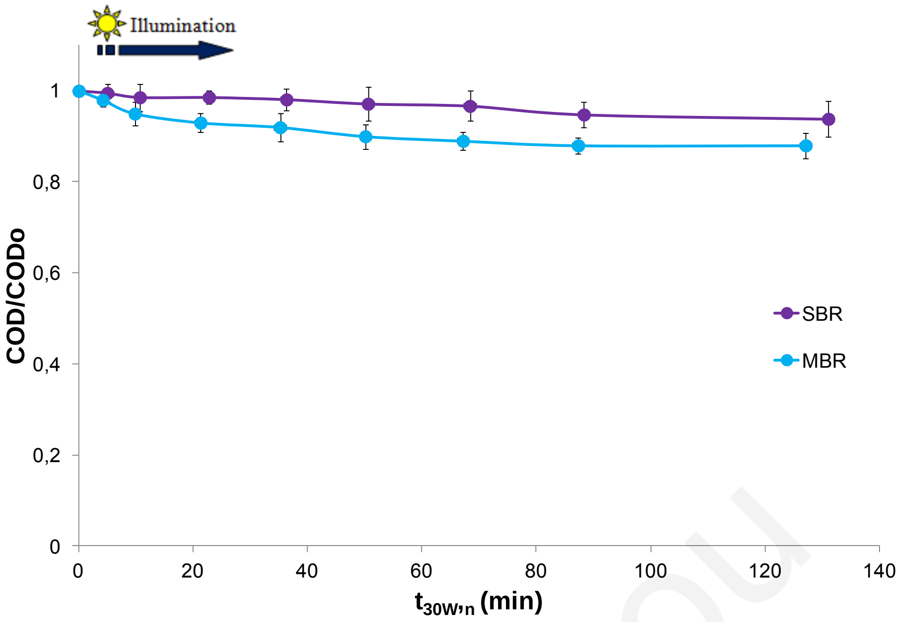


Figure 7.14: % COD removal for the winery effluents pretreated by SBR and MBR, during photolysis experiments (pilot scale); pH=3.0.

7.2.2 Solar Fenton experiments

7.2.2.1 Effect of H_2O_2 concentration

For the pilot-scale solar Fenton experiments, the optimum concentration of iron found at the bench-scale experiments for each of the winery effluent streams was used. A ferrous concentration of 5 mg L^{-1} for the effluent pretreated by SBR, and 3 mg $L^{-1}$for the effluent pretreated by MBR was chosen considering the fact that these concentrations are capable to achieve relatively high COD and DOC reduction rates (as shown in Sections 7.1.1 and 7.1.8), and in order to avoid the requirement for iron removal at the end of the process, according to the existing regulations concerning iron discharge limits (Cyprus Law: 106(I)/2002, Section 7.1.1). Furthermore, considering the fact that hydrogen peroxide has significant economic cost, a system with the lowest possible concentration of the latter was selected, as shown below, so as to be able to establish an economically viable process with high performance in organic compounds removal for both biologically pretreated flows.

In the case of the effluent pretreated by SBR, preliminary solar Fenton experiments were carried out using 5 mg L^{-1} of Fe^{2+} at several doses of H_2O_2 (between 100-1000 mg L^{-1}) in order to establish the optimum peroxide dose for the COD reduction. Additionally, for the effluent pretreated by MBR, the Fe^{2+} concentration was kept constant at 3 mg L^{-1}, while the H_2O_2 concentration ranged from 100 to 750 mg L^{-1} H_2O_2.

Figure 7.15(a,b) shows the COD removal for the winery effluents pretreated by SBR and MBR at various hydrogen peroxide concentrations as a function of the normalized illumination time, $t_{30W,n}$.

As shown in Figure 7.15(a), by increasing the concentration of hydrogen peroxide from 100 to 500 mg L^{-1}, the reduction of COD increased from 50.5±3.5 to 69.6±4.0%, after 180 min of solar Fenton oxidation, for the effluent pretreated by SBR. This can be explained by the effect of the additionally produced hydroxyl radicals (Tamimi et al., 2008). Further increase of the H_2O_2 concentration from 500 to 750 mg L^{-1} seems to cause no significant change in the COD reduction. On the other hand, further increase of H_2O_2 concentration to 1000 mg L^{-1} caused a substantial reduction of the COD removal from 69.6±4.0 to 52.6±3.2%, due to the enhancement of the competition reactions in the case of excess of H_2O_2; mainly the recombination of HO• produced, as well as the reaction of the HO• with H_2O_2, contributing to the hydroxyl radicals scavenging capacity, as described in more detail in previous sections (Section 7.1.2). Summarizing, in the case of the effluent pretreated by SBR, the optimum concentration of hydrogen peroxide was 500 mg L^{-1}, resulting in the maximum COD reduction of approximately 70% after 180 min of solar treatment ($t_{30W,n}$=62 min) (Figure 7.15(a)).

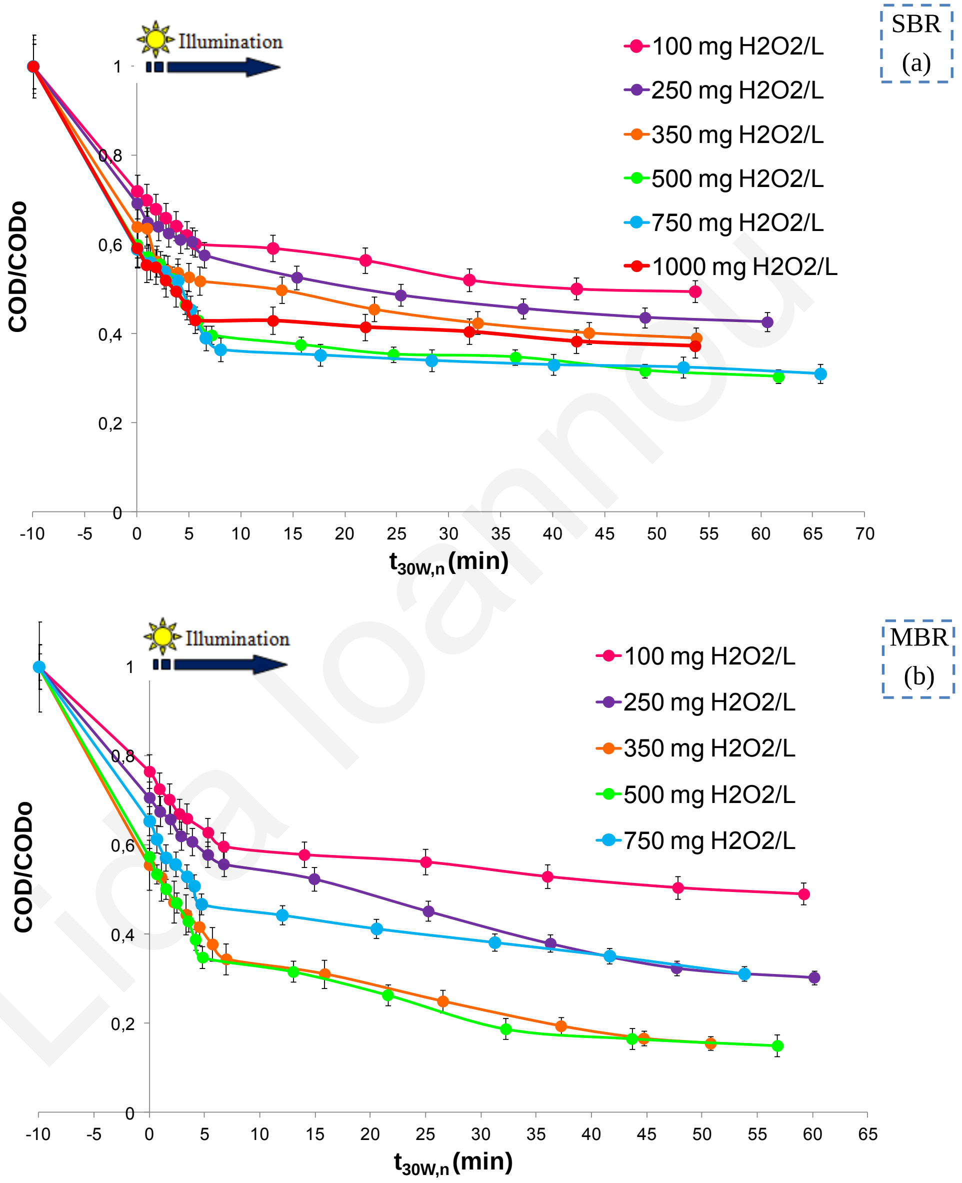


Figure 7.15: COD removal for the winery effluents pretreated by (a) SBR and (b) MBR at various H_2O_2 concentrations, (pilot scale); (pH=3.0) **(a)** COD_0=270 mg L^{-1}, $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=100-1000 mg L^{-1}; **(b)** COD_0=160 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=100-750 mg L^{-1}.

A similar behavior on the COD reduction of the effluent pretreated by MBR at varying doses of H_2O_2 was observed (Figure 7.15(b)). For this effluent, the maximum degradation was observed after 180 min of treatment and was equal to 85±2.4% at $[Fe^{2+}]=3$ mg L^{-1} and $[H_2O_2]=350$ mg L^{-1}. Further increase of the H_2O_2 concentration to 500 mg L^{-1} seems to cause no significant change in COD reduction. However, a higher concentration of H_2O_2 of 750 mg L^{-1} caused a considerable reduction of COD removal from 85±2.4 to 69±1.0% due to the unwanted 'scavenging' reactions which decrease the number of available HO• and ultimately the process efficiency.

Therefore, the concentrations of 500 and 350 mg L^{-1} of H_2O_2 were considered as the optimal dosages for the pilot-scale treatment of the winery effluents pretreated by SBR and MBR, respectively. It should be mentioned that the initial COD values of the effluent pretreated by SBR and MBR were 270 mg L^{-1} and 160 mg L^{-1}, respectively, while the residual COD values after 180 min of solar Fenton oxidation were 82.1±10.0 mg L^{-1} and 24±4.0 mg L^{-1}, respectively.

Moreover, it should be noted, that for both biologically pretreated flows, the degradation of the organic compounds started 10 min before uncovering the reactor ($t_{exp}=-10$ min, dark Fenton reaction), reducing the initial COD value by ~40% (for the optimum experimental conditions), as shown in Figure 7.15. This is very important, since a large part of COD was removed during the first 10 min, and under dark conditions, which means that removal of COD can be obtained in cloudy days. Furthermore, interestingly was the fact that after 90 min of solar treatment the reduction of COD reached a plateau, with a small increase with further increasing of the treatment time to 180 min, for both biologically pretreated flows.

The experiments were repeated with the optimum concentration of Fe^{2+} and H_2O_2 for each one of the effluents, in the absence of solar light (collectors were covered). Figure 7.16(a) shows the results of the dark experiments, at the optimum catalyst and oxidant concentrations determined at the solar Fenton experiments, for both biologically pretreated flows. More specifically, for the effluent pretreated by SBR 5 mg L^{-1} Fe^{2+} and 500 mg L^{-1} H_2O_2 were used, while for the effluent pretreated by MBR the concentrations used were 3 mg L^{-1} Fe^{2+} and 350 mg L^{-1} H_2O_2.

The dark Fenton experiments were performed in order to investigate the efficiency of the process, and thus the possibility of operating the pilot plant overnight. As clearly shown in Figure 7.16(a), a significant reduction of COD under dark conditions was achieved. In the case of the effluent pretreated by SBR the first 10 min of dark reaction 44.4±1.0% of the COD was removed, while with further treatment at 180 min ($t_{30W,n}$=62 min) only a small increase up to 48±1.4% was achieved. The same behavior was also observed for the effluent pretreated by MBR, which at the first 10 min a COD removal of 43±2.0% was observed, while after 180 min of treatment ($t_{30W,n}$=51 min) an increase at 57±1.9% was achieved. The residual COD values after the end of the dark Fenton treatment were 140±3.4 mg L^{-1} and 69±3.0 mg L^{-1} for the effluents pretreated by SBR and MBR, respectively.

For comparison purposes, Figure 7.16(b) depicts the evolution of the COD reduction for both flows, during the solar Fenton oxidation (only at the optimum experimental conditions) vs. the normalized illumination time $t_{30W,n}$. In Figure 7.16(a,b), the H_2O_2 consumption during the dark and solar Fenton oxidation is also presented.

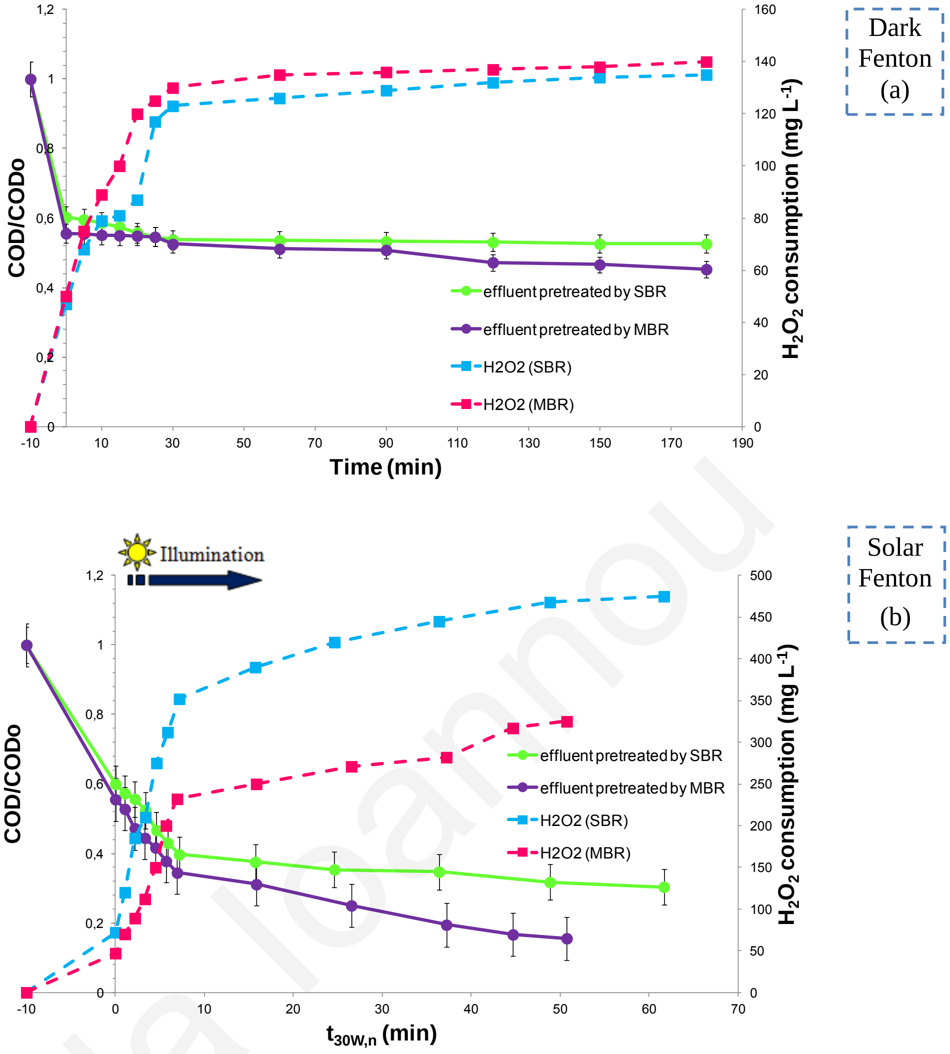


Figure 7.16: COD removal for the winery effluents pretreated by SBR and MBR during (a) dark Fenton and (b) solar Fenton experiments at pilot scale; $[Fe^{2+}(SBR)]_0=5$ mg L^{-1}, $[H_2O_2(SBR)]_0=500$ mg L^{-1}, $[Fe^{2+}(MBR)]_0=3$ mg L^{-1}, $[H_2O_2(MBR)]_0=350$ mg L^{-1} and pH=3.0.

7.2.2.2 Color removal

As already mentioned in detail, (Section 7.1.7) even after the SBR and MBR treatment the winery effluents were still colored (yellowish) due to the natural colors of grapes.

Figure 7.17(a,b) depicts the removal of the color present in the winery effluents pretreated by SBR and MBR vs. the normalized illumination time, $t_{30W,n}$.

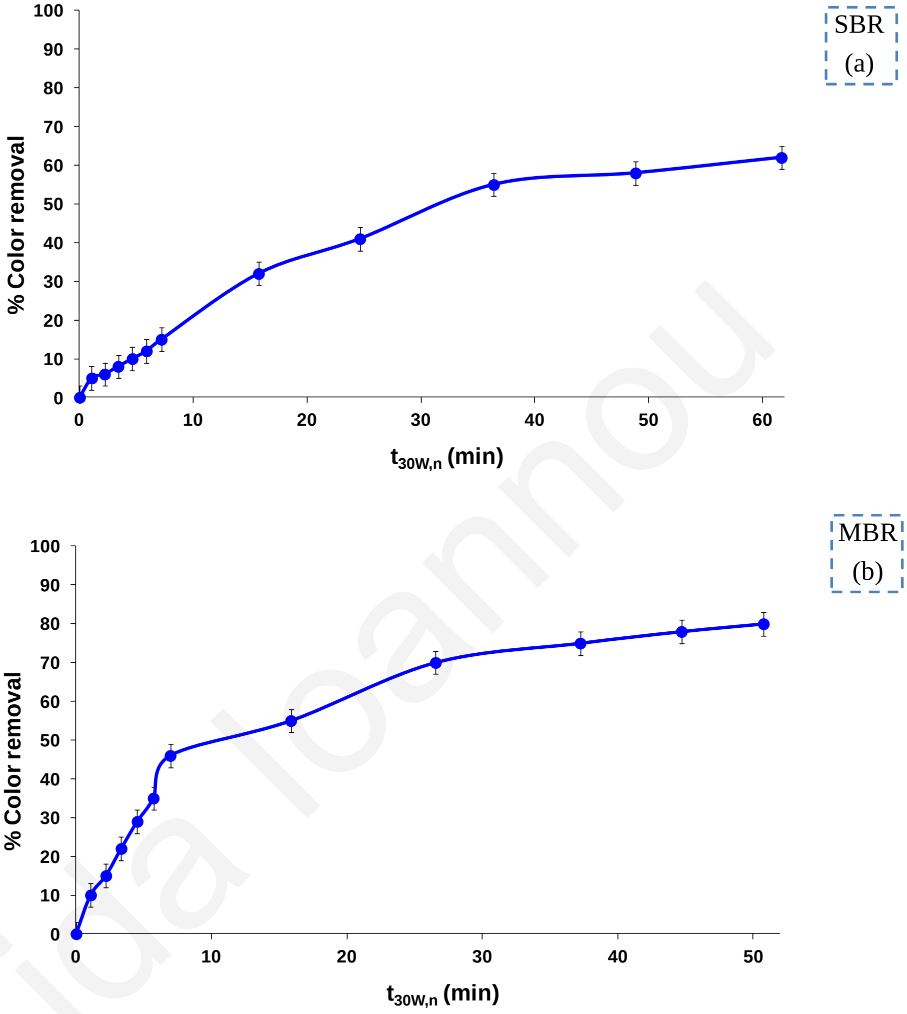


Figure 7.17: % Color removal for the winery effluents pretreated by (a) SBR and (b) MBR vs. the normalized illumination time, $t_{30W,n}$ (pilot scale); (pH=3.0) **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=350 mg L^{-1}.

In the case of the effluent pretreated by SBR, a maximum color removal of 62±3.0% (at the same time the COD removal was 69±4.0%) was observed after 180 min ($t_{30W,n}$=62 min) of treatment. It should be noted that 15±2.8% of the color removal was observed in the first 30 min ($t_{30W,n}$=7.2 min) of the process. On the other hand, a higher maximum color removal was observed for the effluent pretreated by MBR; which was equal to 80±3.2% (at the same time the COD removal was 85±2.4%) after 180 min of treatment ($t_{30W,n}$=51 min); while almost half of the color (35±2.7%) was eliminated in the first 25 min ($t_{30W,n}$=5.7 min).

7.2.2.3 DOC removal (mineralization)

DOC concentrations in the solar Fenton treated winery effluents were measured at constant Fe^{2+} concentration, at varying H_2O_2 concentrations and treatment time. Figure 7.18(a,b) shows the DOC removal as a function of the normalized illumination time, $t_{30W,n}$, which was studied in the range of $[H_2O_2]$=100-1000 mg L^{-1} and at a constant Fe^{2+} concentration of 5 mg L^{-1} for the effluent pretreated by SBR; and in the range of $[H_2O_2]$=100-750 mg L^{-1} and at a constant catalyst concentration of 3 mg L^{-1} for the effluent pretreated by MBR. The consumed H_2O_2 for the solar Fenton process vs. the normalized illumination time, $t_{30W,n}$ (at the optimum conditions: $[Fe^{2+}(SBR)]_0$=5 mg L^{-1}, $[H_2O_2(SBR)]_0$=500 mg L^{-1} and $[Fe^{2+}(MBR)]_0$=3 mg L^{-1}, $[H_2O_2(MBR)]_0$=350 mg L^{-1}) is also presented in the same figure.

In the case of the effluent pretreated by SBR, with an initial DOC equal to 74 mg L^{-1}, the DOC determination showed no substantial mineralization of the organic compounds during the solar Fenton process. The maximum DOC removal was 38±4.5% (residual DOC=46±4.0 mg L^{-1}) after an illumination time of 180 min ($t_{30W,n}$=62 min) and at the optimum hydrogen peroxide concentration of 500 mg L^{-1}. The DOC removal increased gradually by increasing the oxidant concentration from 100 to 500 mg L^{-1}, due to the formation of additional hydroxyl radicals (HO•). However, as clearly seen in Figure 7.18(a), the use of excessive concentrations of peroxide (1000 mg L^{-1}) may have even an adverse effect on the mineralization of the winery effluent.

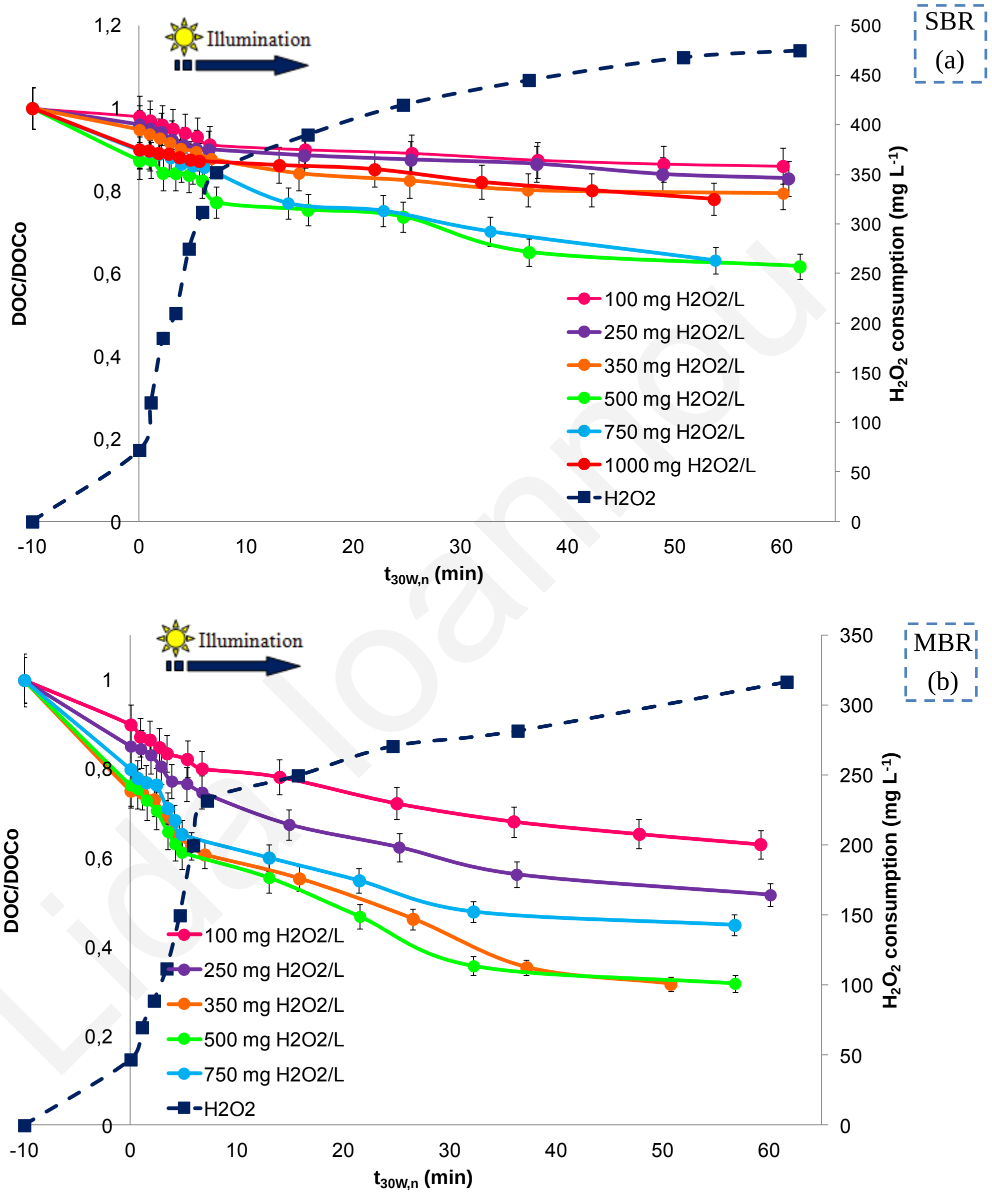


Figure 7.18: Effect of the initial H_2O_2 concentration on the DOC removal of the winery effluents pretreated by (a) SBR and (b) MBR vs. the normalized illumination time, $t_{30W,n}$ (pilot scale); (pH=3.0) **(a)** DOC_0=74 mg L^{-1}, $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=100-1000 mg L^{-1}; **(b)** DOC_0=37.5 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=100-750 mg L^{-1}.

On the other hand, the DOC determination of the effluent pretreated by MBR, with initial DOC equal to 37.5 mg L^{-1}, showed a substantial mineralization of the organic compounds during the solar Fenton process. Similarly, the DOC removal was found to increase by increasing the oxidant concentration from 100 to 350 mg L^{-1}, but further increase to 500 mg L^{-1} caused no additional reduction of the mineralization. However, the use of excessive concentration of peroxide (750 mg L^{-1}) had an adverse effect on the mineralization of the effluent pretreated by MBR, as shown in Figure 7.18(b). The highest DOC removal for the effluent pretreated by MBR was 68±2.0% after an illumination time of 180 min ($t_{30W,n}$=60 min), while the residual DOC was equal to 12±1.0 mg L^{-1}.

It should be mentioned, that the degradation of the organic compounds started 10 min before uncovering the reactor (t_{exp}=-10 min, dark Fenton), thus reducing the initial DOC values by 15 and 23% (for the optimum experimental conditions) of the effluents pretreated by SBR and MBR, respectively.

Moreover, it should be noted that for the effluent pretreated by SBR, the COD removal was found to be much higher than the DOC removal, which means that not all the oxidation products mineralized to CO_2. On the other hand for the effluent pretreated by MBR, the COD removal was found to be slightly higher than the DOC removal, and this means that more compounds present in the winery effluent pretreated by MBR undergo full mineralization to CO_2, compared to the case of the effluent pretreated by SBR.

7.2.2.4 DOC removal kinetics

As described in detail in Section 7.1.9, the winery wastewater DOC removal may be described by a pseudo first-order kinetic expression (Eq. (7.25)) ($C = C_0 \times e^{-k_{app}t}$)

where C and C_0 are the time-dependent concentration of the DOC in the winery wastewater and the initial DOC concentration respectively, and k_{app} is the apparent pseudo-first order rate constant.

The above expression resulted under the assumption that HO• radicals rapidly achieve a constant steady-state concentration in the solution, and can be included into the rate constant ($k_{app} = k \times HO^{\bullet}$), as other authors also have reported (Evgenidou et al., 2007b; Sirtori et al., 2009; Navarro et al., 2011).

The DOC reduction of both biologically pretreated flows, with solar Fenton process during the pilot-scale experiments followed apparent first-order kinetics. This was confirmed by the linear behavior of $-\ln(C/C_o)$ as a function of the normalized illumination time, $t_{30W,n}$, for the optimum experimental conditions, as shown in Figure 7.19.

The rate constant calculated for both biologically pretreated flows, when treated with solar Fenton oxidation in the pilot-scale plant, is summarized in Table 7.2.

Table 7.2: Degradation kinetic parameters for the effluent**s** pretreated by SBR and MBR

Winery effluent	**k_{app} (min^{-1})**	**R^2**
SBR	0.0149	0.9968
MBR	0.0295	0.9976

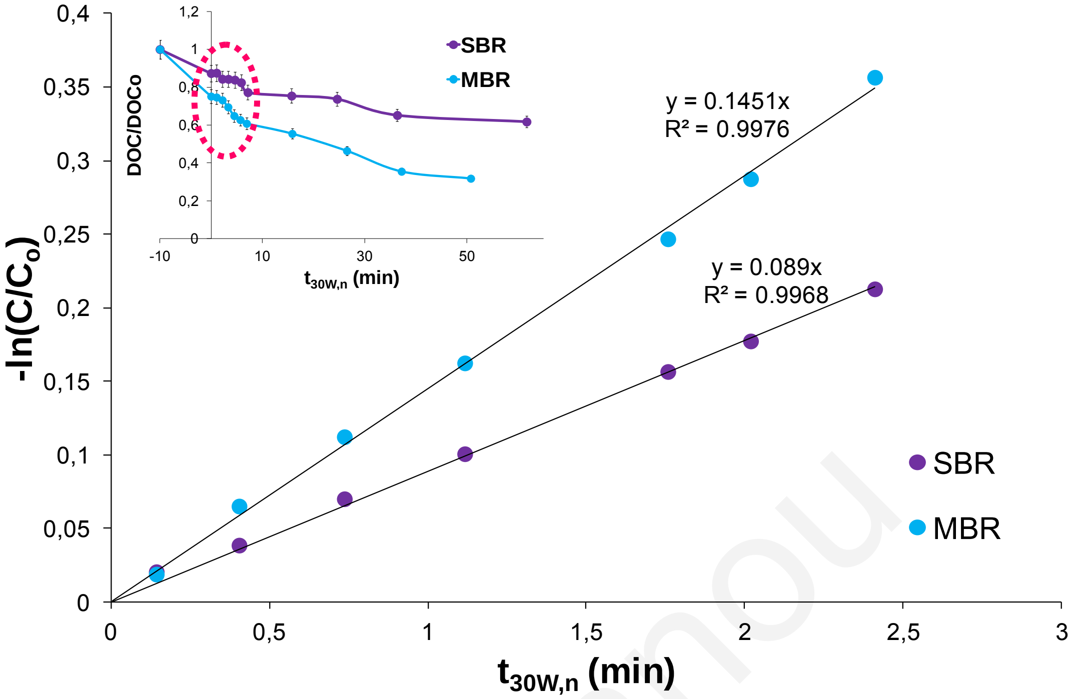


Figure 7.19: DOC removal kinetics for the winery effluents pretreated by SBR and MBR after solar Fenton oxidation at pilot scale, at the optimum experimental conditions; $[Fe^{2+}(SBR)]_0$=5 mg L^{-1}, $[H_2O_2(SBR)]_0$=500 mg L^{-1}, $[Fe^{2+}(MBR)]_0$=3 mg L^{-1}, $[H_2O_2(MBR)]_0$=350 mg L^{-1}, and pH=3.0.

Moreover, the degradation kinetic parameters described above were estimated under different conditions of the oxidant concentration (within the range of 100-1000 mg L^{-1} for the effluent pretreated by SBR, and 100-750 mg L^{-1} for the effluent pretreated by MBR), as described in Section 7.2.2.1. Each mineralization experiment described therein was used to calculate the rate constant by the linear fit, using only the experimental data from the first 15 min of reaction in order to avoid variations as a result of competitive effects of oxidation products, pH changes, etc (Michael et al., 2010). The experimental data under different concentrations of H_2O_2 fit also well the linear kinetic equation, as depicted in Figure 7.20(a,b).

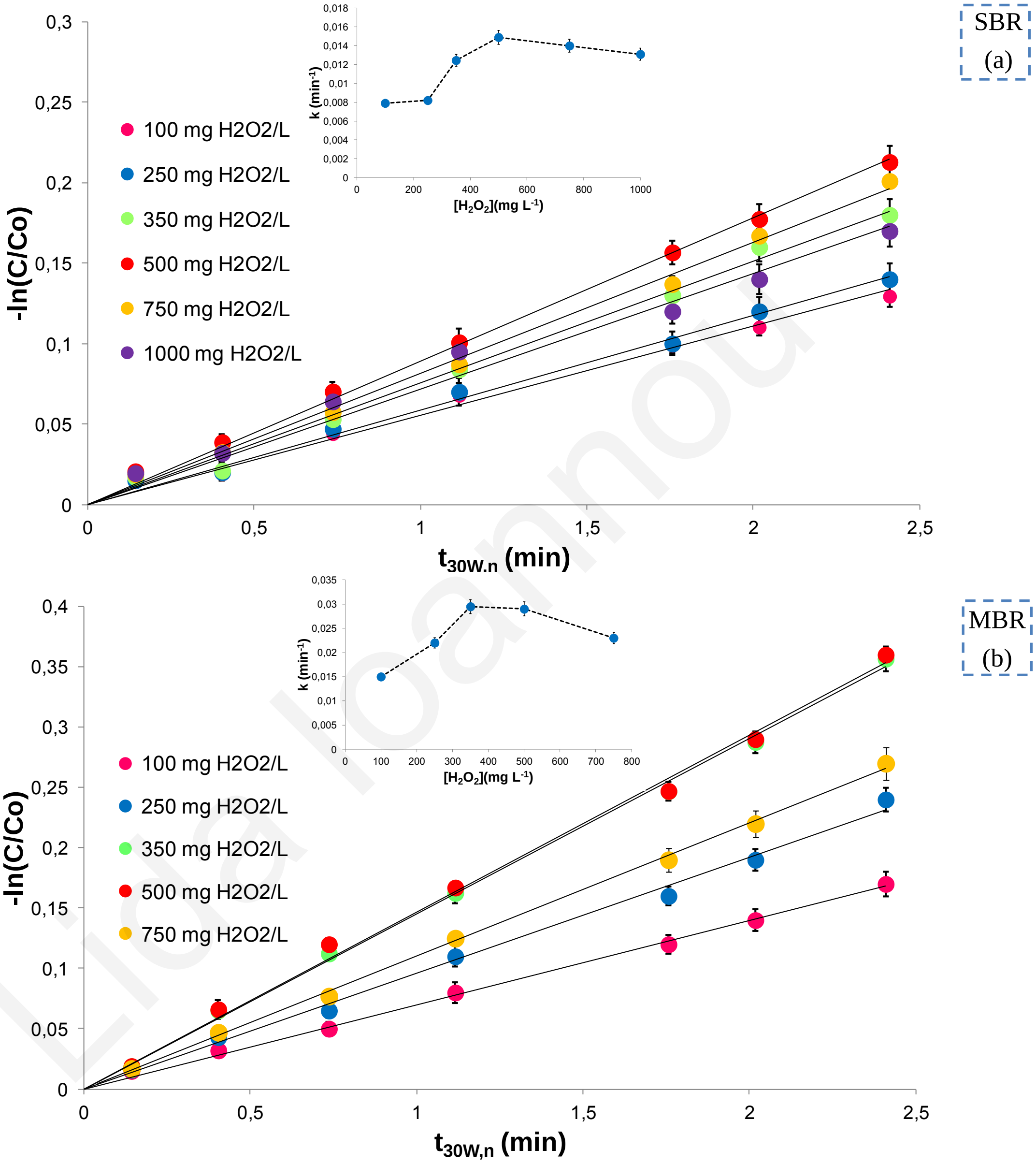


Figure 7.20: Fitting of the experimental data to the linear kinetic model for solar Fenton oxidation at pilot scale, for the winery effluents pretreated by (a) SBR and (b) MBR; Inset graphs: effect of H_2O_2 concentration on the rate constant, k; (pH=3.0) **(a)**$[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=100-1000 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=100-750 mg L^{-1}.

As shown in Figure 7.20 (inset graphs) an increase of the amount of H_2O_2 causes an increase of the reaction rate, since more HO• are produced. This increase of the reaction rate continues up to a level which corresponds to an optimum concentration (Evgenidou et al., 2007b). At higher dosages of H_2O_2, the observed decrease of the reaction rate is due to the HO• scavenging effect of H_2O_2 (Muruganandham and Swaminathan, 2004).

7.2.3 Evaluation of the toxicity

The investigation of the toxicity of the final product is an imperative task, due to the fact that oxidation products resulting during the application of solar Fenton process may be in many cases more toxic than the untreated samples. The use of biological assays can provide a direct and appropriate measure of toxicity, to complement the physicochemical measures of the quality of wastewater. Winery wastewater has a high organic, as well as inorganic load, generally low pH and high salinity, all of which indicating as mentioned already, that these effluents can pose an environmental risk (Mosse et al., 2010). Toxicity tests using *Daphnia magna* and three plant species (*Sinapis alba, Lepidium sativum, Sorghum saccharatum*) were considered suitable to evaluate the toxicity of the treated winery wastewater before its disposal into the environment (i.e. water bodies) and/or its use for agricultural irrigation, respectively, as was done for the bench-scale experimental process, as well.

It should be noted that the results are presented as a function of the actual experimental time, instead of the normalised illumination time ($t_{30W,n}$) for practical purposes and for simplicity as well, in order to use the same experimental time for all toxicity and phytotoxicity experiments.

7.2.3.1. Daphnia magna

Firstly, in order to investigate the effect of the initial winery wastewater concentration to the immobilization of *D. magna*, both biologically pretreated flows were subjected to various dilutions (initial concentrations of 100% (undiluted), 75%, 50% and 25%) and their toxicity was tested. As shown in Figure 7.21 the dilutions of both biologically pretreated flows show a moderate decrease in the toxicity.

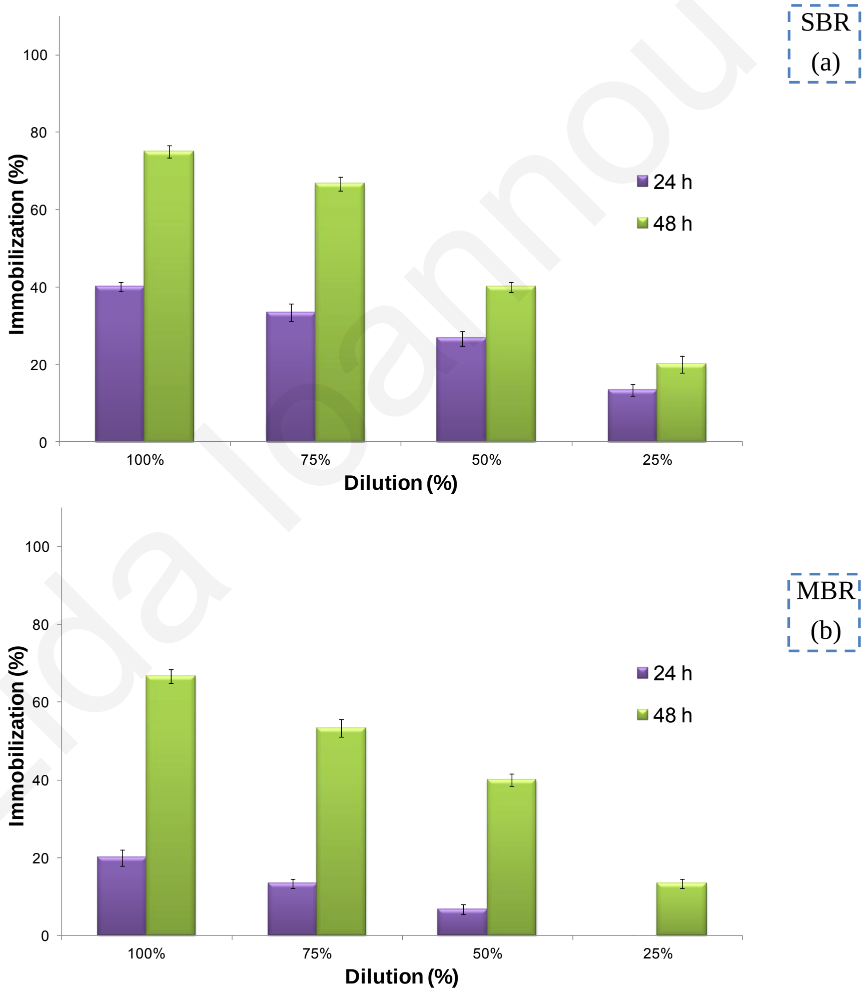


Figure 7.21: Effect of the initial concentration of the winery effluents pretreated by (a) SBR and (b) MBR on toxicity to *D. magna* for 24 and 48 h exposure times (pilot scale).

As shown in Figure 7.21(a) in the case of the effluent pretreated by SBR, the toxicity of the non-diluted sample (100%) was high (75±1.9%). At the concentration of 75% the toxicity was reduced to 33.3±2.1% and 66.7±1.9% after 24 and 48 h of exposure, while at the lower concentration of 25% this was significantly reduced to 20±2.1%, after 48 h of exposure. On the other hand, the non-diluted (100%) effluent pretreated by MBR was toxic to daphnids, only for the exposure of 48 h, with the toxicity being 67±1.7% (Figure 7.21(b)). At 75% concentration, the toxicity decreased to 13.3±1.2 and 53.3±2.3% for 24 and 48 h of exposure, respectively. Interestingly however was the fact that, at the lowest concentration of 25% no toxicity was observed for 24 h of exposure, while a slight toxicity was observed for prolonged exposure time of 48 h (13.3±1.1%).

Moreover, toxicity measurements were performed in samples collected at various times of solar Fenton process without dilutions. In the case of the effluent pretreated by SBR, this was found to be toxic to *D. magna* (45±1.2% and 75±1.9% immobilization after 24 and 48 h of exposure), as shown in Figure 7.22(a). The toxicity of the treated samples taken at 30 min of the solar oxidation was increased, and this increase is attributed to the oxidation products formed, causing toxicity effects on the daphnids especially at longer exposure times. The immobilization after the first 30 min of treatment and for 48 h of exposure was 80±2.2%. After 60 and 90 min a decrease of immobilization was observed, indicating further oxidation of the oxidation products formed at 30 min. However, after 120 min of treatment the toxicity increased again, indicating the formation of more toxic oxidation products. The immobilization after 120 min of treatment was 33±1.5% and 67±1.7% after 24 and 48 h of exposure, respectively. From that time onwards a continued decrease on toxicity was observed. After 180 min of irradiation, a significant decrease on immobilization after 48 h of exposure (13.3±1.8%) was observed, due to the potential formation of less toxic oxidation products. Finally, prolonged solar Fenton oxidation of the effluent at 240 min led to a complete reduction of toxicity.

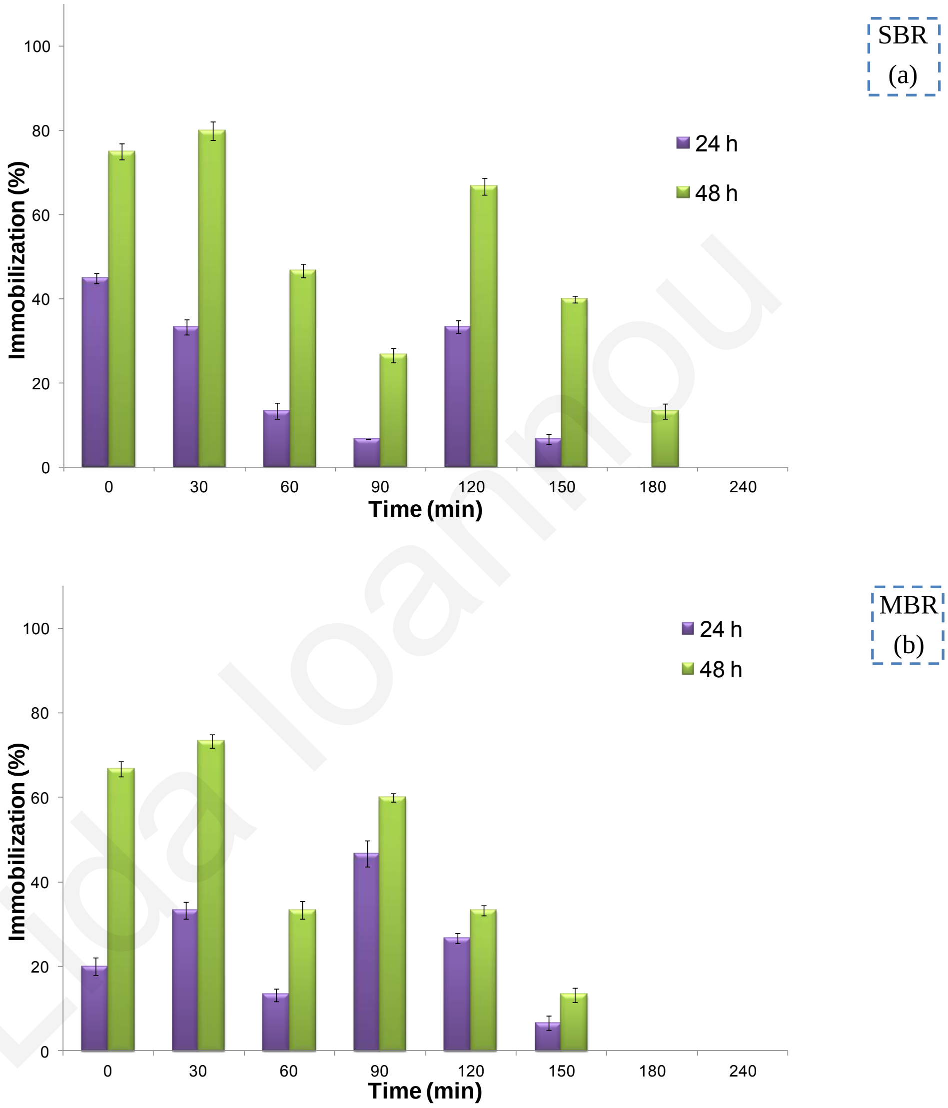


Figure 7.22: Evolution of toxicity to *D. magna* during the solar Fenton oxidation at pilot scale, for the winery effluents pretreated by (a) SBR and (b) MBR; **(a)** $[Fe^{2+}]_0$=5 mg L^{-1}, $[H_2O_2]_0$=500 mg L^{-1}; **(b)** $[Fe^{2+}]_0$=3 mg L^{-1} and $[H_2O_2]_0$=350 mg L^{-1}.

Similar was also the behavior of the winery effluent pretreated by MBR to the immobilization of *D. magna*. As shown in Figure 7.22(b), the raw effluent was found to be toxic towards *D. magna* (20±2.1% and 66.7±1.8% immobilization for 24 and 48 h of exposure), especially for prolonged exposure time. During the first minutes of solar treatment (30 min) the toxicity of the treated effluent slightly increased for both 24 and 48 h of exposure (up to 73.3±1.6%), due to the subsequent formation of toxic oxidation products. A substantial decrease of the toxicity was observed in the next 60 min, indicating the potential oxidation of the products formed at 30 min. After 90 min, the toxicity was again increased reaching the maximum value of 60±1.0% after prolonged exposure time of 48 h; indicating the potential formation of more toxic oxidation products. Further increase of the treatment time reduced the toxicity of the treated effluent. More specifically, the toxicity of the treated samples at 120 min was considerably decreased to 26.7±1.2% and 40±1.2% after 24 and 48 h of exposure, respectively. After 180 min of solar Fenton treatment, no toxicity to *D. magna* was observed for both exposure times.

From all the above, it could be summarized that the toxicity of both winery effluents increases during the first minutes of solar Fenton oxidation, due to the formation of toxic oxidation products; then usually is reduced indicating the potential oxidation of the toxic products formed, while it is possible to increase again, since more or new toxic oxidation products can be formed. Finally, after a sufficient treatment time (usually over 120 min), the toxicity is gradually reduced, and can even be eliminated at the end of the solar treatment.

In summary, the effluent pretreated by SBR seems to be more toxic to *D. magna* than the effluent pretreated by MBR, especially for the short exposure time of 24 h (45% vs. 20% immobilization, respectively). This may be due to the higher remaining organic load during the SBR treatment (as described in Chapter 5, Table 5.3). Additionally, solar Fenton oxidation was proved efficient to completely eliminate the toxicity of both biologically pretreated flows, at the pilot-scale experiments, as also happened during the bench-scale experiments (Section 7.1.10.1). In the case of the effluent pretreated by SBR, 240 min were needed, while for the effluent pretreated by MBR, 180 min were sufficient for the complete reduction of the toxicity.

7.2.3.2 Phytotestkit toxicity test

Phytotoxicity testing was conducted according to the standard testing protocols using three types of plant seeds: (i) mustard (*Sinapis alba*), (ii) dicotyls garden cress (*Lepidium sativum*), and (iii) monocotyl Sorgho (*Sorghum saccharatum*) (as described in more detail in Section 7.1.10.2). The seed germination inhibition (GI), the shoot growth inhibition (SI) and the root growth inhibition (RI) were calculated by applying the abovementioned formula (Eq. (7.27)) (see Section 7.1.10.2).

The phytotoxicity test was conducted on samples taken from the solar Fenton process (at the optimum reagents concentrations, as determined in the Section 7.2.2.1) at various times of treatment (0-240 min) for both biologically pretreated flows. The treated samples displayed a varying toxicity profile to each type of plant by means GI, SI and RI, and the results are depicted in Table 7.3.

Table 7.3: Seed germination inhibition (GI), shoot growth inhibition (SI) and root growth inhibition (RI) during solar Fenton oxidation (pilot scale)

Plant species	Treatment time (min)	Germination inhibition (%GI)*		Shoot inhibition (%SI)		Root inhibition (%RI)	
		SBR	MBR	SBR	MBR	SBR	MBR
Sinapis alba	0	10.0±1.2	10.0±2.2	42.7±3.1	37.1±3.1	47.7±3.4	29±2.2
	30	5.0±2.1	5.0±1.4	46.8±4.3	21.7±2.9	69.9±2.7	3.3±1.1
	60	5.0±1.5	5.0±1.0	18.5±2.7	28.3±2.7	57.4±4.1	24.3±3.1
	90	10.0±2.2	ni	21.6±5.1	14.0±1.8	45.5±2.5	7.9±2.7
	120	ni	ni	17.5±2.3	11.8±3.5	50.9±1.9	9.7±2.2
	150	ni	ni	14.6±2.2	10.1±2.5	17.3±2.2	5.7±1.5
	180	ni	ni	13.3±2.9	9.3±1.7	10.6±3.1	3.1±0.8
	240	ni	ni	6.9±3.4	3.0±1.9	7.3±1.2	2.1±0.5
Lepidium sativum	0	20.0±2.7	15.0±2.7	37.5±3.1	28.8±2.2	22.0±1.6	12.0±2.1
	30	10.0±1.6	5.0±1.1	41.7±4.2	27.9±3.4	55.3±3.4	9.6±2.3
	60	5.0±2.3	10.0±2.3	10.4±1.9	15.6±1.7	44.3±3.7	29.6±2.7
	90	10.0±1.9	10.0±1.8	31.2±2.4	31.4±2.6	24.8±2.6	20.5±3.5
	120	15.0±3.1	5.0±1.4	20.4±2.5	14.6±1.8	37.5±2.1	18.4±3.3
	150	15.0±1.2	ni	16.7±1.7	7.1±2.1	18.6±1.7	12.4±2.2
	180	ni	ni	12.5±3.6	6.2±1.8	11.8±1.1	8.2±1.5
	240	ni	ni	8.8±3.3	5.4±1.4	9.4±1.5	4.2±1.6
Sorghum saccharatum	0	5.0±1.1	ni	60.5±3.0	52.0±3.3	19.3±1.3	31.5±3.7
	30	ni	15.0±2.9	65.8±2.6	37.8±2.2	32.2±3.1	40.7±4.1
	60	ni	10.0±3.7	25.0±1.8	29.9±2.7	20.5±2.2	39.8±2.5
	90	ni	5.0±2.0	21.4±1.1	17.3±1.9	23.2±2.4	33.0±3.0
	120	ni	ni	46.1±2.2	29.9±2.4	23.3±2.9	25.2±2.3
	150	10±2.5	ni	28.9±3.4	17.3±2.0	15.8±3.3	20.1±3.2
	180	ni	ni	17.1±4.1	10.2±1.7	15.7±1.7	15.2±1.9
	240	ni	ni	5.9±1.6	4.7±1.1	7.8±1.2	5.2±1.1

*ni: no inhibition

Experimental conditions: $[Fe^{2+}(SBR)]_0$=5 mg L^{-1}, $[H_2O_2(SBR)]_0$=500 mg L^{-1}, $[Fe^{2+}(MBR)]_0$=3 mg L^{-1} and $[H_2O_2(MBR)]_0$=350 mg L^{-1}.

7.2.3.2.1 Germination inhibition

In general, both biologically pretreated flows did not significantly influence the seed germination, and the inhibition level ranged from 5±1.1 to 20±2.7% for the winery effluent pretreated by SBR, while for the winery effluent pretreated by MBR was between zero and 15±2.7%, for the three plant species, as shown in Table 7.3.

When the solar Fenton was applied, the phytotoxic effects on seed germination varied among the plant species and the treatment time. In the case of the winery effluent pretreated by SBR, the seed germination inhibition (GI) was 10±1.2% for *Sinapis alba*, 20±2.7% for *Lepidium sativum*, and 5±1.1% for *Sorghum saccharatum*. For *Sinapis alba* the GI showed a reduction (5±2.1%) at 30 and 60 min, followed by an increase (10±2.2%) at 90 min, probably due to the formation of toxic oxidation products, and then followed by a zero inhibition. For *Lepidium sativum*, up to 90 min, the GI behavior was more or less the same as that for *Sinapis alba*, while at 120 min an increase up to 15±3.1% was observed, which remained constant until 150 min. This could be explained by the subsequent formation of oxidation products which were found to be toxic to these seeds. Finally at 180 min of treatment, *Lepidium sativum* showed no GI. For *Sorghum saccharatum* the GI observed to the untreated effluent was remarkably lower than that of the other two plant species. Between 30 and 120 min of treatment no GI was observed, with a considerable increase at 150 min (10±2.5%), probably due to the formation of toxic oxidation products, followed by an elimination of GI at the end of the treatment (240 min).

On the other hand, in the case of the winery effluent pretreated by MBR, only a slight GI was observed before solar treatment and only for *Sinapis alba* (10±2.2%) and *Lepidium sativum* (15±2.7%). For *Sinapis alba* the GI showed a reduction at 30 min and 60 min (5±1.4%), while after 120 min no GI was observed. For *Lepidium sativum* at 30 min the GI showed a reduction (5±1.1%) with an increase at 60 and 90 min (10±2.3%). After that time a reduction was again observed with no GI at 150 min of treatment. In the case of the last plant species, *Sorghum saccharatum*, although no GI was observed for the raw effluent, at 30 min a moderate increase (15±2.9%) was determined, indicating that toxic oxidation products were formed. From that time onwards a continued decrease was observed, and the GI was eliminated after 120 min of solar Fenton treatment.

7.2.3.2.2 Shoot growth inhibition

The phytotoxicity in the treated samples expressed as shoot growth inhibition (SI), varied differently compared to the inhibition of seed germination (GI). The growth of the shoots was affected by the solar treated samples, potentially reflecting their own specific mechanisms for growth and adaption to the oxidation products. Interestingly however, was the fact that the shoot inhibition of the treated samples was considerably higher compared to the seed germination inhibition.

In the case of the winery effluent pretreated by SBR, it is noticeable, that the raw effluent showed a remarkable SI for all three species; 42.7±3.1% for *Sinapis alba*, 37.5±3.1% for *Lepidium sativum*, and 60.5±3.0% for *Sorghum saccharatum*. For *Sinapis alba* the SI showed a slightly increase of 46.8±4.3% at 30 min of solar Fenton treatment, due to the formation of toxic oxidation products. After 60 min, a significant decrease of SI was observed, indicating the oxidation of the products formed at 30 min, while a slight increase at 90 min, indicated the formation of more toxic oxidation products. Finally, further increase of the treatment time reduced the SI, reaching at the end of the treatment (240 min) a value lower than 6.9±3.4%. A quite similar behavior for *Lepidium sativum* and *Sorghum saccharatum* SI was observed during the solar Fenton treatment. For both plants species, first the SI showed a slight increase at 30 min, followed by a substantial reduction at 60 min, and a sharply increase at 90 min for *Lepidium sativum* and 120 min for *Sorghum saccharatum*. After that time a continuous reduction was observed which at the end of the treatment (240 min) was 8.8±3.3% for *Lepidium sativum* and 5.9±1.6% for *Sorghum saccharatum*. It is important to note that the SI of all plant species at the end of the solar Fenton treatment was much lower than that of the raw effluent.

In the case of the winery effluent pretreated by MBR, a quite significant SI for all the three species was observed for the raw effluent; 37.1±3.1% for *Sinapis alba*, 28.8±2.2% for *Lepidium sativum* and 52±3.3% for *Sorghum saccharatum*. The SI was then gradually reduced during the solar Fenton process with only a slight increase at 60 min for *Sinapis alba* (28.3±2.7%), at 90 min for *Lepidium sativum* (31.4±2.6%), and at 120 min for *Sorghum saccharatum* (29.9±2.7%), due to the subsequent formation of oxidation products which were toxic to plant species. The SI of all plant species decreased by further increasing of the treatment time from 120 to 240 min, indicating that the toxic oxidation products were further oxidized to less toxic ones. At the end of the process the SI was 3±1.9% for *Sinapis alba*, 5.4±1.4% for *Lepidium sativum*, and 4.7±1.1% for *Sorghum saccharatum*. Moreover, it should be noted that the maximum decrease in the SI was observed for *Sorghum saccharatum*, which at the raw effluent was 52±3.3% and decreased to 4.7±1.1% after 240 min of solar Fenton oxidation. Summarizing, the SI of all plant species at the end of the solar Fenton treatment was significantly lower than that of the raw effluent.

7.2.3.2.3 Root Inhibition

As shown in Table 7.3 the effluent pretreated by SBR, caused an inhibition of the order of 19.3±1.3 to 47.7±3.4% on root growth for the three plant species. At 30 min of solar Fenton treatment the RI of the three plants increased rapidly, and specifically from 47.7±3.4 to 69.9±2.7% for *Sinapis alba*, from 22±1.6 to 55.3±3.4% for *Lepidium sativum*, and from 19.3±1.3 to 32.2±3.1% for *Sorghum saccharatum*, due to the subsequent formation of toxic oxidation products. For *Sinapis alba*, a further increase of the treatment time caused a reduction of the RI, while at 120 min a slight increase was observed, indicating that more toxic oxidation products were formed, whereas after that it was substantially decreased to reach after 240 min a value of 7.3±1.2%. For *Lepidium sativum* and *Sorghum saccharatum*, the RI showed approximately the same trend, yielding an RI at the end of the treatment of 9.4±1.5% for *Lepidium sativum*, and 7.8±1.2% for *Sorghum saccharatum*.

The RI profile of the effluent pretreated by MBR, as can be seen in Table 7.3, is different for the three species. The RI of the raw effluent was relatively low for *Lepidium sativum* (12±2.1%), while for *Sinapis alba* (29±2.2%) and *Sorghum saccharatum* (31.5±3.7%) was higher. For *Sinapis alba* the RI showed a deep reduction at 30 min of treatment with a remarkable increase at 60 min followed by a substantial reduction, reaching 2.1±0.5% at the end of the treatment (240 min). The increase of the RI can be attributed to the oxidation products formed during the early stages of the solar Fenton process. For *Lepidium sativum* at the first 30 min of solar Fenton oxidation, the RI value was more or less the same as that of the raw effluent. At 60 min a sharp increase was observed which gradually decreased to 4.2±1.6% inhibition at the end of the treatment (240 min). For *Sorghum saccharatum* the RI observed in relation to the untreated effluent increased slightly at 30 min of treatment and remained at the same level at 60 min, and was gradually reduced to 5.2±1.1% at 240 min. All the above fluctuations are attributed to the formation and decomposition of the various oxidation products which may, depending on the nature of the product, negatively or positively affect the root development.

In summary, the examined plant species displayed a varying profile concerning their sensitivity due to the presence of the remaining organic load of the two biological pretreated flows, and also the varying response to the oxidation products formed. Seed germination inhibition of the raw wastewater was always higher than that after 180 min of solar Fenton treatment, for both winery effluents. At the end of the solar treatment (240 min) the seed germination decreased to zero for all plant species examined, and for both biologically pretreated flows. As an overall observation, GI, SI and RI evolution can be attributed to the products formed during the oxidation of the organic matters of winery wastewater, indicating the adverse effects on roots and shoots growth of all three species during the first minutes of the solar Fenton process.

In summary, solar Fenton oxidation at a pilot scale was proved to be efficient in removing the organic content of both biologically pretreated flows. Moreover, solar oxidation was able to effectively oxidize the refractory colored organic compounds, and achieve a significant color removal for both biologically pretreated flows. The extent of *mineralization was lower than the COD removal, indicating the formation of oxidation products, especially in the case of the effluent pretreated by SBR, where the difference was bigger. In addition, the DOC removal was found to follow during the solar Fenton oxidation the pseudo first-order kinetic law, in the first 15 minutes of the reaction. It should be noted that linear fitting is valid for reaction times up to 15 min. This criterion was set assuming a steady state production of HO• from hydrogen peroxide decomposition, which is likely to occur mainly during the early stages of the reaction. And finally, the results demonstrated the capacity of the solar Fenton process to significantly reduce and in some cases even completely eliminate the initial toxicity against the aquatic microorganism Daphnia magna and the examined plant species (Sinapis alba, Lepidium sativum and Sorghum saccharatum).*

Considering the fact that the winery wastewater can be discharged into the environment, mainly for vineyards irrigation purposes, it is important to comply with the national environmental limits (Cypriot Regulatory Administrative Act 772/2003: COD < 125 *mg* L^{-1}*, TP* < 2 *mg* L^{-1}*, TN* < 15 *mg* L^{-1} *and TSS* < 35 *mg* L^{-1}*). The combined MBR + solar Fenton process seems to be a more effective technology for winery wastewater treatment than the combined SBR + solar Fenton, since the first one can reduce the organic pollutants in the winery effluent to values well below those included in the Cypriot discharge limits (residual COD* < 30 *mg* L^{-1}*, TN* < 1.6 *mg* L^{-1}*, TP* < 0.4 *mg* L^{-1} *and TSS* < 10 *mg* L^{-1}*). On the other hand, SBR treatment was found to have a significantly higher residual TP value equal to 39.5 mg* L^{-1}*, which is well below the environmental limits. Moreover, the combined SBR + solar Fenton process was characterized by a higher residual COD equal to 82 mg* L^{-1} *(which however is within the* *limits), and a significant higher suspended solids load of 245 mg L*$^{-1}$*, which is a significantly higher concentration than that of the Cypriot discharge limits. As a result a subsequent filtration treatment is needed before its disposal in the environment. As a consequence, the combined MBR + solar Fenton process was proved to be the most efficient process for further upscaling to an industrial application.*

7.3 Industrial-scale solar Fenton treatment using winery effluent pretreated by membrane bioreactor (MBR)

The next objective of this study was the monitoring of the efficiency of solar Fenton as post-treatment of the most efficient biological treatment, which was the MBR, at industrial scale. For this reason an integrated winery wastewater treatment plant (WWWTP) based on a reliable biological treatment stage (MBR) followed by an advanced oxidation process (AOP, solar Fenton treatment) was constructed, in order to achieve maximum removal of pollutants and toxicity from the final effluent, as shown in Schematic 7.1. The design data of the WWWTP constructed are presented in Table 7.4.

Table 7.4: Design data of the integrated winery wastewater treatment plant (MBR followed by solar Fenton)

Design data	**Influent**
Daily Average Flow	4.0 m^3 d^{-1}
Daily peak flow	8.0 m^3 d^{-1}
Feed rate to balance tank	0.2 m^3 h^{-1}

* The design was based on the average quantities produced at an actual winery

The WWWTP consists of the following three parts (Chapter 5 and Schematic 7.1):

- Preliminary treatment (screening, equalization / balancing tank, pH adjustment)
- Biological Treatment (pre-aeration / nitrification, membrane reactor, storage / irrigation tank)
- Advanced Oxidation Process - Solar Fenton (compound parabolic collectors)

Schematic 7.2: Integrated winery wastewater treatment plant (MBR + solar Fenton)

As shown in Schematic 7.2 the solar Fenton treatment was carried out in a compound parabolic collector (solar driven industrial-scale plant) that has been placed side by side with the biological MBR treatment at the premises of the winery.

The industrial-scale study focused on the COD, DOC and color removal, as well as on the evaluation of the toxicity and phytotoxicity (i.e. *Daphnia magna, Sinapis alba, Lepidium sativum, Sorghumm saccharatum*) of the winery effluent pretreated by MBR during the solar Fenton treatment. The design and construction of the winery wastewater treatment plant was funded by the LIFE project WINEC, (Project title: Advanced systems for the enhancement of the environmental performance of WINEries in Cyprus), LIFE08/ENV/000455.

7.3.1 Solar Fenton treatment

At the industrial scale, the solar Fenton operation was carried out using the optimum catalyst and oxidant concentrations determined during the pilot-scale experiments (Fe^{2+}=3 mg L^{-1} and H_2O_2=350 mg L^{-1}) (Section 7.2.1). Figure 7.23 shows the COD and

DOC removal from the winery effluent pretreated by MBR vs. the operational time of the solar driven industrial-scale plant.

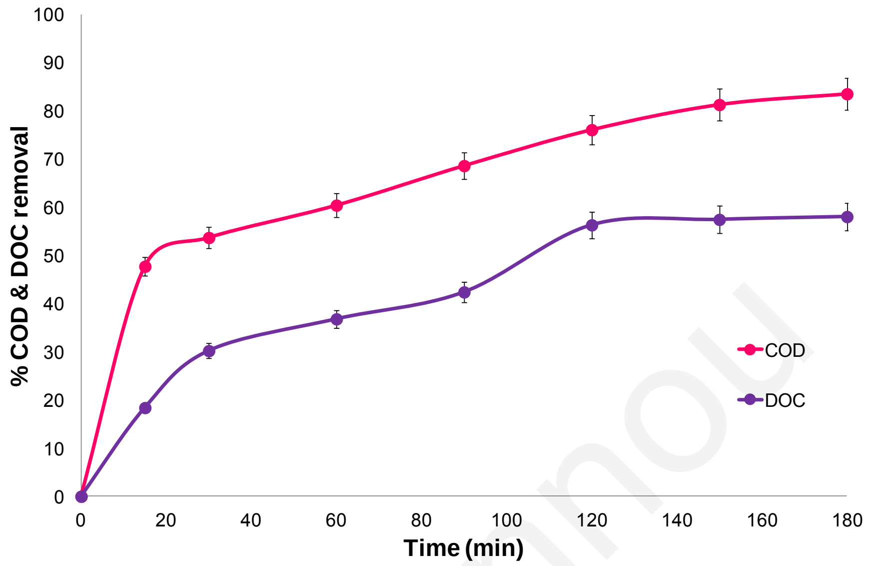


Figure 7.23: % COD and DOC removal for the winery effluent pretreated by MBR during solar Fenton oxidation (industrial scale); COD_0=180 mg L^{-1}, DOC_0=47 mg L^{-1}, $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=350 mg L^{-1}, and pH=3.0.

As shown in Figure 7.23, after 180 min of solar treatment the observed COD removal for the effluent pretreated by MBR was 84±4.0%. This was in agreement with the maximum COD reduction observed at the pilot-scale treatment (85±2.4%) (Section 7.2.2.1). On the other hand, DOC determinations showed that sufficient mineralization of the organics occur during the solar Fenton process. The DOC was reduced to 62±4.5% after illumination for 180 min. The corresponding DOC removal at the pilot-scale experiments was slightly higher (68±2.0%). This probably is the result of the slightly different composition of the initial effluent that was used for the pilot-scale experiments (Chapter 5 (Table 5.3 and 5.4)). It should be mentioned that the pilot- and industrial-scale treatment, were performed in different time periods, (during July to October, 2012), where different processes were taking place in the winery, and thus the winery effluents had quite different qualitative characteristics after the MBR treatment.

Moreover, it should be noted that the initial COD and DOC values of the winery effluent pretreated by MBR were on average equal to 180 mg L^{-1} and 47 mg L^{-1}, respectively, while the residual COD and DOC after the solar Fenton treatment at industrial scale, were equal to 28.8±7.2 mg L^{-1} and 17.9±2.2 mg L^{-1}, respectively.

Due to the fact, that winery wastewater could be discharged into the environment mainly for irrigation purposes, it is important to comply with the national environmental limits. The regulations in Cyprus (Regulatory Administrative Act 772/2003) set maximum limits of 125 mg L^{-1} COD and 35 mg L^{-1} TSS for treated effluents that can be used for irrigation and can be discharged in water dams and other water bodies; the combined MBR + solar Fenton process seems to be an effective technology for winery wastewater treatment, that can reduce these pollutants in the effluent to values well below that of the Cypriot discharge limits (residual COD < 30 mg L^{-1} and residual TSS < 10 mg L^{-1}).

In addition, as mentioned in previous sections even after the MBR treatment the winery effluent was colored (yellowish) due to the natural colors of grapes. Figure 7.24 depicts the removal of the color present in the effluent pretreated by MBR vs. the operational time at the industrial-scale operation. A maximum color removal of 82±4.6% (at the same time the COD removal was 84±4.0%) was obtained after 180 min of treatment. This was also in good agreement with the color removal for the same effluent, in the case of the pilot-scale experiments, where a percentage of 80±3.2% was removed (Section 7.2.2.2).

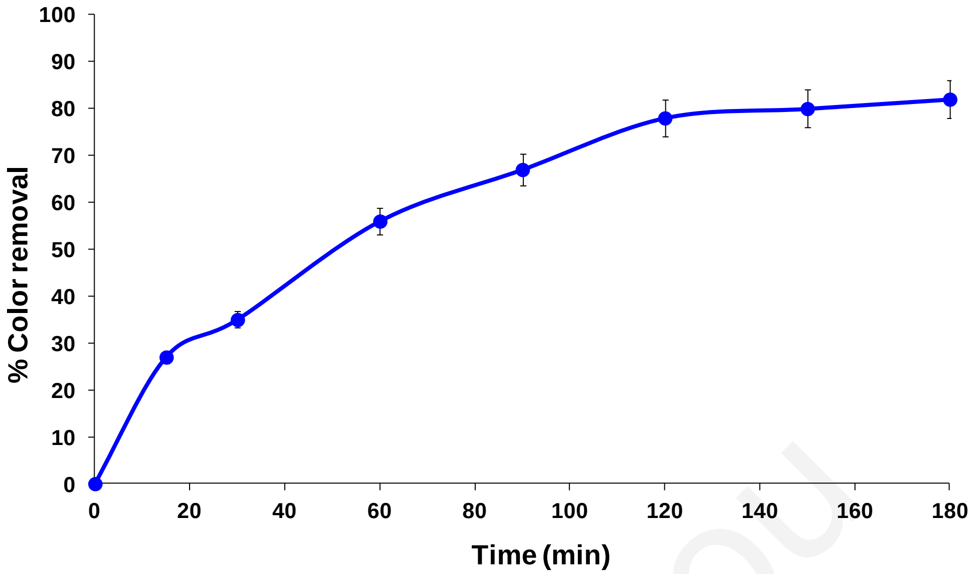


Figure 7.24: % color removal for the winery effluent pretreated by MBR during the solar Fenton treatment (industrial scale); $[Fe^{2+}]_0$=3 mg L^{-1}, $[H_2O_2]_0$=350 mg L^{-1}, and pH=3.0.

7.3.2 Evaluation of the toxicity

The evaluation of the toxicity and phytotoxicity (i.e. *Daphnia magna, Sinapis alba, Lepidium sativum, Sorghumm saccharatum*) was studied, in order to evaluate the potential toxicity of the biologically treated winery effluent, as well as the toxicity of its oxidation products formed during the various stages of the oxidation process.

7.3.2.1 Daphnia magna

In Figure 7.25, the variation of the toxicity during the industrial-scale solar Fenton treatment of the winery effluent pretreated by MBR is presented. As shown, the winery effluent pretreated by MBR was found to be moderately toxic to *D. magna* (26.7±1.2% and 53.3±2.2% immobilization after 24 and 48 h of exposure, respectively). However, the toxicity of the treated effluent at 30 min of solar treatment increased up to 66.7±1.8%

after 48 h of exposure; due to the potential formation of toxic oxidation products. After 60 min of treatment, a decrease on the immobilization of *D. magna* to 40±1.0% was observed for 48 h of exposure, which remained constant up to 90 min of treatment, while from that time onwards a continued decrease was observed. Finally prolonged solar Fenton oxidation of the winery effluent for 240 min led to a complete elimination of toxicity.

The development of the toxicity at the early stages of the solar Fenton process can be attributed to the formation of toxic oxidation products, which decompose to less toxic substances with further treatment.

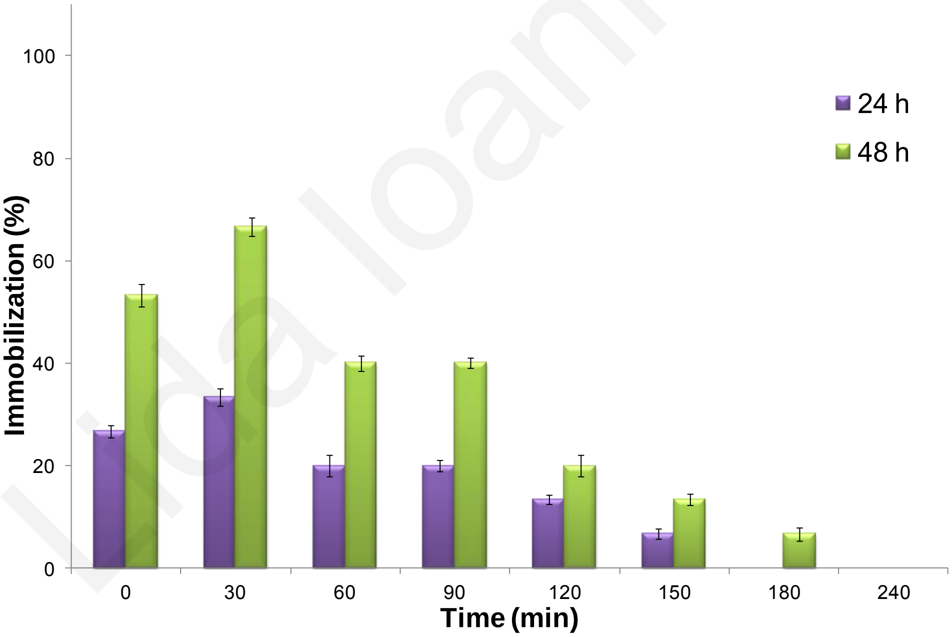


Figure 7.25: Evolution of toxicity to *D. magna* during the solar Fenton oxidation (industrial scale), for the winery effluent pretreated by MBR; $[Fe^{2+}]_0$=3 mg L^{-1} and $[H_2O_2]_0$=350 mg L^{-1}.

7.3.2.2 Phytotestkit toxicity test

Phytotoxicity testing was conducted according to the standard testing protocols using 3 type of plant seeds: (i) monocotyl Sorgho (*Sorghum saccharatum*), (ii) dicotyls garden cress (*Lepidium sativum*), and (iii) mustard (*Sinapis alba*), as already presented in previous Sections (Sections 7.1.10.2 and 7.2.3.2).

The phytotoxicity test was conducted on samples taken from the solar Fenton process at various times of treatment (0-240 min) for the winery effluent pretreated by MBR. The treated samples displayed a varying toxicity profile to each type of plant by means GI, SI and RI and the results are depicted in Table 7.5.

Table 7.5: Seed germination inhibition (GI), shoot inhibition (SI) and root inhibition (RI) of the winery effluent pretreated by MBR during solar Fenton oxidation (industrial scale)

Plant species	Treatment time (min)	Germination inhibition (%GI)*	Shoot inhibition (%SI)	Root inhibition (%RI)
Sinapis alba	0	10.0±2.1	18.2±2.1	27.4±4.1
	30	ni	18.1±1.3	42.3±2.5
	60	5.0±1.1	30.4±3.1	29.2±3.8
	90	15.0±2.3	9.7±1.7	23.3±1.9
	120	15.0±1.8	5.2±1.1	11.6±2.2
	150	5.0±0.9	2.2±0.5	10.1±3.0
	180	ni	1.9±1.0	8.2±2.5
	240	ni	1.1±0.7	6.7±2.2
Lepidium sativum	0	ni	24.0±3.1	33.5±2.7
	30	5.0±1.1	26.8±2.2	39.1±4.2
	60	5.0±1.0	34.2±1.2	20.2±1.5
	90	ni	21.0±1.6	19.7±3.1
	120	ni	10.0±1.7	12.0±4.3
	150	ni	5.9±1.9	7.2±2.5
	180	ni	4.8±1.0	5.4±2.2
	240	ni	2.3±0.6	3.8±1.4
Sorghum saccharatum	0	5.0±1.4	14.2±1.3	14.1±2.2
	30	ni	25.7±2.2	7.2±3.4
	60	ni	56.1±1.7	22.3±1.5
	90	ni	19.6±1.1	6.3±2.8
	120	10.0±2.1	18.0±1.6	5.9±2.0
	150	ni	13.6±1.4	3.9±1.1
	180	ni	9.5±1.9	2.7±1.0
	240	ni	7.5±1.2	1.6±0.5

*ni: no inhibition

Operational conditions: $[Fe^{2+}]_0$=3 mg L^{-1} and $[H_2O_2]_0$=350 mg L^{-1}

7.3.3.2.1 Germination Inhibition

At the industrial-scale solar Fenton application, as shown in Table 7.5, the winery effluent pretreated by MBR showed quite the same GI as that observed with the pilot-scale plant (see Table 7.3). On the other hand the solar Fenton process caused at the beginning a slight negative influence on seed germination, which however disappeared during further treatment, for all the species tested.

More analytically, as shown in Table 7.5, the effluent pretreated by MBR caused a slight inhibition of the order of 0 to 10.0±2.1% on the germination for the three plants species. On the other hand, the solar Fenton process did not exert further influence on seed germination. After 30 min of solar treatment, the *Sinapis alba* and *Sorghum saccharatum* GI were reduced to zero, while *Lepidium sativum* GI was higher than that of the raw effluent (5.0±1.1%), due to the subsequent formation of oxidation products which seems to be more toxic for these seeds. At 90 min no inhibition on seed germination for *Lepidium sativum* and *Sorghum saccharatum* was observed, while for *Sinapis alba* the GI increased to 15.0±2.3%. Interestingly, was the fact that at 120 min an increase GI for *Sorghum saccharatum* was observed (10.0±2.1%), which was zero at the previous times, as well as for *Sinapis alba* (15.0±1.8%), possibly due to the formation of more phytotoxic oxidation products. The GI of all plant species was decreased by further increasing of the treatment time, while no inhibition was detected after 240 min of treatment, indicating that less toxic oxidation products were formed at the end of the process. As clearly shown from the above, the phytotoxic effects on seed germination varied among the plant species and the treatment time.

7.3.3.2.2 Shoot growth inhibition

The growth of shoots was affected by the solar Fenton treated samples, potentially reflecting their own specific mechanisms for growth and adaption to the oxidation products. As can be seen in Table 7.5, the winery effluent pretreated by MBR caused moderate SI, 18.2±2.1% for *Sinapis alba*, 24±3.1% for *Lepidium sativum*, and 14.2±1.3% for *Sorghum saccharatum*.

When the solar Fenton treatment was applied, the phytotoxic effects on SI varied among the plant species and the treatment time. The SI of *Sinapis alba* and *Lepidium sativum* remained almost constant at the first 30 min of solar Fenton treatment, while the SI of *Sorghum saccharatum* was increased yielding 25.7±2.2% inhibition. After 60 min, a significant increase of SI for all plant species was observed, indicating the subsequent formation of phytotoxic oxidation products. More specifically, the SI at 60 min for *Sinapis alba* was increased to 30.4±3.1%, for *Lepidium sativum* to 34.2±1.2%, and for *Sorghum saccharatum* to 56.1±1.7%. The SI of all plant species was decreased by increasing the treatment time from 90 to 240 min, which at the end of the treatment (240 min) was 1.1±0.7% for *Sinapis alba*, 2.3±0.6% for *Lepidium sativum*, and 7.5±1.2% for *Sorghum saccharatum*. It should be noted that the SI of all three plants at the end of the solar treatment was substantially lower than that of the raw effluent, indicating that the toxic oxidation products were further oxidized to less toxic substances.

7.3.3.2.3 Root growth inhibition

The phytotoxicity of the treated samples expressed as root growth inhibition (RI), varied differently compared to the GI and SI. The RI of the raw winery effluent was relatively low for *Sorghum saccharatum* (14.1±2.2%), while for *Sinapis alba* (27.4±4.1%) and *Lepidium sativum* (33.5±2.7%) was higher. After the first 30 min of solar treatment the RI increased rapidly for *Sinapis alba* and *Lepidium sativum*, gained an RI value equal to 42.3±2.5% and 39.1±4.2%, respectively. On the other hand, the RI of *Sorghum saccharatum* was decreased to the half value of the raw effluent at 30 min. A continued decrease was observed for that time onwards for *Sinapis alba* and *Lepidium sativum*, while for *Sorghum saccharatum* an additional increase was observed at 60 min, due to the potential formation of toxic oxidation products. Then the RI was sharply decreased. At the end of solar Fenton treatment the RI was lower than that of the raw effluent for all plant species, indicating the removal or transformation of the toxic products throughout the process. More specifically, the RI at 240 min of treatment was 6.7±2.2% for *Sinapis alba*, 3.8±1.4% for *Lepidium sativum*, and 1.6±0.5% for *Sorghum saccharatum*.

In summary, the biological treatment applied in this industrial-scale study (membrane bioreactor (MBR)) proved to be an efficient solution for organic load, as well as solids removals from winery wastewater, since the biologically treated effluent has a residual COD value equal to 180 mg L^{-1} *and TSS<10 mg* L^{-1}*. However, it should be mentioned that the effluent after the MBR treatment was found to be toxic to D. magna (53% immobilization after 48 h of exposure), while regarding its phytotoxicity, the winery effluent pretreated by MBR was found to cause a moderate inhibition on the seed germination, shoot and root growth of the three plant species tested.*

The combination of the biological treatment (MBR) and solar Fenton oxidation, was proved to be quite beneficial for the treatment of winery wastewater, since the solar Fenton oxidation can lead to elimination of the toxicity to D. magna, high reduction of *phytotoxicity (almost complete, inhibition lower than 7.5%), as well as to further reduction of COD, DOC and color of the order of 84±4.0%, 62±4.5% and 82±4.6%, respectively. The treated effluent resulting from the combined MBR and solar Fenton process has a residual COD of 29 mg* L^{-1} *and can be discharged into the environment, since its characteristics are well below the requirements of the national environmental limits (i.e.* $COD<125$ *mg* L^{-1}*). Moreover, since the final winery effluent was found to be non toxic to D. magna (an aquatic microorganism) and cause minor inhibition to the growth of three plant species compared to the inhibition caused by MBR alone (which was even up to 33.5±2.7%), it can be used for irrigation purposes or for enrichment of surface/groundwater sources more safely.*

7.4 Reverse osmosis (RO) treatment of winery wastewater at pilot scale

As shown from the literature review that was performed in Chapter 3 (Section 3.2), limited studies exist so far for the treatment of winery wastewater by membrane filtration and separation processes (Ferrarini et al., 2001). Thus, in order to have an integrated view of the treatment of winery wastewater, the efficiency of a membrane separation process for the purification of winery wastewater was also investigated in this thesis. More specifically, reverse osmosis (RO) was selected to be used as an alternative to the biological and advanced chemical treatment of winery wastewater, since it is the tightest possible membrane process in liquid/liquid separation, and it is well known that produces the highest water quality of any pressure driven membrane process (Simate et al., 2011).

Due to the very strict feedwater requirements of the RO process, mainly regarding the concentration of the suspended solids that may cause fouling problems to the membrane (Bodalo-Santoyo et al., 2003); the raw winery wastewater was first centrifuged and then filtered through glass microfilter (Whatman, 1 μm) before fed to the RO unit (as described in more detail in Section 5.1.3.1). Although these two pretreatment steps constituted a sufficient and practicable solution for bench and pilot-scale applications, in order to prevent membrane fouling and reduced flux, in full-scale applications these two techniques are not applicable. According to Braz et al. (2010), however, coagulation / precipitation and / or microfiltration (MF) / ultrafiltration (UF) are more appropriate processes for full-scale pretreatment of winery wastewater.

The main objectives of this pilot-scale study performed at the Technical University of Crete (TUC) were the following:

- the assessment of the efficiency of RO to remove the organic load from winery wastewater, by examining firstly, using a single-pass mode, the optimum experimental conditions (i.e. pressure and temperature) of the RO process, during which the maximum volume recovery could be achieved, as well as the toxicity of the permeate resulting from the RO process against one aquatic and three plant species, since the treated winery wastewater can be disposed in the environment or used for irrigation purposes (e.g. vineyards),
- the assessment of the efficiency of the RO treatment of winery wastewater, when used in recirculation mode at the optimum experimental conditions determined during the experiments using the single-pass mode, in order to investigate the potential increase of the volume recovery, as well as the maximum organic load removal that could be achieved,
- the investigation of the concentration of the phenolic compounds presented in the RO concentrate stream of the winery wastewater, with a view to future recovery, due to being high-value natural compounds. For this reason, a high performance liquid chromatography (HPLC) method was developed for studying the phenolic compounds concentration of winery wastewater during the RO process, and finally,
- since RO process does not really destroy the pollutants, but merely concentrates them into a smaller volume of wastewater, the RO concentrate; the investigation of the efficiency of the solar photo-Fenton oxidation for the treatment of the RO concentrate, by examining the organic load removal, as well as the reduction of its toxicity.

7.4.1 Single-pass mode RO experiments

Some of the key factors for evaluating the performance of the RO membranes are the permeate rejection (of volume, COD, TN, TP, phenolic compounds, etc.) and the water flux (Madaeni and Mansourpanah, 2003; Nataraj et al., 2006). In the RO membrane process, the separation ability of the membrane towards specific compounds can be established in terms of the solute rejection coefficient (Rj) defined according to Eq. (7.29), a relationship that has been widely applied (Ferrarini et al., 2001; Nataraj et al., 2006; Cséfalvay et al., 2008; Vourch et al., 2008; Díaz-Reinoso et al., 2009; Conidi et al., 2011).

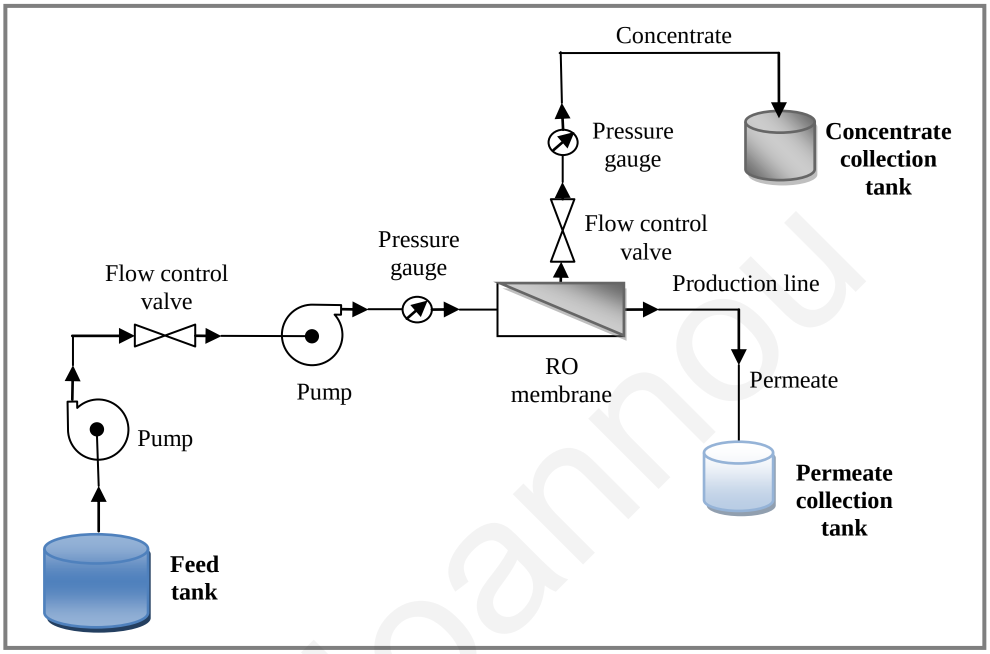
$$Rj = (1 - \frac{C_p}{C_f}) \times 100 \tag{7.29}$$

where C_p represents the concentration of a particular component in the permeate, and C_f is the feed concentration of this particular component. Rj measures the fraction of the solute retained by the membrane (Díaz-Reinoso et al., 2009).

Additionally, flux or water flux (J) is used to express the rate at which water permeates a reverse osmosis membrane; and is typically expressed as volume (V) per membrane area (A) per unit of time (t) as shown in Eq. (7.30) (Madaeni and Mansourpanah, 2003; Nataraj et al., 2006).

$$J = \frac{V}{A \cdot t} \tag{7.30}$$

The simple case of single-pass operation of the RO process is depicted in Schematic 7.3. In this configuration a constant feed rate and composition are utilized. As shown, the concentrate and permeate streams are removed separately from the membrane module. Furthermore, in a single-pass mode the energy requirements, water velocity, and shear force are low compared to those of the recirculation mode (Bourgeous et al., 2001).



Schematic 7.3: Flow diagram of the RO process in single-pass mode.

7.4.1.1 Effect of feed pressure and temperature

Reverse osmosis is a pressure-driven process whereby a semi-permeable membrane rejects dissolved constituents present in the feed water. This rejection is due to size exclusion, charge exclusion and physical-chemical interactions between solute, solvent and membrane. The process efficiency depends on operational parameters (e.g. feed pressure, temperature), as well as on the membrane and feed water properties (Al-Bastaki, 2004; Malaeb and Ayoub, 2011).

The effect of pressure on the volume recovery and the COD rejection through the RO module, while keeping the temperature at 26 ^{o}C, which was the natural winery wastewater temperature, is depicted in Figure 7.26. As shown clearly, the volume recovery increased almost linearly with pressure, which is in agreement with other studies (Ferrarini et al., 2001; Nataraj et al., 2006). Metcalf and Eddy (2008) reported that as the pressure increases the flux increases linearly, and the product quality increases as well. More specifically, the volume recovery increased from 10.8 to 24.3% and to 50.4% when the feed pressure increased from 5 to 7.5 and finally to 10 bar. Since membrane filtration is a pressure driven process as mentioned before, the permeate flux increases with increasing pressure. Additionally, the COD rejection increased from 90 to 96.7% when the feed pressure increased from 5 to 7.5 bar, while only a further slight increase was observed from 7.5 to 10 bar (97.4%). From all the above, 10 bar was identified as the optimum operating pressure, giving the maximum volume recovery (50.4%) and the maximum COD rejection (97.4%) of the winery wastewater.

It should be mentioned that for the specific RO unit, it was not possible to apply a pressure higher than 16 bar (although the pump was capable of providing driving pressures up to 55 bar), due to the polyvinyl chloride (PVC) piping and pre-filter casing pressure limitations.

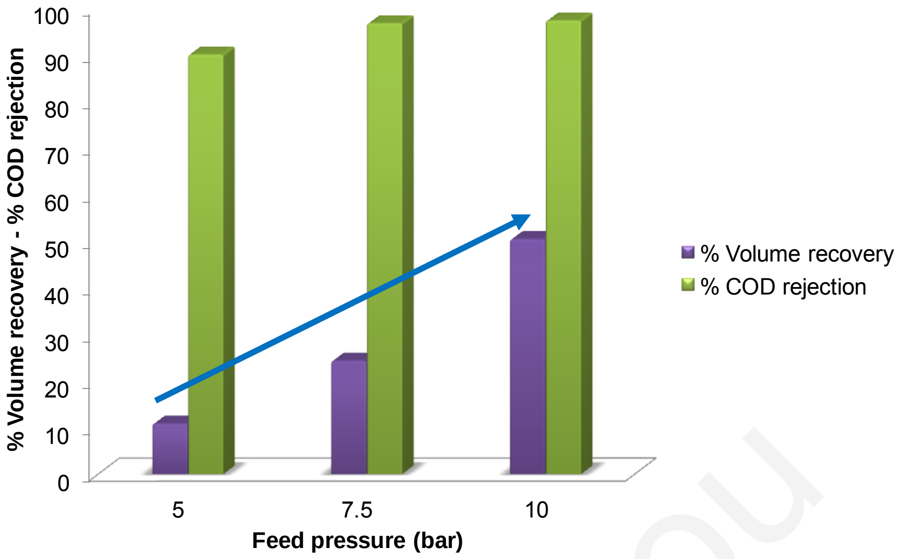


Figure 7.26: Effect of the feed pressure on winery effluents' volume recovery and COD rejection (P=5-10 bar and T=26 ^{o}C).

Moreover, Figure 7.27 shows the effect of temperature on the performance of the RO system operating at the optimum pressure (10 bar). The temperatures tested were 20 ^{o}C and 26 ^{o}C. It should be mentioned that the 26 ^{o}C was the natural temperature of winery wastewater. It was not possible to apply a temperature higher than 26 ^{o}C, due to the technical specifications of the membrane (maximum temperature 29 ^{o}C), and as a consequence, a lower temperature was tested (20 ^{o}C). A slight increase of the volume recovery was observed from 44.6 to 50.4% with increasing the temperature from 20 to 26 ^{o}C, while only a small increase on the COD rejection was also observed (96.5 to 97.4%). These results can be attributed to the reduction of the winery wastewater viscosity with increasing temperature (Schaep et al., 1998; Ferrarini et al., 2001; Metcalf and Eddy, 2008).

It should also be noted, that in order to maintain the wastewater temperature at 20 °C, ice packs were periodically placed in the feed wastewater tank; as other researchers have also applied in order to reduce the temperature of the RO feed (Song et al., 2009). As a consequence, the natural temperature of winery wastewater (26 °C) was identified as the optimum operational temperature, having also the advantage of not needing an extra effort (e.g. ice packs) in order to reach this temperature.

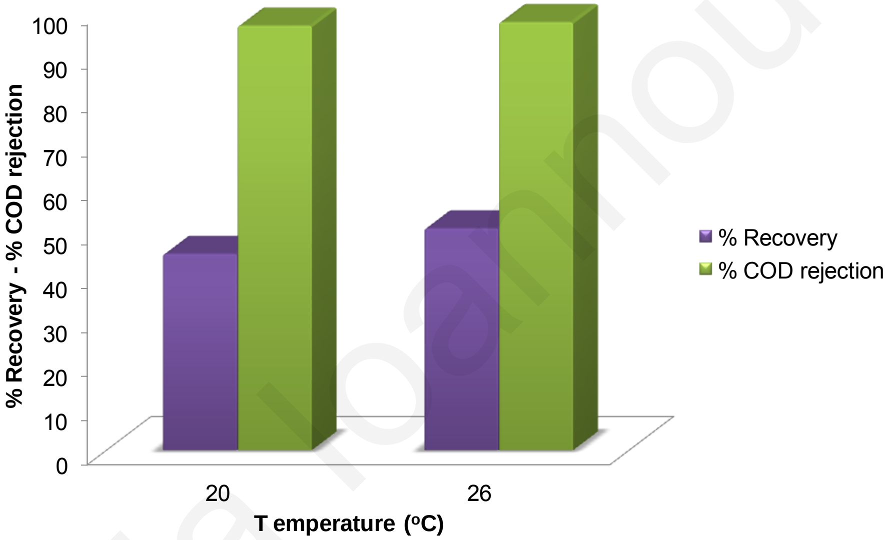


Figure 7.27: Effect of temperature on the RO volume recovery and COD rejection of winery wastewater under the optimum working pressure, (P=10 bar and T=20 and 26 °C).

7.4.1.2 Effect of the optimum operating conditions on the removal of other constituents

At the optimum RO operating conditions of 10 bar and 26 °C, the removal of some additional constituents of winery wastewater were also studied, such as total nitrogen (TN), total phosphorous (TP), total suspended solids (TSS), total solids (TS), and conductivity in order to obtain a more integrated view of the efficiency of RO process towards the purification of winery wastewater.

These chemical characteristics of winery wastewater were selected for further investigation, since these constitute indicators controlled by the existing legislation (Fumi et al., 1995; Petruccioli et al., 2002; Vlyssides et al., 2005).

Table 7.6 depicts the COD, BOD_5, TN, TP, TSS, TS and conductivity removal for the winery wastewater achieved at the optimum operating conditions (10 bar and 26 ^{o}C). As shown, a high level of purification was achieved by the reverse osmosis process. More specifically, the COD removal was 97.4% resulting in a permeate effluent with the COD value equal to 140 mg L^{-1}, the BOD_5 removal was 97.9% (9 mg L^{-1}), the TN removal was 67% (3.3 mg L^{-1}), while the TP removal was 76.2% (0.5 mg L^{-1}). Moreover, the TSS and TS removal was very high (94% (4 mg L^{-1}) and 96% (200 mg L^{-1}), respectively), and the conductivity removal was 94% (0.182 mS cm^{-1}).

Table 7.6: Purification of winery wastewater through reverse osmosis

(discharge limits for the treated effluents released for irrigation and into surface waters corresponding to Cypriot Regulatory Administrative Act 772/2003 and the Greek FEKB΄534/2011)

Constituents	**Feed**	**Concentrate**	**Permeate**		**Discharged limits**	
	Concentration	Concentration	Concentration	% Reduction	Cypriot Law 772/2003	Greek FEKB΄ 534/2011
COD (mg L^{-1})	5353	10290	140	97.4 %	125	-
BOD_5 (mg L^{-1})	424	812	9	97.9%	-	-
TN (mg L^{-1})	10	17	3.3	67 %	15	5
TP (mg L^{-1})	2.1	3.5	0.5	76.2 %	2	-
TSS (mg L^{-1})	66	110	4	93.9 %	35	10
TS (mg L^{-1})	5040	8745	200	96 %	-	-
Conductivity (mS cm^{-1})	3.34	5.12	0.182	94.6 %	-	-

Considering the fact that the treated winery wastewater can be discharged in the environment mainly for irrigation purposes, it is important to comply with the national environmental limits. The regulations in Cyprus and Greece (Regulatory Administrative Act 772/2003 and FEKB΄ 534/2011) set maximum limits of 5-15 mg L^{-1} TN, 125 mg L^{-1} COD, 2 mg L^{-1} TP, and 10-35 mg L^{-1} TSS for treated effluents that can be used for irrigation and/or can be discharged in water dams and other water bodies (Table 7.6). According to the aforementioned results, the RO process seems to be an effective technology for winery wastewater treatment, that can reduce these pollutants in the permeate to values below that of the Cypriot and Greek discharge limits (with the exception of the COD value which slightly exceeded the limit).

7.4.1.3 Evaluation of the toxicity of the three streams of the RO process

The chemistry of winery wastewater is complex and variable, containing numerous organic, as well as inorganic compounds whose individual and combined contribution to toxicity and plant phytotoxicity is not known (Arienzo et al., 2009b; Mosse et al., 2010). Hence, it was imperative to investigate the possible toxicity of the permeate winery effluent of RO process, as well as the initial toxicity of the feed stream, and the possibly increased toxicity of the concentrate stream. This would enable a more complete evaluation of the efficiency and environmental safety of the applied technology.

Toxicity tests using *Daphnia magna* and three plant species (*Sinapis alba, Lepidium sativum, Sorghum saccharatum*) were considered suitable to evaluate the toxicity of the treated winery wastewater before its disposal in the environment.

7.4.1.3.1 *Daphnia magna*

Toxicity measurements on *D. magna* were carried out using the permeate, in order to ensure that a significant reduction of the toxicity through the RO process was achieved. Moreover, for comparison purposes, in order to have an integrated view of the evolution of the toxicity through this process, toxicity measurements were also carried out using the feed wastewater and the concentrate, at varying dilutions, as shown in Figure 7.28.

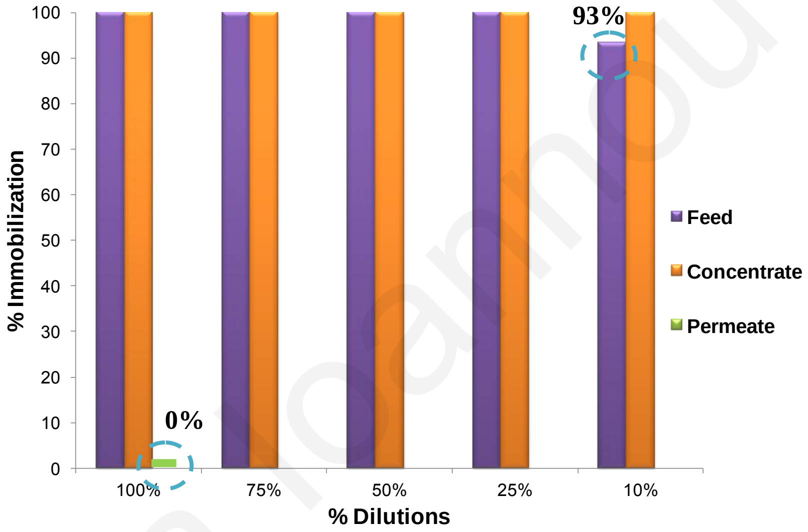


Figure 7.28: Effect of the initial concentration of the three streams of the RO process on the toxicity towards *D. magna* after 24 h of exposure, (P=10 bar and T=26 ^{o}C).

Both the feed wastewater and the concentrate stream even after substantial dilutions (initial winery wastewater concentrations up to 10%) showed high toxicity to *D. magna* (100% immobilization of microorganism) after prolonged exposure of 48 h. Only in the case of the feed wastewater and at the lower concentration of 10%, the toxicity slightly decreased to 93.3% for the short exposure time of 24 h. This high toxicity was expected taking into consideration the high organic content of both winery streams (COD ranges from 5353 to 10290 mg L^{-1}).

In contrast, the undiluted permeate stream proved to be non toxic to *D. magna*, for both times of exposure (0% immobilization); and thus further dilutions for the permeate stream were not required. It should be noted that the experiments were performed in triplicate and the relative standard deviation was always zero. These results show that the toxicity of the winery wastewater towards *D. magna* can be completely eliminated through the RO process (0% permeate immobilization of *D. magna*).

7.4.1.3.2 Phytotestkit toxicity test

Phytotoxicity testing was conducted according to the standard testing protocols using three types of plant seeds: (i) mustard (*Sinapis alba*), (ii) dicotyls garden cress (*Lepidium sativum*), and (iii) monocotyl Sorgho (*Sorghum saccharatum*) (as described in more detail in Section 7.1.10.2). The seed germination inhibition (GI), the shoot growth inhibition (SI) and the root growth inhibition (RI) were calculated by applying the following formula (Eq. (7.27)) ($\%GI, SI \,\&\, RI = \frac{A-B}{A} \times 100$), where A is the average number of germinated seeds or the average shoot length or the average root length in the control water and B the same parameters for the test wastewaters. The GI, SI and RI profile for the various RO streams is shown in Table 7.7.

Table 7.7: % effect of reverse osmosis treated winery streams on the seed germination, shoot and root growth inhibition

Plant species	**FEED**			**CONCENTRATE**			**PERMEATE**		
	%GI	%SI	%RI	%GI	%SI	%RI	%GI*	%SI	%RI
Sinapis alba	5±1.1	66.3±3.4	71.8±4.2	30±2.2	93.1±3.1	93.1±2.0	ni	2.5±0.9	3.9±0.5
Lepidium sativum	15±2.3	46.1±2.9	71.6±3.3	15±3.0	72.1±2.8	85.1±2.5	5±1.1	15.0±1.0	4.7±0.8
Sorghum saccharatum	5±1.5	49.6±3.1	56.4±2.4	10±1.1	81.8±1.6	77.4±3.7	ni	6.7±1.2	21.3±1.4

*ni: no inhibition
Operating conditions: 10 bar, 26 ^{o}C, single-pass mode

As shown in Table 7.7, the feed wastewater caused a moderate GI for all three plants, of the order of 5±1.1 to 15±2.3%, and more specifically 5±1.1% for *Sinapis alba*, 15±2.3% for *Lepidium sativum*, and 5±1.5% *Sorghum saccharatum*. It is noticeable that the feed stream, showed remarkable SI and RI for all three plant species, of the order of 46.1±2.9 to 66.3±3.4%, and 56.4±2.4 to 71.8±4.2%, respectively.

Additionally, the GI of the concentrate stream was increased compared to that of the feed stream, and was 30±2.2% for *Sinapis alba*, and 10±1.1% for *Sorghum saccharatum*, while no difference from the feed wastewater was observed for *Lepidium sativum* (15±3.0%). The SI of the concentrate stream for all three species was also increased compared to that of the feed stream, reaching a maximum value of 93.1±3.1% for *Sinapis alba*. The RI of the concentrate stream however ranged from 77.4±3.7 to 93.1±2.0% for the three plants, which was approximately 20% higher than that of the feed winery wastewater.

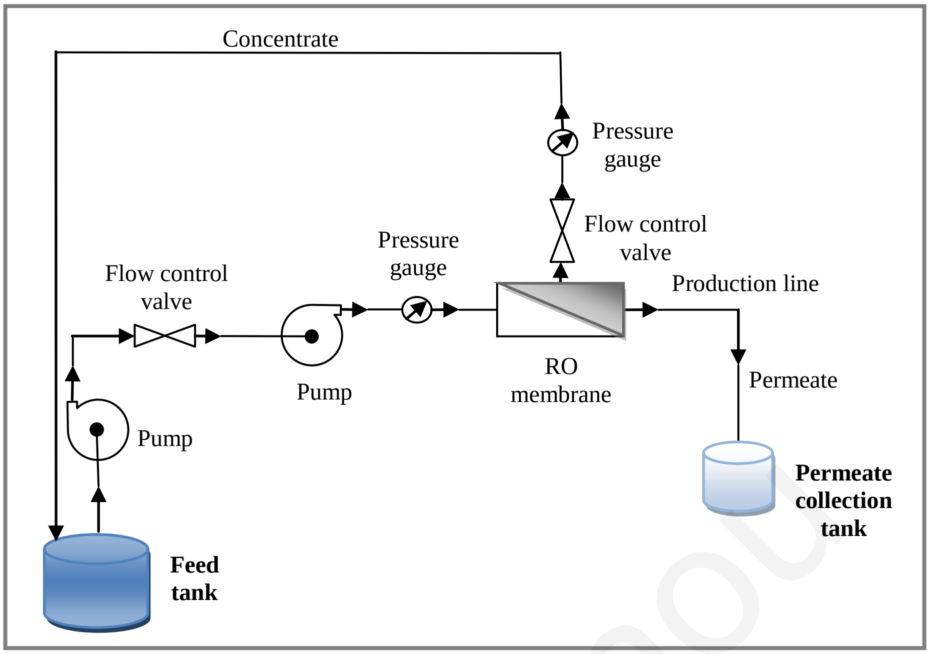
On the contrary, the permeate stream showed no GI for *Sinapis alba* and *Sorghum saccharatum*, while for *Lepidium sativum* the GI was only 5±1.1%. The SI caused by the permeate stream was significantly decreased compared to the feed wastewater, to values equal to 6.7±1.2% for *Sinapis alba*, 15±1.0% for *Lepidium sativum*, and 2.5±0.9% for *Sorghum saccharatum*. Finally, the RI was decreased sharply for the permeate stream compared to that of the feed stream, being 3.9±0.5% for *Sinapis alba*, 4.7±0.8% for *Lepidium sativum*, and 21.3±1.4% for *Sorghum saccharatum*.

Summarizing, it is obvious, that the phytotoxicity (GI, SI and RI) for the three plant species, was considerably reduced after the RO treatment for the permeate stream, compared to the feed winery wastewater, at values lower than 21%.

7.4.2 Recirculation mode RO experiment

The primary reason to use recirculation is to increase the volume of the permeate, and consequently to reduce the volume of the concentrate that needs further treatment, before its disposal into the environment (Bourgeous et al., 2001).

Thus, an experiment in recirculation mode was carried out, at the optimum experimental conditions determined using the single-pass mode (10 bar and 26 ^{o}C), in order to investigate the increase of the volume recovery and the maximum COD removal for winery wastewater that could be achieved. For the recirculation mode, the permeate flow was taken out of the loop, while the concentrate was returned to the feed tank, where it was mixed with the feed wastewater, as shown in Schematic 7.4.



Schematic 7.4: Flow diagram of the RO process in recirculation mode.

More specifically, the permeate flux, the experimental pressure drop (ΔP) and the permeate COD vs. experimental time were studied. Experimental pressure drop was obtained by subtracting the mean measured outlet pressure from the mean measured inlet pressure ($\Delta p=P_{in}-P_{out}$). The volume recovery achieved in the recirculation mode was 65%, while in the single-pass mode was 50%.

Figure 7.29 shows the results of flux measurements of the RO membrane during the experiment in recirculation mode. As shown, the permeate flux was decreased gradually with experimental time.

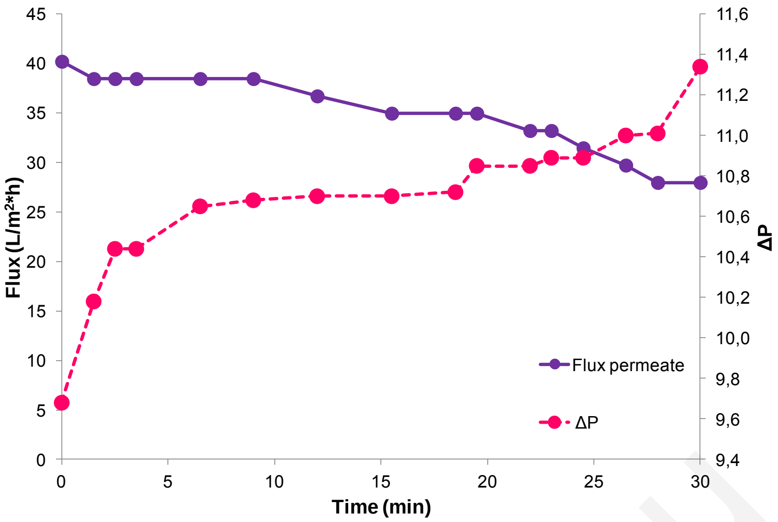


Figure 7.29: Evolution of the permeate flux and ΔP of the RO membrane vs. operation time, in recirculation mode, under the optimum operating conditions, (P=10 bar and T=26 ^{o}C).

According to Ndiaye et al. (2005), during RO with recirculation of concentrate, the concentration of the organic compounds retained by the membrane increased, and the permeate flux decreased slightly with time. This can also be described with the continuous increase of the experimental pressure drop, which increased from 9.7 to 11.3 after 30 min of recirculation, as also shown in Figure 7.29. This is a typical behavior of the membrane processes. It is well known that the flux is controlled by two phenomena, the concentration polarization and the membrane fouling (Madaeni and Mansourpanah, 2003).

Concentration polarization (CP) is one of the most important factors influencing the performance of the membrane separation processes (Mulder, 1991; Kim and Hoek, 2005). Concentration polarization results from the build-up of a boundary layer of highly concentrated solute on the membrane surface. This term is used to describe the accumulation of membrane rejected solutes close to the membrane surface. As water passes through the membrane, the convective flow of solute to membrane surface is much higher than the diffusion of the solute back to the bulk feed solution. Concentration polarization increases the osmotic pressure at the membrane surface, which causes a reduction in water flux. If the concentration of soluble species in the boundary layer exceeds their solubility limits, precipitation or scaling will occur (Madaeni and Mansourpanah, 2003). However, according to Bourgeous et al. (2001), the use of recirculation mode, increases the amount of shear acting on the membrane surface, which lessens fouling caused by extreme concentration polarization when treating concentrated feeds.

Fouling is a term generally used to describe the undesirable formation of deposits on surfaces (Amjad, 1993). Based on the terminology by IUPAC, fouling is ‘a process resulting in loss of performance of a membrane due to the deposition of suspended or dissolved substances on external surface, at its pore openings or within its pores’ (IUPAC, 1996). There are mainly four types of foulants in RO membrane fouling: inorganic (salt precipitations such as metal hydroxides and carbonates), organic (natural organic matters such as humic acid), colloidal (suspended particles such as silica), and biological (such as bacteria and fungi) (Madaeni and Mansourpanah, 2003; Kang and Cao, 2011)

During membrane fouling, foulants accumulate on the membrane surface or within the membrane pores and adversely affect both the quantity (permeate flux) and quality (solute concentration) of the product water (Lee and Elimelech, 2006). As the total accumulated mass of particles increases, the resistance of the foulant deposit layer to water flow increases and, hence, the permeate flux decreases (Zhu and Elimelech, 1997; Holloway et al., 2007), as also shown in Figure 7.29. Membrane fouling is considered a major obstacle for efficient membrane operation due to declining permeate flux, increased operational cost, and shortened membrane life (Xu et al., 2006).

The COD of the RO permeate versus the operation time in the recirculation mode is shown in Figure 7.30. During the experiment, the COD in the RO permeate increased gradually to a certain value and then remained practically constant. This is in agreement with the results obtained by other studies, which report the same behavior of the permeate COD vs. operation time in recirculation mode (Madaeni and Mansourpanah, 2003; Cséfalvay et al., 2008). The increase of the permeate COD was due to the fact that in the feeding stream the COD was increasing also with the time, since the concentrate was continuously returning to the feed tank and mixed with the feed wastewater. The COD of the RO permeate was maintained below 184 mg L^{-1} throughout the experiment, and the organic removal efficiency was over 95%.

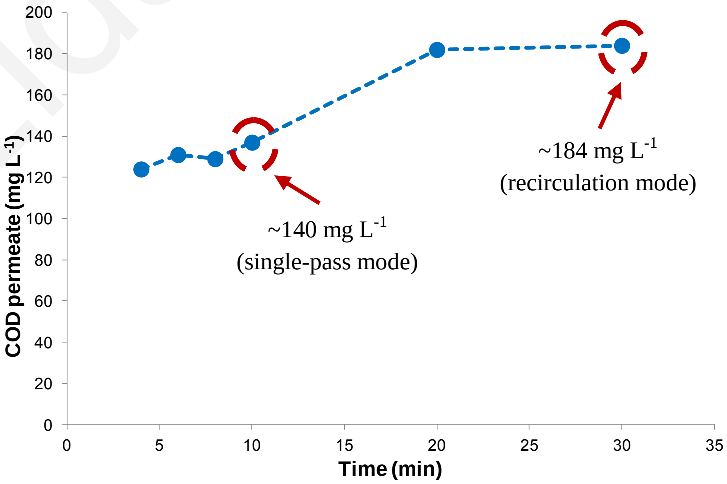


Figure 7.30: Permeate COD vs. operation time, in recirculation mode, under the optimum operating conditions, (P=10 bar and T=26 ^{o}C).

As a consequence, although the recirculation mode was found to increase the volume recovery by 15% compared to the single-pass mode, achieving its primary goal of reducing the volume of concentrate that need further treatment before its disposal in the environment, it was proved to be less efficient than the single-pass mode for the treatment of winery wastewater due to the increase of the permeate COD (by 44 mg L^{-1}).

Given the experimental setup in this work, it was not feasible to extend the duration of the recirculation run beyond 30 min. There were two reasons for this: First, the significant increase in the pressure drop, as mentioned above. The second reason was related to an increase in the temperature of the feed wastewater, due to the continuous recirculation of the concentrate, reaching membranes' maximum recommended operating temperature 29 ^{o}C.

7.4.3 Determination of phenolic compounds in the winery wastewater

According to the literature, the total phenolic compounds content of winery wastewater ranges from 5.8 to 1450 mg L^{-1} (Petruccioli et al., 2002; Bertran et al., 2004; Bustamante et al., 2005; Vlyssides et al., 2005; Mosteo et al., 2008; Arienzo et al., 2009b; Lucas et al., 2009; Mosse et al., 2011). In recent years, more emphasis is being placed upon the recovery of valuable compounds contained in agro-industrial effluents (Nataraj et al., 2006; Russo, 2007; Villanova et al., 2008), and hence to the valorization of the wastewater.

High performance liquid chromatography (HPLC) was employed to monitor the concentration of phenolic compounds in winery wastewater. The detection was achieved through two detectors connected in series, a photo-diode array (PDA) detector and a fluorescence (FL) detector, and the analyses were performed under the conditions described in the experimental Section 5.3.2 (Chapter 5).

Figure 7.31(a,b) shows the separation of the five selected low molecular weight phenolic compounds often determined in wine (gallic acid, vanillic acid, epicatechin, p-coumaric acid and ferulic acid) according various other studies (Nigdikar et al., 1998; Dell'Agli et al., 2004; Hernández et al., 2006; Garcia-Falcón et al., 2007; Lucas et al., 2008; Strong and Burgess, 2008; Mosse et al., 2012); by the method developed during this thesis (Section 5.3.2).

As shown in Figure 7.31, a good separation of the five phenolic compounds was achieved. The detection of gallic acid was done at 271 nm (t_R=5.578 min), vanillic acid at 260 nm (t_R=23.001 min), epicatechin at 278 nm (t_R=26.942 min), p-coumaric acid at 309 nm (t_R=36.277 min), and ferulic acid at 322 nm (t_R=40.134 min).

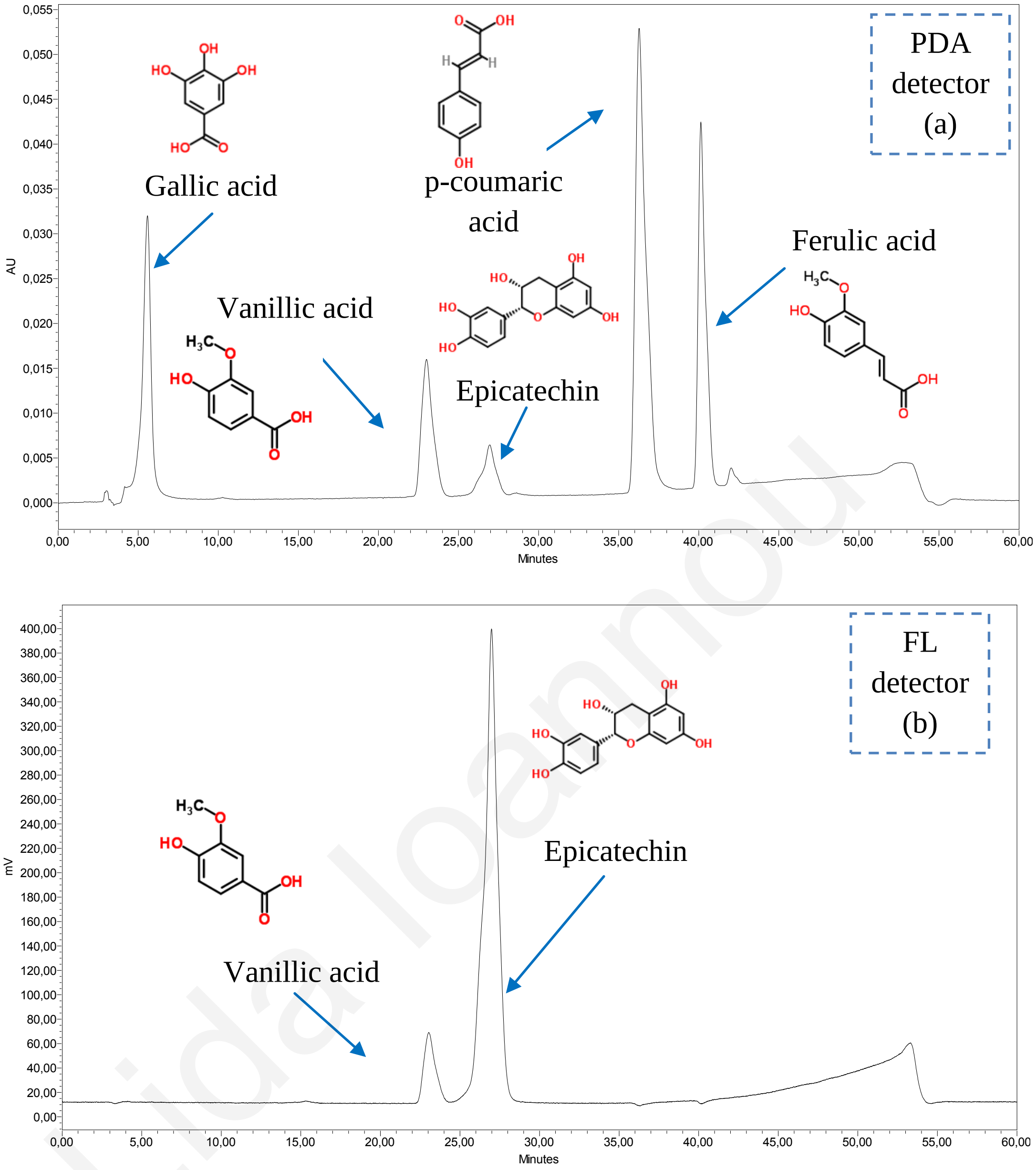


Figure 7.31: Chromatograms of a standard mixture using (a) photo-diode array detection (PDA) (λ: 280 nm), and (b) fluorescence detection (FL) (λ_{ex}: 280 nm and λ_{em}: 320 nm).

Samples of winery wastewater taken from the feed, concentrate and permeate stream of RO process, at the optimum operating conditions (10 bar and 26 ^{o}C) and at single-pass mode, were also analyzed by HPLC (Figure 7.32).

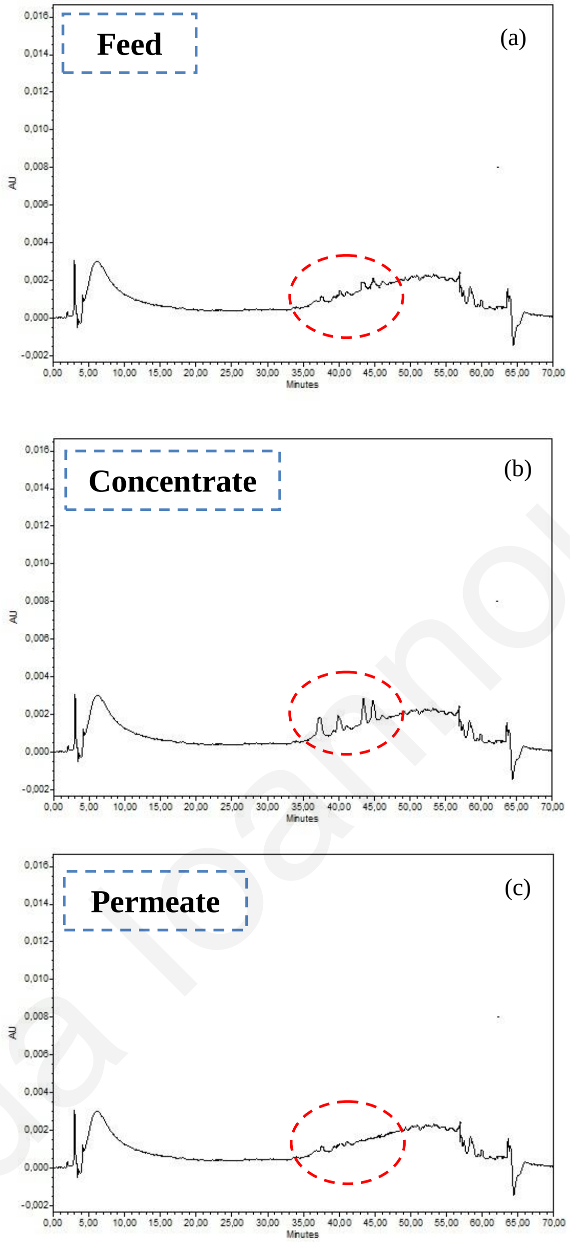


Figure 7.32: HPLC chromatograms of the (a) feed, (b) concentrate, and (c) permeate of the raw winery wastewater, after RO treatment, (P=10 bar, T=26 ^{o}C and single-pass mode).

As can be seen in Figure 7.32, the selected phenolic compounds were not identified in any of the three RO streams tested, not even in the concentrate stream. On the other hand, it should be mentioned that some other peaks existed in the chromatograms (red dashed lines in Figure 7.32), in different retention times (t_R~38-47 min) that probably imply the existence of some unknown phenolic compounds, but at low concentrations.

The absence of the selected phenolic compounds may probably be due to the fact that the particular winery effluent used, was derived mainly from white wine production, which has a low content of phenolic compounds (initial total phenolic compounds of feed wastewater 20 mg L^{-1} measured by Folin-Ciocalteu method (as described in Section 5.3.4.12)).

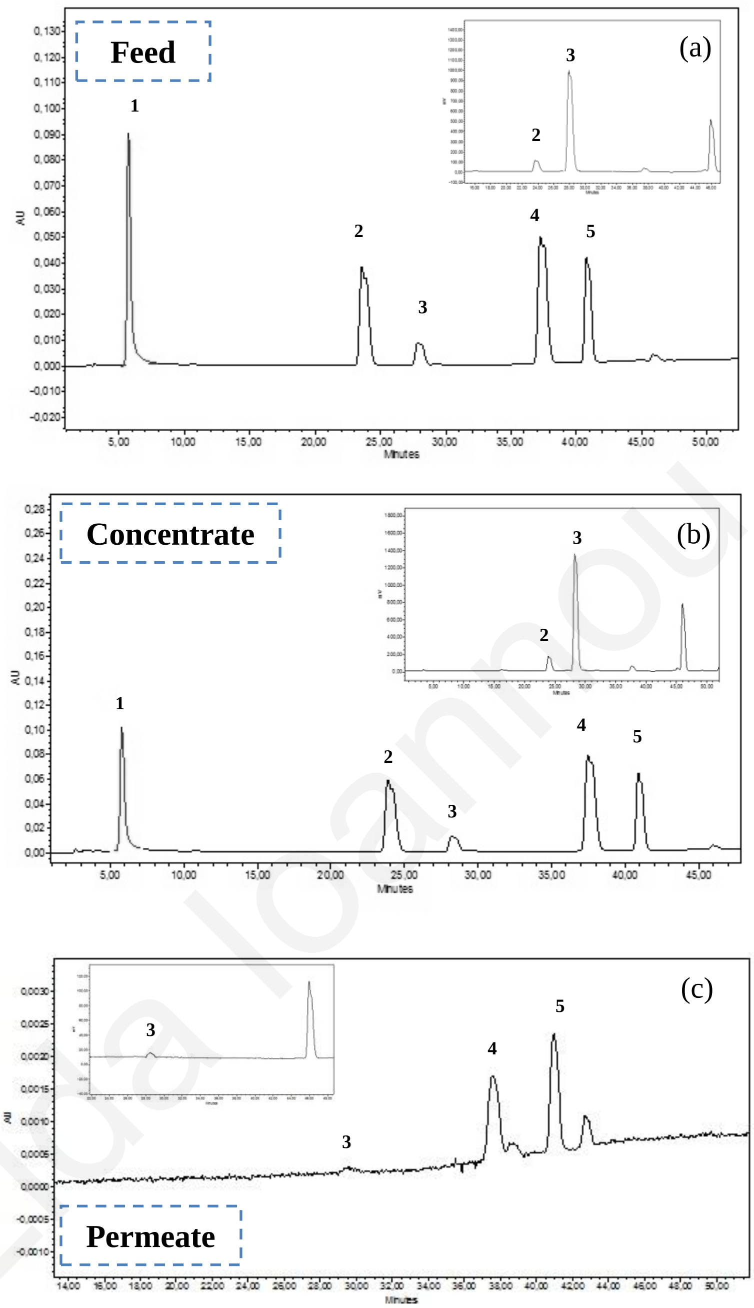
Since the first objective was the evaluation of the potential of the RO process in concentrating phenolic compounds, the feed stream was spiked with 15 mg L^{-1} of each one of the five selected phenolic compounds. More analytically, solutions containing each phenolic compound separately at initial concentration of 15 mg L^{-1} were prepared by dissolving a quantity of the powdered standard in 2 mL of methanol (MeOH, HPLC grade, >99.9%, Sigma Aldrich), and then the solution was stirred to ensure complete dissolution of the compound. The solutions of the five phenolic compounds were then spiked into the raw winery effluent contained in the feed tank (30 L) of the RO unit.

The percentage of the concentration of the phenolic compounds in the RO concentrate stream was calculated according to Eq. (7.29), where C_p is the concentration of phenolic compounds in the permeate stream (mg L^{-1}), and C_F is the concentration of phenolic compounds in the feed stream (mg L^{-1}).

It was concluded, that almost complete concentration of the five phenolic compounds was achieved in the concentrate stream; and more specifically, 98.7% for ferulic acid and p-coumaric acid, 99.1% for epicatechin, and 100% for gallic acid and vanillic acid, as shown in Table 7.8. Figure 7.33 shows the HPLC chromatograms of the three RO streams (feed, concentrate and permeate) taken after the phenolic compounds spiking.

Table 7.8: Concentration of phenolic compounds in the concentrate stream of the reverse osmosis process

Phenolic compounds	Winery wastewater + phenolic compounds % Concentration
Gallic acid	100
Vanillic acid	100
Epicatechin	99.1
p-coumaric acid	98.7
Ferulic acid	98.7



* 1: gallic acid; 2: vanillic acid; 3: epicatechin; 4: p-coumaric acid; 5: ferulic acid

Figure 7.33: HPLC chromatograms of the selected phenolic compounds added in (a) the feed, (b) after one cycle in the concentrate of the RO process and (c) after one cycle in the permeate of the RO process; Inset graphs show the HPLC chromatograms with FL detector, (P=10 bar, T=26 ^{o}C and single-pass mode).

According to our knowledge, although numerous phenolic compounds are present in the winery wastewater (Benitez et al. 2003, Strong and Burgess, 2008; Lucas et al. 2009), there is no study so far concerning the concentration of these compounds for further valorization.

Moreover, there is limited literature concerning the investigation of the exact phenolic compounds existing in winery wastewater. Mosse et al. (2012) investigated the phenolic compounds present in the influents and effluents of four winery wastewater treatment plants (WWWTP) (1: pond treatment, 2: Passive treatment, 3: constructed wetland treatment, and 4: sequencing batch reactor (SBR)). Several phenolic compounds were detected at significant levels in the influent samples, including gallic acid, tyrosol, hydrotyrosol, ferulic acid, cis and trans-resveratrol, epicatechin, and catechin, at concentrations up to 145 mg L^{-1}. Only very low amounts (<10 mg L^{-1}) of some of these phenolic compounds were detected in the effluents of pond, passive and SBR treatment systems. And more specifically, tyrosol was detected in the effluents of pond (2.5 mg L^{-1}) and passive treatment systems (5.5 mg L^{-1}); trans-resveratrol was detected only within the passive treatment effluent (2 mg L^{-1}); and ferulic acid was detected in the effluent from the SBR treatment (3.8 mg L^{-1}).

Moreover, in the study of Anastasiadi et al. (2009), the quantitation of the main phenolic compounds and the assessment of the total phenolic content (TPC) in polyphenol-rich extracts of vinification by-products were investigated. The samples studied were seeds, pomace and stems obtained from representative varieties of red and white wine. Several phenolic compounds were detected in the vinification by-products, among these gallic acid, epicatechin, catechin, ferulic acid, caffeic acid, p-coumaric acid, resveratrol, etc.

Seeds are particularly rich in catechin (up to 174 mg L^{-1}) and epicatechin (up to 38 mg L^{-1}), as well as they also have high TPC, averaging from 325 to 812 mg g $^{-1}$ gallic acid equivalents. On the other hand, grape pomace and stems were found to contain considerable amounts of diverse phenolic compounds, such as flavonoids (up to 7.2 mg L^{-1}), stilbenes (up to 31.4 mg L^{-1}), and phenolic acids (up to 42 mg L^{-1}).

Although, as mentioned before, literature on the membrane based recovery of phenolic compounds from winery wastewater does not exist, there are other relevant studies for the recovery of phenolic compounds from other agro-industrial wastewater, and mainly from olive mill wastewater (OMW).

Villanova et al. (2008) described a process for the recovery of tyrosol and hydroxytyrosol from olive mill wastewater (OMW). The process suggested for the recovery of tyrosol and hydroxytyrosol from polyphenolic fractions or their purified components of OMW comprised rough filtration (RF), microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) units followed by column chromatography. The sequential stages of RF, MF, UF, NF and RO with different modules of different molecular weight cut-offs provide the recovery of at least 1 g L^{-1} hydroxytyrosol and 0.6 g L^{-1} tyrosol. The concentration ratio of feed/extract in the stages of MF, UF, NF and RO is reported to be preferably higher than 8. In the chromatographic separation, a preparatory column filled with a macroporous co-polymer of divinylbenzene and N-vinylpyrrolidone is used to isolate tyrosol, hydroxytyrosol, trihydroxybenzoic acid, catechol, etc., available in the extract of RO, and in particular to prepare hydroxytyrosol with a high level of purity and free from catechol, which is toxic for humans. The chromatographic separation affords tyrosol with a yield higher than 84% (purity > 80%), and hydroxytyrosol with a yield higher than 80% (purity > 80%).

Additionally, the purpose of the study of Garcia-Castello et al. (2010) was to analyse the potentialities of an integrated membrane system for the recovery, purification and concentration of phenolic compounds from OMW. The proposed system included some well-known membrane operations, such as microfiltration (MF) and nanofiltration (NF), as well as an additional not yet investigated for this specific application, such as osmotic distillation (OD). The OMW was directly submitted to a MF operation without preliminary centrifugation. This step allowed to achieve a 78% recovery of the initial content of phenolic compounds in the permeate stream. The HPLC phenolic analyses of the MF permeate show that hydroxytyrosol is the main compound being the 54% of the total phenolic compounds, with a concentration of 88.7 mg L^{-1}. The MF permeate was then submitted to a NF treatment, and almost all phenolic compounds were recovered in the produced permeate solution. In particularly, the rejection of the NF membrane towards low molecular weight phenolic compounds was about 5%. A concentrated solution enriched in phenolic compounds was obtained by treating the NF permeate by OD. In particular, a solution containing about 0.5 g L^{-1} of free low molecular weight phenolic compounds, with hydroxytyrosol representing 56% of the total, was produced by using a calcium chloride dihydrate solution as brine. The obtained solution is of interest for preparing formulations to be used in food, cosmetic and pharmaceutical industry.

In the study of Conidi et al. (2011), a membrane-based process was investigated for the separation and concentration of phenolic compounds in the bergamot juice, in order to develop a natural product enriched in phenolic compounds suitable for nutraceutical applications. The process was based on the initial clarification of bergamot juice by ultrafiltration (UF), devoted to the removal of suspended solids. The clarified juice was then submitted to different UF and NF processes in order to evaluate the effect of the nominal molecular weight cut-off (MWCO) on the rejection of the membranes towards sugars and phenolic compounds. Phenolic compounds were recovered in the concentrate stream of the NF process and separated from sugar (around 52% of sugar is recovered on the permeate side). The rejection of the NF membrane towards flavonoids (narirutin, naringin, hesperidin and neohesperidin) was in the range of 91-99%.

7.4.4 Solar photo-Fenton treatment of the RO concentrate generated

The RO technology employs semi-permeable membranes, that allow separating a solution into two streams: permeate, containing the purified water that passes through the membrane, and concentrate, the portion that contains salts and retained compounds, and therefore needs a suitable and environmentally friendly management option (Mauguin and Corsin, 2005). The characteristics of the waste stream, named concentrate or retentate, depend on the quality of the feed water, the quality of the produced water (recovery varies from 35% to 85%), the pretreatment method (e.g. added chemicals), and the cleaning procedures used (Watson, 1990; Squire et al., 1997; Greenlee et al., 2009; Chelme-Ayala et al., 2009; Wang et al., 2012). Additionally, according to Chelme-Ayala et al. (2009) and Pérez-González et al. (2012), the constituent concentrations in the concentrate were found to be higher or even double than that in the feed wastewater.

Even though much research effort has been successfully devoted to reduce the generated concentrate, by increasing the recovery efficiency of RO systems (Ning and Troyer, 2009;

Greenlee et al., 2010), this trend inevitably results in a progressive higher concentration of pollutants in the concentrate fraction, making more difficult the appropriate treatment of that effluent (Hermosilla et al., 2012).

Hence, since the RO treatment plants generate significant volumes of concentrates containing all the retained compounds constituting a potentially serious threat to ecosystems, there is an urgent need for environmentally friendly management options of RO concentrates (Pérez-González et al., 2012). A lot of researches have addressed this objective in the last decade (Dialynas et al., 2008; Westerhoff et al., 2009; Bagastyo et al., 2011; Zhou et al., 2011a; Wang et al., 2012) pointing out the prominent role of a special class of oxidation techniques defined as advanced oxidation processes (AOPs), which usually operate at or near ambient temperature and pressure (Fernández-Alba et al., 2002).

As a consequence, in the case of the winery wastewater studied in this thesis, the RO treatment produced a quantity of concentrate, which was 50% of the feed volume (single-pass mode), with increased amounts of organic matter (10290 mg L^{-1}, expressed as COD and 812 mg L^{-1}, expressed as BOD_5) (Section 7.4.1). For the treatment of the concentrate stream, an alternative treatment method was tested, to reduce its COD. From the various AOPs that have been adopted for the wastewater treatment, photo-Fenton oxidation was selected, due to the fact that is a technology with many advantages including the possible use of solar light to facilitate the oxidation (Malato et al., 2002; Nogueira et al. 2005; Tamimi et al., 2008; Malato et al., 2009).

To obtain the optimal H_2O_2 concentration, experiments were conducted at several combinations of oxidant concentrations (750-1500 mg H_2O_2 L^{-1}) and at a constant catalyst concentration of 20 mg Fe^{2+} L^{-1}, as shown in Figure 7.34. The ferrous concentration of 20 mg L^{-1} was used based on similar running experiments in our laboratory concerning the treatment of other agro-industrial wastewater, at the same level of COD, as well as according to other relevant studies (for a winery wastewater with initial COD of 11000 mg L^{-1}, 20 mg L^{-1} of ferrous and a ratio of H_2O_2/Fe^{2+}=15 were used by Lucas et al. (2009b); for actual agro-industrial wastewater with initial COD of 7000 mg L^{-1}, 30 mg L^{-1} of ferrous and 3 g L^{-1} of H_2O_2 were used by Ahmed et al. (2011)). Taking into consideration the high initial organic load of the RO concentrate stream (>10000 mg L^{-1}), it was decided to use a higher ferrous dosage despite the existing limit of 5 mg L^{-1} (Cyprus Law: 106(I)/2002), in order to increase the efficiency of the process; ignoring the potential need of further treatment for iron removal.

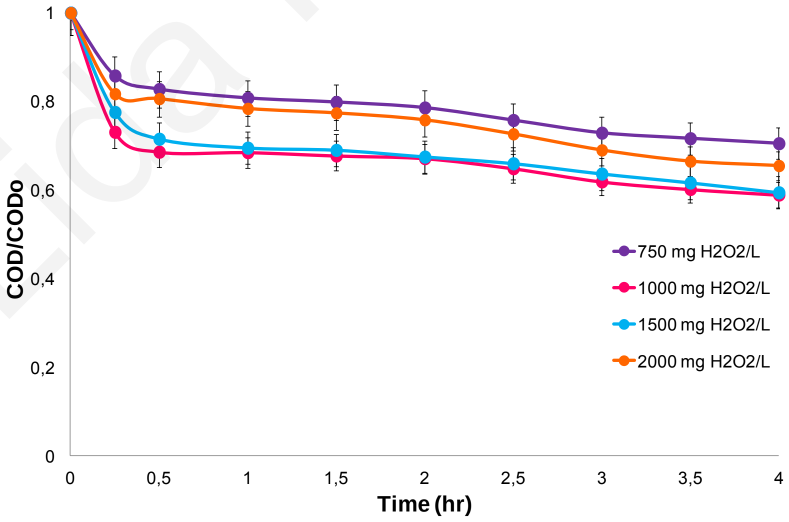


Figure 7.34: Effect of the initial hydrogen peroxide concentration on the COD removal of the RO concentrate stream; COD_0=10290 mg L^{-1}, $[Fe^{2+}]_0$=20 mg L^{-1}, $[H_2O_2]_0$=750-2000 mg L^{-1}, pH=3.0 and T=25±0.1 °C.

The results reveal that the COD removal increases with increasing hydrogen peroxide concentration up to an optimum value (i.e. 1000 mg L^{-1} H_2O_2), which brings about 41±2.4% COD removal after 4 h of solar photo-Fenton treatment; while at higher oxidant concentration (1500 mg L^{-1} H_2O_2) no significant change in the COD reduction is observed (Figure 7.34). On the other hand, further increase of the concentration of H_2O_2 to 2000 mg L^{-1} caused a substantial reduction in the COD removal from 41±2.4% to 34±2.9%.

An increase in the oxidant concentration is well known to induce the generation of higher amounts of HO• thus enhancing the degradation efficiency; however, excessive concentrations of H_2O_2 (over the optimum value) may lead to unwanted ‘scavenging’ reactions which may even decrease the number of available hydroxyl radicals, and ultimately the process efficacy (Tamimi et al., 2008; Malato et al., 2009). Therefore, 1000 mg L^{-1} of H_2O_2 was considered as the optimal dosage at the conditions studied.

Extra experiments were performed at the optimum H_2O_2 concentration of 1000 mg L^{-1}, and at a lower and at a higher ferrous concentration of 5 mg L^{-1} and 30 mg L^{-1}, in order to ensure that 20 mg L^{-1} was really the optimum dose. As shown in Figure 7.35, the lower ferrous dosage of 5 mg L^{-1} led to a significant lower COD removal (28±1.8%) compared to 41±2.4% COD removal achieved by 20 mg L^{-1} Fe^{2+} after 4 h of solar treatment. On the other hand, a further increase of the concentration of ferrous ion from 20 to 30 mg L^{-1} did not cause any significant change in the COD reduction (42±1.6%). As a result, a ferrous dosage of 20 mg L^{-1} was required for the treatment of the RO concentrate, since it remarkably increased the efficiency of solar photo-Fenton oxidation for the purification of winery RO concentrate.

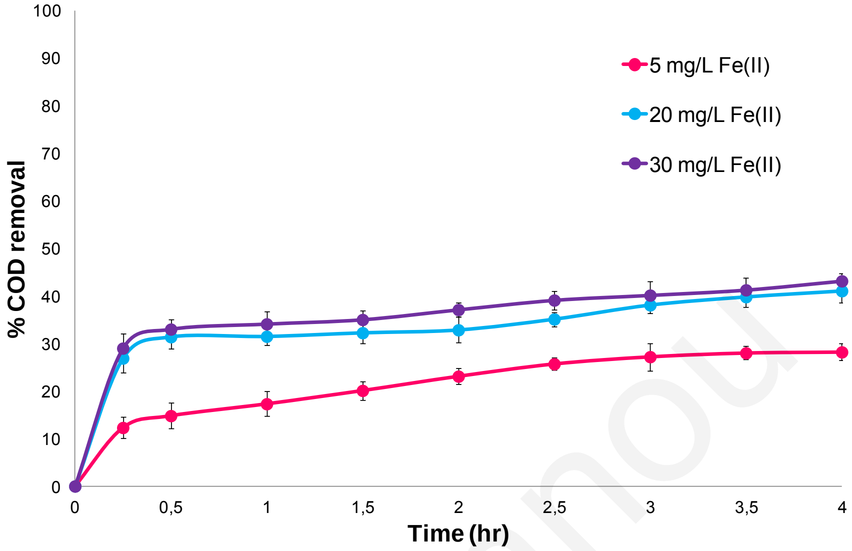


Figure 7.35: Effect of the initial ferrous concentration on the COD removal for the winery RO concentrate stream; COD_0=10290 mg L^{-1}, $[Fe^{2+}]_0$=5, 20 and 30 mg L^{-1}, $[H_2O_2]_0$=1000 mg L^{-1}, pH=3.0 and T=25±0.1 °C.

In order to achieve further reduction of the COD of the concentrate winery effluent, an additional experiment was performed at the optimum ferrous (20 mg L^{-1}) and H_2O_2 concentrations (1000 mg L^{-1}), where extra additions of oxidant were taken place during the reaction, so that the hydrogen peroxide was always available (i.e. H_2O_2 in excess). Moreover, it should be noted that the experimental time was extended to 28 h, in order to demonstrate that no further reduction of the COD have been achieved.

At the beginning of the solar-photo Fenton experiment the initial pH was adjusted to 3, the catalyst loading was 20 mg L^{-1} and a dose of 1000 mg L^{-1} H_2O_2 was added. The concentration of H_2O_2 was monitored every hour with hydrogen peroxide test strips

(Quantofix peroxide, 1-100 mg L^{-1}, Sigma Aldrich), and when this was consumed to values below 25 mg L^{-1}, a subsequent dose (1000 mg L^{-1}) was added. During the experimental time, the H_2O_2 was consumed twice; at 10 h and at 24 h. As shown in Figure 7.36, the COD of the concentrate stream, was decreased until a certain value after 18 h of solar treatment (70±2.7%), and then reached a steady state, while a slight reduction (~5%), almost negligible, was observed with the addition of the last dose of H_2O_2 at 24 h until the end of the treatment (28 h). Summarizing all the above, the solar photo-Fenton oxidation removed after 28 h of treatment 75±2.5% of the COD presented in the concentrate stream, with a residual COD equal to 2573±257 mg L^{-1}.

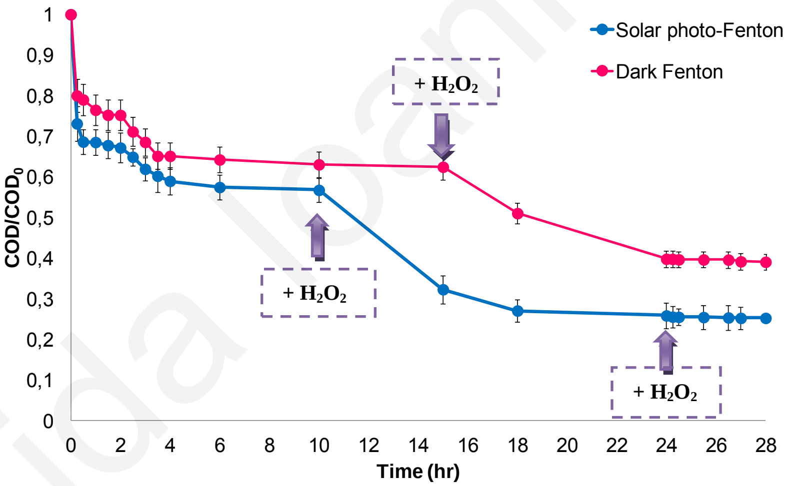


Figure 7.36: COD removal for the RO concentrate stream vs. operation time of the dark Fenton and solar photo-Fenton oxidation, under the optimum experimental conditions; COD_0=10290 mg L^{-1}, $[Fe^{2+}]_0$=20 mg L^{-1}, $[H_2O_2]_{dose}$=1000 mg L^{-1}, pH=3.0 and T=25±0.1 ^{o}C.

Additionally, a dark experiment (Fenton process) in the optimum experimental conditions and under prolonged treatment time, with H_2O_2 in excess, was conducted to assess the oxidation power of Fenton reagent in the absence of solar light. During the dark Fenton oxidation process, the COD of the concentrate stream was removed by 61±2.2% after 28 h of treatment, and with the addition of a consequent extra dose of H_2O_2 (1000 mg L^{-1}) after the first dose was consumed to a value lower than 25 mg L^{-1} (15 h), as shown in Figure 7.36.

7.4.4.1 Evaluation of the toxicity of the solar photo-Fenton treated RO concentrate

Solar photo-Fenton oxidation was proved to be an efficient method for the significant reduction (even elimination) of the toxicity of the winery effluents pretreated by SBR and MBR (as described in Section 7.1.10). As a consequence, the investigation of the efficiency of the solar photo-Fenton oxidation on the reduction of the toxicity of the RO concentrate winery stream (after 28 h of solar irradiation), was considered as an imperative task. Toxicity tests using *Daphnia magna* and three plant species (*Sinapis alba, Lepidium sativum, Sorghum saccharatum*) were used to evaluate the toxicity of the solar photo-Fenton treated winery RO concentrate effluent before its disposal in the environment.

7.4.4.1.1 *Daphnia magna*

Toxicity measurements were carried out both on the raw concentrate stream (before solar treatment) and the solar photo-Fenton treated concentrate effluent (after 28 h of treatment) at varying dilutions. As described before (section 7.4.1.3.1) the untreated concentrate stream even after substantial dilutions showed high toxicity towards *D. magna* (100% immobilization of microorganism) after both exposure times (Figure 7.28). On the other hand, the solar photo-Fenton treated concentrate stream was found to be very toxic to daphnids at the 100% concentration (undiluted effluent) (93.3±0.8% and 100±0.0% immobilization after 24 and 48 h of exposure); while at the concentration of 50% the toxicity was reduced to 46.6±2.7 and 73.3±2.4% after 24 and 48 h of exposure, respectively. At the lower concentration of 25% the toxicity was significantly reduced to 33.3±2.1 and 45±1.8% after 24 and 48 h of exposure, as shown in Figure 7.37. As a consequence, the combination of solar photo-Fenton oxidation and further dilution was able to significantly reduce the toxicity of the winery RO concentrate stream.

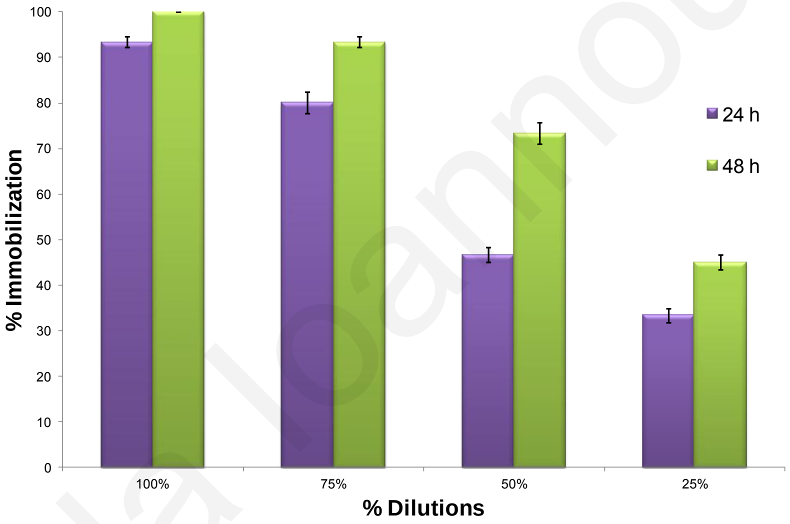


Figure 7.37: Effect of the initial concentration of the concentrate treated by solar photo-Fenton process on toxicity towards *D. magna* for 24 and 48 h of exposure times.

7.4.4.1.2 Phytotestkit toxicity test

Following, the concentrate treated by solar photo-Fenton process was tested for phytotoxicity. The seed germination inhibition (GI), shoot growth inhibition (SI) and root growth inhibition (RI) profile for the concentrate and the solar-photo Fenton treated concentrate streams are shown in Table 7.9.

Table 7.9: % effect of the reverse osmosis concentrate stream before and after the solar photo-Fenton treatment on the seed germination, shoot and root growth inhibition

Species	Concentrate			Concentrate after solar photo-Fenton oxidation		
	%GI	%SI	%RI	%GI	%SI	%RI
Sinapis alba	30±2.4	93.1±1.9	93.1±2.2	2.5±0.9	30.1±4.1	32.0±3.9
Lepidium sativum	15±3.1	72.1±3.7	85.1±3.0	7.5±1.8	21.1±3.5	29.2±2.6
Sorghum saccharatum	10±1.5	81.8±2.5	77.4±2.9	2.5±1.5	22.5±3.3	20.6±4.2

Experimental conditions: $[Fe^{2+}]$=20 mg L^{-1}, $[H_2O_2]_{dose}$=1000 mg L^{-1}, pH=3.0 and T=25±0.1 ^{o}C

As shown in Table 7.9, comparing the GI of the concentrate stream after solar photo-Fenton treatment to the initial concentrate stream, this was significantly reduced from 30±2.4 to 2.5±0.9% for *Sinapis alba*, from 15±3.1 to 7.5±1.8% for *Lepidium sativum*, and from 10±1.5 to 2.5±1.5% for *Sorghum saccharatum*.

It is noticeable that the concentrate stream after 28 h of solar photo-Fenton treatment showed a 4-fold SI reduction compared to the initial concentrate stream. More specifically, the SI of the concentrate wastewater after solar photo-Fenton treatment was reduced from 93.1±1.9 to 30.1±4.1% for *Sinapis alba*, from 72.1±1.8 to 21.1±3.5% for *Lepidium sativum*, and from 81.8±2.5 to 22.5±3.3% for *Sorghum saccharatum*.

Moreover, the winery concentrate effluent after the solar photo-Fenton treatment caused RI of the order of 20.6±4.2 to 32±3.9% for the three plants, which was reduced ca. 3-fold compared to the inhibition of the initial concentrate wastewater (77.4±2.9 to 93.1±2.2%).

Additionally, it must be noted that the GI, SI and RI of the concentrate stream after solar photo-Fenton treatment, were also lower (almost half for all three plant species) than the GI, SI and RI of the feed winery wastewater (Table 7.7).

From all the results obtained, RO can be considered as a promising process for both the purification of the raw winery wastewater and the concentration of phenolic compounds in the concentrate stream, since all selected phenolic compounds were almost completely concentrated in the resulting RO stream, demonstrating the capacity of the process purifying and valorizing winery wastewater. Reverse osmosis achieved high levels of purification of the winery wastewater and a water fraction (permeate) which can be discharged in aquatic systems or to be reused in winery cleaning processes. The toxicity to D. magna was reduced to zero in the permeate, while the phytotoxicity was substantially reduced. Moreover, it is therefore apparent that solar photo-Fenton oxidation can be considered as an effective process for the treatment of the RO concentrate stream of the winery wastewater, due to the significant reduction of both the organic load and toxicity.

CHAPTER 8: COST ESTIMATION FOR THE WINERY WASTEWATER TREATMENT TECHNOLOGIES DEVELOPED AND APPLIED IN THE PRESENT STUDY

On a worldwide level, the wastewater technology has been improved substantially during the last few decades. However, in several parts of the world, decisions on the more efficient use of the appropriate technology, are often made not on the basis of the general benefit, but are dictated by various ‘factors’, which often result in wrong directions and decisions, and of course high cost installation (Tsagarakis et al., 2003).

The high cost (for construction, maintenance, and operation) of most of the widely applied treatment processes, has brought about economic pressures to societies even in the developed countries, and has forced engineers to search for creative, cost-effective and environmentally sound ways to control water pollution (Verlinden et al., 2008). At the present time, according to Tsagarakis et al. (2003), it is recognised that even in the developed world complete municipal wastewater treatment systems covering 100% of the population, may never be possible to be implemented, unless wastewater management is based on selecting the cost-effective technologies. Therefore, it is clear that the cost for construction, operation and maintenance of a wastewater treatment plant (WWTP) has a great importance in the wastewater management strategies in most parts of the world (Verlinden et al., 2008).

When selecting a system to treat industrial and/or municipal wastewater, initially all processes are theoretically competitive. To determine the best option three major analyses should be undertaken. First, the required effluent quality should be achieved, according to the existing legislations. Then a number of aspects, which could restrict the applicability of some processes, should be examined. These aspects are economic, institutional and political, climatic, environmental, land availability and property, socio-cultural, etc. Finally, a cost-effective analysis should be carried out to determine the optimum economically viable solution as the selection criterion (Tsagarakis et al., 2001).

Comparison of the utility and treatment costs associated with different technologies is a subject of major significance. In many studies, various aspects of the treatment costs (investment and/or operational costs) are discussed, but very few studies focus specifically on this topic. Even in these cases, it is very difficult to compare technologies from different studies, as the cost analyses are often based on different assumptions (Beltran et al., 2002; Vidal et al., 2002) and, consequently, they can lead to very different treatment costs. For example, literature data concerning the cost associated with the Fenton treatment are in the range of 0.2 to 17.7 € m^{-3} (Cañizares et al., 2009). This very big range does not mean that estimations are incorrect, but simply that very different assumptions are made in the cost analysis. The same trend is observed for other AOPs such as electrochemical oxidation, ozonation, etc. (Awad and Abuzaid, 1997; Vlyssides and Israilides, 1997; De Lucas et al., 2002). Therefore, several items must be considered in order to estimate the cost of a wastewater treatment process, the most important of which are equipment amortization cost, raw materials cost, energy cost and labour cost (Cañizares et al., 2009).

Hence, in spite of the intense scientific effort carried out by several research groups to study the technical feasibility of a wide variety of treatment techniques, the lack of information on accurate cost estimation (at least under well-defined conditions) complicates any comparisons and, consequently, the choice of the best available technology not entailing excessive cost (BATNEEC) to treat the wastewater generated in industrial processes is more difficult (Cañizares et al., 2009).

The aim of this task was to compare the treatment cost of the three processes applied in this study for the treatment of winery wastewater, and more specifically, the biological process that was selected as the most efficient for industrial-scale application (membrane bioreactor, MBR), the advanced oxidation process (solar Fenton), and the membrane separation process (reverse osmosis, RO).

8.1 Cost estimation of the MBR process

Over the last two decades, implementation of membrane bioreactors (MBRs) has increased due to their superior effluent quality and low plant footprint (Judd, 2008). However, they are still viewed as a high-cost option, both with regard to capital and operating expenditure (capex and opex), mainly due to membrane installation and replacement costs, as well as the higher energy demand compared to conventional activated sludge systems. However, quantification of such impacts is constrained by the availability of credible data (Verrecht et al., 2010).

An overview of literature investment cost data (McAdam and Judd, 2008; Figure 8.1) over a range of reported plant sizes reveals costs to increase exponentially with decreasing plant size, and that a large variation in required capex arises according to assumptions made and costs included. DeCarolis et al. (2004) provided a comprehensive overview of costing data in terms of capex and opex, both for the MBR system alone (based on quotes from four leading suppliers), and for the complete installation (based on preliminary plant design and assumptions about the location-specific contribution of land costs, contractor overheads, engineering, legal costs, etc.). Cote et al. (2004) compared capex and opex of an MBR to a conventional activated sludge (CAS) system with tertiary filtration for effluent reuse purposes, demonstrating an integrated MBR to be less expensive than a combination of CAS and tertiary filtration - a conclusion subsequently corroborated by Brepols et al. (2009) for German wastewater plants. The latter authors showed energy demand to increase for plants with significant in-built contingency, since the average plant utilisation is low. This has recently led Maurer (2009) to introduce the specific net value (SNPV), which takes into account the average plant utilisation over its lifetime and so reflects the cost per service unit.

Notwithstanding the above, no in-depth analysis has been produced quantifying the impact of key-design and operating parameters on both capex and opex over the lifetime of an installation.

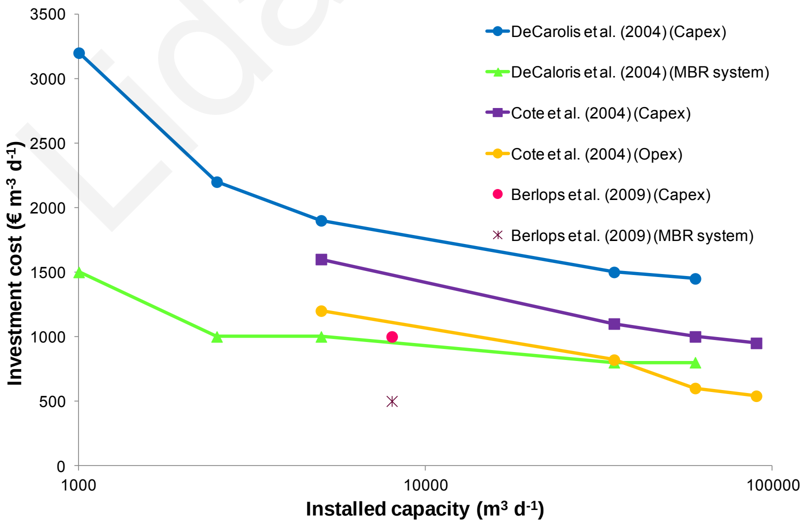


Figure 8.1: Specific investment vs. installed plant capacity based on literature data

Sources: McAdam and Judd, 2008; Verrect et al., 2010

8.1.1 MBR system sizing

In this study, a membrane bioreactor was used for the treatment of winery wastewater consisting as mentioned in previous Sections (Chapter 5 and 7). The treatment consisted of the following two parts:

- Preliminary treatment (screening, equalization / balancing tank, pH adjustment)
- Biological Treatment (pre-aeration / nitrification, membrane reactor, storage / irrigation tank)

In general, in order to maintain permeability and improve performance, a pretreatment device such as a fine screen or a cloth-media filter is installed ahead of the membrane unit (Metcalf and Eddy, 2008). The preliminary treatment used in this study, consists of the following steps:

Screening and grid removal: Wineries are known to produce wastewater with high solid loads. A large portion of the solids present in winery wastewater are organic in nature. This organic matter is partly (but not entirely) responsible for the high BOD levels, and can hinder the effectiveness of subsequent treatment processes. For this reason, it is beneficial to remove large particles prior to treatment. This is performed by screening (using static, rotating or auger screens) and grid removal. In this study, a static basket screen with an opening of 2 mm is used to remove large solids, in order to avoid also abrasion problems with grape seeds circulating between the membranes.

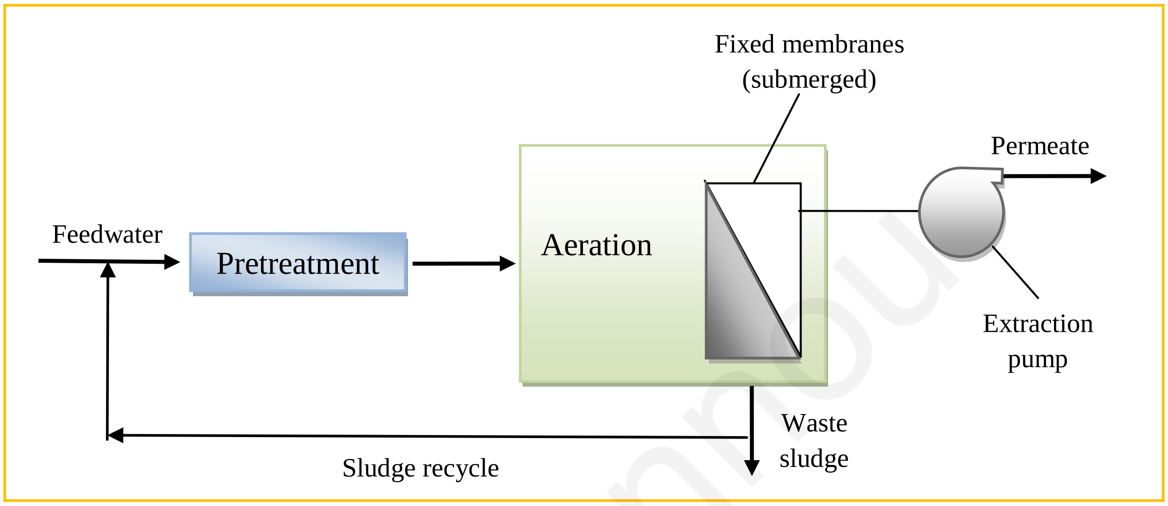
Equalization Tank: Depending on the various production activities being undertaken at any time during the winemaking process, wastewater flows from wineries may vary significantly over a short period of time. In order to reduce the impact of peak flows and to homogenize the winery effluent before this is introduced to the next treatment stages an equalization tank is required. Mixing of the tank content is achieved by mechanical mixers or air diffusion. Feed pumps in combination to a flow control system are used to ensure constant and adjusted hydraulic feed rate to the next stages.

pH adjustment: Generally, the pH of winery wastewater is acidic, but periodically it may be alkaline, as a result of cleaning operations with the use of caustic products (e.g. caustic soda). For this reason, it is important to adjust pH prior to any biological treatment.

Nutrient addition: Winery wastewater presents seasonal deficiencies in nutrients concentrations in relation to total carbon concentrations. It is therefore necessary to adjust the balance of nutrients that are necessary for the growth of microorganisms in the biological treatment stage. The addition of nitrogen and phosphate is introduced with the use of appropriate dosing systems.

The MBR process is very similar to the conventional activated sludge process, in that both have mixed liquor solids in suspension in an aeration tank. The difference in the two processes lies in the method of separation of bio-solids. In the MBR process, the bio-solids are separated by means of a polymeric membrane based on microfiltration or ultrafiltration unit, as against the gravity settling process in the secondary clarifier in conventional activated sludge process.

MBR design consideration - The items for consideration in the design of an MBR are the aeration tank capacity and dimensions, the aeration facilities, the membrane filtration and recycle, and the excess sludge wasting, as shown in Schematic 8.1.



Schematic 8.1: Integrated submerged membrane bioreactor (MBR)

Source: Metcalf and Eddy, 2008

Aeration Tank - The volume of aeration tank is calculated on the basis of F/M ratio (food to microorganism ratio) that is the loading from organic pollutants with respect to the concentration of activated sludge, the hydraulic retention time (HRT) and the mixed liquid suspended solids (MLSS) concentration. In general, the aeration volumes are relatively high in comparison to other advanced treatment processes. According to Verrecht et al. (2010) the average total aeration energy in kWh d^{-1} could be obtained by summing blower power consumption for both membrane and biology blowers and integrating over the 365 day simulation period.

MBR - In the various types of MBR systems, the key component is the MF or UF membrane. The membranes may be either pressure driven or vacuum driven. In the MBR system used in this study, vacuum-driven MF membranes (0.4 μm pore size) are immersed directly into the activated sludge reactor. The membranes are subjected to a vacuum (less than 50 kPa) that draws water (permeate) through the membrane, while retaining solids in the reactor or the membrane separation tank. To clean the exterior of the membranes, air is introduced below the membranes. As the air bubbles rise to the surface, scouring of the membrane surface occurs and rejected material is returned to the mixed liquor (Metcalf and Eddy, 2008).

Sludge Recycle - The MLSS concentration in the aeration tank is controlled by the sludge recirculation rate and the sludge settleability.

Excess Sludge Wasting - The sludge in the aeration tank has to be wasted to maintain a steady level of MLSS in the system. The excess sludge quantity will increase with increasing F/M and decrease with increasing temperature. Excess sludge may be wasted either from the sludge return line or directly from the aeration tank as mixed liquor.

8.1.2 Estimation of the investment, operation and maintenance cost of MBR

The cost benefit analysis presented below is based on a typical medium-size winery that is expected to produce an average of 50 m^3 of wastewater per day. As winery wastewater treatment is considered a plant consisting of a pretreatment stage and a biological stage (MBR) able to produce a high quality effluent. Cost estimation is based on the initial investment cost and the operation and maintenance cost for five years time.

In general, the costs of construction normally include those for civil works, mechanical works, buildings, engineering designs and supervision of on-site infrastructure, start-up costs and working capital. The construction cost depends on a number of parameters. The main ones are the level of wastewater treatment required (the greater the level of treatment the higher the cost), and the capacity of the installation. A number of other individual local factors may arise, which increase the construction cost. The most common ones are special site preparations, quality of materials used, tender procedure, housing of unit processes other than preliminary works, etc. (Tsagarakis et al., 2003).

In this study, the construction costs are major contributors to the total investment costs that relate to the necessary tanks used as the processing units and the necessary service buildings, as described below. In addition, other direct and indirect investment costs are required to establish a complete system.

The most important investment costs for the construction of an MBR can be classified as following:

- Tanks and other concrete or steel constructions
- Service building including machinery room and storage room
- Supply and installation of equipment
- Control system and instrumentation
- Necessary materials for electromechanical installations
- Utilities such as power supply, tap water supply, etc.
- Engineering cost for the design, supervision and system start-up
- Labour costs for the installation

Table 8.1 shows the total investment cost for the construction of the MBR based on 2013 prices. This refers to a treatment capacity of 50 m^3 of wastewater per day that is considered representative for a medium production capacity winery. This investment cost is based on the readjustment of the actual costs needed for the construction of the smaller MBR unit (treatment capacity of 4-8 m^3 d^{-1}), that was described in Chapter 7, and was constructed and funded by the LIFE project (WINEC). Moreover, it should be noted that the purchase of land is not included in the cost estimation.

Table 8.1: Investment cost of the MBR system based on 2013 prices

Cost Element	MBR in €
Detail design (process design, civil engineering design for infrastructure, detail engineering design for electromechanical installations)	6000
Civil works (site preparation, excavations, backfilling, concrete works, reinforcement, waterproof insulation, construction of services building)	45000
Supply of equipment (equipment sizing selection and cost of supply)	64000
Electromechanical installations (Engineering supervision and Labour)	16000
Other expenses (Utilities: water and power supply, test and commissioning)	4000
Total Investment Cost	**135000**

In Table 8.2, the total operation costs of the MBR system for a five-year period are presented. It should be mentioned that for the calculation of the operating costs the seasonal variations in the flow have been taken into consideration. The analysis of the operation and maintenance expenses includes:

- Personnel salaries for maintenance and operation,
- Cost of electrical energy based on 0.25 € kWh^{-1}, and
- Chemicals' consumption and consumables

To calculate electricity costs, air flows to keep the membranes clean and for the biomass are considered. The flow needed for the membranes is given by the manufacturer and the air needed by the biomass is calculated by means of an oxygen balance (Judd, 2010; Pérez et al., 2013).

A typical membrane cleaning protocol and frequency, based on literature data (Brepols et al., 2008; Judd, 2010) were assumed to provide chemicals' consumption data. The protocol comprised a weekly cleaning in place (CIP) with 500 mg L^{-1} NaOCl and 2000 mg L^{-1} citric acid, and a cleaning out of place (COP) with 1000 mg L^{-1} NaOCl and 2000 mg L^{-1} citric acid, conducted twice yearly (Verrecht et al., 2010). Representative prices for bulk chemicals were obtained from local chemical suppliers.

Table 8.2: Cost of operation of the MBR system for a five-year period based on 2013 prices

Cost Element	MBR in €
Operation (Engineering supervision, operator, technical support)	22750
Monitoring of performance (on-site and lab analyses)	2400
Cost of electricity (based on 0.25€ kWh^{-1})	20100
Equipment maintenance and repairs (lubrication, maintenance, spare parts)	13200
Chemicals' consumption (Chemical cleaning of membranes)	650
Total Operational Cost for a five-year period	**59100**

For the calculation of the operating costs the seasonal variations in flow have been taken into consideration. In Table 8.3 the overall cost of the ownership of the MBR system with a capacity of 50 m^3 per day is presented.

Table 8.3: Total cost estimation of the MBR system for a five-year operational period based on 2013 prices

Cost element	MBR in €
Initial Investment Cost	135000
Total Operational Cost for a five-year period	59100
Total Ownership Cost for a five-year period	**194100**

The total cost of the full-scale MBR unit for the treatment of 50 m^3 of winery effluent per day was estimated at 194100 € for a five-year operation, which corresponds to 38820 € yr^{-1}, and to 2.1 € m^{-3}, while the total operational cost was found to be 0.65 € m^{-3}.

From the point of view of economical analysis, an operational cost estimation of the MBR plant was performed by Valderrama et al. (2012), based on energy and chemicals' consumption, considering these as the main components of the total operational cost. The results showed that total operational costs were 0.40 € m^{-3} for an MBR plant with a capacity of 6 m^3 winery wastewater per day.

Moreover, a cost estimation analysis for an MBR on the basis of winery wastewater generation of 1000 m^3 d^{-1} and a payback of five years for the capital investment was estimated by Artiga et al. (2007). The total annualized cost was found to be 0.36 € per m^3

of treated wastewater, which is similar to 0.38 € m^{-3} that was obtained by Lubello et al. (2003) for the operation of a similar MBR. In these studies, as capital costs only the civil works, the membrane, and the pumps were considered; and as operational costs only the chemicals' and the energy consumption have been taken into account.

Finally, the total cost of an MBR for the treatment of 10 m^3 d^{-1} of highly ecotoxic industrial wastewater containing pesticides, was found to be 0.48 € m^{-3}, according to Pérez et al. (2013). It should be noted that the total costs included the operating costs (i.e. personnel, chemicals, maintenance and energy consumption) plus the amortization costs (when the plant works for 300 days per year and its life cycle is considered to be 20 years) in € m^{-3}.

As shown from the above, it is very difficult to compare technologies from different studies, as the cost analyses are based on different assumptions, and as a result they can lead to very different investment and operational costs.

8.2 Cost estimation of the solar Fenton process

The use of solar Fenton oxidation as a post-treatment stage after the biological treatment is one on the major innovations of this thesis with respect to the wastewater treatment process. Apart from the proven ability to remove residual pollutants after the pretreatment and biological treatment stages, the solar Fenton oxidation proved its ability to completely remove the toxicity remained after the biological process.

Even nowadays, there is limited literature (Lucas et al., 2010; Krichevskaya et al., 2011; Jordá et al., 2011) dealing with the wastewater treatment process economics at pilot scale, although this aspect is a very important issue. At the same time, there is hardly any exhaustive report, addressing the issue of economic feasibility of the use of advanced oxidation for wastewater treatment at industrial scale (Mahamuni and Adewuyi, 2010).

The main drawback of advanced oxidation processes (AOPs) is their high cost in comparison to the biological treatment processes. This cost difference is caused not only by the type of treatment itself, but also by the nature of the effluent and the treatment volume, which is usually on a smaller scale for AOPs (Pérez et al., 2013). For instance, the estimated cost for treating wastewater containing 500 mg L^{-1} of alpha-methyl-phenylglycine (MPG) applying solar photo-Fenton has been found to be 14.1 € m^{-3} (Muñoz et al., 2008) in contrast to 0.25 € m^{-3} for the activated sludge treatment cost with nutrient removal for domestic wastewater (Hernandez-Sancho et al., 2011). The higher costs of AOPs have caused many authors to study the possibility of a combined process, coupling a less expensive biological stage followed by a more costly photocatalytic treatment for example (de Heredia et al., 2005b; Anastasiou et al., 2009; Lucas et al., 2009b).

8.2.1 Solar Fenton system sizing

The solar Fenton system has the following treatment stages:

Buffer tank - This is an effluent storage tank following the biological treatment stage with adequate volume to accommodate the flow during the period that the solar Fenton is not in operation. It also serves as a mixing tank to establish homogeneous characteristics of the stored liquid before this is introduced to the solar reactors.

Feeding system to the Solar Oxidation reactors - The hydraulic feed rate is a prime operating parameter of the oxidation process as it is directly proportional to the contact time, and therefore the exposure duration of the wastewater to UV light. For this purpose a precise adjustment of feed rate is necessary.

Chemicals dosing systems - Necessary chemicals are dosed to the desired concentrations (e.g. 3 mg L^{-1} Fe^{2+} and 350 mg L^{-1} H_2O_2). Ferrous sulphate, hydrogen peroxide and sulphuric acid are prepared as concentrated solutions into the designated solution tanks. Dosing is performed by special dosing pumps. These pumps are controlled via the measuring sensors and controllers. Adjustment of the desired concentration is possible via the computerized control system. The sizing of the different dosing systems was based on the required chemicals' concentrations and the feed rate to the treatment unit. Chemicals' concentrations are relatively low and standard diaphragm type dosing pumps of 0.08 kW can easily satisfy the dosing rates required.

CPC (Compound parabolic collector) Solar Reactors - These are the main system components, where the solar oxidation process takes place. A number of CPC assemblies are installed to provide sufficient contact time for the photocatalytic degradation of the targeted polluting compounds. These are connected in series and recirculation of effluent is possible with the piping network configuration. Flow measurement of the recirculation flow is monitored with the use of a designated electromagnetic flow meter.

Control System - A computerised control system is installed to control and monitor the operation of the solar Fenton unit.

8.2.2 Estimation of the investment, operation and maintenance cost of solar Fenton process

Based on the experimental and operational data recorded during the operational period for the solar driven industrial-scale plant unit, and the basic design parameters applied, a scaling up extrapolation was carried out. The aim of this task was to establish the overall cost for a full-scale installation operating for a period of five years. For this purpose, a simple methodology was developed based on the investment cost estimation along with the operational (including electric power and consumables), and the maintenance cost.

In order to estimate the cost of the full-scale treatment plant the following were considered:

- The hydraulic capacity of the upscaled unit is based on an expected effluent flow from a membrane bioreactor (MBR) serving a typical medium-size winery with a winery wastewater production of about 50 m^3 per day.

- The mean yearly illuminated hours per day, 10 h, were considered as the working hours. This was based on the findings of the operation of the existing pilot unit and on literature data (Malato et al., 1999; Pérez et al., 2013).

Moreover, it should be mentioned that this investment cost is based on the readjustment of the actual costs needed for the construction of the smaller solar Fenton plant (treatment capacity of 4-8 m^3 d^{-1}), that was described in Chapter 7, and was constructed and funded by the LIFE project (WINEC).

In Table 8.4 the total cost of the investment of a solar Fenton post-treatment system is presented, based on a capacity of 50 m^3 per day. As mentioned already, it should be noted that the purchase of land is not included in the cost estimation.

Table 8.4: Investment cost of a solar Fenton system based on 2013 prices

Cost Element	Solar Fenton system in €
Detail design (process design, civil engineering design for infrastructure, detail engineering design for electromechanical installations)	2000
Civil works (site preparation, excavations, backfilling, concrete works, reinforcement, waterproof insulation, construction of services building)	2500
Supply of equipment (equipment sizing selection and cost of supply)	53850
Electromechanical installations (Engineering supervision and Labour)	4000
Other expenses (Utilities: water and power supply, test and commissioning)	500
Total Investment Cost	**62850**

In Table 8.5, the total cost of operation of the solar Fenton post-treatment system for a five-year period is presented. The analysis of the operation and maintenance expenses includes:

- Personnel salaries for maintenance and operation,
- Cost of electrical energy based on 0.25 € kwh^{-1}, and
- Chemicals' consumption and consumables

Maintenance costs included one or more of the following: regular repairs (mechanical, electrical, electronic and civil parts); minor or major replacements (small or large parts for pumps, blowers, motors, etc.). Quantities of spare parts kept in stock and purchasing deals influence the total maintenance cost (Tsagarakis et al., 2003). The maintenance cost (inspection, replacement and repair) was estimated as a percentage of the total investment cost. For this type of plants, 1.5% of the initial investment cost per annum can be considered as representative. This approach leads to an estimated maintenance cost of approximately 1000 € over the period of five years, as shown in Table 8.5.

Additionally, the chemicals' cost considers the concentration of the hydrogen peroxide needed to achieve the necessary mineralization, the concentration of the iron salt used as the catalyst, and the amount of sulfuric acid and the sodium hydroxide used to adjust the pH to 3.0 at the beginning of the process and to 7 at the end, before its disposal in the environment. The unitary chemicals' costs considered are: 1.10 € Kg^{-1} H_2O_2, 1.30 € Kg^{-1} $FeSO_4{\cdot}7H_2O$, 2 € Kg^{-1} H_2SO_4, and 0.55 € Kg^{-1} NaOH.

The energy cost is estimated by calculating the power required to pump water in the system applying Eq. (8.1):

$$C_E = \frac{P_E \cdot W \cdot t_W}{V_t} \tag{8.1}$$

where W is the power required to pump water in the system, estimated through energy balance; P_E is the power cost, V_t is the capacity of the plant in m^3 d^{-1} and t_W is the number of working hours a day.

Table 8.5: Cost of operation of a solar Fenton system for a five-year period based on 2013 prices

Cost Element	Solar Fenton system in €
Operation (Engineering supervision, operator, technical support)	5200
Monitoring of performance (on-site and lab analyses)	1000
Cost of electricity (based on 0.25 € kWh^{-1})	3500
Equipment maintenance and repairs (lubrication, maintenance, spare parts)	1000
Chemicals' consumption (Reagents for solar Fenton treatment (H_2SO_4, $FeSO_4 \cdot 7H_2O$, H_2O_2))	93150
Total Operational Cost for five-year period	**103850**

In Table 8.6 the overall cost of ownership of a solar Fenton system with a capacity of 50 m^3 per day is presented.

Table 8.6: Total cost estimation of a solar Fenton system for a five-year operational period

Cost element	**Solar Fenton system in €**
Initial Investment Cost	62850
Total Operational Cost for five-year period	103850
Total Ownership Cost for five-year period	**166700**

The total cost of the full-scale solar Fenton unit for the treatment of 50 m^3 of winery effluent pretreated by MBR per day was found to be 166700 € for five-year operation, which corresponds to 33340 € yr^{-1}, and to 1.83 € m^{-3}. This value is in agreement with a recent study by Pérez et al. (2013) for the treatment of 10 m^3 d^{-1} of industrial wastewater containing pesticides, which reaches a value up to 2.04 € m^{-3}. On the other hand, lower costs of photo-Fenton process at pilot scale were estimated for the treatment of hospital wastewater with a capacity of 350 m^3 d^{-1} (1.09 € m^{-3}) (Kajitvichyanukul and Suntronvipart, 2006); for the degradation of paracetamol in water (0.74 € m^{-3}) (Jordá et al., 2011); and for the degradation of antibiotics at low concentration level (μg L^{-1}) in secondary treated domestic effluents (0.85 € m^{-3}) with a maximum daily flow of 150 m^3 d^{-1} (Michael et al., 2012).

8.3 Cost estimation of the reverse osmosis process

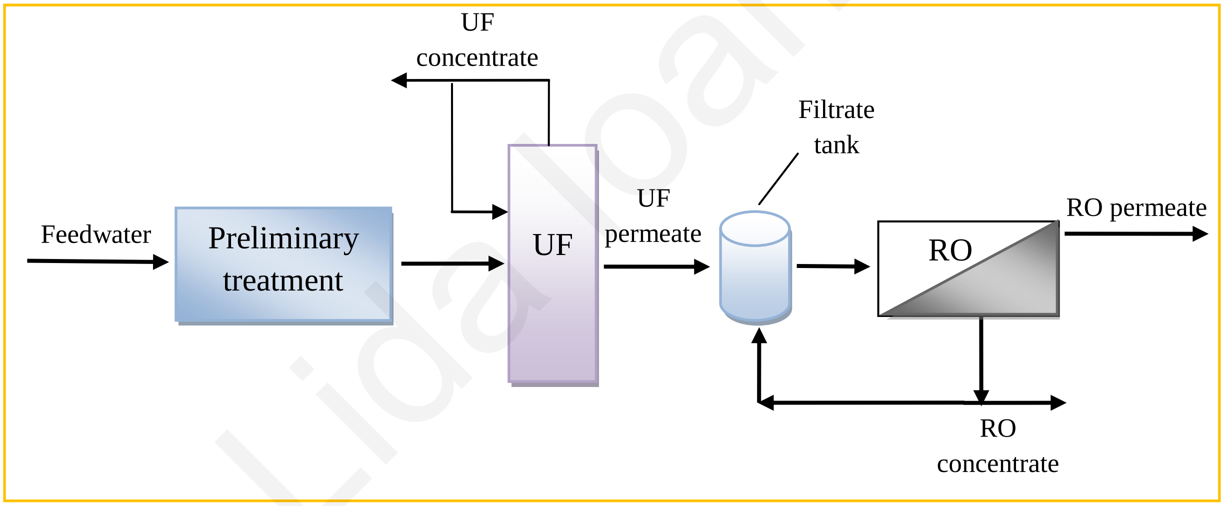
The use of a membrane separation process, such as reverse osmosis (RO) for the treatment of winery wastewater was the last objective of this study. RO membranes are very sensitive to foulant materials such as colloids, inorganic scale and biofilm development (biofouling) (Bonnelye et al., 2008). For this reason, membrane fouling is an important consideration in the design and operation of membrane systems as it affects pretreatment needs, cleaning requirements, operating conditions, cost, and performance (Metcalf and Eddy, 2008). As a consequence, the need for appropriate pretreatment to ensure the optimum performance of RO systems is of great importance (Lorain et al., 2007).

Ultrafiltration (UF) has been generally accepted as the proper pretreatment stage before RO process, because it removes, in only one physical filtration stage, all the suspended solids and biological materials of feedwater (Wolf et al., 2005; Knops et al., 2007; Lorain et al., 2007). The alternative, according to Knops et al. (2007), would require an extensive train of several treatment steps with associated high investment and operating costs, while RO feedwater quality cannot be guaranteed 100% of the time. On the other hand, the UF guarantees a constant water quality at low cost, virtually independent of the feedwater quality (Knops et al., 2007). Moreover, blocking of RO elements and biological fouling are avoided. Furthermore, in most cases, hydraulic performance of the RO plant is increased when it is fed with such high quality water (i.e. UF permeate). Thus, chemical cleaning frequency is reduced and life-time of the RO membranes is increased. All these advantages involve important cost savings on both capital investment and operation of the RO plants, according to Lorain et al. (2007).

Additionally, it should be mentioned that according to Metcalf and Eddy (2008), the high pressure RO systems require significant energy costs, high capital costs for the high pressure pumps, as well as high maintenance costs. Moreover, after the membrane replacement, energy is the next major operating expense of the RO systems.

8.3.1 Reverse osmosis system sizing

The items for consideration in the design of an integrated reverse osmosis system are the preliminary treatment (screening, equalization / balancing tank, pH adjustment), the UF pretreatment, the RO treatment, and the UF and RO concentrate management, as shown in Schematic 8.2.



Schematic 8.2: Integrated reverse osmosis system

Generally, membrane systems are very sensitive and care must be provided to optimize their performance. Depending on the wastewater to be treated, pretreatment may consist of fine screening; surface filtration; or chemical neutralization, conditioning, or precipitation (Metcalf and Eddy, 2008). In more detail, the preliminary treatment used in this study, consists of the following steps:

Screening and grid removal: Firstly, the large particles of winery effluents need to be removed, prior to further treatment. This is performed by screening (using static, rotating or auger screens) and grid removal. In this study, a static basket screen is used to remove large solids, in order to avoid abrasion problems with grape seeds circulating between the membranes.

Equalization Tank: Depending on the various production activities being undertaken at any time during the winemaking process, wastewater flows from wineries may vary significantly over a short period of time. In order to reduce the impact of peak flows and to homogenize the winery effluent before this is introduced to the next treatment stages an equalization tank is required. Mixing of the tank content is achieved by mechanical mixers or air diffusion. Feed pumps in combination to a flow control system are used to ensure constant and adjusted hydraulic feed rate to the next stages.

pH adjustment: Generally, the pH of winery wastewater is acidic, but periodically it may be alkaline, as a result of cleaning operations with the use of caustic products (e.g. caustic soda). For this reason, it is important to adjust pH prior to membrane filtration treatment, in order to avoid any damages to the membranes.

Then a filtration process, ultrafiltration (UF), is also used as a pretreatment stage of RO feedwater. UF relies on the membrane separation technique that requires low pressure to force good quality filtered water through the membrane, thus rejecting harmful contaminates, including micro-organisms (Ebrahim et al., 2001). The feedwater is pumped to the UF module with a feed pump. The permeate is collected in the filtrate tank. Backwash is periodically performed with filtered water (from the filtrate tank). During each backwash, air scouring is provided in the feed side. Typically backwash occurs every 40-60 min of membrane operation, and the duration is approximately 60 sec, according to previous studies (Lorain et al., 2007). Irreversible fouling or long-term fouling, typically adsorption and precipitation, needs to be cleaned chemically. The frequency of chemical cleaning depends of the feed quality; however, according to Lorain et al. (2007), one month is commonly encountered between two chemical cleanings. After cleaning, the membrane permeability is restored as its initial value.

Pilots have shown that with UF as pretreatment, the RO cleaning frequency can be virtually reduced. Because of the reduced RO fouling and because of the reduced chemical attack due to RO cleaning, the RO membrane life-time will be increased, when UF is being selected. According to Knops et al. (2007) it is safe to say that typical RO life can be increased to at least seven years (conservative), with eight years or longer being realistic.

Finally, a reverse osmosis unit completes the system, which operates in recirculation mode. For the recirculation mode, the permeate flow is taken out of the loop, while the concentrate is returned to the feed tank (filtrate tank), where it is mixed with the feed wastewater, as shown in Schematic 8.2.

It should be noted that for a treatment capacity of 50 m^3 of winery wastewater per day, the UF concentrate was estimated to be 5 m^3 d^{-1}, which corresponds to a UF volume recovery equal to 90%, while the RO concentrate was estimated to be 9 m^3 d^{-1}, which corresponds to an RO volume recovery equal to 80%.

8.3.2 Estimation of the investment, operation and maintenance cost of reverse osmosis system

The cost benefit analysis presented below is based on a typical medium-size winery that is expected to produce an average of 50 m^3 of wastewater per day. As a winery wastewater treatment is considered a plant consisting of a preliminary stage treatment (screening, balance tank, etc.), a UF pretreatment and an RO unit able to produce a high quality effluent. Cost estimation is based on the initial investment cost and the operation and maintenance cost for five-year period. Moreover, it should be reiterated that the purchase of land is not included in the cost estimation.

In Table 8.7 the total cost of the investment of an integrated reverse osmosis system based on 2013 prices is presented, for a capacity of 50 m^3 of winery wastewater per day.

Table 8.7: Investment cost of an integrated reverse osmosis system based on 2013 prices

Cost Element	Integrated RO system in €
Detail design (process design, civil engineering design for infrastructure, detail engineering design for electromechanical installations)	5000
Civil works (site preparation, excavations, backfilling, concrete works, reinforcement, waterproof insulation, construction of services building)	18000
Supply of equipment preliminary treatment (screening, primary sludge pumps, feed pumps and mixers in balancing tanks)	16600
Supply of equipment UF (equipment sizing selection and cost of supply)	25000
Supply of equipment RO (equipment sizing selection and cost of supply)	28000
Electromechanical installations (Engineering supervision and Labour)	8000
Other expenses (Utilities: water and power supply, test and commissioning)	4000
Total Investment Cost	**104600**

In Table 8.8, the total cost of operation of the integrated RO system for a five-year period is presented. The analysis of the operation and maintenance expenses includes:

- Personnel salaries for maintenance and operation,
- Cost of electrical energy based on 0.25 € kwh^{-1}, and
- Chemicals' consumption and consumables

Maintenance costs include one or more of the following: regular repairs (mechanical, electrical, electronic and civil parts) and minor or major replacements (pumps, motors, etc.).

Additionally, the chemicals' cost considers mainly the chemicals used for the cleaning of the membranes (UF and RO). The cleaning process must remove deposits and restore the normal capacity and separation characteristics of the system. The choice of cleaning method depends on the module configuration, membrane resistance and nature of the foulants (Madaeni and Mansourpanah, 2004). Membrane manufacturers usually recommend conducting a cleaning when:

- the normalized permeate flow drops 10%, and
- the normalized pressure drop (feed pressure minus concentrate pressure) increases 10-15% (Kolk et al., 2013).

However, in field operation these recommendations are often not kept with, but cleaning is done not before there is a much higher impact on the key performance indicators, for different reasons. If a cleaning is delayed too long, it can be difficult to remove the foulants completely from the surface. This can make things worse in the long-run because remaining deposits can enhance new fouling, which in the end will increase the necessary membrane cleaning frequency (Kolk et al., 2013).

Finally, to calculate the energy needs of the system, the total power requirements for the different parts of the system were calculated, based on 2013 prices, given the maximum operation hours of the system (Mohamed and Papadakis, 2004).

Table 8.8: Cost of the operation of an integrated reverse osmosis system for a five-year period based on 2013 prices

Cost Element	Integrated RO system in €
Operation (Engineering supervision, operator, technical support)	22800
Monitoring of performance (on-site and lab analyses)	12000
Cost of electricity (based on 0.25 € kWh^{-1})	55000
Equipment maintenance and repairs (lubrication, maintenance, spare parts)	16800
Chemicals' consumption (Chemical cleanings of membranes)	21200
Total Operational Cost for five-year period	**127800**

In Table 8.9 the overall cost of ownership of an integrated RO system with a capacity of 50 m^3 per day is presented.

Table 8.9: Total cost estimation of an integrated reverse osmosis system for a five-year operational period

Cost element	Integrated RO system in €
Initial Investment Cost	**104600**
Total Operational Cost for five-year period	**127800**
Total Ownership Cost for five-year period	**232400**

The total cost of the integrated RO system for the treatment of 50 m^3 of winery effluent per day was found to be 232400 € for a five-year operation, which corresponds to 46480 € yr^{-1}, and to 2.55 € m^{-3}, while the total operational cost was found to be 1.4 € m^{-3}.

Since no literature data regarding the ownership cost of similar RO systems for the treatment of winery or other agro-industrial effluents exist so far, the costs of reverse osmosis seawater desalination units can be compared. The water production cost obtained from the study of Mohamed and Papadakis (2004), using an RO desalination unit for a capacity of 0.5 m^3 seawater per h (12 m^3 d^{-1}), was 5.21 € m^{-3}. Moreover, the total cost of ownership of the seawater reverse osmosis (SWRO) desalination plant, with conventional pretreatment was approximately 1.15 € m^{-3} (Knops et al., 2007). According to Akgul et al. (2008) the capital cost for a SWRO desalination plant was obtained to 0.46 € m^{-3} at the capacity of 250 m^3 d^{-1}, while the operating cost was 0.27 € m^{-3}. In this study, it was noticed that the capacity of the treatment system decreases the capital and total production costs, and that the energy costs constitute the greatest part of the operating costs (70%) (Akgul et al., 2008).

From all the cost estimations obtained, the most expensive system for the treatment of the effluents of a typical medium-size winery that is expected to produce an average of 50 m^3 of wastewater per day, seems to be the RO system (2.55 € m^{-3}), followed by the MBR system (2.1 € m^{-3}), while the less expensive technology seems to be the solar Fenton system (1.83 € m^{-3}), which was used as post-treatment of the biological treatment. However, it should be noted that both the MBR and the RO system need to be followed by the solar Fenton system (as post-treatment), either as a polishing step (e.g. for the elimination of effluents' organic content and its toxicity), or as a further treatment for the concentrate stream before its disposal in the environment. As a consequence, the optimum and the most cost-effective integrated system for the treatment of winery wastewater seems to be the MBR + solar Fenton process, since is the less expensive (based on both *the capital and operational cost), and is also the system with the higher efficiency, able to reduce the organic pollutants in the winery effluent to values well below those included in the Cypriot discharge limits.*

CHAPTER 9: CONCLUSIONS

9.1 General conclusions

The main conclusions of this thesis are divided in six sections. In the first section, the conclusions concerning the environmental impacts related to the wine production process in Cyprus are discussed. In the second section the main conclusions concerning the bench-scale study of solar photo-Fenton oxidation of winery effluents pretreated by SBR and MBR are reported. The third section includes the main conclusions derived from the solar Fenton pilot-scale experiments, while in the fourth section the main conclusions of the solar Fenton industrial-scale operation are presented. The fifth section includes the main findings that can be concluded derived from the pilot-scale reverse osmosis treatment of winery wastewater. Finally, the last section includes the main conclusions withdrawn from the cost estimation of the processes applied in this study for the treatment of winery wastewater (i.e. MBR, solar Fenton and reverse osmosis).

9.1.1 Environmental problems related to the wine production process in Cyprus

The identification of the exact environmental impacts related to the wine production process in Cyprus were investigated through a review, which was prepared based on the relevant available literature, a questionnaire designed specifically for collecting relevant information from the operators (Annex), site visits to wineries in Cyprus, and personal communication with the owners of numerous wineries. The main conclusions derived from this research are the following:

- Large wineries pay more attention on the environmental impacts resulting from their produced wastewater as evidenced by the monitoring of the produced wastewater and the operation of a private wastewater treatment plant by 3 out of 4 wineries. Moreover, all 4 large wineries are aware of the relevant national legislation, while only 60% of small wineries are.
- The questionnaire review evidenced that most of the small wineries dispose their wastewater with no or minimal treatment either for irrigation or in water receivers, resulting in the pollution of sensitive mountainous areas, groundwater and soil resources.
- Wineries consume a lot of water in their everyday cleaning practices, and only a 36% of small wineries and 75% of large wineries implement water saving measures.
- The use of solid organic waste produced by the operation of wineries as a fertilizer (i.e. compost), as reported by the majority of wineries participating in this study, if utilized appropriately, may significantly reduce the impacts resulting from the extensive use of synthetic fertilizers.
- With regard to the packaging waste produced, most of the wineries do collect them for recycling (especially glass and paper), but the amount of collected packaging waste by all wineries could be further increased.
- Most of the wineries in Cyprus (29 out of the 46 wineries, 63%) implement a number of measures mainly related to insulation, pumping of outside cold air for cellar cooling, utilization of energy released by the chiller for heating and introduction of renewable energy sources (e.g. solar photovoltaic panels). However, more wineries could apply such and even additional measures, in order to reduce the amount of energy consumed.

- The amount of chemicals used in the wineries for cleaning and disinfection purposes can be reduced by the introduction of environmentally friendly methods, such as the use of only water and steam, as well as biodegradable cleaning products. Moreover, the quantities of chemicals consumed need to be monitored and recorded by all wineries.
- In addition organic cultivation certification should be endeavoured, as only 8 out of the 46 wineries are currently certified as “organic” wineries.
- The environmental impacts of a winery can be significantly reduced through the relevant training of the personnel on environmental and safety issues. In order to effectively establish environmental management practices and provide for emergency action, winery managers need to be aware of their potential environmental impacts, the generation of potential pollutants, as well as alternative solutions in order to minimize their impacts. The Best Practice guide that was developed in the framework of this thesis, provides information to wine industry owners on key environmental issues. These include energy and water conservation, wastewater and solid waste management, and use of chemicals. If the winery owners follow the simple steps that are included in this guide, it will significantly enhance their environmental performance, and as a consequence their public image in the marketplace will be improved, by adopting pollution prevention as a part of their management policy.

9.1.2 Solar photo-Fenton bench-scale experiments

The solar photo-Fenton oxidation was applied against the bioresistant fractions of winery wastewater. Winery effluents already treated by two different biological processes: (a) a Sequencing Batch Reactor (SBR) and (b) a Membrane Bioreactor (MBR), were subjected to further purification by solar photo-Fenton oxidation process (hv/Fe^{2+}/H_2O_2). Bench-scale experiments using a solar simulator and a photochemical batch reactor were carried out to evaluate the influence of various operational parameters on the purification of these winery effluents.

The effect of various operating variables, such as the catalyst and oxidant concentration, as well as the initial pH and temperature on the abatement of chemical oxygen demand (COD), dissolved organic carbon (DOC), color, toxicity and phytotoxicity has been assessed. The main conclusions drawn from the bench-scale study are summarized as follows:

- Solar photo-Fenton has been demonstrated to be a highly effective method for the purification of both biologically pretreated flows (SBR and MBR), reaching a COD removal of approximately 70%; while the residual COD values were 82.7±8.2 mg L^{-1} and 33±3.6 mg L^{-1} for the winery effluents pretreated by SBR and MBR, respectively.
- The kinetic parameters were found to be influenced by the operating conditions employed, such as ferrous and hydrogen peroxide concentration, the initial pH of the solution and the temperature.
- In the presence of higher iron concentration, the process was slightly accelerated mainly by the photocatalytic regeneration of ferrous from ferric iron, resulting in the rapid regeneration of additional hydroxyl radicals.
- The COD removal for both biologically pretreated winery flows was increased when increasing the concentration of hydrogen peroxide up to a value. However, at higher oxidant concentration, an adverse effect on the organic matter removal was observed,

due to the recombination of HO• produced, as well as the reaction of HO• with H_2O_2, contributing to the HO• scavenging capacity.

- As already proved by various other studies, our investigation confirmed once again the fact that during the solar photo-Fenton process, the pH range should be kept between the optimum range of 2.8-3.0.
- The photolysis (solar irradiation alone) and the combined action of solar irradiation and Fe^{2+} at acidic conditions, showed no significant COD removal for both winery effluents. Under the presence of H_2O_2, 25±2.0% and 29±3.3% of COD was removed after 120 min of treatment, from the effluents pretreated by SBR and MBR, respectively; due to the photochemical cleavage of H_2O_2 to yield HO• by solar light absorption. Dark Fenton experiments showed 45±2.0 and 49±1.8% COD reduction for the effluents pretreated by SBR and MBR, respectively. The COD reduction was strongly accelerated by the solar photo-Fenton process, and after 120 min of treatment was almost 70% for both effluents, from which ~59% was removed during the first 30 min of solar photo-Fenton process.
- Solar photo-Fenton process efficiency increases with temperature, due to the fact that the regeneration of ferric to ferrous ions is faster at higher temperatures (e.g. 45 ^{o}C).
- MBR treatment seems to be an efficient process to completely remove the phenolic compounds. On the other hand, the total phenolic compounds were considerably reduced but not eliminated after the SBR treatment (from 52 to 4.3 mg L^{-1}), proving that some of those compounds are not readily biodegradable. After 120 min of solar photo-Fenton oxidation, a high reduction of the total phenolic compounds was observed (71±5.0%), corresponding to a residual total phenolic concentration of

1.2±0.2 mg L^{-1}, and to an overall total phenolic compounds reduction of 97.7±2.0% (biological (SBR) + solar photo-Fenton).

- Raw winery effluents are considerably colored (dark red) mostly due to the natural colors of grapes. It is known, that after the biological treatment, some of the colored compounds are still present in the winery effluents, because of their resistance to biodegradation. Solar photo-Fenton oxidation proved suitable for effectively oxidizing these refractory organics and it significantly removed the color from winery wastewater (up to 75±2.2%).
- The extent of mineralization (as assessed by DOC) for both biologically treated flows was lower than the COD decrease, implying thus, that partial oxidation reactions of the organic content prevail over total oxidation to carbon dioxide and water.
- The removal of the DOC for both biologically pretreated flows, was found to follow the pseudo first-order law.
- The toxicity towards *D. magna* increases during the first minutes of solar photo-Fenton oxidation, due to the formation of toxic oxidation products. Then it is usually reduced, indicating the potential oxidation of the toxic products formed, while it is possible to increase again, since more toxic oxidation products can be formed. Finally, after a longer treatment time (usually over 90 min) the toxicity is gradually reduced, and can even be eliminated at the end of the solar treatment.
- Solar photo-Fenton was proved to be an efficient method considering the wastewater reuse for agricultural irrigation purposes and recharging groundwater aquifers. The phytotoxicity of all plant species (*Sinapis alba*, *Lepidium sativum* and *Sorghum saccharatum*) at the end of solar photo-Fenton treatment was significantly lower than that of the raw effluent, for both biologically pretreated flows.

9.1.3 Solar Fenton pilot-scale experiments

This study investigated the efficiency of the solar Fenton process for the purification of two biologically pretreated winery effluents at a pilot scale. A compound parabolic collector (CPC) pilot plant was used for the photocatalytic experiments. The effect of catalyst and oxidant concentration on the abatement of chemical oxygen demand (COD), dissolved organic carbon (DOC), color, and ecotoxicity have been assessed.

The main conclusions drawn from the pilot-scale study of the solar Fenton process are listed below:

- The results obtained from the preliminary photolytic experiments (solar irradiation alone) indicated that the photolysis was not responsible for the observed significant COD reduction of winery effluents, when those were irradiated in the presence of Fenton reagent.
- Solar Fenton oxidation at a pilot scale was proved to be highly efficient in organic content removal from winery wastewater, reaching 69±4.0% and 85±2.4% COD removal for the effluent pretreated by SBR and MBR, respectively. Moreover, it should be noted, that for both biologically pretreated flows, the degradation of the organic compounds started 10 min before uncovering the reactor (dark Fenton reaction), thus reducing the initial COD value by ~40%. This is very important, since a large part of COD was removed during the first 10 min, and under dark conditions.
- A significant reduction of COD under dark conditions was achieved, and more specifically, a maximum COD removal of 48±1.4% and 57±1.9% was observed after 180 min of treatment, for the effluents pretreated by SBR and MBR, respectively.

- The solar Fenton oxidation was able to effectively oxidize the refractory colored organic compounds, and achieved a significant color removal for both biologically pretreated flows (up to 82±4.6%).
- The extent of mineralization was lower than the COD removal, indicating the formation of oxidation products, especially in the case of the effluent pretreated by SBR, where the difference was bigger.
- The DOC removal was also found to follow the pseudo first-order kinetic law in the first 15 min of the reaction, for both biologically pretreated flows.
- The results demonstrated the capacity of the solar Fenton process to significantly reduce, and in some cases even completely eliminate the initial toxicity against the aquatic microorganism *Daphnia magna* and the examined plant species (*Sinapis alba*, *Lepidium sativum* and *Sorghum saccharatum*).
- Considering the fact that the winery wastewater can be discharged into the environment, mainly for vineyards irrigation purposes, it is important to comply with the national environmental limits (Cypriot Regulatory Administrative Act 772/2003: COD < 125 mg L^{-1}, TP < 2 mg L^{-1}, TN < 15 mg L^{-1} and TSS < 35 mg L^{-1}). The combined MBR + solar Fenton process seems to be a more effective technology for winery wastewater treatment than the combined SBR + solar Fenton, since it can reduce the organic pollutants in the winery effluent to values well below than that of the Cypriot discharge limits (residual COD < 30 mg L^{-1}, TN < 1.6 mg L^{-1}, TP < 0.4 mg L^{-1} and TSS < 10 mg L^{-1}). On the other hand, the SBR treatment was found to have a significantly high residual TP value equal to 39.5 mg L^{-1}, which is well off the environmental limits. Moreover, the combined SBR + solar Fenton process has a higher residual COD equal to 82 mg L^{-1} (which however is within the

limits), and a significantly higher suspended solids load of 245 mg L^{-1}, which is significantly higher than the Cypriot discharge limits. As a result a subsequent filtration treatment is needed before its disposal into the environment. As a consequence, the combined MBR + solar Fenton combination was determined as the most efficient process, and thus selected for the industrial-scale application.

9.1.4 Industrial-scale solar Fenton treatment

In this study, the monitoring of the efficiency of solar Fenton oxidation as post-treatment of the most efficient biological treatment, which was the MBR, at an industrial scale was performed. For this reason an integrated winery wastewater treatment plant (WWWTP) based on a reliable biological treatment stage (MBR) followed by an AOP (solar Fenton treatment) was constructed, in order to achieve maximum removal of pollutants and toxicity from the final effluent. The main conclusions drawn from the industrial-scale operation are summarized as follows:

- Biological treatment (MBR) at industrial scale proved to be an efficient solution for the organic load as well as solids removals, since the biologically treated effluent had a residual COD equal to 180 mg L^{-1} and TSS< 10 mg L^{-1}. It should be mentioned that the effluent after the MBR treatment is toxic to *Daphnia magna* (53% immobilization); while regarding its phytotoxicity, it was found to cause a moderate inhibition on the three plant species tested.
- On the other hand, the combined biological treatment and solar Fenton oxidation, proved to be a more efficient process for the treatment of winery wastewater, since the latter can lead to overall elimination of toxicity to *D. magna*, high reduction of

phytotoxicity (almost complete, inhibition lower than 7.5%), as well as high additional reductions of COD, DOC and color of the order of 84±4.0%, 62±4.5% and 82±4.6%, respectively.

- The final effluent of the combined MBR and solar Fenton process had a residual COD of 28.8±7.2 mg L^{-1}, and can be discharged into the environment, or used for irrigation purposes, since its qualitative characteristics are well below those of the national environmental limits (i.e. COD $\leq$ 125 mg L^{-1}).
- Furthermore, the upscaling from the pilot to the industrial reactor resulted in the same high efficiency for the COD and DOC removal, and achieved the same level of elimination of the toxicity and phytotoxicity as well, proving once again that solar Fenton oxidation can be effectively used in a larger scale.

9.1.5 Pilot-scale reverse osmosis experiments

In order to have an integrated view for the treatment of winery wastewater, the efficiency of a membrane-based method for the purification of winery wastewater was also investigated. More specifically, reverse osmosis (RO) was selected to be used as an alternative to the biological and advanced treatment of winery wastewater, since is the tightest possible membrane process in liquid/liquid separation, and it is well known that produces the highest water quality of any pressure driven membrane process. The experiments were carried out in a pilot-scale reverse osmosis (RO) unit, which was equipped with a spiral-wounded polyamide membrane, installed at the Laboratory of Environmental Technology and Management of the Department of Environmental Engineering, at the Technical University of Crete (TUC), Greece. The RO study included a detailed evaluation of a number of process parameters for the RO streams (feed, concentrate and permeate). Moreover, since RO does not really destroy the pollutants, but merely concentrate them into smaller volumes of wastewater, the RO concentrate, solar photo-Fenton oxidation was used for the purification of the produced concentrate, in order to achieve an integrated treatment of winery wastewater.

The main conclusions drawn from the pilot-scale study of the reverse osmosis treatment of winery wastewater are listed below:

- Reverse osmosis proved to be a successful process for the treatment of the winery effluents. The removal of COD by the RO process reached a maximum value of 97%, resulting in a permeate with residual COD level lower than 150 mg L^{-1}.
- The effect of the operating pressure and temperature on the volume recovery and the COD rejection through the RO module was also investigated. It is well known that as the pressure increases, the flux increases linearly, and the product quality increases as well. As a consequence, the 10 bar working pressure, and the temperature of 26 ^{o}C were identified as the optimum, giving the maximum volume recovery (50%), and the maximum COD rejection (97.4%), in a single-pass mode.
- Both the feed wastewater and the concentrate stream even after tremendous dilutions (initial winery wastewater concentrations up to 10%) showed high toxicity towards *D. magna* (100% immobilization of microorganism) after prolonged exposure of 48 h. In contrast, the undiluted permeate stream proved to be non toxic to *D. magna*, for both times of exposure (0% immobilization); indicating that the toxicity to *D. magna* was completely eliminated through the RO process.
- The phytotoxicity (GI, SI and RI) for all the three plant species, was considerably reduced after the RO treatment, for the permeate stream, compared to the feed

wastewater, at values lower than 21%; while on the other hand, was significantly increased at the concentrate stream, even up to 30% higher than that of the feed wastewater.

- Although the recirculation mode could increase the volume recovery by 15% compared to the single-pass mode, achieving thus its primary goal of reducing the volume of concentrate that need further treatment before its disposal in the environment, it was proved to be less efficient than the single-pass mode for the treatment of winery wastewater due to the increase of the permeate COD (by 44 mg L^{-1}).
- The concentration of five selected phenolic compounds was also studied in the concentrate stream with HPLC analysis, with a view to future recovery, and was found to be higher than 98.7% for the five phenolic compounds examined (i.e. gallic acid, vanillic acid, epicatechin, p-coumaric acid and ferulic acid).
- For the treatment of the concentrate stream, an alternative treatment method (solar photo-Fenton oxidation) was tested, in order to reduce its organic content. The effect of various operating variables, such as the catalyst and oxidant concentration, as well as the use of hydrogen peroxide in excess and with or without the use of the solar irradiation on the abatement of chemical oxygen demand (COD), toxicity and phytotoxicity have been assessed.
  - In the presence of higher iron concentration (up to 20 mg L^{-1}), the process was accelerated mainly by photocatalytic regeneration of ferrous from ferric iron resulting in the rapid regeneration of additional hydroxyl radicals.
  - An increase in the oxidant concentration is well known to induce the generation of higher amounts of HO• thus enhancing the degradation efficiency (up to

41±2.4% after 4 h of solar treatment); however, excessive concentrations of H_2O_2 (over the optimum value of 1000 mg L^{-1}) may lead to unwanted 'scavenging' reactions, which may even decrease the number of available hydroxyl radicals and ultimately the process efficacy.

- The combination of the solar photo-Fenton oxidation (after 28 h of solar irradiation) and further dilution was able to significantly reduce the toxicity of the concentrate stream to values lower than 45±1.8%.
- Moreover, the concentrate stream after 28 h of solar photo-Fenton treatment showed a 3- to 4-fold phytotoxicity reduction comparing to the initial concentrate stream. Additionally, it must be noted that the GI, SI and RI of the concentrate stream after solar photo-Fenton treatment, were also lower (almost half for all three plant species) than the GI, SI and RI of the RO feed winery wastewater.

9.1.6 Cost estimation

A detail estimation of the total ownership cost for five-year operation of (a) a membrane bioreactor (MBR), (b) a solar Fenton system, and (c) an integrated reverse osmosis (RO) treatment plant, with treatment capacity of 50 m^3 of winery wastewater per day was calculated. For this purpose, the investment cost along with the operational cost (including mainly electric power and consumables), and the maintenance cost were estimated.

The main conclusions drawn from the cost estimations of the three processes applied for the treatment of winery wastewater are listed below:

- The construction cost of an MBR is a major contributor to the total investment cost that relate to the necessary tanks used as the processing units and the necessary service buildings. The operation and maintenance expenses include the personnel salaries for maintenance and operation, the cost of electrical energy, and the chemicals' consumption for the cleaning of membranes. The total cost of the full-scale MBR unit for the treatment of 50 m^3 of winery effluent per day was estimated to be 194100 € for five-year operation, which corresponds to 38820 € yr^{-1}, and to 2.1 € m^{-3}; while the total operational cost was found to be 0.65 € m^{-3}.
- The solar Fenton system consisting of a storage tank, a feeding system to solar oxidation reactors, the three chemical dosing systems (H_2O_2, $FeSO_4 \cdot 7H_2O$ and H_2SO_4), the compounds parabolic collectors and the control panel. The total cost of the full-scale solar Fenton unit for the treatment of 50 m^3 of winery effluent per day was found to be 166700 € for a five-year operation, which corresponds to 33340 € yr^{-1}, and to 1.83 € m^{-3}; while the total operational cost was found to be 1.13 € m^{-3}.
- The items for consideration in the design of an integrated reverse osmosis system are the preliminary treatment (screening, equalization / balancing tank, pH adjustment), the UF pretreatment, the RO treatment, and the UF and RO concentrate management. The total cost of the full-scale integrated RO system for the treatment of 50 m^3 of winery effluent per day was found to be 232400 € for five-year operation, which corresponds to 46480 € yr^{-1}, and to 2.55 € m^{-3}, while the total operational cost was found to be 1.4 € m^{-3}

Taking into consideration that both the MBR and the RO system need to be followed by the solar Fenton system (as post-treatment), either as a polishing step (e.g. for the elimination of effluents' organic content and its toxicity), or as a further treatment for the concentrate stream before its disposal in the environment, the optimum and the most cost-effective integrated system for the treatment of winery wastewater seems to be the MBR + solar Fenton process. This is because of being the less expensive (both in relation to the capital and operational cost), and is also the system with the higher efficiency, able to reduce the organic pollutants in the winery effluent to values well below those included in the Cypriot discharge limits.

9.2 Future work

In this section selected important future research directions related to the advanced treatment of winery wastewater that worth further investigation are presented.

- Evaluation of the toxicity potential of the solar Fenton treated effluents with regard to irrigation of crops (*e.g* vegetables).
- Evaluation of the soil toxicity of winery wastewater that originates after biological + solar Fenton treatment.
- Identification of the kinetics of solar photo-Fenton process based on the consumption of hydrogen peroxide.
- Identification of the unknown phenolic compounds that were found to possibly exist in the raw winery wastewater with the use of an Ultra Performance Liquid Chromatography - tandem mass spectrometric (UPLC/MS/MS), as well as of the phenolic compounds that may possibly remained after the various treatments applied (MBR, solar Fenton and RO).
- Recovery of the valuable phenolic compounds that can be reused in other industries (e.g. pharmaceuticals, food, etc.) and are contained in high concentrations in the RO concentrate stream of the winery wastewater, through various processes like solvent extraction, adsorption onto resins, selective membranes, supercritical fluid extraction, etc.
- Estimation of the total cost (investment and operational) of the technologies developed and applied in this thesis per bottle of the wine produced (€ bottle^{-1}).

CHAPTER 10: REFERENCES

Abdessalem, A. K., Bellakhal, N., Oturan, N., Dachraoui, M., Oturan, M. A. 2010. Treatment of a mixture of three pesticides by photo-and electro-Fenton processes. *Desalination* 250 (1), 450-455.

Abegglen, C., Ospelt, M., Siegrist, H. 2008. Biological nutrient removal in a small-scale MBR treating household wastewater. *Water Research* 42 (1), 338-346.

Agüera, A., Mezcua, M., Hernando, D., Malato, S., Cáceres, J., Fernández-Alba, A. 2004. Application of GC-MS and GC-AED to the evaluation of by-products formed by solar photo-fenton degradation of Methyl tert-butyl ether in water. *International Journal of Environmental Analytical Chemistry* 84 (1-3), 149-159.

Ahmed, B., Limem, E., Abdel-Wahab, A., Nasr, B. 2011. Photo-Fenton treatment of actual agro-industrial wastewaters. *Industrial & Engineering Chemistry Research* 50 (11), 6673-6680.

Akdemir, E. O. and Ozer, A. 2009. Investigation of two ultrafiltration membranes for treatment of olive oil mill wastewater. *Desalination* 249 (2), 660-666.

Akgul, D., Çakmakcı, M., Kayaalp, N., Koyuncu, I. 2008. Cost analysis of seawater desalination with reverse osmosis in Turkey. *Desalination* 220 (1), 123-131.

Al-Bastaki, N. 2004. Removal of methyl orange dye and Na_2SO_4 salt from synthetic waste water using reverse osmosis. *Chemical Engineering and Processing: Process Intensification* 43 (12), 1561-1567.

Al-Rekabi, W. S., Qiang, H., Qiang, W. W. 2007. Review on sequencing batch reactors. *Pakistan Journal of nutrition* 6 (1), 11-19.

Amjad, Z. (1993) Reverse Osmosis, Van Nostrand Reinhold, New York, USA.

Anastasiadi, M., Chorianopoulos, N. G., Nychas, G. E., Haroutounian, S. A. 2009. Antilisterial activities of polyphenol-rich extracts of grapes and vinification byproducts. *Journal of Agricultural and Food Chemistry* 57 (2), 457-463.

Anastasiou, N., Monou, M., Mantzavinos, D., Kassinos, D. 2009. Monitoring of the quality of winery influents/effluents and polishing of partially treated winery flows by homogeneous Fe (II) photo-oxidation. *Desalination* 248 (1), 836-842.

Anderson, K. (2001) The globalization and regionalization of wine. Centre for International Economic Studies, Adelaide University, SA5005, Australia.

Anderson, K. and Norman, D. (2003) Global wine production, consumption and trade, 1961 to 2001: a statistical compendium. Centre for International Economic Studies, University of Adelaide, Australia.

Anderson, K., Norman, D., Wittwer, G. 2003. Globalisation of the world's wine markets. *The World Economy* 26 (5), 659-687.

Andreottola, G., Foladori, P., Nardelli, P., Denicolo, A. 2005. Treatment of winery wastewater in a full-scale fixed bed biofilm reactor. *Sustainable Viticulture and Winery Wastes Management* 51 (1), 71-79.

Andreozzi, R., Caprio, V., Insola, A., Marotta, R. 1999. Advanced oxidation processes (AOP) for water purification and recovery. *Catalysis Today* 53 (1), 51-59.

Ang, W. S., Lee, S., Elimelech, M. 2006. Chemical and physical aspects of cleaning of organic-fouled reverse osmosis membranes. *Journal of Membrane Science* 272 (1), 198-210.

APHA, (1998) Standard Methods for the Examination of Water and Wastewater, American Public Health Association, 20th edn, Washington, DC, USA.

Aranda, A., Zabalza, I., Scarpellini, S. 2005. Economic and environmental analysis of the wine bottle production in Spain by means of life cycle assessment. *International Journal of Agricultural Resources, Governance and Ecology* 4 (2), 178-191.

Ardente, F., Beccali, G., Cellura, M., Marvuglia, A. 2006. POEMS: A Case Study of an Italian Wine-Producing Firm. *Environmental Management* 38 (3), 350-364.

Arcese, G., Lucchetti, M. C., Martucci, O. 2012. Analysis of Sustainability Based on Life Cycle Assessment: An Empirical Study of Wine Production. *Journal of Environmental Science and Engineering B* 1, 682-689.

Arienzo, M., Christen, E. W., Quayle, W. C. 2009b. Phytotoxicity testing of winery wastewater for constructed wetland treatment. *Journal of Hazardous Materials* 169 (1), 94-99.

Arienzo, M., Christen, E. W., Quayle, W., Stefano, N. D. 2009a. Development of a low-cost wastewater treatment system for small-scale wineries. *Water Environment Research* 81 (3), 233-241.

Artiga, P., García-Toriello, G., Méndez, R., Garrido, J. 2007. Use of a hybrid membrane bioreactor for the treatment of saline wastewater from a fish canning factory. *Desalination* 221 (1), 518-525.

Artiga, P., Ficara, E., Malpei, F., Garrido, J., Mendez, R. 2005. Treatment of two industrial wastewaters in a submerged membrane bioreactor. *Desalination* 179 (1), 161-169.

Arvanitoyannis, I. S., Ladas, D., Mavromatis, A. 2006a. Potential uses and applications of treated wine waste: a review. *International Journal of Food Science & Technology* 41 (5), 475-487.

Arvanitoyannis, I. S., Ladas, D., Mavromatis, A. 2006b. Wine waste treatment methodology. *International Journal of Food Science & Technology* 41 (10), 1117-1151.

Asami, D. K., Hong, Y-J., Barrett, D. M., Mitchell, A. E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry* 51 (5), 1237-1241.

Asses, N., Ayed, L., Bouallagui, H., Sayadi, S., Hamdi, M. 2009. Biodegradation of different molecular-mass polyphenols derived from olive mill wastewaters by Geotrichum candidum. *International Biodeterioration & Biodegradation* 63 (4), 407-413.

Awad, Y. M. and Abuzaid, N. S. 1997. Electrochemical treatment of phenolic wastewater: Efficiency, design considerations and economic evaluation. *Journal of Environmental Science & Health Part A* 32 (5), 1393-1414.

Azbar, N., Yonar, T., Kestioglu, K. 2004. Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. *Chemosphere* 55 (1), 35-43.

Badani, Z., Ait-Amar, H., Si-Salah, A., Brik, M., Fuchs, W. 2005. Treatment of textile waste water by membrane bioreactor and reuse. *Desalination* 185 (1), 411-417.

Badawy, M. I., Ali, M. E. M. 2006. Fenton's peroxidation and coagulation processes for the treatment of combined industrial and domestic wastewater. *Journal of Hazardous Materials* 136 (3), 961-966.

Bae, T., Han, S., Tak, T. 2003. Membrane sequencing batch reactor system for the treatment of dairy industry wastewater. *Process Biochemistry* 39 (2), 221-231.

Bahnemann, D. 2004. Photocatalytic water treatment: solar energy applications. *Solar Energy* 77 (5), 445-459.

Bagastyo, A. Y., Keller, J., Poussade, Y., Batstone, D. J. 2011. Characterisation and removal of recalcitrants in reverse osmosis concentrates from water reclamation plants. *Water Research* 45 (7), 2415-2427.

Bandara, J., Pulgarin, C., Peringer, P., Kiwi, J. 1997. Chemical photoactivated coupling of biological homogeneous degradation of p-nitrotoluene sulfonic acid in a flow reactor. *Journal of Photochemistry and Photobiology A: Chemistry* 111 (1), 253-263.

Barb, W., Baxendale, J., George, P., Hargrave, K. 1951a. Reactions of ferrous and ferric ions with hydrogen peroxide. Part I. The ferrous ion reaction. *Transactions of the Faraday Society* 47, 462-500.

Barb, W., Baxendale, J., George, P., Hargrave, K. 1951b. Reactions of ferrous and ferric ions with hydrogen peroxide. Part II.-The ferric ion reaction. *Transactions of the Faraday Society* 47, 591-616.

Barlow, K., Bond, W., Holzapfel, B., Smith, J., Hutton, R. 2009. Nitrogen concentrations in soil solution and surface run-off on irrigated vineyards in Australia. *Australian Journal of Grape and Wine Research* 15 (2), 131-143.

Baughman A. T., Brown E. J., Brummet W., Dramko J. M., Goldstein J. H., Hooper B. E. (2000). California Winemaking Impact Assessment, Master Thesis, University of California, Santa Barbara.

Beck-Friis, B., Smars, S., Jönsson, H., Kirchmann, H. 2001. Gaseous emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. *Journal of Agricultural Engineering Research* 78 (4), 423-430.

Beddow, V. 2011. Best practice water management in wineries. (Accessed 13/3/2012), Available from World Wide Web: <http://www.iwawaterwiki.org/xwiki/bin/view/ Articles/ WaterManagementinWineries> .

Bellona, C., Drewes, J. E., Xu, P., Amy, G. 2004. Factors affecting the rejection of organic solutes during NF/RO treatment-a literature review. *Water Research* 38 (12), 2795-2809.

Beltran, F. J., García-Araya, J. F., Alvarez, P. M. 2000. Continuous flow integrated chemical (ozone)-activated sludge system treating combined agro-industrial - domestic wastewater. *Environmental Progress* 19 (1), 28-35.

Benitez, J. F., Francisco R. J., Acero, J. L., Garcia, J., Sanchez, M. 2003. Kinetics of the ozonation and aerobic biodegradation of wine vinasses in discontinuous and continuous processes. *Journal of Hazardous Materials* 101 (2), 203-218.

Benito, Y. and Ruiz, M. L. 2002. Reverse osmosis applied to metal finishing wastewater. *Desalination* 142 (3), 229-234.

Benner, J., Salhi, E., Ternes, T., von Gunten, U. 2008. Ozonation of reverse osmosis concentrate: Kinetics and efficiency of beta blocker oxidation. *Water Research* 42 (12), 3003-3012.

Bennett, L. E. and Drikas, M. 1993. The evaluation of colour in natural waters. *Water Research* 27 (7), 1209-1218.

Berg, S. and Lindholm, E. 2005. Energy use and environmental impacts of forest operations in Sweden. *Journal of Cleaner Production* 13 (1), 33-42.

Bertran, E., Sort, X., Soliva, M., Trillas, I. 2004. Composting winery waste: sludges and grape stalks. *Bioresource Technology* 95 (2), 203-208.

Bisson, L. F., Waterhouse, A. L., Ebeler, S. E., Walker, M. A., Lapsley, J. T. 2002. The present and future of the international wine industry. *Nature* 418 (6898), 696-699.

Bixio, D., Thoeye, C., De Koning, J., Joksimovic, D., Savic, D., Wintgens, T., Melin, T. 2006. Wastewater reuse in Europe. *Desalination* 187 (1), 89-101.

Bodalo-Santoyo, A., Gómez-Carrasco, J., Gómez-Gómez, E., Maximo-Martin, F., Hidalgo-Montesinos, A. 2003. Application of reverse osmosis to reduce pollutants present in industrial wastewater. *Desalination* 155, 101-108.

Boland, A.M. and Tee, E. (2005) Good Environmental Management Guidelines: Vineyard Pest and Chemical Management, Cooperative Research Centre for Viticulture.

Bolzonella, D. and Rosso, D. 2007. Winery wastewater characterisation and biological treatment options. *Water Science and Technology* 56 (2) 79-87.

Bonnelye, V., Sanz, M.A., Durand, J-P., Plasse, L. Gueguen, F., Mazounie P. 2004. Reverse osmosis on open intake seawater: pre-treatment strategy. *Desalination* 167, 191-200.

Bories, A., Sire, Y., Colin, T. 2005. Odorous compounds treatment of winery and distillery effluents during natural evaporation in ponds. *Sustainable Viticulture and Winery Wastes Management* 51 (1), 129-136.

Bossmann, S. H., Oliveros, E., Göb, S., Siegwart, S., Dahlen, E. P., Payawan Jr, L., Straub, M., Wörner, M., Braun, A. M. 1998. New evidence against hydroxyl radicals as reactive intermediates in the thermal and photochemically enhanced Fenton reactions. *The Journal of Physical Chemistry A* 102 (28), 5542-5550.

Bourgeous, K. N., Darby, J. L., Tchobanoglous, G. 2001. Ultrafiltration of wastewater: effects of particles, mode of operation, and backwash effectiveness. *Water Research* 35 (1), 77-90.

Braz, R., Pirra, A., Lucas, M. S., Peres, J. A. 2010. Combination of long term aerated storage and chemical coagulation/flocculation to winery wastewater treatment. *Desalination* 263 (1), 226-232.

Breeuwsma, A. and Silva, S. (1992) Phosphorus fertilisation and environmental effects in the Netherlands and the Po region (Italy). DLO The Winand Staring Centre.

Brentrup, F., Küsters, J., Kuhlmann, H., Lammel, J. 2004. Environmental impact assessment of agricultural production systems using the life cycle assessment methodology: I. Theoretical concept of a LCA method tailored to crop production. *European Journal of Agronomy* 20 (3), 247-264.

Brepols, C., Drensla, K., Janot, A., Trimborn, M., Engelhardt, N. 2008. Strategies for chemical cleaning in large scale membrane bioreactors. *Water Science and Technology* 57 (3), 457-463.

Broome, J.C. and Warner, K.D. 2008. Agro-environmental partnerships facilitate sustainable wine-grape production and assessment. Official Webpage (online) (Accessed 20/3/2012), Available from World Wide Web: <http://California Agriculture.ucop.edu>.

Brown, D.T. and Pollard, K. (2009) Waste management in the wine industry, Sustainable Integrated Waste Management. Brock University of Canada, America.

Brucculeri, M., Bolzonella, D., Battistoni, P., Cecchi, F. 2005. Treatment of mixed municipal and winery wastewaters in a conventional activated sludge process: a case study. *Water Science & Technology* 51 (1), 89-98.

Bustamante, M., Paredes, C., Morales, J., Mayoral, A., Moral, R. 2009. Study of the composting process of winery and distillery wastes using multivariate techniques. *Bioresource Technology* 100 (20), 4766-4772.

Bustamante, M. A., Paredes, C., Moral, R., Moreno-Caselles, J., Pérez-Espinosa, A., Pérez-Murcia, M. D. 2005. Uses of winery and distillery effluents in agriculture: characterisation of nutrient and hazardous components. *Water Science & Technology* 51 (1), 145-151.

Bustamante, M., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M. 2008. Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. *Waste Management* 28 (2), 372-380.

Bustamante, M., Pérez-Murcia, M., Paredes, C., Moral, R., Pérez-Espinosa, A., Moreno-Caselles, J. 2007. Short-term carbon and nitrogen mineralisation in soil amended with winery and distillery organic wastes. *Bioresource Technology* 98 (17), 3269-3277.

Buyukkamaci, N. 2004. Biological sludge conditioning by Fenton's reagent. *Process Biochemistry* 39 (11), 1503-1506.

Canepa, P., Marignetti, N., Rognoni, U., Calgari, S. 1988. Olive mills wastewater treatment by combined membrane processes. *Water Research* 22 (12), 1491-1494.

Cañizares, P., Paz, R., Sáez, C., Rodrigo, M. A. 2009. Costs of the electrochemical oxidation of wastewaters: A comparison with ozonation and Fenton oxidation processes. *Journal of Environmental Management* 90 (1), 410-420.

Cieśla, P., Kocot, P., Mytych, P., Stasicka, Z. 2004. Homogeneous photocatalysis by transition metal complexes in the environment. *Journal of Molecular Catalysis A: Chemical* 224 (1), 17-33.

Capasso, R., Cristinzio, G., Evidente, A., Scognamiglio, F. 1992. Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. *Phytochemistry* 31 (12), 4125-4128.

Capelo, J., Lavilla, I., Bendicho, C. 2001. Ultrasonic extraction followed by sonolysis-ozonolysis as a sample pretreatment method for determination of reactive arsenic toward sodium tetrahydroborate by flow injection-hydride generation AAS. *Analytical Chemistry* 73 (15), 3732-3736.

Cassano, D., Zapata, A., Brunetti, G., Del Moro, G., Di Iaconi, C., Oller, I., Malato, S., Mascolo, G. 2011. Comparison of several combined/integrated biological-AOPs setups for the treatment of municipal landfill leachate: Minimization of operating costs and effluent toxicity. *Chemical Engineering Journal* 172 (1), 250-257.

Cater, S. R., Stefan, M. I., Bolton, J. R., Safarzadeh-Amiri, A. 2000. UV/H_2O_2 treatment of methyl tert-butyl ether in contaminated waters. *Environmental Science & Technology* 34 (4), 659-662.

Chacón, J. M., Leal, T., Sánchez, M., Bandala, E. R. 2006. Solar photocatalytic degradation of azo-dyes by photo-Fenton process. *Dyes and Pigments* 69 (3), 144-150.

Chang, C. H. and Hao, O. J. 1996. Sequencing batch reactor system for nutrient removal: ORP and pH profiles. *Journal of Chemical Technology and Biotechnology* 67 (1), 27-38.

Chapagain, A. K. and Orr, S. 2009. An improved water footprint methodology linking global consumption to local water resources: A case of Spanish tomatoes. *Journal of Environmental Management* 90 (2), 1219-1228.

Chapagain, A. K. and Hoekstra, A. Y. 2008. The global component of freshwater demand and supply: an assessment of virtual water flows between nations as a result of trade in agricultural and industrial products. *Water International* 33 (1), 19-32.

Chapman, J. A., Correll, R. L., Ladd, J. N., 1995. Removal of soluble organic carbon from winery and distillery wastewaters by application to soil. *Australian Journal of Grape and Wine Research* 1 (1), 39-47.

Chatzilazarou, A., Katsoyannos, E., Gortzi, O., Lalas, S., Paraskevopoulos, Y., Dourtoglou, E., Tsaknis, J., 2010. Removal of polyphenols from wine sludge using cloud point extraction. *Journal of the Air & Waste Management Association* 60 (4), 454-459.

Chelme-Ayala, P., El-Din, M. G., Smith, D. W. 2010. Treatability Study on Membrane Concentrate Containing Pesticides Using Advanced Oxidation Processes. *Ozone: Science & Engineering* 32 (1), 16-24.

Chiron, S., Fernandez-Alba, A., Rodriguez, A., Garcia-Calvo, E. 2000. Pesticide chemical oxidation: state-of-the-art. *Water Research* 34 (2), 366-377.

Ghiselli, A., Nardini, M., Baldi, A., Scaccini, C. 1998. Antioxidant activity of different phenolic fractions separated from an Italian red wine. Journal of Agricultural and Food Chemistry 46 (2), 361-367.

Cieśla, P., Kocot, P., Mytych, P., Stasicka, Z. 2004. Homogeneous photocatalysis by transition metal complexes in the environment. *Journal of Molecular Catalysis A: Chemical* 224 (1), 17-33.

Coetzee, G., Malandra, L., Wolfaardt, G., Viljoen-Bloom, M. 2004. Dynamics of a microbial biofilm in a rotating biological contactor for the treatment of winery effluent. *Water SA* 30 (3), 407-412.

Colin, T., Bories, A., Sire, Y., Perrin, R. 2005. Treatment and valorisation of winery wastewater by a new biophysical process (ECCF). *Water Science Technology* 51 (1), 99-106.

Colman, T. and Paster, P. (2007) Red, white and 'green': the cost of carbon in the global wine trade. American Association of wine economics.

Conidi, C., Cassano, A., Drioli, E. 2011. A membrane-based study for the recovery of polyphenols from bergamot juice. *Journal of Membrane Science* 375 (1), 182-190.

Comninellis, C., Kapalka, A., Malato, S., Parsons, S. A., Poulios, I., Mantzavinos, D. 2008. Advanced oxidation processes for water treatment: advances and trends for R&D. *Journal of Chemical Technology and Biotechnology* 83 (6), 769-776.

Cornellus, S.O. 1997. Chemicals used in Making Wine. *Chemical Engineering News* 65(1), 19-28.

Coskun, T., Debik, E., Demir, N. M. 2010. Treatment of olive mill wastewaters by nanofiltration and reverse osmosis membranes. *Desalination* 259 (1), 65-70.

Cote, P., Masini, M., Mourato, D. 2004. Comparison of membrane options for water reuse and reclamation. *Desalination* 167, 1-11.

Crites, R. W., Reed, S. C., Bastian, R. K. (2000) Land treatment systems for municipal and industrial wastes. McGraw-Hill New York.

Cronje, A., Barker, A., Guy, S., Turner, C., Williams, A. 2002. Ammonia emissions and pathogen inactivation during composting. In: Proceedings of the 2002 International Symposium Composting and Compost Utilization. JG Press, Emmaus, 845-856.

Caéfalvay, E., Imre, P.M., Mizsey, P. 2008. Applicability of nanofiltration and reverse osmosis for the treatment of wastewater of different origin. *Central European Journal of Chemistry* 6 (2), 277-283.

Cypriot Wine Products Council, 2013. Annual Report 2011, Official webpage (Accessed 12/4/2013), Available from World Wide Web: <http://www.wpc.org.cy/>.

Cyprus Law 160(I)/2002, 2002 on the pollution of water bodies.

Cyprus Law 32(I)/2002, 2002 on packaging and packaging waste.

Cyprus Regulatory Administrative Act 706/2004, 2004 on Specific Areas and Appellation of Controlled Origin Wine Production Criteria.

Cyprus Regulatory Administrative Act 772/2003, 2003 on the pollution of water bodies (urban wastewaters discharges).

Daffonchio, D., Colombo, M., Origgi, G., Sorlini, C., Andreoni, V. 1998. Anaerobic digestion of winery wastewaters derived from different wine making processes. *Journal of Environmental Science & Health Part A* 33 (8), 1753-1770.

Dai, H., Yang, X., Dong, T., Ke, Y., Wang, T. 2010. Engineering application of MBR process to the treatment of beer brewing wastewater. *Modern Applied Science*, 4 (9), 103.

de Heredia, J. B., Dominguez, J., Partido, E. 2005a. Physico-chemical treatment for the depuration of wine distillery wastewaters (vinasses). *Sustainable Viticulture and Winery Wastes Management* 51 (1), 159-166.

de Heredia, J. B., Torregrosa, J., Dominguez, J. R., Partido, E. 2005b. Degradation of wine distillery wastewaters by the combination of aerobic biological treatment with chemical oxidation by Fenton's reagent. *Sustainable Viticulture and Winery Wastes Management* 51 (1), 167-174.

De Laat, J., Truong Le, G., Legube, B. 2004. A comparative study of the effects of chloride, sulfate and nitrate ions on the rates of decomposition of H_2O_2 and organic compounds by Fe(II)/H_2O_2 and Fe(III)/H_2O_2. *Chemosphere* 55 (5), 715-723.

De Lucas, A., Cañizares, P., Rodrigo, M., Garcia-Gomez, J. 2002. Electrochemical treatment of aqueous phenol wastes: A preliminary economical outlook. *Waste Management*, 161-170.

DeCarolis, J., Adham, S., Grounds, J., Pearce, B., Wasserman, L. 2004. Cost analysis of MBR systems for water reclamation. *Proceedings of the Water Environment Federation* 2004 (10), 401-411.é

DellaGreca, M., Monaco, P., Pinto, G., Pollio, A., Previtera, L., Temussi, F. 2001. Phytotoxicity of low-molecular-weight phenols from olive mill waste waters. *Bulletin of Environmental Contamination and Toxicology* 67 (3), 352-359.

Dell'Agli, M., Buscialà, A., Bosisio, M. 2004. Vascular effects of wine polyphenols, *Cardiovascular Research* 63, 593-602.

Dhaouadi, H. and Marrot, B. 2008. Olive mill wastewater treatment in a membrane bioreactor: Process feasibility and performances. *Chemical Engineering Journal* 145 (2), 225-231.

Dialynas, E. and Diamadopoulos, E. 2008. Integration of immersed membrane ultrafiltration with coagulation and activated carbon adsorption for advanced treatment of municipal wastewater. *Desalination* 230 (1), 113-127.

Dialynas, E., Mantzavinos, D., Diamadopoulos, E. 2008. Advanced treatment of the reverse osmosis concentrate produced during reclamation of municipal wastewater. *Water Research* 42 (18), 4603-4608.

Díaz-Reinoso, B., González-López, N., Moure, A., Domínguez, H., Parajó, J. C. 2010. Recovery of antioxidants from industrial waste liquors using membranes and polymeric resins. *Journal of Food Engineering* 96 (1), 127-133.

Dickens, P.S. (1993) Making Pollution Prevention Work, 48th Purdue Industrial Waste Conference Proceedings, Lewis Publications, Inc. Chelsea, Michigan, 39-42.

Dogruel, S., Olmez-Hanci, T., Kartal, Z., Arslan-Alaton, I., Orhon, D., Dixon, D., Mantzavinos, D., Kassinos, D., Parsons, S. 2009. Effect of Fenton's oxidation on the particle size distribution of organic carbon in olive mill wastewater. *Water Research* 43 (16), 3974-3983.

Dong, M.W. (2006) Modern HPLC for practicing scientists. John Wiley & Sons, Inc., Publication.

Ebrahim, S., Abdel-Jawad, M., Bou-Hamad, S., Safar, M. 2001. Fifteen years of R&D program in seawater desalination at KISR Part I. Pretreatment technologies for RO systems. *Desalination* 135 (1), 141-153.

Eiro, M. J., Heinonen, M. 2002. Anthocyanin color behavior and stability during storage: Effect of intermolecular copigmentation. *Journal of Agricultural and Food Chemistry* 50 (25), 7461-7466.

Elwell, D., Hong, J., Keener, H. 2002. Composting hog manure/sawdust mixtures using intermittent and continuous aeration: Ammonia emissions. *Compost Science & Utilization* 10 (2), 142-149.

Environmental Protection Agency (EPA), (2002) Environmental management at the wineries in California.

Environmental Protection Agency (EPA), (2006) Best practice guide for water and waste management in the Quennsland wine industry.

Environmental Protection Authority (EPA) Victoria, 2011. Official Webpage (online) (Accessed 23/2/2013), Available from World Wide Web: < http://www.epa.vic.gov.au/search?q=wineEMS >.

Eren, H. A. 2006. Afterclearing by ozonation: a novel approach for disperse dyeing of polyester. *Coloration Technology* 122 (6), 329-333.

European Environmental Agency (2009) Water, Official Webpage (online) (Accessed 20/5/2013) available from World Wide Web: <http://www.eea.europa.eu/themes/water>.

European Union (2010) Water Scarcity and Drought in the European Union, Official Webpage (online) (Accessed 14/5/2013) available on European Commission's water scarcity & droughts page: <http://ec.europa.eu/ environment/water/quantity/scarcity_en.htm>.

Eusebio, A., Petruccioli, M., Lageiro, M., Federici, F., Duarte, J. 2004. Microbial characterisation of activated sludge in jet-loop bioreactors treating winery wastewaters. *Journal of Industrial Microbiology & Biotechnology* 31 (1), 29-34.

Evgenidou, E., Bizani, E., Christophoridis, C., Fytianos, K. 2007a. Heterogeneous photocatalytic degradation of prometryn in aqueous solutions under UV-Vis irradiation. *Chemosphere* 68 (10), 1877-1882.

Evgenidou, E., Konstantinou, I., Fytianos, K., Poulios, I. 2007b. Oxidation of two organophosphorous insecticides by the photo-assisted Fenton reaction. *Water Research* 41 (9), 2015-2027.

Falkenmark, M. 2008. Water and sustainability: a reappraisal. *Environment: Science and Policy for Sustainable Development* 50 (2), 4-17.

Farré, M. and Barceló, D. 2003. Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis. *TrAC Trends in Analytical Chemistry* 22 (5), 299-310.

FAO, Food and Agriculture Organization of the United Nations (2013). FAO statistical Yearbook 2012 Europe and Central Asia Food and agriculture, Official Webpage (online) (Accessed 14/4/2013) Available from World Wide Web: < http://www.fao.org/docrep/017/i3138e/i3138e00.htm>.

Fentona, K. and Chemizmu, L. 2009. Fenton reaction-controversy concerning the chemistry. *Ecological Chemistry and Engineerings* 16 (3), 347-358.

Fernández-Alba, A., Hernando, D., Agüera, A., Cáceres, J., Malato, S. 2002. Toxicity assays: a way for evaluating AOPs efficiency. *Water Research* 36 (17), 4255-4262.

Ferrarini, R., Versari, A., Galassi, S. 2001. A preliminary comparison between nanofiltration and reverse osmosis membranes for grape juice treatment. *Journal of Food Engineering* 50 (2), 113-116.

Ferrer, J., Páez, G., Mármol, Z., Ramones, E., Chandler, C., Marın, M., Ferrer, A. 2001. Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology* 76 (1), 39-44.

Forsyth, K., Oemcke, D., Michael, P. (2008) Greenhouse Gas Accounting Protocol for the International Wine Industry. Provisor Pty Ltd.Glen Osmond, Australia.

Foster, M. and Spencer, D. (2002) World wine market. Barriers to Increasing Trade, Abare Economics Report for the Grape and Wine Research and Development Corporation. Australian Bureau of Agricultural and Resource Economics, Australia

Fritzmann, C., Löwenberg, J., Wintgens, T., Melin, T. 2007. State-of-the-art of reverse osmosis desalination. *Desalination* 216 (1), 1-76.

Fumi, M. D., Parodi, G., Parodi, E., Silva, A., Marchetti, R. 1995. Optimisation of long-term activated-sludge treatment of winery wastewater. *Bioresource Technology* 52 (1), 45-51.

Galitsky, C., Worrell, E., Radspieler, A., Healy, P., Zechiel, S. (2005). BEST Winery Guidebook: Benchmarking and Energy and Water Savings Tool for the Wine Industry. Ernest Orlando Lawrence Berkeley National Laboratory, California.

Ganesh, R., Rajinikanth, R., Thanikal, J. V., Ramanujam, R. A., Torrijos, M. 2010. Anaerobic treatment of winery wastewater in fixed bed reactors. *Bioprocess and Biosystems Engineering* 33 (5), 619-628.

Gao, B-Y., Wang, Y., Yue, Q-Y., Wei, J-C., Li, Q. 2007. Color removal from simulated dye water and actual textile wastewater using a composite coagulant prepared by ployferric chloride and polydimethyldiallylammonium chloride. *Separation and Purification Technology* 54 (2), 157-163.

Gao, Y., Yang, M., Hu, J., Zhang, Y. 2004. Fenton's process for simultaneous removal of TOC and Fe^{2+} from acidic waste liquor. *Desalination* 160, 123-130.

Garcia-Castello, E., Cassano, A., Criscuoli, A., Conidi, C., Drioli, E. 2010. Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system. *Water Research* 44 (13), 3883-3892.

García-Falcón, M., Pérez-Lamela, C., Martínez-Carballo, E., Simal-Gándara, J. 2007. Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. *Food Chemistry* 105 (1), 248-259.

García-Montaño, J., Torrades, F., García-Hortal, J. A., Domenech, X., Peral, J. 2006. Degradation of Procion Red H-E7B reactive dye by coupling a photo-Fenton system with a sequencing batch reactor. *Journal of Hazardous Materials* 134 (1), 220-229.

García-Montaño, J., Pérez-Estrada, L., Oller, I., Maldonado, M. I., Torrades, F., Peral, J. 2008. Pilot plant scale reactive dyes degradation by solar photo-Fenton and biological processes. *Journal of Photochemistry and Photobiology A: Chemistry* 195 (2-3), 205-214.

Garrido, J., Omil, F., Arrojo, B., Mendez, R., Lema, J. 2001. Carbon and nitrogen removal from a wastewater of an industrial dairy laboratory with a coupled anaerobic filter-sequencing batch reactor system. *Sequencing Batch Reactor Technology II* 43 (3), 249-256.

Gernjak, W., Fuerhacker, M., Fernández-Ibañez, P., Blanco, J., Malato, S. 2006. Solar photo-Fenton treatment-Process parameters and process control. *Applied Catalysis B: Environmental* 64 (1), 121-130.

Gernjak, W., Krutzler, T., Glaser, A., Malato, S., Caceres, J., Bauer, R., Fernández-Alba, A. 2003. Photo-Fenton treatment of water containing natural phenolic pollutants. *Chemosphere* 50 (1), 71-78.

Ghaly, M. Y., Härtel, G., Mayer, R., Haseneder, R. 2001. Photochemical oxidation of p-chlorophenol by UV/H_2O_2 and photo-Fenton process. A comparative study. *Waste Management* 21 (1), 41-47.

Gil, J., Túa, L., Rueda, A., Montaño, B., Rodríguez, M., Prats, D. 2010. Monitoring and analysis of the energy cost of an MBR. *Desalination* 250 (3), 997-1001.

Gimeno, O., Rivas, F. J., Beltrán, F. J., Carbajo, M. 2007. Photocatalytic ozonation of winery wastewaters. *Journal of Agricultural and Food Chemistry* 55 (24), 9944-9950.

Göb, S., Oliveros, E., Bossmann, S., Braun, A., Nascimento, C., Guardani, R. 2001. Optimal experimental design and artificial neural networks applied to the photochemically enhanced Fenton reaction. *Water Science & Technology* 44 (5), 339-345.

Cravotto, G., Di Carlo, S., Tumiatti, V., Roggero, C., Bremner, H. D. 2005. Degradation of persistent organic pollutants by Fenton's reagent facilitated by microwave or high-intensity ultrasound. *Environmental Technology* 26 (7), 721-724.

Greek Law FEKB΄534/2011, 2011 on the determination of measures, conditions and procedures for the reuse of treated wastewater and other provisions.

Greenlee, L. F., Testa, F., Lawler, D. F., Freeman, B. D., Moulin, P. 2010. Effect of antiscalants on precipitation of an RO concentrate: Metals precipitated and particle characteristics for several water compositions. *Water Research* 44 (8), 2672-2684.

Gregory, J. (2005) Particles in water: properties and processes. CRC, Press.

Grunditz, C. and Dalhammar, G. 2001. Development of nitrification inhibition assays using pure cultures of nitrosomonas and nitrobacter. *Water Research* 35 (2), 433-440.

Guillot, J., Desauziers, V., Avezac, M., Roux, J. 2000. Characterization and treatment of olfactory pollution emitted by wastewater in wineries of mediterranean region. *Fresenius Environmental Bulletin* 9 (5), 243-250.

Guivarch, E., Trevin, S., Lahitte, C., Oturan, M. A. 2003. Degradation of azo dyes in water by electro-Fenton process. *Environmental Chemistry Letters* 1 (1), 38-44.

Haber, F. and Weiss, J. 1934. The catalytic decomposition of hydrogen peroxide by iron salts. Proceedings of the Royal Society of London. Series A, *Mathematical and Physical Sciences* 147 (861), 332-351.

Hai, F. I., Yamamoto, K., Nakajima, F., Fukushi, K. 2011. Bioaugmented membrane bioreactor (MBR) with a GAC-packed zone for high rate textile wastewater treatment. *Water Research* 45 (6), 2199-2206.

Haug, R. T. (1993) The practical handbook of compost engineering. CRC, Press.

Hermosilla, D., Merayo, N., Ordóñez, R., Blanco, Á. 2012. Optimization of conventional Fenton and ultraviolet-assisted oxidation processes for the treatment of reverse osmosis retentate from a paper mill. *Waste Management* 32, 1236-1243.

Hernández, T., Estrella, I., Carlavilla, D., Martín-Álvarez, P. J., Moreno-Arribas, M. 2006. Phenolic compounds in red wine subjected to industrial malolactic fermentation and ageing on lees. *Analytica Chimica Acta* 563 (1), 116-125.

Hernandez-Sancho, F., Molinos-Senante, M., Sala-Garrido, R. 2011. Cost modelling for wastewater treatment processes. *Desalination* 268 (1), 1-5.

Hernando, M. D., Fernández-Alba, A., Tauler, R., Barceló, D. 2005. Toxicity assays applied to wastewater treatment. *Talanta* 65 (2), 358-366.

Holloway, R. W., Childress, A. E., Dennett, K. E., Cath, T. Y. 2007. Forward osmosis for concentration of anaerobic digester centrate. *Water Research* 41 (17), 4005-4014.

Huang, H., Schwab, K., Jacangelo, J. G. 2009. Pretreatment for low pressure membranes in water treatment: a review. *Environmental Science & Technology* 43 (9), 3011-3019.

Huang, R., He, J., Zhao, J., Luo, Q., Huang, C. 2011. Fenton–biological treatment of reverse osmosis membrane concentrate from a metal plating wastewater recycle system. *Environmental technology* 32 (5), 515-522.

ISO 14040:2006. Environmental Management - Life Cycle Assessment - Principles and Framework. Geneva, Switzerland.

ISO 6332:1996. Water quality-Determination of iron-Spectrometric method using 1,10-phenanthroline.

ISO 6341, 2010. Water quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test.

ISO 8245:2000. Water quality-Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).

Jakopović, H. K., Matošić, M., Ecimović, T., Mijatović, I. 2008. Treatment of food industry wastewaters in membrane bioreactor. *Faculty of Food Technology and Biotechnology*, 4, 121-127.

Jing, Z. and Cao, S. 2012. Combined Application of UV Photolysis and Ozonation with Biological Aerating Filter in Tertiary Wastewater Treatment. *International Journal of Photoenergy*. doi:10.1155/2012/140605.

Johnson, H. and Robinson, J. (1994) The world atlas of wine. Simon & Schuster.

Jones, P., Trenberth, K., Ambenje, P., Bojariu, R., Easterling, D., Klein, A., Tank, D., Renwick, J., Rahimzadeh, F., Rusticucci, M. 2007. Observations: surface and atmospheric climate change. *IPCC, Climate change*, 235-336.

Jordá, L., Martín, M. M., Gómez, E. O., Reina, A. C., Sánchez, I. M., López, J. L., Pérez, J. A. 2011. Economic evaluation of the photo-Fenton process. Mineralization level and reaction time: The keys for increasing plant efficiency. *Journal of Hazardous Materials* 186 (2), 1924-1929.

Judd, S. (2010) The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment. Butterworth-Heinemann.

Judd, S. 2008. The status of membrane bioreactor technology. *Trends in Biotechnology* 26 (2), 109-116.

IUPAC, 1996. Terminology for membranes and membrane processes. *Pure and Applied Chemistry* 72 (10), 1851-2082.

Kajitvichyanukul, P. and Suntronvipart, N. 2006. Evaluation of biodegradability and oxidation degree of hospital wastewater using photo-Fenton process as the pretreatment method. *Journal of Hazardous Materials* 138 (2), 384-391.

Kajitvichyanukul, P., Lu, M-C., Jamroensan, A. 2008. Formaldehyde degradation in the presence of methanol by photo-Fenton process. *Journal of Environmental Management* 86 (3), 545-553.

Kallel, M., Belaid, C., Mechichi, T., Ksibi, M., Elleuch, B. 2009. Removal of organic load and phenolic compounds from olive mill wastewater by Fenton oxidation with zero-valent iron. *Chemical Engineering Journal* 150 (2), 391-395.

Kaludjerski, M. and Gurol, M. D. 2004. Assessment of enhancement in biodegradation of dichlorodiethyl ether (DCDE) by pre-oxidation. *Water Research* 38 (6), 1595-1603.

Kalyuzhnyi, S., Gladchenko, M., Sklyar, V., Kurakova, O., Shcherbakov, S. 2000. The UASB treatment of winery wastewater under submesophilic and psychrophilic conditions. *Environmental Technology* 21 (8), 919-925.

Kalyuzhnyi, S. V., Gladchenko, M. A., Sklyar, V. I., Kizimenko, Y. S., Shcherbakov, S. S. 2001. One-and two-stage upflow anaerobic sludge-bed reactor pretreatment of winery wastewater at 4-10 ^{o}C. *Applied Biochemistry and Biotechnology* 90 (2), 107-124.

Kammerer, D., Kljusuric, J. G., Carle, R., Schieber, A. 2005. Recovery of anthocyanins from grape pomace extracts (Vitis vinifera L. cv. Cabernet Mitos) using a polymeric adsorber resin. *European Food Research and Technology* 220 (3-4), 431-437.

Kammerer, D., Claus, A., Carle, R., Schieber, A. 2004. Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry* 52 (14), 4360-4367.

Kang, G. and Cao, Y. 2011. Development of anti-fouling reverse osmosis membranes for water treatment: A review. *Water Research* 46, 584-600.

Kang, S., Liao, C., Chen, M. 2002. Pre-oxidation and coagulation of textile wastewater by the Fenton process. *Chemosphere* 46 (6), 923-928.

Kang, S., Liao, C., Po, S. 2000. Decolorization of textile wastewater by photo-Fenton oxidation technology. *Chemosphere* 41 (8), 1287-1294.

Kao, C., Chou, M., Fang, W., Liu, B., Huang, B. 2001. Regulating colored textile wastewater by 3/31 wavelength admi methods in Taiwan. *Chemosphere* 44 (5), 1055-1063.

Kara, S., Gürbulak, E., Eyvaz, M., Yüksel, E. 2013. Treatment of winery wastewater by electrocoagulation process. *Desalination and Water Treatment*, DOI: 10.1080/19443994.2013.770223.

Katayon, S., Megat Mohd Noor, M., Ahmad, J., Abdul Ghani, L., Nagaoka, H., Aya, H. 2004. Effects of mixed liquor suspended solid concentrations on membrane bioreactor efficiency for treatment of food industry wastewater. *Desalination* 167, 153-158.

Kavitha, V. and Palanivelu, K. 2004. The role of ferrous ion in Fenton and photo-Fenton processes for the degradation of phenol. *Chemosphere* 55 (9), 1235-1243.

Kazakevich and McNair (2000) Basic Liquid Chromatography. Wiley-interscience, John Wiley & Sons, Inc., publication.

Keyser, M., Witthuhn, R., Ronquest, L., Britz, T. 2003. Treatment of winery effluent with upflow anaerobic sludge blanket (UASB)-granular sludges enriched with Enterobacter sakazakii. *Biotechnology Letters* 25 (22), 1893-1898.

Kim, J., Cai, Z., Benjamin, M. M. 2008. Effects of adsorbents on membrane fouling by natural organic matter. *Journal of Membrane Science* 310 (1), 356-364.

Kim, S. and Hoek, E. M. V. 2005. Modeling concentration polarization in reverse osmosis processes. *Desalination* 186 (1-3), 111-128.

Kirzhner, F., Zimmels, Y., Shraiber, Y. 2008. Combined treatment of highly contaminated winery wastewater. *Separation and Purification Technology* 63 (1), 38-44.

Klamerth, N., Malato, S., Maldonado, M. I., Agüera, A., Fernández-Alba, A. 2011. Modified photo-Fenton for degradation of emerging contaminants in municipal wastewater effluents. *Catalysis Today* 161 (1), 241-246.

Klamerth, N., Rizzo, L., Malato, S., Maldonado, M. I., Agüera, A., Fernández-Alba, A. R. 2010. Degradation of fifteen emerging contaminants at mg L^{-1} initial concentrations by mild solar photo-Fenton in MWTP effluents. *Water Research* 44 (2), 545-554.

Klavarioti, M., Mantzavinos, D., Kassinos, D. 2009. Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environment International* 35 (2), 402-417.

Knops, F., van Hoof, S., Futselaar, H., Broens, L. 2007. Economic evaluation of a new ultrafiltration membrane for pretreatment of seawater reverse osmosis. *Desalination* 203 (1), 300-306.

Kolk, C., Hater, W., Kempken, N. 2013. Cleaning of reverse osmosis membranes. *Desalination and Water Treatment* 51 (1-3), 343-351.

Kremer, M. L. 1999. Mechanism of the Fenton reaction. Evidence for a new intermediate. *Physical Chemistry Chemical Physics 1* (15), 3595-3606.

Krichevskaya, M., Klauson, D., Portjanskaja, E., Preis, S. 2011. The Cost Evaluation of Advanced Oxidation Processes in Laboratory and Pilot-Scale Experiments. *Ozone: Science & Engineering: The Journal of the International Ozone Association* 33 (3), 211-223.

Krupa, S. V. 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environmental Pollution* 124 (2), 179-221.

Kurniawan, T. A., Chan, G. L., Wai-Hung; B. S. 2006. Physico–chemical treatment techniques for wastewater laden with heavy metals. *Chemical Engineering Journal* 118 (1), 83-98.

LaPara, T.M. and Alleman, J.E. 1999. Thermophilic aerobic biological wastewater treatment. *Water Research* 33(4), 895-908.

Larson, R. A., Schlauch, M. B., Marley, K. A. 1991. Ferric ion promoted photodecomposition of triazines. *Journal of Agricultural and Food Chemistry* 39 (11), 2057-2062.

Lee, C. and Yoon, J. 2004. Temperature dependence of hydroxyl radical formation in the hv/Fe^{3+}/H_2O_2 and Fe^{3+}/H_2O_2 systems. *Chemosphere* 56 (10), 923-934.

Lee, H., Chen, G., Yue, P. 2001. Integration of chemical and biological treatments for textile industry wastewater: a possible zero-discharge system. *Water Science and Technology* 44 (5), 75-83.

Lee, H. and Shoda, M. 2008. Removal of COD and color from livestock wastewater by the Fenton method. *Journal of Hazardous Materials* 153 (3), 1314-1319.

Lee, S. and Elimelech, M. 2006. Relating organic fouling of reverse osmosis membranes to intermolecular adhesion forces. *Environmental Science & Technology* 40 (3), 980-987.

Lee, Y., Lee, C., Yoon, J. 2003. High temperature dependence of 2,4-dichlorophenoxyacetic acid degradation by Fe^{3+}/H_2O_2 system. *Chemosphere* 51 (9), 963-971.

Legrini, O., Oliveros, E., Braun, A. 1993. Photochemical processes for water treatment. *Chemical Reviews* 93 (2), 671-698.

Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek* 67 (1), 3-28.

Li, N.N., Fane, A.G., Ho, W.W., Matsuura, T. (2008) Advanced membrane technology and applications. John Wiley and Sons Publishing.

Li, X. and Zhang, R. 2002. Aerobic treatment of dairy wastewater with sequencing batch reactor systems. *Bioprocess and Biosystems Engineering* 25 (2), 103-109.

Liang, S., Liu, C., Song, L. 2007. Soluble microbial products in membrane bioreactor operation: Behaviors, characteristics, and fouling potential. *Water Research* 41 (1), 95-101.

Ling, L. and Lo, K. 1999. Brewery wastewater treatment using suspended and attached growth sequencing batch reactors. *Journal of Environmental Science & Health Part A* 34 (2), 341-355.

Linlin, H., Jianlong, W., Xianghua, W., Yi, Q. 2005. The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules. *Process Biochemistry* 40 (1), 5-11.

Litter, M. 2005. Introduction to photochemical advanced oxidation processes for water treatment. *Environmental Photochemistry Part II*, 2, 325-366.

Lopez-Velez, M., Martinez-Martinez, F., Valle-Ribes, C. D. 2003. The study of phenolic compounds as natural antioxidants in wine. *Critical Reviews in Food Science and Nutrition* 43 (3), 233-244.

Lorain, O., Hersant, B., Persin, F., Grasmick, A., Brunard, N., Espenan, J.M., 2007. Ultrafiltration membrane pre-treatment benefits for reverse osmosis process in seawater desalting. Quantification in terms of capital investment cost and operating cost reduction. *Desalination* 203 (1), 277-285.

Lubello, C., Gori, R., De Bernardinis, A., Simonelli, G. 2003. Ultrafiltration as tertiary treatment for industrial reuse. *Water Science and Technology: Water Supply*, 161-168.

Lucas, M. S., Peres, J. A., Li Puma, G. 2010. Treatment of winery wastewater by ozone-based advanced oxidation processes (O_3, O_3/UV and O_3/UV/H_2O_2) in a pilot-scale bubble column reactor and process economics. *Separation and Purification Technology* 72 (3), 235-241.

Lucas, M. S., Mosteo, R., Maldonado, M. I., Malato, S., Peres, J. A. 2009a. Solar photochemical treatment of winery wastewater in a CPC reactor. *Journal of Agricultural and Food Chemistry* 57 (23), 11242-11248.

Lucas, M. S., Mouta, M., Pirra, A., Peres, J. A. 2009b. Winery wastewater treatment by a combined process: long term aerated storage and Fenton's reagent. *Water Science and Technology* 60 (4), 1089.

Lucas, M. S., Dias, A. A., Bezerra, R. M., Peres, J. A. 2008. Gallic acid photochemical oxidation as a model compound of winery wastewaters. *Journal of Environmental Science and Health Part A* 43 (11), 1288-1295.

Lucas, M. S. and Peres, J. A. 2007. Degradation of Reactive Black 5 by Fenton/UV-C and ferrioxalate/H_2O_2/solar light processes. *Dyes and Pigments* 74 (3), 622-629.

Lucas, M. S. and Peres, J. A. 2006. Decolorization of the azo dye Reactive Black 5 by Fenton and photo-Fenton oxidation. *Dyes and Pigments* 71 (3), 236-244.

Lunar, L., Sicilia, D., Rubio, S., Pérez-Bendito, D., Nickel, U. 2000. Degradation of photographic developers by Fenton’s reagent: condition optimization and kinetics for metol oxidation. *Water Research* 34 (6), 1791-1802.

Mace, S. and Mata-Alvarez, J. 2002. Utilization of SBR technology for wastewater treatment: an overview. *Industrial and Engineering Chemistry Research* 41 (23), 5539-5553.

Maciel, R., Sant'Anna, G. L., Dezotti, M. 2004. Phenol removal from high salinity effluents using Fenton's reagent and photo-Fenton reactions. *Chemosphere* 57 (7), 711-719.

Madaeni, S. and Mansourpanah, Y. 2003. COD removal from concentrated wastewater using membranes. *Filtration & Separation* 40 (6), 40-46.

Madaeni, S.S. and Mansourpanah, Y. 2004. Chemical cleaning of reverse osmosis membranes fouled by whey. *Desalination* 161 (1), 13-24.

Mahamuni, N. N. and Adewuyi, Y. G. 2009. Optimization of the synthesis of biodiesel via ultrasound-enhanced base-catalyzed transesterification of soybean oil using a multifrequency ultrasonic reactor. *Energy & Fuels* 23 (5), 2757-2766.

Mahamuni, N. N. and Adewuyi, Y. G. 2010. Advanced oxidation processes (AOPs) involving ultrasound for waste water treatment: a review with emphasis on cost estimation. *Ultrasonics Sonochemistry* 17 (6), 990-1003.

Mahvi, A. 2008. Sequencing batch reactor: a promising technology in wastewater treatment. *Iranian Journal of Environmental Health Science & Engineering* 5 (2), 79-90.

Malaeb, L. and Ayoub, G. M. 2011. Reverse osmosis technology for water treatment: State of the art review. *Desalination* 267 (1), 1-8.

Malandra, L., Wolfaardt, G., Zietsman, A., Viljoen-Bloom, M. 2003. Microbiology of a biological contactor for winery wastewater treatment. *Water Research* 37 (17), 4125-4134.

Malato Rodríguez, S., Blanco Gálvez, J., Maldonado Rubio, M. I., Fernández Ibáñez, P., Alarcón Padilla, D., Collares Pereira, M., Farinha Mendes, J., Correia de Oliveira, J. 2004. Engineering of solar photocatalytic collectors. *Solar Energy* 77 (5), 513-524.

Malato, S., Blanco, J., Richter, C., Milow, B., Maldonado, M. 1999. Pre-industrial experience in solar photocatalytic mineralization of real wastewaters. Application to pesticide container recycling. *Water Science and Technology* 40 (4), 123-130.

Malato, S., Fernández-Ibáñez, P., Maldonado, M., Blanco, J., Gernjak, W. 2009. Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends. *Catalysis Today* 147 (1), 1-59.

Malato, S., Blanco, J., Vidal, A., Alarcón, D., Maldonado, M.I., Cáceres, J., Gernjak, W. 2003. Applied studies in solar photocatalytic detoxification: an overview. *Solar Energy* 75 (4), 329-336.

Malato, S., Blanco, J., Vidal, A., Richter, C. 2002. Photocatalysis with solar energy at a pilot-plant scale: an overview. *Applied Catalysis B: Environmental* 37 (1), 1-15.

Malato, S., Caceres, J., Agüera, A., Mezcua, M., Hernando, D., Vial, J., Fernández-Alba, A. R. 2001. Degradation of imidacloprid in water by photo-Fenton and TiO2 photocatalysis at a solar pilot plant: a comparative study. *Environmental Science and Technology* 35 (21), 4359-4366.

Mantzavinos, D. and Psillakis, E. 2004. Enhancement of biodegradability of industrial wastewaters by chemical oxidation pre-treatment. *Journal of Chemical Technology and Biotechnology* 79 (5), 431-454.

Maranon, E., Vazquez, I., Rodriguez, J., Castrillón, L., Fernández, Y., López, H. 2008. Treatment of coke wastewater in a sequential batch reactor (SBR) at pilot plant scale. *Bioresource Technology* 99 (10), 4192-4198.

Marhuenda-Egea, F., Martínez-Sabater, E., Jordá, J., Moral, R., Bustamante, M., Paredes, C., Pérez-Murcia, M. 2007. Dissolved organic matter fractions formed during composting of winery and distillery residues: evaluation of the process by fluorescence excitation–emission matrix. *Chemosphere* 68 (2), 301-309.

Martín, M., Casas López, J., Oller, I., Malato, S., Sánchez Pérez, J. 2010. A comparative study of different tests for biodegradability enhancement determination during AOP treatment of recalcitrant toxic aqueous solutions. *Ecotoxicology and Environmental Safety* 73 (6), 1189-1195.

Martín, M., Sánchez Pérez, J., Casas López, J., Oller, I., Malato Rodríguez, S. 2009. Degradation of a four-pesticide mixture by combined photo-Fenton and biological oxidation. *Water Research* 43 (3), 653-660.

Marshall, R. S., Akoorie, M. E., Hamann, R., Sinha, P. 2010. Environmental practices in the wine industry: An empirical application of the theory of reasoned action and stakeholder theory in the United States and New Zealand. *Journal of World Business* 45 (4), 405-414.

Marshall, R. S., Cordano, M., Silverman, M. 2005. Exploring individual and institutional drivers of proactive environmentalism in the US wine industry. B*usiness Strategy and the Environment* 14 (2), 92-109.

Martins, R. C., Pinto, F. L., Castro-Silva, S., Quinta-Ferreira, R. M. 2011. Flocculation, Ozonation, and Fenton's Process in the Treatment of Distillery Effluents. *Journal of Environmental Engineering* 139 (1), 110-116.

Martins, R. C., Rossi, A. F., Quinta-Ferreira, R. M. 2010. Fenton's oxidation process for phenolic wastewater remediation and biodegradability enhancement. *Journal of Hazardous Materials* 180 (1), 716-721.

Martins, R. C., Abegao, F. J., Silva, A. M., Quinta-Ferreira, R. M. 2009. Integrated Strategy for Treatment of Winery Wastewaters Using Flocculation, Ozonation and Fenton's Oxidation. *Journal of Advanced Oxidation Technologies* 12 (2), 255-264.

Mauguin, G. and Corsin, P. 2005. Concentrate and other waste disposals from SWRO plants: characterization and reduction of their environmental impact. *Desalination* 182 (1), 355-364.

Maurer, M. 2009. Specific net present value: an improved method for assessing modularisation costs in water services with growing demand. *Water Research* 43, 2121-2130.

McAdam, E. and Judd, S. 2008. Immersed membrane bioreactors for nitrate removal from drinking water: Cost and feasibility. *Desalination* 231 (1), 52-60.

McCorkle, K. (2009) Verbal communication. Why Business Is Embracing Sustainability. Unified Wine & Grape Symposium, Sacramento, CA.

Mcmanus, P. 2008. Mines, wines and thoroughbreds: towards regional sustainability in the Upper Hunter, Australia. *Regional Studies* 42 (9), 1275-1290.

Medina-Remón, A., Barrionuevo-González, A., Zamora-Ros, R., Andres-Lacueva, C., Estruch, R., Martínez-González, M-Á., Diez-Espino, J., Lamuela-Raventos, R. M. 2009. Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Analytica Chimica Acta* 634 (1), 54-60.

Melamane, X., Tandlich, R., Burgess, J. 2007. Anaerobic digestion of fungally pre-treated wine distillery wastewater. *African Journal of Biotechnology* 6 (17).

Mert, B., Yonar, T., Yalili Kiliç, M., Kestioğlu, K. 2010. Pre-treatment studies on olive oil mill effluent using physicochemical, Fenton and Fenton-like oxidations processes. *Journal of Hazardous Materials* 174 (1), 122-128.

Metcalf, E. and Eddy, H. (2003) Wastewater engineering: treatment and reuse. McGraw-Hill, New York.

Metcalf, E. and Eddy H. (2008) Water reuse issues, technologies and applications. McGraw-Hill, New York.

Michael, I., Hapeshi, E., Michael, C., Fatta-Kassinos, D. 2010. Solar Fenton and solar TiO_2 catalytic treatment of ofloxacin in secondary treated effluents: Evaluation of operational and kinetic parameters. *Water Research* 44 (18), 5450-5462.

Michael, I., Hapeshi, E., Michael, C., Varela, A. R., Kyriakou, S., Manaia, C. M., Fatta-Kassinos, D. 2012. Solar photo-Fenton process on the abatement of antibiotics at a pilot scale: degradation kinetics, ecotoxicity and phytotoxicity assessment and removal of antibiotic resistant enterococci. Water Research 46 (17), 5621-5634.

MicroBioTests Inc. For determination of the direct effects of chemicals on seed germination and early growth of plants. Bench protocol.

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., Hairston, N. G. 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society B: Biological Sciences* 279 (1735), 1873-1882.

Mohamed, E.S. and Papadakis, G. 2004. Design, simulation and economic analysis of a stand-alone reverse osmosis desalination unit powered by wind turbines and photovoltaics. *Desalination* 164 (1), 87-97.

Mohan, S. V., Rao, N. C., Prasad, K. K., Madhavi, B. T. V., Sharma, P. N. 2005. Treatment of complex chemical wastewater in a sequencing batch reactor (SBR) with an aerobic suspended growth configuration. *Process Biochemistry* 40 (5), 1501-1508.

Mohana, S., Acharya, B. K., Madamwar, D. 2009. Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials* 163 (1), 12-25.

Moletta, R. 2005. Winery and distillery wastewater treatment by anaerobic digestion. *Sustainable Viticulture and Winery Wastes Management* 51 (1), 137-144.

Molina, F., Ruiz-Filippi, G., Garcia, C., Roca, E., Lema, J. 2007. Winery effluent treatment at an anaerobic hybrid USBF pilot plant under normal and abnormal operation. *Water Science Technology* 56, 25-31.

Monagas, M., Bartolome, B., Gomez-Cordoves, C. 2005. Updated knowledge about the presence of phenolic compounds in wine. *Critical Reviews in Food Science and Nutrition* 45 (2), 85-118.

Montalvo, S., Guerrero, L., Rivera, E., Borja, R., Chica, A., Martín, A. 2010. Kinetic evaluation and performance of pilot-scale fed-batch aerated lagoons treating winery wastewaters. *Bioresource Technology* 101 (10), 3452-3456.

Monteagudo, J. M., Durán, A., Corral, J. M., Carnicer, A., Frades, J. M., Alonso, M. A. 2012. Ferrioxalate-induced solar photo-Fenton system for the treatment of winery wastewaters. *Chemical Engineering Journal* 181-182, 281-288.

Mortatti, J., Krug, F. J., Pessenda, L. C. R., Zagatto, E. A. G., Jørgensen, S., 1982. Determination of iron in natural waters and plant material with 1, 10-phenanthroline by flow injection analysis. *Analyst* 107, 659-663,

Mosse, K. P., Verheyen, T. V., Cruickshank, A. J., Patti, A. F., Cavagnaro, T. R. 2012. Soluble organic components of winery wastewater and implications for reuse. *Agricultural Water Management* 180, 63-70.

Mosse, K. P., Patti, A. F., Christen, E. W., Cavagnaro, T. R. 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. *Journal of Hazardous Materials* 180 (1), 63-70.

Mosse, K., Patti, A., Christen, E., Cavagnaro, T. 2011. Review: Winery wastewater quality and treatment options in Australia. *Australian Journal of Grape and Wine Research* 17 (2), 111-122.

Mosteo, R., Sarasa, J., Ormad, M. P., Ovelleiro, J. 2008. Sequential solar photo-Fenton-biological system for the treatment of winery wastewaters. *Journal of Agricultural and Food Chemistry* 56 (16), 7333-7338.

Mosteo, R., Ormad, P., Mozas, E., Sarasa, J., Ovelleiro, J. L. 2006. Factorial experimental design of winery wastewaters treatment by heterogeneous photo-Fenton process. *Water Research* 40 (8), 1561-1568.

Mourato, S., Ozdemiroglu, E., Foster, V. 2000. Evaluating health and environmental impacts of pesticide use: implications for the design of ecolabels and pesticide taxes. *Environmental Science & Technology* 34 (8), 1456-1461.

Mulder, M. (1996) Basic principles of membrane technology. Springer.

Muñoz, I., Malato, S., Rodriguez, A., Domenech, X. 2008. Integration of environmental and economic performance of processes. Case study on advanced oxidation processes for wastewater treatment. *Journal of Advanced Oxidation Technologies* 11 (2), 270-275.

Musee, N., Lorenzen, L., Aldrich, C. 2007. Cellar waste minimization in the wine industry: a systems approach. *Journal of Cleaner Production* 15(5), 417-431.

Muruganandham, M. and Swaminathan, M. 2004. Decolourisation of Reactive Orange 4 by Fenton and photo-Fenton oxidation technology. *Dyes and Pigments* 63 (3), 315-321.

Mwebi, N.O. (2005) Fenton & Fenton-like Reactions: The Nature of Oxidizing Intermediates Involved. Ph.D Dissertation submitted to the Faculty of the Graduate School of the University of Maryland.

Nair, C. I., Jayachandran, K., Shashidhar, S. 2008. Biodegradation of phenol. African *Journal of Biotechnology* 7 (25), 4951-4958.

Nataraj, S.K., Hosamani, K.M., Aminabhavi, T.M. 2006. Distillery wastewater treatment by the membrane-based nanofiltration and reverse osmosis processes. *Water Research* 40 (12), 2349-2356.

Navarro, P., Sarasa, J., Sierra, D., Esteban, S., Ovelleiro, J. 2005. Degradation of wine industry wastewaters by photocatalytic advanced oxidation. *Water Science & Technology* 51 (1), 113-120.

Navarro, S., Fenoll, J., Vela, N., Ruiz, E., Navarro, G. 2011. Removal of ten pesticides from leaching water at pilot plant scale by photo-Fenton treatment. *Chemical Engineering Journal* 167 (1), 42-49.

Ndon, U. J. and Dague, R. R. 1997. Effects of temperature and hydraulic retention time on anaerobic sequencing batch reactor treatment of low-strength wastewater. *Water Research* 31 (10), 2455-2466.

Ndiaye, P. I., Moulin, P., Dominguez, L., Millet, J. C., Charbit, F. 2005. Removal of fluoride from electronic industrial effluentby RO membrane separation. *Desalination* 173 (1), 25-32.

Neczaj, E., Okoniewska, E., Kacprzak, M. 2005. Treatment of landfill leachate by sequencing batch reactor. *Desalination* 185(1), 357-362.

Nicoletti, G.M., Notarnicola, B., Tassielli. G. 2001. Comparison of Conventional and Organic Wine. Proceedings: International Conference on LCA in Foods. Gothenburg, Sweden. SIK Document 143.

Niccolucci, V. G. A., Kitzes, J., Pulselli, R. M., Borsa, S., Marchettini, N. 2008. Ecological footprint analysis applied to the production of two Italian wines. *Agriculture, Ecosystems and Environment* 128, 162-166.

Nielsen, M. and Rank, J. 1994. Screening of Toxicity and Genotoxicity in Wastewater by the use of the Use of the Allium Test. *Hereditas* 121 (3), 249-254.

Nigdikar, S. V., Williams, N. R., Griffin, B. A., Howard, A. N. 1998. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. *The American Journal of Clinical Nutrition* 68 (2), 258-265.

Ning, R. Y. and Troyer, T. L. 2009. Tandom reverse osmosis process for zero-liquid discharge. *Desalination* 237 (1), 238-242.

Nogales, R., Cifuentes, C., Benitez, E. 2005. Vermicomposting of winery wastes: a laboratory study. *Journal of Environmental Science and Health Part B* 40 (4), 659-673.

Nogueira, R. F. P., Silva, M. R. A., Trovó, A. G. 2005. Influence of the iron source on the solar photo-Fenton degradation of different classes of organic compounds. *Solar Energy* 79 (4), 384-392.

Norcross, K.L. 1992. Sequencing batch reactors-an overview. *Water Science & Technology* 26(9-11), 2523-2526.

Notarnicola, B., Tassielli, G., Nicoletti, G., Mattsson, B., Sonesson, U. 2003. Life cycle assessment (LCA) of wine production. *Environmentally-friendly food processing*, 306-326.

Obaja, D., Mace, S., Costa, J., Sans, C., Mata-Alvarez, J. 2003. Nitrification, denitrification and biological phosphorus removal in piggery wastewater using a sequencing batch reactor. *Bioresource Technology* 87 (1), 103-111.

OIV, International Organization of Vine and Wine, Intergovermental Organization, 1999. Index Folin-Ciocalteu, Method OIV-MA-AS2-10. Official Webpage (Online) (Accessed 21/4/2013), Available from World Wide Web: < http://www.oiv.int/oiv/ index>.

OIV, International Organization of Vine and Wine, Intergovermental Organization, 2013. Statistical report on world vitiviniculture 2012. Official Webpage (Online) (Accessed 17/3/2013), Available from World Wide Web: < http://www.oiv.int/ oiv/index>.

Oliveira M. and Duarte E. (2010) Guidelines for the management of winery wastewaters. Treatment and use of non conventional organic residues in agriculture. Institute Superior to Agronomy, Portugal.

Oliveira, M., Queda, C., Duarte, E. 2009. Aerobic treatment of winery wastewater with the aim of water reuse. *Water Science and Technology* 60 (5), 1217-1223.

Oller, I., Malato, S., Sánchez-Pérez, J. 2011. Combination of advanced oxidation processes and biological treatments for wastewater decontamination-a review. *Science of the Total Environment* 409 (20), 4141-4166.

Oller, I., Gernjak, W., Maldonado, M. I., Pérez-Estrada, L. A., Sánchez-Pérez, J. A., Malato, S. 2006. Solar photocatalytic degradation of some hazardous water-soluble pesticides at pilot-plant scale. *Journal of Hazardous Materials* 138 (3), 507-517.

Ormad, M. P., Mosteo, R., Ibarz, C., Ovelleiro, J. L. 2006. Multivariate approach to the photo-Fenton process applied to the degradation of winery wastewaters. *Applied Catalysis B: Environmental* 66 (1), 58-63.

Pagans, E., Barrena, R., Font, X., Sánchez, A. 2006. Ammonia emissions from the composting of different organic wastes. Dependency on process temperature. *Chemosphere* 62 (9), 1534-1542.

Pala, A. and Tokat, E. 2002. Color removal from cotton textile industry wastewater in an activated sludge system with various additives. *Water Research* 36 (11), 2920-2925.

Papaphilippou, P. C., Yiannapas, C., Politi, M., Daskalaki, V. M., Michael, C., Kalogerakis, N., Mantzavinos, D., Fatta-Kassinos, D. 2012. Sequential coagulation-flocculation, solvent extraction and photo-Fenton oxidation for the valorization and treatment of olive mill effluent. *Chemical Engineering Journal* 224, 82-88.

Pariente, M. I., Martínez, F., Melero, J. A., Botas, J. Á., Velegraki, T., Xekoukoulotakis, N. P., Mantzavinos, D., 2008. Heterogeneous photo-Fenton oxidation of benzoic acid in water: Effect of operating conditions, reaction by-products and coupling with biological treatment. *Applied Catalysis B: Environmental* 85 (1), 24-32.

Parsons, S. (2004) Advanced oxidation processes for water and wastewater treatment. International Water Association (IWA), London, UK.

Pepi, M., Lampariello, L. R., Altieri, R., Esposito, A., Perra, G., Renzi, M., Lobianco, A., Feola, A., Gasperini, S., Focardi, S. E. 2010. Tannic acid degradation by bacterial strains Serratia spp. and Pantoea sp. isolated from olive mill waste mixtures. *International Biodeterioration & Biodegradation* 64 (1), 73-80.

Peres, J. A. S., Melo de Carvalho, L. H., Boaventura, R. A. R., Costa, C. A. V. 2004. Characteristics of p-hydroxybenzoic acid oxidation using Fenton's reagent. *Journal of Environmental Science and Health, Part A* 39 (11-12), 2897-2913.

Pérez, M., Torrades, F., Domènech, X., Peral, J. 2002. Removal of organic contaminants in paper pulp effluents by AOPs: an economic study. *Journal of Chemical Technology and Biotechnology* 77(5), 525-532.

Pérez, J. A. S., Sánchez, I. M. R., Carra, I., Reina, A. C., López, J. L. C., Malato, S. 2013. Economic evaluation of a combined photo-Fenton/MBR process using pesticides as model pollutant. Factors affecting costs. *Journal of Hazardous Materials* 244-245, 195-203.

Pérez-Estrada, L. A., Maldonado, M. I., Gernjak, W., Agüera, A., Fernández-Alba, A. R., Ballesteros, M. M., Malato, S. 2005. Decomposition of diclofenac by solar driven photocatalysis at pilot plant scale. *Catalysis Today* 101 (3-4), 219-226.

Pérez-González, A., Urtiaga, A., Ibáñez, R., Ortiz, I. 2011. State of the art and review on the treatment technologies of water reverse osmosis concentrates. *Water Research* 46, 267-283.

Pérez-Serradilla, J. and de Castro, M. 2008. Role of lees in wine production: A review. *Food Chemistry* 111 (2), 447-456.

Perry, R. H. and Green, D. W. (2008) Perry's chemical engineers' handbook. McGraw-Hill New York.

Petruccioli, M., Cardoso Duarte, J., Eusebio, A., Federici, F. 2002. Aerobic treatment of winery wastewater using a jet-loop activated sludge reactor. *Process Biochemistry* 37 (8), 821-829.

Petruccioli, M., Duarte, J., Federici, F. 2000. High-rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge. *Journal of Bioscience and Bioengineering* 90 (4), 381-386.

Pignatello, J. J. 1992. Dark and photoassisted iron (3)-catalyzed degradation of chlorophenoxy herbicides by hydrogen peroxide. *Environmental Science & Technology* 26 (5), 944-951.

Pignatello, J. J., Di Liu, a., Huston, P. 1999. Evidence for an additional oxidant in the photoassisted Fenton reaction. *Environmental Science & Technology* 33 (11), 1832-1839.

Pimentel, D. and Levitan, L. 1986. Pesticides: amounts applied and amounts reaching pests. *Bioscience* 36 (2), 86-91.

Pizzigallo, A., Granai, C., Borsa, S. 2008. The joint use of LCA and emergy evaluation for the analysis of two Italian wine farms. *Journal of Environmental Management* 86 (2), 396-406.

Poltak, R.F. (2005) Sequencing batch reactor design and operational considerations. New England Interstate Water Pollution Control Commission, UK.

Radjenovic, J., Bagastyo, A., Rozendal, R. A., Mu, Y., Keller, J., Rabaey, K. 2011. Electrochemical oxidation of trace organic contaminants in reverse osmosis concentrate using RuO_2/IrO_2-coated titanium anodes. *Water Research* 45 (4), 1579-1586.

Radjenovic, J., Petrovic, M., Barceló, D. 2007. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. *Analytical and Bioanalytical Chemistry* 387 (4), 1365-1377.

Radjenović, J., Matošić, M., Mijatović, I., Petrović, M., Barceló, D. 2008. Membrane bioreactor (MBR) as an advanced wastewater treatment technology. *The Handbook of Environmental Chemistry* 5, 37-101.

Radjenović, J., Sirtori, C., Petrović, M., Barceló, D., Malato, S. 2009. Solar photocatalytic degradation of persistent pharmaceuticals at pilot-scale: Kinetics and characterization of major intermediate products. *Applied Catalysis B: Environmental* 89 (1-2), 255-264.

Reimann, W. 1997. Influence of organic matter from waste water on the permeability of membranes. *Desalination* 109 (1), 51-55.

Rektor, A., Kozak, A., Vatai, G., Bekassy-Molnar, E. 2007. Pilot plant RO-filtration of grape juice. *Separation and Purification Technology* 57 (3), 473-475.

Ridoutt, B. G. and Pfister, S. 2010. A revised approach to water footprinting to make transparent the impacts of consumption and production on global freshwater scarcity. *Global Environmental Change* 20 (1), 113-120.

Rizzo, L., Lofrano, G., Belgiorno, V. 2010. Olive mill and winery wastewaters pre-treatment by coagulation with chitosan. *Separation Science and Technology* 45 (16), 2447-2452.

Robinson, T., McMullan, G., Marchant, R., Nigam, P. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology* 77 (3), 247-255.

Roche, P. and Prados, M. 1995. Removal of pesticides by use of ozone or hydrogen peroxide/ozone. *Ozone: Science & Engineering* 17 (6), 657-672.

Roche, P., Volk, C., Carbonnier, F., Paillard, H. 1994. Water oxidation by ozone or ozone/hydrogen peroxide using the “ozotest” or “peroxotest” methods. *Ozone: Science & Engineering: The Journal of the International Ozone Association.* 16 (2), 135-155.

Rodrıguez-Delgado, M. A., Malovaná, S., Pérez, J. P., Borges, T., Garcıa Montelongo, F. J. 2001. Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *Journal of Chromatography A* 912 (2), 249-257.

Romero, E., Plaza, C., Senesi, N., Nogales, R., Polo, A. 2007. Humic acid-like fractions in raw and vermicomposted winery and distillery wastes. *Geoderma* 139 (3), 397-406.

Ruggieri, L., Cadena, E., Martínez-Blanco, J., Gasol, C. M., Rieradevall, J., Gabarrell, X., Gea, T., Sort, X., Sánchez, A. 2009. Recovery of organic wastes in the Spanish wine industry. Technical, economic and environmental analyses of the composting process. *Journal of Cleaner Production* 17 (9), 830-838.

Ruiz, C., Torrijos, M., Sousbie, P., Martinez, J. L., Moletta, R., Delgenes, J. P., van Lier, J., Lubberding, H., 2002. Treatment of winery wastewater by an anaerobic sequencing batch reactor. Conference paper *Water Science and Technology* 45 (10), 219-224.

Russo, C. 2007. A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from vegetation waters (VW). *Journal of Membrane Science* 288 (1), 239-246.

Rytwo, G., Rettig, A., Gonen, Y. 2011. Organo-sepiolite particles for efficient pretreatment of organic wastewater: Application to winery effluents. *Applied Clay Science* 51 (3), 390-394.

Saadi, I., Laor, Y., Raviv, M., Medina, S. 2007. Land spreading of olive mill wastewater: effects on soil microbial activity and potential phytotoxicity. *Chemosphere* 66 (1), 75-83.

Sagawe, G., Lehnard, A., Lübber, M., Bahnemann, D. 2001. The insulated solar Fenton hybrid process: fundamental investigations. *Helvetica Chimica Acta* 84 (12), 3742-3759.

Santos, A. B., Cervantes, F. J., van Lier, J. B. 2007. Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. *Bioresource Technology* 98 (12), 2369-2385.

Saravanan, P., Pakshirajan, K., Saha, P. 2008. Biodegradation of phenol and m-cresol in a batch and fed batch operated internal loop airlift bioreactor by indigenous mixed microbial culture predominantly Pseudomonas sp. *Bioresource Technology* 99 (18), 8553-8558.

Satyawali, Y. and Balakrishnan, M. 2008b. Treatment of distillery effluent in a membrane bioreactor (MBR) equipped with mesh filter. *Separation and Purification Technology* 63 (2), 278-286.

Schaep, J., Van der Bruggen, B., Uytterhoeven, S., Croux, R., Vandecasteele, C., Wilms, D., Van Houtte, E., Vanlerberghe, F. 1998. Removal of hardness from groundwater by nanofiltration. *Desalination* 119 (1), 295-301.

Schwarzenbeck, N., Erley, R., Mc Swain, B. S., Wilderer, P. A., Irvine, R. L. 2004. Treatment of malting wastewater in a granular sludge sequencing batch reactor (SBR). *Acta Hydrochimica et Hydrobiologica* 32 (1), 16-24.

Selcuk, H. 2005. Decolorization and detoxification of textile wastewater by ozonation and coagulation processes. *Dyes and Pigments* 64 (3), 217-222.

Seruga, M., Novak, I., Jakobek, L., 2011. Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chemistry 124 (3), 1208-1216.

Shaw, C., Carliell, C., Wheatley, A. 2002. Anaerobic/aerobic treatment of coloured textile effluents using sequencing batch reactors. *Water Research* 36 (8), 1993-2001.

Shemer, H., Kunukcu, Y. K., Linden, K. G. 2006. Degradation of the pharmaceutical Metronidazole via UV, Fenton and photo-Fenton processes. *Chemosphere* 63 (2), 269-276.

Shepherd, H. L., Grismer, M. E., Tchobanoglous, G. 2001. Treatment of high-strength winery wastewater using a subsurface-flow constructed wetland. *Water Environment Research* 73 (4), 394-403.

Shin, H., Lee, S., Seo, I., Kim, G., Lim, K., Song, J. 1998. Pilot-scale SBR and MF operation for the removal of organic and nitrogen compounds from greywater. *Water Science and Technology* 38 (6), 80-88.

Shin, J., Lee, S., Jung, J., Chung, Y., Noh, S. 2005. Enhanced COD and nitrogen removals for the treatment of swine wastewater by combining submerged membrane bioreactor (MBR) and anaerobic upflow bed filter (AUBF) reactor. *Process Biochemistry* 40 (12), 3769-3776.

Silverman, M., Marshall, R.S., Cordano, M. 2005. The greening of the California wine industry: Implications for regulators and industry associations. *Journal of Wine Research* 16 (2), 151-169.

Simate, G. S., Cluett, J., Iyuke, S. E., Musapatika, E. T., Ndlovu, S., Walubita, L. F., Alvarez, A. E. 2011. The treatment of brewery wastewater for reuse: State of the art. *Desalination* 273 (2), 235-247.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology* 299, 152-178.

Singleton, V. L., and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16 (3), 144-158,

Sipowich, M. (2008) Winery cleaning and Sanitation. Texas Cooperative Extension, the Texas A&M University System.

Sirtori, C., Zapata, A., Oller, I., Gernjak, W., Aguera, A., Malato, S. 2009. Solar photo-Fenton as finishing step for biological treatment of a pharmaceutical wastewater. *Environmental Science and Technology* 43 (4), 1185-1191.

Smakhtin, V. 2008. Basin closure and environmental flow requirements. *Water Resources Development* 24 (2), 227-233.

Smyth, M., Nesbitt A. (2012) Energy and English Wine Production. Wine Department, United Kingdom.

Solozhenko, E., Soboleva, N., Goncharuk, V. 1995. Decolourization of azodye solutions by Fenton's oxidation. *Water Research* 29 (9), 2206-2210.

Song, H., Orr, O., Hong, Y., Karanfil, T. 2009. Isolation and fractionation of natural organic matter: evaluation of reverse osmosis performance and impact of fractionation parameters. *Environmental Monitoring and Assessment* 153 (1), 307-321.

Sourirajan, S. (1977) Reverse osmosis and synthetic membranes: theory, technology, engineering. Publications/National Research Council Canada.

Souza, B. S., Moreira, F. C., Dezotti, M. W., Vilar, V. J., Boaventura, R. A. 2013. Application of biological oxidation and solar driven advanced oxidation processes to remediation of winery wastewater. *Catalysis Today* 209 (15), 201-208.

Sreethawong, T. and Chavadej, S. 2008. Color removal of distillery wastewater by ozonation in the absence and presence of immobilized iron oxide catalyst. *Journal of Hazardous Material* 155 (3), 486-493.

Squire, D., Murrer, J., Holden, P., Fitzpatrick, C. 1997. Disposal of reverse osmosis membrane concentrate. *Desalination* 108 (1), 143-147.

Sreethawong, T. and Chavadej, S. 2008. Color removal of distillery wastewater by ozonation in the absence and presence of immobilized iron oxide catalyst. Journal of hazardous materials 155 (3), 486-493.

Sridhar, S., Kale, A., Khan, A. 2002. Reverse osmosis of edible vegetable oil industry effluent. *Journal of Membrane Science* 205 (1), 83-90.

Stasinakis, A. 2008. Use of selected advanced oxidation processes (AOPs) for wastewater treatment-A mini review. *Global Nest Journal* 10 (3), 376-385.

Stefan, M. I., Hoy, A. R., Bolton, J. R. 1996. Kinetics and mechanism of the degradation and mineralization of acetone in dilute aqueous solution sensitized by the UV photolysis of hydrogen peroxide. *Environmental Science & Technology* 30 (7), 2382-2390.

Strong, P. J. and Burgess, J. E. 2008. Treatment methods for wine-related and distillery wastewaters: a review. *Bioremediation Journal* 12 (2), 70-87.

Tabrizi, G. B. and Mehrvar, M., 2004. Integration of advanced oxidation technologies and biological processes: Recent developments, trends, and advances. *Journal of Environmental Science and Health, Part A,* 39 (11-12), 3029-3081.

Tam, L.S., Tang, T.W., Lau, G.N., Sharma, K.R., Chen, G.H. 2007. A pilot study for wastewater reclamation and reuse with MBR/RO and MF/RO systems. *Desalination* 202 (1), 106-113.

Tamimi, M., Qourzal, S., Barka, N., Assabbane, A., Ait-Ichou, Y. 2008. Methomyl degradation in aqueous solutions by Fenton's reagent and the photo-Fenton system. *Separation and Purification Technology* 61 (1), 103-108.

Tekin, H., Bilkay, O., Ataberk, S. S., Balta, T. H., Ceribasi, I. H., Sanin, F. D., Dilek, F. B., Yetis, U. 2006. Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater. *Journal of Hazardous Materials* 136 (2), 258-265.

Tian, J., Liang, H., Li, X., You, S., Tian, S., Li, G. 2008. Membrane coagulation bioreactor (MCBR) for drinking water treatment. *Water Research* 42 (14), 3910-3920.

Tofflemire, T.J. 1972. Survey of Methods of Treating Wine and Grape Wastewater. *American Journal of Enology and Viticulture* 23 (4), 165-172.

Torrijos, M. and Moletta, R. 1997. Winery wastewater depollution by sequencing batch reactor. *Water Science and Technology* 35 (1), 249-257.

Trovó, A. G., Melo, S. A. S., Nogueira, R. F. P. 2008. Photodegradation of the pharmaceuticals amoxicillin, bezafibrate and paracetamol by the photo-Fenton process-Application to sewage treatment plant effluent. *Journal of Photochemistry and Photobiology A: Chemistry* 198 (2), 215-220.

Tsagarakis, K., Mara, D., Angelakis, A. 2003. Application of cost criteria for selection of municipal wastewater treatment systems. *Water, Air, and Soil Pollution* 142 (1-4), 187-210.

Valderrama, C., Ribera, G., Bahí, N., Rovira, M., Giménez, T., Nomen, R., Lluch, S., Yuste, M., Martinez-Lladó, X. 2012. Winery wastewater treatment for water reuse purpose: Conventional activated sludge versus membrane bioreactor (MBR): A comparative case study. *Desalination* 306, 1-7.

Van Aken, P., Van Eyck, K., Luyten, J., Degréve, J., Liers, S. 2010. Advanced treatment of the reverse osmosis concentrate by the Fenton and O_3/UV oxidation processes. *Desalination* 22, 14-19.

Van der Bruggen, B., Lejon, L., Vandecasteele, C. 2003. Reuse, treatment, and discharge of the concentrate of pressure-driven membrane processes. *Environmental Science and Technology* 37 (17), 3733-3738.

Vancouver, B.C. (1997) Technical pollution prevention guide for brewery and wine operations in the lower Frasen Basin. Environment Canada, Canada, USA.

Van Schoor, L. (2005) Guidelines for the management of wastewater and solid waste at existing wineries. Wine Industry Network of Expertise and Technology, Winetech.

Verlinden, B., Duflou, J., Collin, P., Cattrysse, D. 2008. Cost estimation for sheet metal parts using multiple regression and artificial neural networks: A case study. *International Journal of Production Economics* 111 (2), 484-492.

Verrecht, B., Maere, T., Nopens, I., Brepols, C., Judd, S. 2010. The cost of a large-scale hollow fibre MBR. *Water Research* 44 (18), 5274-5283.

Vidal, G., Nieto, J., Mansilla, H. D., Bornhardt, C. 2004. Combined oxidative and biological treatment of separated streams of tannery wastewater. *Water Science and Technology* 49, 287-292.

Viets, F. and Lunin, J. 1975. The environmental impact of fertilizers. *Critical Reviews in Environmental Science and Technology* 5 (4), 423-453.

Villanova, L., Villanova, L., Fasiello, G., Merendino, A. 2008. Process for the recovery of tyrosol and hydroxytyrosol from oil mill wastewaters and catalytic oxidation method in order to convert tyrosol in hydroxytyrosol. *European Patent Application* 2, 1-15.

Vinci, G., Eramo, S., Nicoletti, I., Restuccia, D. 2008. Influence of environmental and technological parameters on phenolic composition in red wine. *Journal of Commodity, Science, Technology and Quality* 47 (1-4), 245-266.

Vlyssides, A. G. and Israilides, C. J. 1997. Detoxification of tannery waste liquors with an electrolysis system. *Environmental Pollution* 97 (1), 147-152.

Vlyssides, A.G., Barampouti, E.M., Mai, S. 2005. Wastewater characteristics from Greek wineries and distilleries. *Water Science and Technology* 51 (1), 53-60.

Vourch, M., Balannec, B., Chaufer, B., Dorange, G. 2008. Treatment of dairy industry wastewater by reverse osmosis for water reuse. *Desalination* 219 (1), 190-202.

Walling, C. 1975. Fenton's reagent revisited. *Accounts of Chemical Research* 8 (4), 125-131.

Walsdorff, A., Van Kraayenburg, M., Barnardt, C. 2007. A multi-site approach towards integrating environmental management in the wine production industry. *Water SA* 30 (5), 82-87.

Walsdorff, A., van Kraayenburg, M., Barnardt, C. 2005. A multi-site approach towards integrating environmental management in the wine production industry. *Water Science & Technology* 51 (1), 61-69.

Wang, X., Wang, Y., Chunsheng, Y., Wang, L., Han, S. 2002. Mechanism-based quantitative structure-phytotoxicity relationships comparative inhibition of substituted phenols on root elongation of Cucumis sativus. *Archives of Environmental Contamination and Toxicology* 42 (1), 29-35.

Wang, Y., Li, X., Zhen, L., Zhang, H., Zhang, Y., Wang, C. 2012. Electro-Fenton treatment of concentrates generated in nanofiltration of biologically pretreated landfill leachate. *Journal of Hazardous Materials* 229-230, 115-121.

Watson, I. 1990. Characterization of desalting concentrates. *Desalination* 78 (1), 5-9.

Weber M.M., and Bahner M. (1999) Pollution prevention for the wine industry. Research Triangle Institute, North Carolina, United States, America.

Wen, X., Ding, H., Huang, X., Liu, R. 2004. Treatment of hospital wastewater using a submerged membrane bioreactor. *Process Biochemistry* 39 (11), 1427-1431.

Westerhoff, P., Moon, H., Minakata, D., Crittenden, J. 2009. Oxidation of organics in retentates from reverse osmosis wastewater reuse facilities. *Water Research* 43 (16), 3992-3998.

Wilderer, P. A., Irvine, R. L., Goronszy, M. C. (2001) Sequencing batch reactor technology. International Water Association (IWA) Publishing, London, UK.

Williams, R. D. and Hoagland, R. E. 1982. The effects of naturally occurring phenolic compounds on seed germination. *Weed Science* 30 (2), 206-212.

Wine Institute, 2013. Wine statistics, World wine production, consumption and vineyard area by country. Official Webpage (Online) (Accessed 15/1/2013), Available from World Wide Web: <http://www.wineinstitute.org/ resources/statistics>.

Wittwer, G., Berger, N., Anderson, K. 2003. A model of the world's wine markets. *Economic Modelling* 20 (3), 487-506.

Wolf, P.H., Siverns, S., Monti, S. 2005. UF membranes for RO desalination pretreatment. *Desalination* 182 (1), 293-300.

Xing, C., Qian, Y., Wen, X., Wu, W., Sun, D. 2001. Physical and biological characteristics of a tangential-flow MBR for municipal wastewater treatment. *Journal of Membrane Science* 191 (1), 31-42.

Xu, P., Drewes, J. E., Kim, T-U., Bellona, C., Amy, G. 2006. Effect of membrane fouling on transport of organic contaminants in NF/RO membrane applications. *Journal of Membrane Science* 279 (1), 165-175.

Xu, X., Li, X., Li, X., Li, H. 2009. Degradation of melatonin by UV, UV/H_2O_2, Fe^{2+}/H_2O_2 and UV/Fe^{2+}/H_2O_2 processes. *Separation and Purification Technology* 68 (2), 261-266.

Yang, G. C. C., Long, Y-W. 1999. Removal and degradation of phenol in a saturated flow by in-situ electrokinetic remediation and Fenton-like process. *Journal of Hazardous Materials* 69 (3), 259-271.

Yang, W., Cicek, N., Ilg, J. 2006. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. *Journal of Membrane Science* 270 (1), 201-211.

Yapijakis, C. 1992. Planning for Success in Industrial Pollution Prevention Programs, 47th Purdue Industrial Waste Conference Proceedings, Lewis Publications, Inc. Chelsea, Michigan, 21-26.

Yavuz, Y., Savas, K. A., Bakir, Ö. Ü. 2007. Phenol removal through chemical oxidation using Fenton reagent. *Chemical Engineering Technology* 30 (5), 583-586.

You, S.J., Tseng, D.H., Ou, S.H., Chang, W.K. 2007. Performance and microbial diversity of a membrane bioreactor treating real textile dyeing wastewater. *Environmental Technology* 28 (8), 935-941.

Zalidis, G., Stamatiadis, S., Takavakoglou, V., Eskridge, K., Misopolinos, N. 2002. Impacts of agricultural practices on soil and water quality in the Mediterranean region and proposed assessment methodology. *Agriculture, Ecosystems & Environment* 88 (2), 137-146.

Zapata, A., Malato, S., Sánchez-Pérez, J., Oller, I., Maldonado, M. 2010. Scale-up strategy for a combined solar photo-Fenton/Biological system for remediation of pesticide-contaminated water. *Catalysis Today* 151 (1), 100-106.

Zapata, A., Velegraki, T., Sánchez-Pérez, J., Mantzavinos, D., Maldonado, M., Malato, S. 2009. Solar photo-Fenton treatment of pesticides in water: Effect of iron concentration on degradation and assessment of ecotoxicity and biodegradability. *Applied Catalysis B: Environmental* 88 (3), 448-454.

Zhang, M., Jiang, S., Tanuwidjaja, D., Voutchkov, N., Hoek, E. M. V., Cai, B. 2011. Composition and Variability of Biofouling Organisms in Seawater Reverse Osmosis Desalination Plants. *Applied and Environmental Microbiology* 77 (13), 4390-4398.

Zhang, Z. Y., Jin, B., Bai, Z. H., Wang, X. Y. 2008. Production of fungal biomass protein using microfungi from winery wastewater treatment. *Bioresource Technology* 99 (9), 3871-3876.

Zhao, W., Huang, X., Lee, D. 2009. Enhanced treatment of coke plant wastewater using an anaerobic–anoxic–oxic membrane bioreactor system. *Separation and Purification Technology* 66 (2), 279-286.

Zhou, M., Tan, Q., Wang, Q., Jiao, Y., Oturan, N., Oturan, M. A. 2012. Degradation of organics in reverse osmosis concentrate by electro-Fenton process. *Journal of Hazardous Materials* 215-216, 287-293.

Zhou, M., Liu, L., Jiao, Y., Wang, Q., Tan, Q. 2011a. Treatment of high-salinity reverse osmosis concentrate by electrochemical oxidation on BDD and DSA electrodes. *Desalination* 277 (1), 201-206.

Zhou, T., Lim, T., Chin, S., Fane, A. 2011b. Treatment of organics in reverse osmosis concentrate from a municipal wastewater reclamation plant: Feasibility test of advanced oxidation processes with/without pretreatment. *Chemical Engineering Journal* 166 (3), 932-939.

Zhu, X. and Elimelech, M. 1997.Colloidal fouling of reverse osmosis membranes: measurements and fouling mechanisms. *Environmental Science and Technology* 31 (12), 3654-3662.

ANNEX

Questionnaire

WINERY DETAILS

- ❖ Annual quantity of grapes received (tones/year) – number declared at the Wine Products Council

2009…………………………………………………………………………………

ENVIRONMENTAL MEASURES

- ❖ Do you implement environmental measures in your winery? ☐ YES ☐ NO

If yes, in which areas? Please specify:

☐ Wastewater………………………………………………………………………

☐ Solid waste……………………………………………………………………….

☐ Energy……………………………………………………………………………

☐ Air emissions…………………………………………………………………….

☐ Water consumption……………………………………………………………...

☐ Odours……………………………………………………………………………

WINERY WASTEWATER

- ❖ Is the volume of winery wastewater monitored? ☐ YES ☐ NO

What is the annual volume of wastewater produced in your winery? …………….

(Specify wastewater volume if monitored. Otherwise, please estimate)

- ❖ Are all wet-work areas (i.e. those subject to contact with grape, juice, wine or cleaning products) served by <u>only one</u> wastewater drainage/collection system? ☐ YES ☐ NO
- ❖ Is wastewater mixed with rainwater? ☐ YES ☐ NO

- ❖ How do you treat winery wastewater?
  - A) Septic tank ☐ YES ☐ NO
  - B) Sealed tank ☐ YES ☐ NO
  - C) Private wastewater treatment plant ☐ YES ☐ NO

D) Other ☐ YES ☐ NO

- ❖ Are you aware of the local legislation on the treatment and disposal of winery wastewater? ☐ YES ☐ NO

- ❖ How is wastewater disposed of?

☐ Vineyard irrigation

☐ Field

☐ Water receiver (e.g. river)

☐ Tree irrigation

☐ Other

Is wastewater disposed of before or after treatment? ☐ YES ☐ NO

- ❖ Is the area where the wastewater is disposed of owned or managed by the winery? ☐ YES ☐ NO

- ❖ Is the area where the wastewater is disposed of environmentally sensitive (e.g. protected)? ☐ YES ☐ NO

- ❖ Is the main wastewater collection sump at or near the lowest point of the site? ☐ YES ☐ NO

Does the system rely on pumping and if so what are the likely consequences of an equipment or power failure?

…………………………………………………………………………………………

…………………………………………………………………………………………

SOLID WASTE

- ❖ How organic waste produced in your winery are managed (e.g. alcohol recovery, composting)?

Please specify:…………………………………………………………………………

…………………………………………………………………………………………

- ❖ How packaging waste (e.g. glass, paper, plastic) produced in your winery is managed?

Please specify:……………………………………………………………………………

- ❖ Are you a member of Green dot Cyprus? ☐ YES ☐ NO

- ❖ How is wine sludge managed?

Please specify:…………………………………………………………………….

- ❖ Is the annual quantity of solid waste produced in your winery monitored? ☐ YES ☐ NO

If YES please specify……………………………………………………………………

AIR EMISSIONS AND ODOURS

- ❖ Are the air emissions produced by your winery monitored? ☐ YES ☐ NO

If YES please specify the air emissions and the way these are monitored.

……………………………………………………………………………………..

NOISE

- ❖ Is intense noise observed during the operation of your winery? ☐ YES ☐ NO

If YES, where does this come from?

☐ Transportation

☐ Cooling equipment

☐ Grinding and pressing procedure

☐ Other…………………………………………………………………………

- ❖ Are noise measurements made to confirm whether the winery is generating excessive noise? ☐ YES ☐ NO

Please specify how this is achieved:……………………………………………..

- ❖ Do you implement specific measures for noise minimisation/elimination? ☐ YES ☐ NO

If **yes**, please specify:………………………………………………………………

WATER CONSUMPTION

❖ Is monthly consumption of water monitored? ☐ YES ☐ NO

Please specify how this is achieved

❖ Have water spills/leaks been noticed in your winery? ☐ YES ☐ NO

If YES has the problem been resolved? ☐ YES ☐ NO

❖ Do you implement water saving measures in your winery (e.g. water saving systems for irrigation, washing, economical flushers and mixers) ☐ YES ☐ NO

If YES please specify:………………………………………………………………

ENERGY CONSUMPTION

❖ Is monthly energy consumption monitored? ☐ YES ☐ NO

❖ Have you noticed any excessive energy consumption values? ☐ YES ☐ NO

If YES are these monitored/controlled? ☐ YES ☐ NO

❖ Do you implement specific measures for energy saving? (e.g. use of renewable energy sources, insulation etc) ☐ YES ☐ NO

If YES please specify:………………………………………………………………

CHEMICALS

❖ Which chemicals are used used in the winery?

☐ Chemicals used for cleaning / disinfection

☐ caustic soda, ☐ citric acid, ☐ oxonium,☐ sulfurous, ☐ other………..

☐ Chemicals used in distillation

☐ Pesticides

☐ Fertilizers

❖ Are the quantities of chemicals used monitored? ☐ YES ☐ NO

- ❖ Which are the measures implemented in your effort to limit the use of chemicals? …………………………………………………………………...

- ❖ Do you use environmentally friendly cleaning products? ☐ YES ☐ NO

Please specify:………………………………………………………………………

- ❖ How often do you spray your vineyard per year?

Please specify:………………………………………………………………………

Do you examine whether spraying is or is not necessary? ☐ YES ☐ NO

- ❖ Do you monitor the quantity of fertilisers that are used for vine cultivation? ☐ YES ☐ NO

If YES please specify:………………………………………………………………

- ❖ Do you use organic fertilizers? ☐ YES ☐ NO

- ❖ Are you registered in an organization for the certification of organic cultivation? ☐ YES ☐ NO

If YES please specify the area of your organic vineyard:…………………………..

- ❖ Do you maintain an archive (files) with the specifications of chemical products, fertilizers e.t.c., from your suppliers? ☐ YES ☐ NO

TRAINING ON ENVIRONMENTAL AWARENESS

- ❖ Is your permanent staff trained on environmental awareness and safety issues? ☐ YES ☐ NO

If YES please specify the areas covered by the training

☐ Environmental Management

☐ Environmental Impacts resulting from the winery wastewater

☐ Solid Waste Management

☐ Energy and water consumption saving

☐ Other

- ❖ Are temporary employees trained through seminars on environmental management issues? ☐ YES ☐ NO

- ❖ Is the personnel informed about the environmental legislation related to the operation of the wineries? ☐ YES ☐ NO

- ❖ Do you maintain up to date records for staff training? ☐ YES ☐ NO

<u>END OF QUESTIONNAIRE</u>

………………………………………………………………………………………………

………………………………………………………………………………………………

………………………………………………………………………………………………

………………………………………………………………………………………………

………………………………………………………………………………………………