ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

INTEGRATED MEMBRANE FILTRATION ENHANCED WITH CHEMICAL CONDITIONING AND BIOLOGICAL TREATABILITY OF OLIVE MILL WASTEWATER

M.Sc. THESIS

Soodeh SALIMI KHALIGH

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Soodeh SALIMI KHALIGH
(501111812)

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Thesis Advisor: Prof. Dr. Seval SÖZEN

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ZEYTİN KARASUYUNUN KİMYASAL ŞARTLANDIRMA DESTEKLİ ENTEGRE MEMBRAN FİLTRASYONU SONRASINDA BİYOLOJİK ARITILABİLİRLİĞİNİN ARAŞTIRILMASI

YÜKSEK LİSANS TEZİ

Soodeh SALİMİ KHALİGH
(501111812)

Çevre Bilimleri ve Mühendisliği Anabilim Dalı

Çevre Biyoteknoloji Programı

Tez Danışmanı: Prof. Dr. Seval SÖZEN

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Soodeh SALIMI KHALIGH, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology,** student ID 501111812, successfully defended the **thesis** entitled "**INTEGRATED MEMBRANE FILTRATION ENHANCED WITH CHEMICAL CONDITIONING AND BIOLOGICAL TREATABILITY OF OLIVE MILL WASTEWATER**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Seval SÖZEN** Istanbul Technical University	....
Jury Members :	**Prof. Dr. Bülent KESKİNLER** Gebze Technical University	....
	Assoc. Prof. Dr. Nevin YAĞCI Istanbul Technical University	....

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To my family,

FOREWORD

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MAY 2015 Soodeh Salimi Khaligh

TABLE OF CONTENTS

Page

ABBREVIATIONS

$\hat{\mu}_H$	: Maximum heterotrophic growth rate
ASM	: Activated Sludge Model
b_H	: Endogenous decay coefficient
BOD_5	: Biochemical Oxygen Demand
COD	: Chemical Oxygen Demand
C_S	: Total biodegradable COD
C_{T1}	: Total initial COD
F/M	: Initial food to microorganism ratio
IOOC	: International Olive Oil Council
k_h	: Maximum hydrolysis rate
K_S	: Half saturation constant for growth
MLSS	: Mixed Liquor Suspended Solids
MLVSS	: Mixed Liquor Volatile Suspended Solids
OMW	: Olive Mill Wastewater
OUR	: Oxygen Uptake Rate
PSD	: Particle Size Distribution
S_p	: Soluble microbial product
S_{S1}	: readily biodegradable COD
X_H	: Heterotrophic biomass concentration
X_{S1}	: slowly biodegradable COD

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTEGRATED MEMBRANE FILTRATION ENHANCED WITH CHEMICAL CONDITIONING AND BIOLOGICAL TREATABILITY OF OLIVE MILL WASTEWATER

SUMMARY

Environmental biotechnology has been as area where extensive scientific efforts have been devoted to realize the characteristics and biodegradation of complex pollutants in different wastewaters and illuminate related biochemical reactions. Biological treatment has been traditionally based on the activated sludge process involve primary settling, aeration and secondary settling.

However, the obligation to comply with new concepts of environmental sustainability, which require reducing the use of recourses and making the most energy efficient systems, underline the need for recognizing the full potential from the new and emerging technological options in wastewater treatment.

Olive oil wastewater is one of the most complex and strong agro-industrial wastewaters which cause significant pollution problems in environments if appropriate treatment is not applied before disposal. The disposal and treatment of this wastewater is an important subject specially for the Mediterranean countries as most of olive oil producers in the world are located in this region. Due to specific characterization of this wastewater, especially high amount of organic, toxic and recalcitrant compounds, and on the other hand it's seasonally generation, it is necessary to find most appropriate method to have the best treatment efficiency. The most abundant organic substance in olive mill wastewater are polyphenols which are generally cause the high strength characteristic of wastewater, responsible for antimicrobial and toxic attribute of it, and are barriers in the wastewater biological treatment.

This study, intended to provide the starting move towards the olive mill wastewater treatment by using the function of chemical conditioning and membrane filtration in reduction of the organic compounds of wastewater to the lowest level, that allows domestic biological treatment plant to be operated as final treatment system.

For this application, in this work a significant amount of COD has been removed by means of *chemical conditioning* and membrane filtration using *ultrafiltration* and *nanofiltration* processes.

In this framework, the olive oil wastewater with an initial COD of 106500 mg/L was subjected first to a coagulation - flocculation process, applying PAC supplemented with anionic polyelectrolyte yielded excellent effluent quality with COD level of 40800mg/L under optimum condition after coarse filtration (62 % overall COD removal).

The particle size distribution analysis was conducted before and after chemical conditioning. This method is a beneficial tool for wastewater characterization that supplies valuable data about COD fractions for different size intervals and shows the relation between these COD fractions and particle size distribution of the wastewater. It can also compare different treatment technologies as a function of Particle Size Distribution. The results revealed that the chemical precipitation effectively removed the particulate fraction of COD and the highest COD removal efficiency was achieved on this fraction which appeared as the major fraction of raw wastewater.

While after coagulation flocculation, the most COD portion remained on soluble fraction below 2 nm, but also with a similar significant removal from 27.500 mg/L in raw wastewater down to 21.000 mg/L. This level represented 53% of the remaining COD after chemical conditioning, which gives clear indication that major change occurred in the size range below 2 nm particularly important for membrane filtration.

Applying ultrafiltration and nanofiltration yielded significant results in reducing COD. First step was started with a raw wastewater with a COD level of 51000 mg/L after coarse filtration. Ultra filtration using UC010 was able to reduce this level down to 33300 mg/L, and the nanofiltration step further reduced the COD concentration to 8300 mg/L. , with a final overall COD removal efficiency of % 92.

Second step was started with chemical conditioning prior to membrane filtration, the effluent COD decrease to 29300 mg/L after ultrafiltration and finally to 5870 mg/L after nanofiltration steps.

The third step involved the sequence of ultrafiltration, chemical conditioning and nanofiltration; a final COD of 6200 mg/L was obtained after the final nanofiltration step, which is a great efficiency in COD removal for this strong wastewater.

The next part was the biological treatability of the integrated chemically- physically treated wastewater. A laboratory-scale sequencing batch reactor with a net volume of 5L was conducted with activated sludge taken from a domestic wastewater treatment plant. The system was fed with synthetic substrate (peptone mixture) with the same characteristics of domestic wastewater, in terms of organic carbon content and COD fractions with various biodegradation characteristics. It was operated for 2 months at steady state condition and a sludge age of 8 days, to get acclimated biomass.

Respirometric measurements were used to determine the possible inhibitory effect of olive oil wastewater on the biodegradation of domestic sewage and to choose best mixture ratio of domestic sewage and olive mill wastewater for the biological treatment. Biodegradability of the olive mill wastewater when mixed into a domestic wastewater was essentially tested on the basis of oxygen uptake rate (OUR) profiles generated in respirometric measurements**.** Respirometric tests were conducted in 2L batch reactor started with biomass taken from SBR system at endogenous respiration stage.

The first OUR measurement was conducted with synthetic wastewater or peptone mixture only, as control to evaluate biodegradation characteristics of domestic sewage. In tests 2 and 3 the biodegradability of pretreated olive oil and peptone mixture with mixing ratios of 15% and 50% were evaluated to determine the effect of olive oil wastewater in different concentrations on combined biological treatment. In 4th measurement, pretreated olive oil wastewater with no additional peptone was applied to assess its characteristics and behavior in biodegradation. In last OUR measurement, raw olive oil wastewater with total COD of 200mg/L in respirometric vessel was applied to understand the biodegradation characteristic od olive oil wastewater before any pretreatment.

The results of model calibration show two major COD fractions in pretreated OMW: the readily biodegradable fraction, and the slowly biodegradable COD fraction. Three different COD fractions were shown for peptone: readily biodegradable, rapidly hydrolysable and slowly hydrolysable COD. Two hydrolysable fractions undergo hydrolysis to breaking down into readily biodegradable COD, then utilized by microorganisms.

The respirometric results displayed the recalcitrant nature of row olive mill wastewater, without proper pretreatment. Significantly tampered and reduced OUR profile obtained with raw olive wastewater dosing in last OUR measurement, shows only limited and partial biodegradation. According to basic mass balance between oxygen consumed and substrate utilized in a biochemical reaction, the magnitude of the COD utilized in the test was 46 mg COD/L, corresponding to only 21% of the 200 mg COD/L dose used in the experiment.

Also results indicated that olive oil wastewater becomes fully biodegradable after a pretreatment scheme involving chemical conditioning and membrane filtration with an ultrafiltration/nanofiltration sequence, due to removal of nonbiodegradable COD fraction. Biodegradation was tested in mixture with peptone, which properly simulates and approximates the biodegradation characteristics of sewage after appropriate dilution of OMW. Results indicate complete removal of OMW together with peptone mixture in all conducted OUR measurements.

Olive mill wastewater dosing, even at highest dosage did not exert any appreciable impact on the biodegradation of the peptone mixture as growth and hydrolysis kinetics basically remained the same in all respirometric measurements. Also there were no inhibitory/toxic impact of OMW dosing on the biomass, as the amount of active biomass remained the same in all tests.

Full biodegradation of OMW was achieved by biomass acclimated only to peptone and without any acclimation to olive oil wastewater.

ZEYTİN KARASUYUNUN KİMYASAL ŞARTLANDIRMA DESTEKLİ ENTEGRE MEMBRAN FİLTRASYONU SONRASINDA BİYOLOJİK ARITILABİLİRLİĞİNİN ARAŞTIRILMASI

ÖZET

Çevre Biyoteknolojisi çeşitli karmaşık yapıda kirletici içeren atıksuların özellikleri, biyokimyasal reaksiyonları ve arıtılabilirlikleri araştırmak üzere gelişmiş teknikleri kullanan bir bilimsel alandır. Aktif çamur biyolojik arıtma teknikleri içerisinde günümüzde en yaygın olarak kullanılan proseslerden biridir.

Günümüzde, çevresel sürdürülebilirliğin sağlanması amacına yönelik olarak, kaynak kullanımının azaltılması ve enerji verimli sistemlerin kullanılması gerekliliği atıksu arıtma tesislerinde yenilikçi yaklaşımları ortaya koymuştur.

Zeytinyağı üretimi sonucu oluşan atıksular, yaygın olarak kullanılan adı ile zeytin karasuyu, karmaşık ve kuvvetli tarıma dayalı bir endüstriyel atıksu olup, uygun ve yeterli arıtma yapılmaması durumunda önemli çevre problemlerine yol açmaktadır. Zeytin karasuyunun arıtılabilirliği ve uzaklaştırılması zeytin ve zeytin yağı üretiminin bu bölgede yoğunlaşması nedeniyle özellikle Akdeniz ülkeleri için önemli bir sorun teşkil etmektedir. Karasudaki yüksek organik madde içeriği ve toksik maddelerin varlığı ile birlikte bu atıksuların ancak mevsimsel üretimi nedeniyle arıtılmasında teknik ve ekonomik zorluklarla karşılaşılmakta ve bu hususlar en uygun arıtılabilirlik yönteminin araştırılmasını zorunlu kılmaktadır.

Bu çalışma zeytinyağı üretimi atıksularının kimyasal şartlandırma ve membran filtrasyonu ünitelerinden oluşan bir entegre arıtma şeması ile arıtılarak organik madde içeriğini mümkün olabilecek en düşük düzeye getirmeyi ve bu atıksuların daha sonra evsel atıksu arıtma tesisinde evsel atıksular ile nihai arıtılabilirliğini ortaya koymayı amaçlamaktadır. Üretimin mevsimsel olması nedeniyle karasuyun hangi oranlarda evsel atıksular ile karıştırılmak suretiyle biyolojik arıtılabilirlik üzerinde herhangi bir olumsuz etki yaratmayacağı araştırılacaktır.

Bu çerçevede zeytin karasuyunun kimyasal arıtmayı takip eden bir membran filtrasyonu uygulaması ile daha sonra nihai arıtma yöntemi olarak değerlendirilecek biyolojik arıtmaya olumsuz etkisi olmayacak düzeylerde arıtılması hedeflenmiştir. Kimyasal arıtmada çamur oluşumunu minimize eden PAC kullanılmış ve PAC ile birlikte optimum polielektrolit türü ve konsantrasyonu belirlenmiştir. Kimyasal arıtmanın KOI'yi oluşturan dağılımın hangi aralığında daha etken olduğunu belirleyebilmek amacıyla gerek ham suda gerekse kimyasal şartlandırılmış atıksuda partikül boyut dağılımı saptanmıştır.

Ham suda yapılan partikül boyut dağılımı analizleri atıksuda mevcut olan 106500 mg/l KOI'nin % 57'sinin 1600 nm'den büyük % 26'sının ise 2 nm'den küçük olduğunu ortaya koymuştur. PAC ve anyonik polielektrolit kullanılmak suretiyle uygulanan kimyasal arıtma neticesinde KOI konsantrasyonu 40800 mg /l'ye düşürülerek % 62 giderim verimi sağlanmıştır. Kimyasal şartlandırma sonrasında partikül boyut dağılımı incelendiğinde KOI'nin (21000 mg/l KOI) %53'ünün 2 nm'nin altında olduğu görülmüştür.

Ham atıksuya kaba filtrasyon uygulaması ile 106500 mg/l olan KOI konsantrasyonunun 51000 mg/L'ye düştüğü görülmüştür. Kaba filter ertesinde ultrafiltrasyon ve nanofiltrasyon uygulaması yapılmış, UC010 ile (ultrafiltrasyon)

33300 mg KOI/L, NF90 ile (nanofiltrasyon) 8300 mg KOI/L elde edilmiştir. Kaba filtrasyon sonrasında kimyasal arıtma yapılmaksızın membran filtarsyonu uygulaması ile % 92 giderim verimi sağlanabilmektedir.

İkinci adımda kaba filtre sonrasında kimyasal arıtma uygulaması ile KOI konsantrasyonu 40800 mg/L'ye düşürülmüştür. Bu akıma ultrafiltrasyon uygulaması ile 29300 mg KOI/L ve nanofiltrasyon ile nihai düzey olan 5870 mg KOI/L elde edilmiştir.

Kimyasal şartlandırmanın optimum uygulama yerini belirlemek amacıyla üçüncü adımda ultrafiltrasyondan geçirilen karasu kimyasal arıtılmış ve daha sonra nanofiltrasyon uygulanmıştır. Bu süreçte nihai olarak KOI 6200 mg/L olarak taspit edilmiştir.

Bir sonraki aşamada entegre kimyasal arıtma ve membran filtrasyonu prosesinden geçirilmiş olan karasuyun biyolojik arıtılabilirliğe olan etkisini belirlemek amacıyla farklı oranlarda karasu ve evsel atıksu karışımları oluşturularak respirometrik deneyler yürütülmüş, biyolojik arıtma üzerinde olumsuz etki yaratmayan en uygun karasu-evsel atıksu karışım oranı saptanmıştır.

Biyolojik arıtılabilirlik deneyleri, evsel atıksuyu yansıtmak üzere, organik karbon içeriği ve KOİ fraksyonları evsel atıksu ile eşdeğer olan sentetik pepton kullanılmak suretiyle yürütülmüştür. Biyokütle aklimasyonunu sağlamak amacıyla çamur yaşı 8 gün olan bir doldur-boşalt reaktör 2 ay boyunca kararlı hal koşullarında çalıştırılmıştır.

Zeytin karasuyunun evsel atıksuyun biyolojik ayrışabilirliğini inhibe edici etkisi olup olmadığını ortaya koyabilmek üzere respirometrik testlerden yararlanılmıştır. Deneyler 2L kesikli olarak işletilen reaktörlerde gerçekleştirilmiştir. Çeşitli oranlarda zeytin karasuyu ve evsel atıksu karışımı OUR profilleri değerlendirilmiştir.

Evsel atıksuyun biyolojik olarak ayrışabilirliğini karakterize etmek üzere kontrol amaçlı bir set deney sadece pepton ile yürütülmüş ve OUR ölçümleri yapılmıştır. 2. ve 3. Deney setlerinde ise ön arıtılmadan geçirilmiş zeytinyağı sırasıyla % 15 ve %50 oranlarında pepton ile karıştırılmıştır. 4. Deney seti ise biyolojik olarak ayrışabilme karakterini tayin edebilmek için yalnızca ön arıtılmış zeytinyağı atıksuyu ile yürütülmüştür.

Elde edilen OUR profilleri modelleme yaklaşımı ile irdelendiğinde, karasuyun kolay ayrışabilen ve yavaş ayrışabilen iki KOİ fraksiyonundan oluştuğu, peptonun ise kolay ayrışabilen, hızlı hidroliz olabilen ve yavaş hidroliz olabilen organik maddeler ile karakterize edilebileceği saptanmıştır. Gerek hızlı, gerekse yavaş hidroliz olabilen organik maddeler ancak kolay ayrışabilen organik maddeye dönüştürüldükten sonra çoğalmada kullanılmaktadır.
Ham zeytinyağı atıksuyunun zor ayrışabilirliği respirometrik ölçümler sonucu görülmüştür. Ham zeytinyağı atıksuyu ile yapılan OUR ölçümünde gözlenen düşük OUR profili sınırlı ve kısmen ayrışabilen KOİ miktarının olduğuna işaret etmektedir. Biyokimyasal reaksiyonda kullanılan ve harcanan oksijenin kütle dengesine göre deney setinde elde edilen KOİ değeri toplam KOİ değeri olan 200mg/L'nin yalnızca %21'i olan 46mg/L ile sınırlı kalmıştır.

Yapılan değerlendirmede kimyasal şartlandırma ve ultrafiltrasyon/nanofiltrasyondan adımlarından oluşan ön arıtmadan geçirilmiş karasuyun evsel atıksu niteliğinde pepton ile karışımının, denenen iki farklı karışım oranında da, biyolojik arıtmayı olumsuz olarak etkilemediği görülmüştür. Modelleme çalışmaları karasuyun yüksek karışım oranında dahi mikroorganizmaların maksimum spesifik çoğalma hızı ve hidroliz katsayılarının etkilenmediğini, bir başka ifade ile ön arıtmadan geçirilmiş karasuyun çoğalma ve hidroliz kinetiğine olumsuz bir etkisi olmadığını ortaya koymuştur. Modelleme sonuçları sadece pepton ve sadece karasu setleri için de benzerlik göstermiştir. Bu durum ön arıtma adımının karasu içindeki biyolojik ayrışmayı olumsuz yönde etkileyecek tüm maddeler ile birlikte biyolojik olarak ayrışamayan maddelerin de uzaklaştırıldığını göstermektedir. Aktif biyokütle miktarı her set deneyde hemen aynı kaldığından herhangi bir inhibisyon/ toksik etki söz konusu değildir.

1. INTRODUCTION

Olive oil production is one of the vital industries for many Mediterranean countries. This industry is generating one of the highest amounts of wastewater. The olive oil production usually yields around 20% olive oil, 30% semi solid waste and 50% liquid waste (Fiestas Ros De Ursinos and Borja-Padilla, 1992). The origin of liquid waste is vegetation water and soft parts of olives. Combination of this liquid waste with the water that is used during various steps of olive oil production, makes a wastewater generally named “Olive Mill Wastewater (OMWW)”. Also water comes from olives and equipment washing are included in this wastewater.

Olive oil producers always deal with the problem of disposal of their wastewater and this wastewater is one of the most important difficulties of the producers in terms of its characterization and also its extent. It is one of the critical wastewaters in terms of quality and also quantity. Characteristics of Olive Mill Wastewater is variable depends on some factors such as ripeness of olives, region of cultivation, methods of cultivation, climate conditions, oil extraction methods, etc. (Greca et al, 2004; Bazoti et al, 2006). The wastewater contains different organic substances such as tannins, sugars, polyphenolic compounds, pectins, polyalcohols and lipids (D’Annibale et al., 1998). The most abundant organic substances are polyphenols which are generally cause the high strength characteristic of wastewater, responsible for antimicrobial and toxic attribute of it (Bisignano et al, 1999; De Marco et al, 2007) and significant barriers in olive oil wastewater biological treatment.

The environmental pollution from wastewater of olive oil producers with production and disposal of around 30 million m^3 of Olive Mill Wastewater in year, makes various important problems, specially for Mediterranean countries who produce around 97% of olive oils in the world. It contains high amount of organic compounds which are difficult to degrade with inhibitory effect on biodegradation. Polyphenols have strong antioxidant activity (Obied et al, 2005; DeMarco et al, 2007) and are recalcitrant compounds, which are resistant to biodegradation. This characterization together with high percentage of suspended solids and dissolved minerals cause significant pollution problems in the environment if appropriate treatment is not applied before disposal.

Due to seasonal (November to February) production of Olive Mill Wastewater, it requires storage of the wastewater, or assign specific treatment plant to it which is not economic. On the other hand, high amount of its organic compounds that are not biodegradable (Canizares et al, 2007; Njjar et al, 2007), makes it necessary to find most appropriate method to have best treatment. Various conventional treatment methods have been conducted for treatment. Non of methods alone is able to solve the problem of olive mill waste water treatment to a complete extent and an effectively way, as olive oil producers are still facing serious problems due to lack of economic and feasible solution to control their olive oil wastes. Therefore, development of a practical, efficient and cost effective solution to management of wastewater produced during olive oil production is required.

The focus of this study is to evaluate a novel wastewater management approach for the olive oil industry effluents. The approach is essentially involved a pre-treatment stage consisting of chemical conditioning as a well-known method followed by membrane filtration. It also envisaged feeding the effluent with appropriate dilution to a municipal treatment plant for joint treatment and final disposal. By means of municipal treatment plant, there is no need to storage or allocate specific treatment plant seasonally for olive mill wastewater. In this context, the biodegradability of the treated effluent was then tested with appropriate dilutions when mixed with domestic sewage. Particle size distribution analyses were performed to reveal the mechanistic impact of chemical conditioning on the efficiency of membrane filtration. Poly-aluminum chloride (PAC) was used as the optimum synthetic coagulant. Chemically conditioned wastewater was tested with a sequence of ultrafiltration and nanofiltration steps after coarse filtration. OUR profile was used as an experimental tool for evaluating the biodegradation characteristics of olive mill wastewater mixed with domestic wastewater. Results indicated complete biodegradation of the olive oil wastewater after pretreatment.

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2. LITERATURE REVIEW

2.1 Process Scheme

Olive oil is produced from olive trees, with yield of around 15-40 kg olive per year for each olive tree. The olive oil tree includes 30 species but only one of them is edible with the name of Olea europeaea. Among different varieties of olive oil trees, IOCC published 139 of them in the World Catalogue of Olive Varieties. (Niaounakis M. and Halvadakis CP, 2006).

In EU the olive sectors includes about 2.5 million producers that is around one-third of EU farmers. Due to seasonal production of olive oil this industry has the advantage of seasonal employment in winter, as a complementary activity with other agricultural tasks. Major of olive mills are not big sectors and work as small enterprises.

The world production of olives for the year 2012 was 16,402,619 metric tones (FAOSTAT, 2012). World production of olive oil for the harvesting year 2013/2014 was 3,199,500 tons (IOOC, 2014). The majority of olive oil production belongs to Mediterranean countries with 97% of olive oil production (Paraskeva and Diamadopoulos, 2006). The biggest olive oil producer in the world is *Spain* with production of 1,775,800 tons in harvesting year 2013/2014. For the same period *Italy* with 461,200 tons is the second producer, followed by *Turkey* and *Greece* with production of 190,000 tons and 131,900 tons (IOCC, 2014), Tunisia, Portugal, Morocco and Algeria. Olive oil is also produced out of Mediterranean region like Middle East, the USA, Australia and Argentina. The 4th producer in the world is Turkey (Paraskeva and Diamadopoulos, 2006).

Most of the olive oil industries are located in south and west of Turkey as shown in Fig.2.1.

The annual olive oil production in Turkey is around 100,000-250,000 tons (Akar et al, 2009) and the most frequently used system to ultimate disposal is evaporation lagoons (Erses Yay et al, 2012). Generally this method causes environmental pollution as the basin of lagoons are not isolated and ground and groundwater became polluted due to penetration of the wastewater.

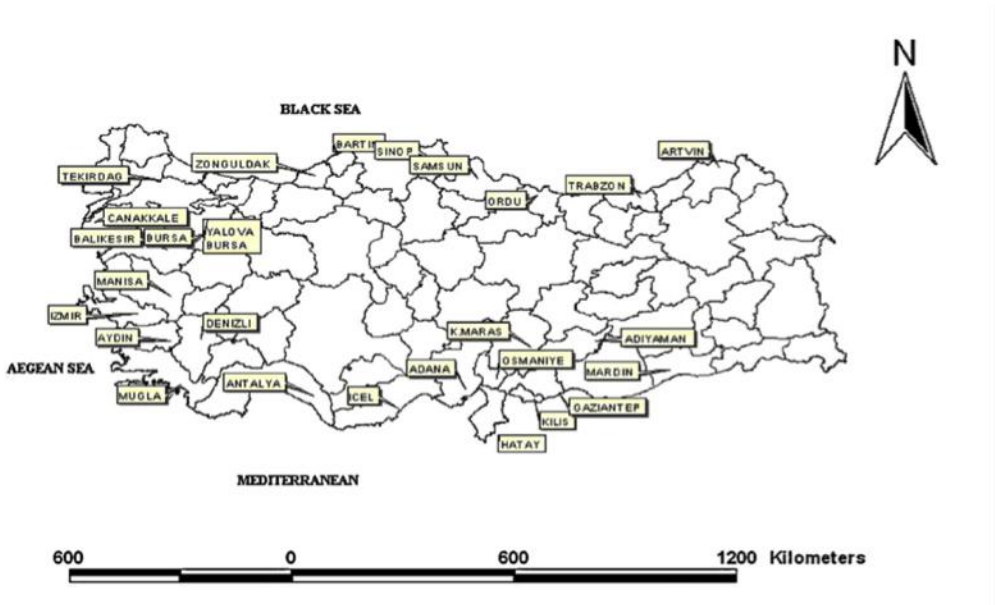

Figure 2.1 : Olive oil industries located in Turkey (Erses Yay et al, 2012).

Many scientists tried to achieve most efficient and cost effective olive mill wastewater treatment technique by different methods. This chapter will explain the literature finding related with olive oil extraction techniques, olive oil wastewater characterization and its treatment options that have been investigated to have the safe disposal and their drawback.

Olive oil production from olive in olive mills is by two methods: discontinuous pressing method or batch method; and continuous centrifugation method (Sabbah et al, 2004; Masghouni and Hassairi, 2000). In discontinuous press method or traditional system, oil is extracting by pressure and generated wastewater is less but more concentrated. In this method there in no need to add water to the olive paste. If it was difficult to separate the oil phase from the other phase, or if the olive fruits are difficult to process, small quantity of water may be required during the process. Depending on the separation degree of the oil from vegetation water at the end of the process, the oil can be perfectly clear or turbid. Existence of particles in the oil will cause less stable oil and rancidity. Generally the oil produced by this method has high quality due to low temperature during the extraction. The quality of oil also strongly depending to hygienic processing condition. The pressing method is costly in terms of manual labor and requirement of filtering equipment.

The traditional discontinuous pressing method replaced by continuous centrifuge system at the end of nineteenth century. It is based on separation through centrifuge named decanters and was developed to decrease the labor costs and increase the yield of olive oil. This method involved crushing of olive fruits, mixing the paste of olive, and centrifuging. Centrifuge may be with or without adding water depends on “three-phase” or “two-phase” system, respectively.

In three-phase continuous system, water is required during the process. After the process olive pomace, wastewater and oil are produced. Olive oil production using this system is 3-4 times more than pressing method also with lower labor needed. While usage of water during production is 50% more (around 80-100 L water per 100kg olive processed), but the wastewater generation is twice more (1.3-2 L/kg olive processed in comparison with 0.5-1 L/kg in traditional method) due to more water consumption in the process. In three phase method some useful components such as natural antioxidant is going to water phase not oil phase, so the oil quality is not as high as in the traditional method. There is also the problem of wastewater disposal in this method due to high amount of generated wastewater (Paraskeva and Diamadopoulos, 2006). Most of the producers in Turkey are using three phase system.

In Two phase method which is developed recently, there is no process water and just two phases are generated: moisturize olive pomace as the solid phase and oil as liquid phase (Masghouni and Hassairi, 2000; De la Casa et al, 2009). This method is interested where the generated wastewater must be decreased or when the water supply is limited. The construction of centrifuge system in this method is not as complicated and expensive as three-phase system, and the cost of operation is lower. Produced olive oil has higher quality in terms of its oxidation stability and organoleptic characteristics.

This method has also some disadvantages. Although the wastewater generated is less, but the semi solid form of effluent needs to be disposed, and it needs some expensive pretreatments to be able to burn or compost it. This wastewater has 55-70% moisture, while the olive cake in pressing method has 20-25% moisture and 40-45% in three phase system. This high moisture together with solids content of the wastewater, makes it difficult to store, handling and transport. It contains high amount of pulp/stone ratio, smaller quantity than the other methods but more concentrated, heavier, with higher amount of phenols, fat, dry residue, COD and turbidity.

The first country used this developed technology was Spain, afterward it was spread in some other countries.

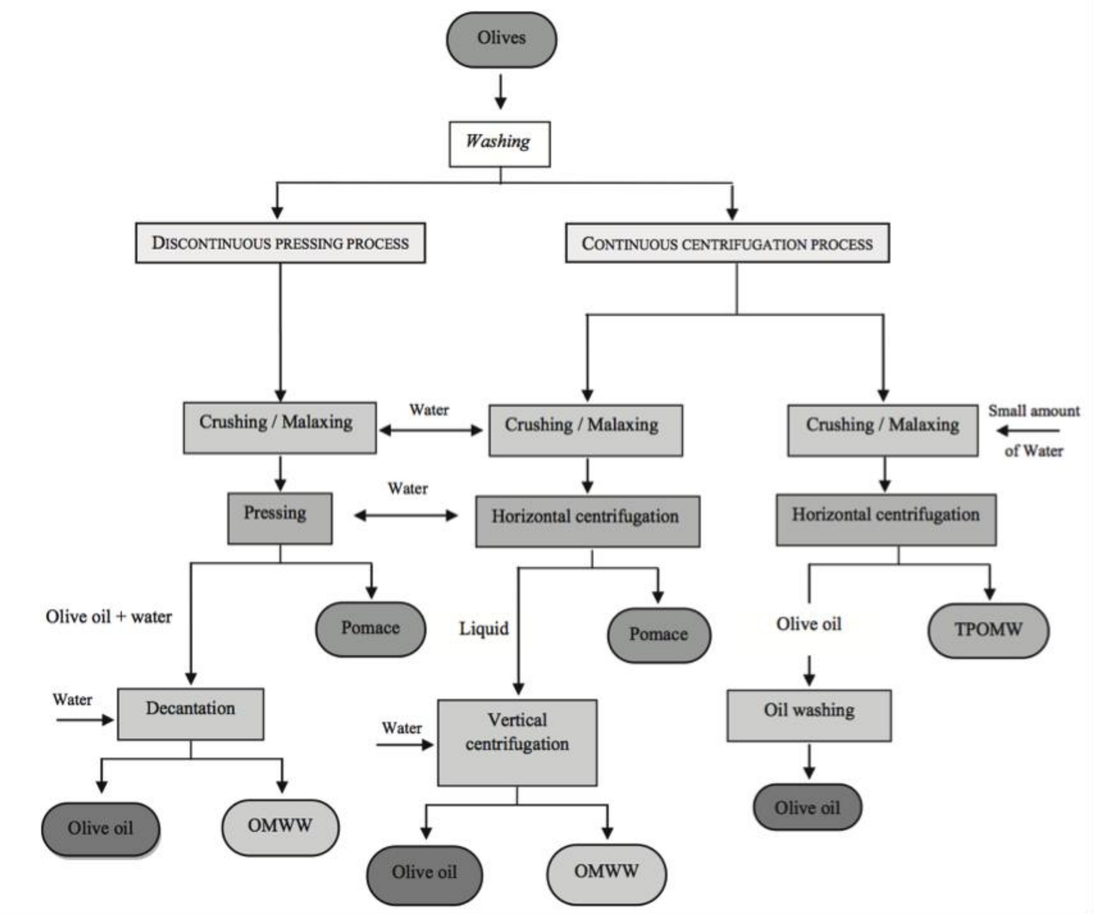

Figure 2.2 : Olive oil extraction processes (Dermeche et al, 2013).

Recently the olive oil manufacturers improve their techniques to two phase process in which usage of water during production is much less than three phase method, and most of the water using in the oil extraction and also water used in vegetation enter the solid waste, therefore the wastewater generated is very low but the amount of solid waste is considerable (De la Casa et al, 2009; Azbar et al 2004).

Additionally, results also show that olive oil produced by two-phase method has higher quality. Decreasing of processing cost, and reduction of hot water utilization and electrical energy, is another advantages of this method. Different olive oil production processes are shown in Figure 2.2.

Generally, olive mill wastewater originated from pressing or traditional method has higher COD. Wastewater with the lowest COD belongs to two-phase method.

2.2 Olive Oil Wastewater Characterization

Characteristic of Olive Mill Wastewater is variable in terms of quality and quantity, and depends on olive type, ripeness of olive fruit, method of cultivation, vegetation water composition, type of oil extraction and storage time of wastewater.

Olive Mill Wastewater contains very high organic matter concentration and various complex organic compounds that are non-biodegradable. Generally, the composition of OMWW is 80-83% water, 15-18% organic compounds, 2% inorganic compounds commonly phosphates and potassium salts (Niaounakis M. and Halvadakis CP, 2006).

It has high organic content in terms of BOD_5 (35-110 gr/L), and includes proteins, polysaccharides, mineral salts, and useful components for agriculture, like humic acids. Besides these beneficial substances, it also contains some substances which are barriers in biodegradation, with their phytotoxisity and biotoxicity effect. These characteristics belongs to its phenolic content causes this wastewater to be less biodegradable. As both of pulp and stone of olive fruit contain phenolic compounds, they released in water and oil phase during processing of olives, as these substances are more soluble in water major fraction of them release in water phase.

Olive Mill Wastewater has considerable negative effects on aquatic life with toxicity effect which can reduces oxygen transfer in water body, and changes natural water color (Suna Erses Yay et al, 2011). The most important obstacle in biological treatment of this wastewater is high concentration of organic compound up to 220gr/L COD values (Balice V. et al, 1990; Sarika et al, 2005) and also high amount of suspended solids and phenolic compounds (0.5-24 gr/L) with inhibitory effect, phytotoxicity and antimicrobial effect on microorganisms (Moreno et al, 1978; Antoniou et al, 2002; Hashwa and Mhanna, 2008).

According to Bettazzi et al. (2006), Some of the main olive mill wastewater characterization are dark brown or black color related to high concentration of lignins and tannins (52,270-180,000 mg/L Pt-Co unit), specific smell of olive oil, acidic pH between 3 and 6, high organic matter concentration, high amount of inert COD, and toxic effect on plants. Due to waste disposal into the environment, odor nuisance is created and deterioration of natural water bodies is occurred by coloring and oily shine.

Olive Mill Wastewater contains high amount of potassium, some nitrogen, calcium, phosphorus, magnesium, and iron. The predominant inorganic material is potassium.

Toxic content of it are phenols, tannins and dyes. Phenolic substances includes at least 30 compounds and their antibacterial activity is higher on Gram-positive than Gram-negative bacteria.

Various and considerable number of bacteria and fungi are identified in Olive Mill Wastewater. Several species of Acinetobacter, Pseudomonas, Enterobacter and different genera of Chalara, Penicillium and Fusarium are found in it.

2.3 Legislation

High rate application of OMW even the treated one will cause serious effects on quality of receiving water body, plant growth and soil characteristics. Different parameters need to be considered in OMW disposal such as its optimum application rate, application frequency, type of soil, type of plants cultivated on the soil and aquifer depth. High concentration of polyphenol has inhibitory effect on microorganism enzymes, fishes and their nervous system, if it is applied on sensitive water body or sandy soil and specific care needs to be taken for final disposal.

The important problem of olive processing waste is absence of a common policy between the countries producing olive oil. Every country has its own regulation or legislation to olive mill waste disposal but it is nessesory to have a common and international unified strategy to manage the olive mill waste (Suna Erses Yay et al, 2012).

2.3.1 Olive oil waste legislation in Turkey

There is no specific legislation related to OMW discharge in Turkey. It seems that Turkish olive oil producers like other European countries will face serious problems, if a feasible and economic solution for OMW disposal is not found. The Turkish water pollution control regulation, controls and protects water resources and put standards for olive oil producer effluents and receiving water body. The main difficulty for safe disposal of OMW in Turkey is that olive oil producers are not big and distributed as small units in a large geographical area (Niaounakis M. and Halvadakis CP, 2006).

Ministry of environment in Turkey authorize combustion of dried solid waste of olive oil producers from the beginning of 2013, with limitation for gass emision (Azbar et al, 2004).

2.3.2 Olive oil waste legislation in other Mediterranean countries

The first olive oil producer in the world is *Spain*. At 1981, discharge of OMW into the receiving media is banned by the Spain Government after contamination problem in most of rivers. Nowadays, this country generate less amount of OMW by application of two phase oil extraction technique as vegetation water and process water goes to the solid waste (De la Casa et al, 2009).

In Italy, there is guidline for disposal of OMW on agricultural lands (Rana et al, 2003). Land spreading of OMW is also permitted in *Portugal* recently (Cheng, 2006).

The goverment of Italy permited land spreading of OMW up to 50 or 80 m^3/ha.year from pressing or continuous centrofugation method, respectively (Altieri & Esposito, 2008).

In Greece also there is no specific legislation for OMW discharge. Each prefecture in Greece has its own policy. However, there is no guideline for effluent reuse and land application in Greece (Pedrero et al, 2010, Kapellakis et al, 2006).

In Tunisia, every year the generated wastewater is collected in big evaporation ponds close to domestic wastewater treatment plants (Ammar & Ben Rouina, 1999).

According to the European wastewater policy, the effluent of OMW needs to be disposed on receiving water media only when its characteristics is according to standards and conditions required by Directive 271/91 on Urban Wastewater Treatment, amended later by Directive 15/98.

2.4 Wastewater Treatment, Disposal Alternatives and Drawbacks

In this section, some of the most commonly used olive oil wastewater treatment methods, their advantages and drawbacks are summarized based on the literature findings.

2.4.1 Physicochemical treatment

Some methods such as dilution, evaporation, filtration, sedimentation and centrifugation have been applied on Olive Mill Wastewater. But none of them alone can decrease the toxicity and organic content to a sufficient limit. One of the commonly used techniques is dilution prior to biological treatment in order to decrease the toxicity effect of wastewater on the microorganisms which are doing biodegradation in treatment plant. Sedimentation and also evaporation can concentrate OMW around 70-75% (Paraskeva & Diamadopoulos, 2006).

These methods have some disadvantages. COD reduction mostly is not related to biodegradation and is due to dehydration or separation of different phases of wastewater. The concentrated portion and also the supernatant need further treatment in sedimentation process.

In the case of evaporation further treatment on the distillate and the paste is required also. Moreover, in open areas under sedimentation or evaporation, there is odor problem.

Some thermal methods like combustion and co-combustion also have been used, which have some advantages like decreasing the amount of waste and energy recovering. But it needs expensive equipment with high power consumption, and there is emission of toxics into the atmosphere, and need of mixing olive mill wastewater with the other wastes (Niaunakis & Halvadakis, 2004).

Filtration and centrifuge processes have the ability of removing organic substances and increase the conductivity and pH of the effluent. More organic matter removal is achievable by combination of physical process with adsorption or coagulation-flocculation method.

Results show 21% COD removal by combination of sedimentation, centrifugation and filtration (Al-Malah et al, 2000).

COD removal of 83% and phenol removal of 94% was achieved by combination of settling, centrifugation, filtration and adsorption by activated carbon (Azzam et al, 2004).

More than 40% COD removal, 62-73% phenol and 95% oil and grease removal was obtained using lime treatment after coagulation-flocculation, sedimentation and filtration process (Aktas et al, 2001).

Effect of Alum and Ferric chloride as coagulant were also examined. 95% COD removal and 90% phenol removal was achieved after acid cracking followed by application of 3gr/L Ferric chloride. 94% COD reduction and 91% phenol reduction was obtained by 6gr/L Alum (Sarika et al, 2005; Kestioglu et al, 2005).

30% COD removal was obtained by means of adsorption by Granular Activated Carbon (GAC) after coagulation, flocculation and sedimentation by 50 kg carbon/m^3 effluent (Kestioglou et al, 2005).

Electro coagulation can also remove COD of olive mill wastewater. Studies indicate 76% COD removal, 91% phenol removal and 95% color reduction after 25min treatment in the optimum pH of 4-6 which is pH range of OMW. The advantage of this method is low sludge generation in comparison with classic coagulation treatment method (Adhoum & Monser, 2004).

2.4.2 Biological treatment

One of the worldwide applications for treatment of wastewaters is biological treatment. In this process microorganism are employing to break down biodegradable contaminations of wastewater. Type of microorganisms depends on the type of treatment method (aerobic or anaerobic). In this treatment method, the biotechnological usage of microorganisms plays a key role in removing the pollutant of wastewater. Biological treatment could be applied as anaerobic biological treatment system (Beccari et al, 1996; Filidei et al, 2003) or aerobic biological treatment system (Bertin et al, 2001; Hashwa and Mhanna, 2008).

Anaerobic process involves different microbial reactions to convert organic substances to methane and carbon dioxide in absence of molecular oxygen. It has three main steps as hydrolysis, acidogenesis and methanogenesis. The most important step is the last one due to methane formation.

Anaerobic treatment is feasible for treatment of high organic load wastewater, with consumption of low energy, less sludge production and generation of methane which is rich in terms of energy. The process is mostly running by bacteria and is strongly affected by temperature, retention time, pH, partial pressure of H_2 and existence of recalcitrant and toxic substances such as phenolic compounds in the composition of wastewater. Additional nutrient, adjusting the alkalinity and dilution are needed in this method. In OMW treatment with anaerobic biological treatment plant, an acclimation period should be considered because of inhibitory substances and high organic load of the wastewater, by introducing the rate of organic load step by step and also collecting the microbial culture which are resistant to inhibition (Sabbah et al, 2005).

One of the most important drawbacks is its high sensitivity to any variation occurring in operational parameters and also toxic substances that may exist in the wastewater. Methanogenic bacteria has very slow growth rate and are so sensitive to any small change in temperature in their environment, which causes reducing their maximum specific growth rate. Also pH has strong effect on methanogenesis bacteria. In normal condition, acidogens bacteria are producing organic acid that causes decreasing pH of the system. Methanogens produce bicarbonate that has buffering capacity and protect the system from changing pH. But in abnormal environmental conditions, buffering capacity of the system could be disturbed. Therefore, due to pH decreasing, methane production will be stopped also. Because of this sensitivity, pretreatment of olive mill wastewater is also required with the aim of removing toxic and recalcitrant substances.

70-80% COD reduction by anaerobic treatment system was observed in OMW with initial COD of 22.6-97 gr/L, with different organic load of 0.83-21.9 kg COD/L.day and Hydraulic retention time of 2-5 days. Laboratory scale upflow anaerobic sludge bed (UASB) reactor was used, initial effluent was diluted and alkalinity and nutrient were adjusted. Methane was produced with a ratio of 0.30-0.35 m^3/kg COD removed (Ubay G. & Ozturk I, 1997).

80% COD reduction was observed in an anaerobic sequencing batch reactor with an average organic loading rate of 5.3 kg COD/m^3.day and HRT of 3 days with no additional nutrient but alkalinity adjustment to fix pH at 7. Initial COD was 97 gr/L and it was diluted to 16 gr/L (Ammary BY, 2005).

Results show that applying a single anaerobic treatment system can not remove the organic content of olive mill wastewater to a sufficient extent. Apart from adjusting nutrient and alkalinity and diluting the influent, some pretreatments are required. Even with 80% COD removal, the remained COD still contain high organic matter and its disposal is not safe.

Aerobic process is also one of the well-known systems in wastewater treatment. One of the main representatives of aerobic system is biological activated sludge system. It is the most common system to stabilize the organic matter in the wastewater which is based on microorganisms that grow under aerobic condition in presence of oxygen. In this system microorganisms grow on the organic substances which are oxidizable in wastewater.

For aerobic treatment of OMW, microorganism responsible for biodegradation need to be acclimated to the waste. Experiments show 80-85% COD removal with 20-25 days HRT. 58-68% COD removal was achieved using a completely mixed batch activated sludge reactor with acclimated biomass and initial COD of 65-98 gr/L. For

initial COD of 22 gr/L, 81-84% COD removal was observed. 90% phenol removal was obtained in all of these studies (Benitez et al, 1997).

74% COD and 83.4% phenol removal was observed in a pilot scale subsurface horizontal flow constructed wetland system with dilution ratio of 1:10, OMW was pretreated physic-chemically (Del Bubba et al, 2004).

Generally, the traditional aerobic methods for treatment of Olive Mill Wastewater could not reach to acceptable results due to recalcitrant contents of the wastewater such as phenolic compounds, which have inhibitory effect on enzyme of microorganism in treatment systems and hinder the aerobic treatment.

However, Biological treatment of olive mill wastewater to a sufficient extent could be achieved in activated sludge treatment system, if pretreatment is applied as first step prior to any biological treatment, or if the wastewater diluted with an easily biodegradable wastewater such as domestic wastewater. This technique is appropriate in the areas, which equipped with biological treatment plants.

In such areas, with both Olive oil production sites and municipal wastewater treatment plants, olive oil wastewater can be first pretreated by employing chemical coagulation-flocculation or physical process such as membrane filtration and in next step, mixed with domestic sewage. Treatment in existing biological treatment plant would be the next step to remove biodegradable content of wastewater. In this case, pretreated Olive Mill Wastewater needs to be transported to the municipal treatment plant.

2.4.3 Combined biological processes

Single aerobic or anaerobic treatment processes are not able to treat olive mill wastewater sufficiently even with their maximum efficiency. Therefore, combination of these systems has been examined.

COD removal of 45% and phenol removal of 60% was obtained in a combined system of GAC-packed anaerobic reactore with upflow silica bead-packed bed aerobic biofilm reactor. With an aerobic post treatment, additional 23% COD removal and 21% phenol removal was acheived that leads to final COD removal of 59% and phenol removal of 76% (Bertin et al, 2004 & 2005).

In an integrated system including two anaerobic reactor in series 83% COD removal was gained in the first reactor with an OLR of 11kg COD/m^3.day and 8% COD removal in the second one with 0.19kg COD/m^3.day OLR. Phenol reduction was 75% in the first one and 45% in the second reactor with biogass production of 2.1-2.2 L/gr (Dalis et al, 1996).

2.4.4 Co-digestion

Combined treatment of olive mill wastewater with another wastewater is possible instead of dilution. This co-treatment has some advantages. Alkalinity and pH and nutrient can be adjusted by additional wastewater. Moreover, it is more economical to treat two wastewaters together in a single treatment plant than separate treatment.

Co-digestion of OMW with manure in a UASB reactor showed COD removal of 85% for low organic loading rate and around 65% for 70 kg/m^3.day OLR. Dilution ratio of 1:2 was applied with no additional alkalinity or nitrogen source (Angelidaki et al, 2002).

Experiments show the complete treatment of OMW diluted with primary municipal wastewater in combined treatment system of anaerobic/aerobic. The anaerobic system was high rate UASB reactor and dilution ratio was 1:17-1:33. The aerobic system was a bench-scale activated sludge system. The influent COD was between 1800-4800 mg/L, but the effluent didn’t show great variation and was between 400-600 mg/L with COD removal of 70-90%. Results show biogass generation of 0.3

m^3/kg removal COD. Final COD of 85-175 mg/L was acheived after aerobic treatment with BOD below 20mg/L (Gizgis et al, 2005).

2.4.5 Composting

Organic substances can be degraded by aerobic decomposition process to a granular humus-like product during some weeks. These product can be used as soil conditioner or fertilizer. A high quality compost can be produced by OMW which can be applied to corps instead of chemical fertilizers. It contains high amount of mineral nutrient, with no heavy metals (Paraskeva & Diamadopoulos, 2006).

Agricultural wastes, straw or sewage sludge have the ability of absorbing OMW and generate a high quality compost in 2 months (Tomati et al., 2003; Paredes et al, 2003).

A good end product was achieved by mixing OMW with waste from juice production sector, sewage sludge and poultry manure in different ratios. An advantage of this process is decreasing the germination index (GI) by phytotoxicity effect of OMW. However the results show still a GI of more than 50%, which is a proper compost for soil conditioning (Paredes et al, 2001).

2.4.6 Land applications

In some researches, land spreading of raw Olive Mill Wastewater on the agricultural lands, has been suggested. It has positive impacts on the crops and also olive plants as the wastewater is rich of nutrient. All of environmental impacts should be considered in this method. For instance, applying 1000 m^3/ha in a year or more of olive mill wastewater on Spanish soil consist of limestone, can increase its organic content, phosphorus, total and soluble nitrogen, and salt.

When the low volume of wastewater like 100 m^3/ha is applied, the soil can works as a biofilter to treatment. But also there is a risk of increasing heavy metals mobility and percolation of nitrate and sodium into soil, which have contamination effect on soil and underground water (Azbar et al, 2004).

2.4.7 Evaporation Lagoons

The most common technique in Turkey is applying evaporation lagoon. It has low cost and no need to specialized workers. Lands with large surfaces are required in this method. For example for 2 tons of processed olive, a volume of $1m^3$ lagoon is needed to store and evaporation in Izmir, Turkey (Azbar et al, 2004).

This system has different disadvantages. Depending on the climate condition of region, long time is needed for natural evaporation. If the isolation of lands is not correct, groundwater contamination will occur. Bad smell and insect existence are the other backwards of this methods (Paredes et. al, 1998; Rozzo A. and Malepi F, 1996).

In this method, the liquid phase is removing via evaporation and after evaporation the solid phase is collected to use as fertilizer (Duarte et al, 1996).

2.4.8 Chemical Treatment

Chemical coagulation flocculation using polyelectrolytes (Olmez-Hanci et al, 2008) is one of the important methods for wastewater treatment that is employed worldwide to get rid of some part of organic substances in a hard wastewaters such olive mill wastewater. Its aim is to achieve envelopment of suspended particles in wastewater in form of flocs with help of chemical force; and prepare a condition for flocs to be removed through following process which is sedimentation or filtration or both (Water treatment plant design, 1969; Rizzo et al, 2010).

In coagulation process, small particles in liquid suspension are aggregated through destabilization mechanism. This destabilization occurs by neutralization of surface charge of particles with the help of metal based coagulants which are mainly iron or aluminum (Gao et al, 2002; Duan & Gregory, 2003; Rizzo et al, 2005) and have opposite charge of particle’s surface charge. Flocs formation through particle aggregation, leads to trapping particle forms of COD and adsorbing soluble form of it, which could be separated from liquid phase at the end of the process. Sedimentation is the next step after coagulation flocculation. In this step, settling or sedimentation basin is used. Wastewater should be left to be settled and then settled solids will be removed from down part of basin.

95% COD reduction was observed by adding alum and chitosan as coagulant supplement. While ferric chloride and starch were not able to remove COD of olive mill wastewater (Meyssami & Kasaeian, 2005).

In another study, two anionic and four cationic polyelectrolytes were applied on OMW. Two references were used as lime and ferric chloride as basis of comparison. Lime and ferric chloride had the ability of removing TSS, but the COD removal were lower than polyelectrolytes (Sarika et al, 2005).

10-40% COD and 30-80% phenol reduction was obtained in another experiment by adding inorganic coagulants such as lime or ferrous sulphate together with cationic polyelectrolytes (Ginos et al, 2006).

Different parameters effect coagulation and flocculation, such as electric charges of the particles, size and concentration of particles, pH, temperature of water, and concentration of electrolyte. Yet, these parameters vary from case to case and it is important to know the behavior of the subjected wastewater. However, this technique

can not remove organic substances from Olive mill Wastewater lonely, to a sufficient extent (Arslan-Alaton et al, 2009) and further treatment required to remove COD of Olive Mill Wastewater.

2.4.9 Membrane Techniques

Membrane technology is also of particular interest between different methods of wastewater treatment (Arevalo et al, 2009; Karagunduz & Dizge, 2013). This technique such as Ultrafiltration, generally is conducting after application of some pretreatments on olive mill wastewater. By means of this technique the wastewater is separated to two phases as liquid phase or pretreated wastewater, and concentrated phase that can be incinerated or sent to landfill sites (Azbar et al, 2004). Ultrafiltration as a physical method is able to remove 94% of pollutants in olive mill wastewater, but due to existence of some substances in the permeate, some further treatments are still required (Drouiche et al, 2004).

This method has significant importance and advantages, such as less space requirement, no chemical consumption, great ability to remove particle materials from liquid phase, and its action to keep microorganisms (Tchobanoglous et al, 1998; Del Pino & Durham, 1999; Gomez et al, 2006).

Its main disadvantage is the rapid fouling which consequently increases consumption of energy and decrease process performance (Dizge et al, 2011), so proper cleaning of membranes should be considered in this process. Ultrafiltration can be applied to improve the quality of effluent by its high efficiency in suspended solids filtration and decreasing the COD in wastewater treatment.

2.4.10 Oxidation and advanced oxidation processes

Recently, there is increasing interest in various advanced treatment techniques for treatment of industrial wastewaters. Employing strong oxidative substance can remove the organics from wastewater by breakdown the toxic compounds and recalcitrant substances. Complete mineralization of polluting substances is possible depending on oxidation potential of oxidative agent and its contact time with the wastewater. Ozone is a strong oxidative agent with the ability of attacking aromatic compounds with ring and double bound structure. High reduction in aromatic compounds can be obtained by ozonation of olive mill wastewater (Panagiota and Diamadopoulos, 2006).

Advanced oxidation processes (AOPs) include generation of hydroxyl groups. These compounds are not stable and are generated by source of oxygen and energy. Generally the source of oxygen is ozone (O_3) or hydrogen peroxide (H_2O_2) and energy source is solar energy or UV.

99% COD and phenol removal was obtained applying H_2O_2/UV with hydrogen peroxide dosage of 750-1000mg/L (Aktas et al, 2001).

If oxidation process by oxygen is conducted under high pressure and temperature, it is called wet air oxidation. It can elevate the mineralization and biodegradation of olive mill wastewater. 30% COD removal and 80% phenolic reduction was achieved on OMW with dilution ratio of 1:10 with synthetic municipal wastewater at 180^oC, 7MPa pressure, and moderate acidic condition. While in neutral condition, 52% COD removal was obtained with no change in phenol removal. COD removals increases to 99% by adding radical promoters to the system (Rivas et al, 2001).

H_2O_2/UV also can be used to remove the color, total organic carbon, lignin and phenol from olive mill wastewater (Uğurlu and Kula, 2007).

80-90% COD reduction was observed by applying O_3/H_2O_2/UV on olive mill wastewater (Beltran et al, 1999).

Chemical oxidation such as Fenton’s oxidation process can be applied on OMW. Studies show COD removal of 47% at pH 3 (Dogruel et al, 2009).

Also COD removal of more than 80% and total phenol removal of more than 85% was shown using Fenton and Fenton-like processes (Kiril Mert et al., 2010).

2.4.11 Combined processes

High total COD removal of 70% and phenol reduction of more than 90%was reported by combing to ozonation and aerobic treatment which is higher than COD removal of each mentioned method alone. Ozonation is able to elevate the biodegradabilty of the effluent. For contact time of up to 2.5h and inlet ozone concentration of 10-45 mg/L, COD reduction of up to 70-80% and phenol removal of 40-50% was shown. It followed by an aerobic treatment system including acclimated activated sludge and further 60-80% COD reduction was obtained (Rivas et al, 2000).

The biodegradabilty of olive mill wastewater is increased also with Fenton's reagent. Fenton oxidation following with aerobic treatment, resulted total COD and phenol reduction of 70% and 90%, respectivley (Beltran-Heredia et al, 2001).
Combined ultrafiltration with UV/H_2O_2 have been examined also. Ultrafiltration with tubular polysulfone module of 20,000Da molecular wight cut off was able to remove 94% of COD at pressure of 0.15 MPa. Following UV/H_2O_2 system with contact time of 35min had the ability of decreasing COD to 52 mg/L with complete color removal (Drouiche et al, 2004).

2.5 Modeling of Carbon Removal

A significant milestone in wastewater biological treatment is attribution of Chemical Oxygen Demand (COD) as a substrate parameter to be able to identify different fractions of COD with different biodegradation characteristics (Ekama et al, 1986; Orhon and Ubay Çokgör, 1997; Henze et al, 2000). It leads to use COD in process modeling as main component and its fractions to establish the electron equivalence between utilized substrate, generated biomass and oxygen consumed (Henze et al, 1987; Orhon and Artan 1994).

COD includes different fractions and each fraction has its biodegradation kinetic (Wentzel et al, 1999). Total COD of wastewater has two main parts: total biodegradable COD and total inert COD or nonbiodegradable COD.

The biodegradable part is divided into readily biodegradable and slowly biodegradable fractions. The inert part is divided into soluble inert COD and particulate inert COD fractions. Soluble inert COD can not be degraded biologically in biological treatment plant, and leave the system without any biological reaction. While the particulate inert COD is accumulating in the system and is removed by sludge wasting.

In a wastewater, evaluation of readily biodegradable COD (S_{S1}) is significant. As this substrate has direct relation with microbial growth and also it leads to calculate the other COD fractions such as slowly biodegradable COD (X_{S1}), which is an important model component in modeling and also design of activated sludge systems for industrial wastewaters (Çokgör et al, 1998).

Characterization of different fractions of COD in terms of biodegradation and consequently, biological treatabilty sudies are assessed by respirometric test (Carvalho et al, 2001), where the biodegradable fractions of wastewater are determined.

Principally, the respirometer is a device for BOD measurement. Decreasing of oxygen concentration is measuring in a certain time interval by using a DO probe during substrate degradation, in a mixed sample and anaerobic environment.

Following equation, raised from endogenous model (Orhan and Artan, 1994), is used to calculate S_{S1} according to OUR curve:

$$S_{S1} = \frac{\Delta S_0}{1 - f x \, Y_H} \quad \textbf{(2.1)}$$

In this equation, ΔS_0 is the area above the second plateau in the OUR profile, as it is indicated in Figure 2.3.

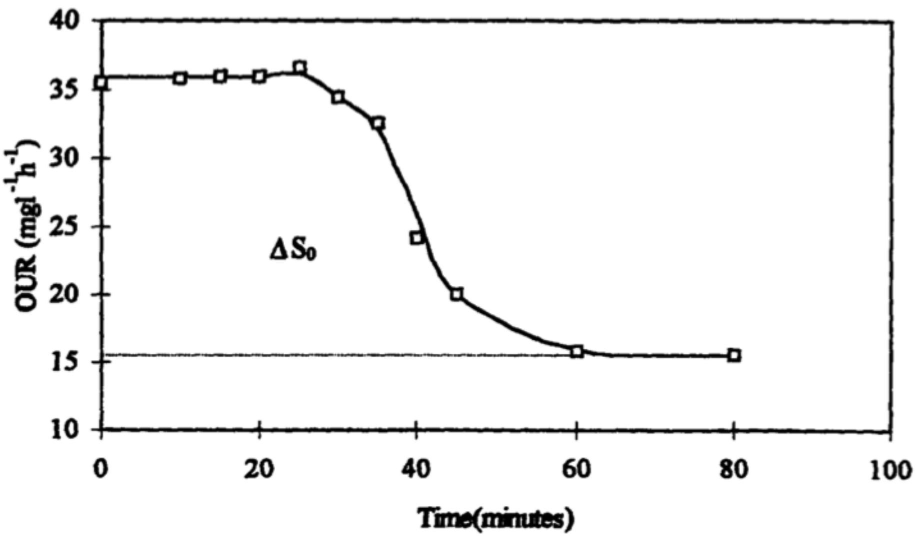

Figure 2.3 : An OUR profile to assess the readily biodegradable substrate.

OUR profile is an experimental tool for evaluating the biodegradation characteristics of a wastewater (Kappeler et al, 1992; Vanrolleghem et al, 1999). This method usually uses modeling based on conducting dissolved oxygen as model component.

OUR profile or changes in oxygen consumption rate during the substrate utilization in wastewater gives specific stoichiometric and kinetics of the biodegradation process.

In the OUR curve of a batch reactor, different parts reflect different sensitivity levels to the various biological processes, which enable the kinetic evaluation.

The main biochemical processes and stoichiometry of processes begins with studies of Ekama & Marais (1979); Dold et al, (1980); Van Haandel et al. (1981), and followed by IAW (International Association on Water Quality) which formed a task group and developed Activated Sludge Model No. 1 (ASM1), a modeling for design and operation of biological wastewater treatment system. Orhon and Artan (1994) developed ASM1 by introducing endogenous decay and residual metabolic production.

ASM1 was extended later to ASM2 to include processes like biological phosphorus removal (ASM2, Henze et al, 1995 and ASM2d, Henze et al, 1999) and storage phenomena (ASM3, Henze et al, 2000).

According to ASM1 models, all the substrates are hydrolyzed before being used by microorganisms in biomass growth. Biodegradable substrate in the influent wastewater includes two fractions: readily biodegradable COD and slowly biodegradable COD. The readily biodegradable COD (S_S) consists of simple components pass through the cell wall rapidly and used by organisms for synthesis. The slowly biodegradable COD (X_S) includes complex and large components, at first adsorbed by activated sludge and then by means of extracellular enzymes breaking down (hydrolysis) then passing through cell wall and used in metabolism.

The ASM3 model assumes that firstly the readily biodegradable substrate is utilized by microorganism and stored in the cell before using in growth process. Then in the absence of external substrate, internal polymers are consumed in biomass growth. The notion of death regeneration described in ASM1, is replaced by endogenous respiration process in this model.

OUR profile is also an instrument for evaluation the inhibitory effect on activated sludge. The biological treatment by activated sludge is mostly involved with toxic and inhibitory substances resistance to biodegradation. Measurement of inhibitory and toxic effect is achievable by deriving a numerical value as an indication of inhibition. Evaluation of these data could leads to expect the impact of these substances on receiving water body and helps to put quality criteria for discharge.

A practical method to reach this numerical data is OUR measurement which can describe the inhibition physically and mathematically in terms of parameters associated to process rates and mass balances of activated sludge. Kinetic and stoichiometric coefficients of microbial processes can reflect the inhibitory effect of substances on the activated sludge. In other words, the impact of inhibitory substances can be evaluated in terms of adverse effect on substrate utilization (Insel et al., 2006).

3. MATERIAL & METHODS

3.1 Experimental Design

Wastewater samples were obtained from a three-phase olive oil mill and a table olive processing facility located in an *Organized Industrial District***,** which would houses both types of industrial activities. Experiments were conducted on a composite sample prepared as a mixture composed of 80% olive oil and 20% table olive processing wastewaters; the ratio was selected to represent to the extent possible.

Two different wastewaters were used in this experimental study as (i) Mixture composed of 80% olive mill wastewater and 20% table olive processing wastewater which was simply named olive mill wastewater, (ii) Synthetic domestic wastewater.

Olive mill wastewater is collected without any pretreatment such as settling, filtration, etc. The mixture was prepared in the laboratory and was stored at 4^{o}C before using in all of the experiments.

Synthetic domestic wastewater was prepared using peptone-meat extract mixture, and was used to evaluate the organic carbon removal performance of mixture of olive oil and domestic wastewater by respirometry analysis.

Aside from chemical analyses, characterization studies also included assessment of the particle size distribution (PSD) profiles of COD identified as major polluting parameters in the wastewater mixture. This approach was crucially significant for the study, mainly because it reflected the effect and limitations of the adopted steps of chemical conditioning and separation technologies on major parameters in each size fragment.

The experimental study was emphasize and assess (i) the potential of membrane separation, including *ultrafiltration* and *nanofiltration* technologies in the treatment of olive processing wastewater, (ii) the merit of chemical conditioning in improving the removal potential of membrane treatment, and (iii) the treatability of pre-treated olive mill wastewater in a municipal treatment plant with domestic wastewater.

3.2 Chemical Settling (Coagulation-Flocculation)

In this study, coagulation flocculation test was carried out in a standard *Jar Test* apparatus at room temperature as shown in Fig.3.1.

Figure 3.1 : Jar test Apparatus.

Polyaluminium Chloride (PAK-FP 17D) from Akkim as coagulant, supplemented with anionic polyelectrolyte (WET-Treat 7053) from Green Chemicals was applied. Polyelectrolyte was prepared before, as 1gr/L and continuously mixed for 24 hours according to manufacturer instruction. The process includes adding chemical coagulant, rapid mixing, flocculation, slow mixing, and settling or filtration.

Coagulation-flocculation started by adding coagulant to the wastewater. Jar test was adjusted to mix rapidly 1 minute at constant speed of 150rpm after adding PAC. Rapid mixing was applied in order to have equal coagulant dispensation in the wastewater. In the next step, coagulated particles were driving together in form of flocs, by means of polyelectrolytes. This step (flocculation) was very slower than coagulation and the jar test was adjusted to mix rapidly for 30 minutes on 15rpm. At

the end, the sample was introduces to coarse filter and the filtrate was measured in terms of COD concentration. Due to high concentration of suspended solids and poor settling of olive mill wastewater, coarse filtration (circle Whatman filter paper, 110mm, grade 113, pore size of 30 μm) was applied to separate settled part from the supernatant.

Figure 3.2. shows the olive mill wastewater after 60 minutes settlement.

Experiment was conducted under optimum dosages of coagulant and flocculent.

To determine the best operating condition or optimum condition of coagulation flocculation, different amount of PAC and anionic polyelectrolyte were applied to 100ml of wastewater.

Firstly, nine different dosages of PAC as 0.1, 0.5, 1, 2, 3, 4, 6, 8, 10ml/L were applied. Jar test adopted to mix wastewater rapidly for 1 minute after adding PAC and 30 minutes slow mixing. After 30 minutes, the wastewater passed through coarse filter and the COD concentration of filtrate was measured. The PAC with highest COD removal in wastewater was selected as optimum dosage.

The next step involved selection of polyelectrolyte group as coagulant aid, to be used with PAC. For this purpose, four sets of Jar Test experiments were carried out with selected optimum PAC doses, supplemented with 0.5 ml/L of polyelectrolyte; each PAC dose was tested twice, the first with anionic polyelectrolyte and the other with cationic polyelectrolyte.

To determine the optimum dosage of polyelectrolyte, different concentration of selected polyelectrolyte were applied to the wastewater (in the range of 0.5-1.5ml/L). In each step, routine sequence of coagulation, flocculation and filtration was conducted separately and at the end of analysis, COD of filtrates were measured.

After defining the optimum condition, coagulation flocculation was carried out on 1L olive mill wastewater using Jar test apparatus step by step. After coarse filtration, COD of permeate was measured and recorded as “initial” COD value in the starting point of next experiment which was COD-based PSD fractionations of chemically treated wastewater.

Figure 3.2 : Settled olive mill wastewater after coagulation flocculation.

3.3 COD-based Particle Size Distribution (PSD analysis)

Particle Size Distribution is a beneficial tool to represent characterization of wastewater and the performance of alternative pretreatment processes on the wastewater. This method provides a profile for organic fraction of wastewater in size ranges between 1600 nm and 2 nm. It is a method of wastewater characterization that supplies valuable data about COD fractions for different size intervals and shows the relation between the COD fractions and particle size distribution of the wastewater. It can also compare different treatment technologies as a function of Particle Size Distribution (Sophonsiri and Morgenroth, 2004; Dulekgürgen et al., 2006). The analysis was conducted based on methodology explained by Dülekgürgen et al. (2006).

In this experiment, raw Olive Mill Wastewater and chemically treated wastewater were passed through sequential filtration/ultrafiltration. The amount of organic carbon of each step's filtrate was measured in terms of COD. Then the profile of wastewater in terms of PSD were assessed to define the efficiency of selected chemical treatment (Arslan-Alaton et al, 2009). The sequential filtration ultrafiltration procedure is shown in Fig. 3.3

COD fractionation after Particle Size Distribution specified two main section of wastewater. One of them was the soluble portion includes the dissolved components with particle size less than 2 nm, and the other one was the particulate portion with particle size of bigger than 1600 nm that retain on the first filter (AP40 glass fiber filter).

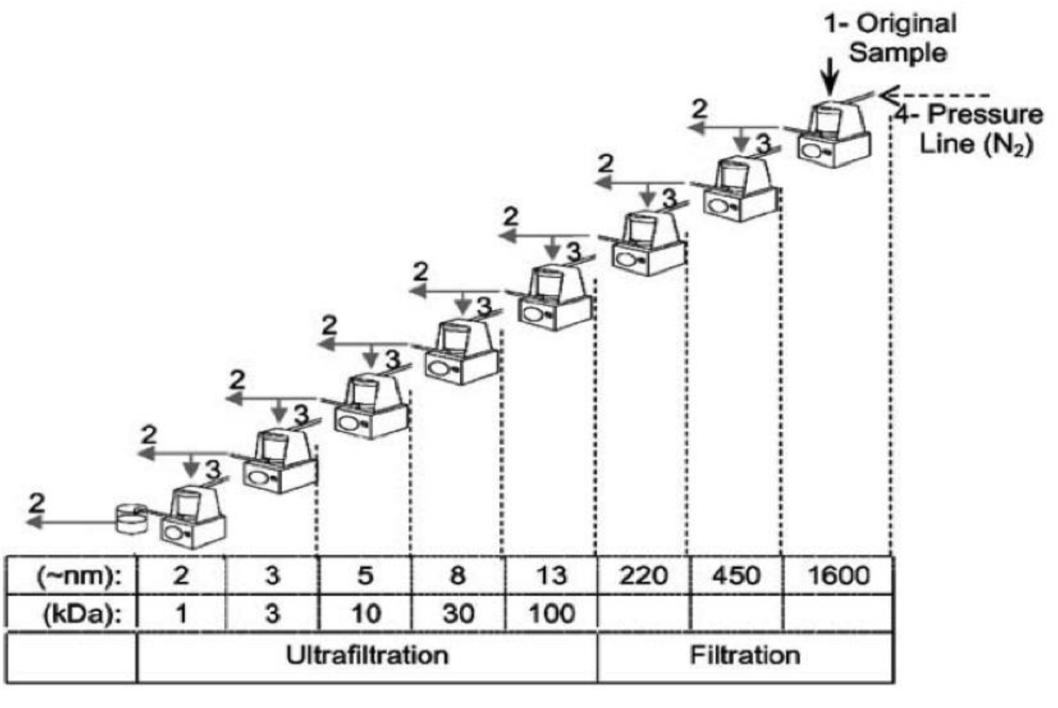

Figure 3.3 : The sequential filtration ultrafiltration procedure (Dülekgürgen et al, 2006).

In this study, 500ml continuously stirred wastewater with known COD concentration was used in consecutive filtration/ultrafiltration experiments. Filtration was begun by filtering raw and treated olive mill wastewater samples with conventional disposal filter of pore size of 1600nm (Millipore AP40, glass fiber). Then it was passed through filter of 450 nm (Durapores HV, polyvinylidene fluoride [PVDF]) and 220 nm (Durapores GV, PVDF). The filtrate of last filter was used as feed of next filters which were ultrafilters.

In ultrafiltration experiment membrane disks were used and the nominal molecular weight cut-offs (MWCO) were 100, 30, 10, 3 and 1 kDa (PL series, Millipore MA). COD of permeate collected after each step of filtration/ultrafiltration was measured.

Filtration with Durapore disposal filters was carried out at 0.35 atm pressure and under the temperature limit of 85°C . Ultrafiltration was performed at pressure of 0.6 atm for membrane filter size of 100 kDa and 1.2 atm for the other membrane filters. According to the manufacturer guideline, cleaning, preserving and pretesting of the cells of ultrafiltration set were considered to do not face incorrect results due to contaminating or adsorption (Dülekgürgen et al., 2006).

Conventional disposal filters and ultrafiltration membrane discs are dissimilar in terms of size units. According to Cheryan (1986) and McGregor (1986) approximate MWCO values in nanometers are 13, 8, 5, 3, 2 nm consequently.

3.4 Membrane Filtration

Membrane filtration was performed using lab scale dead-end membrane filtration system (Sterlitech Brand HP4750, with stirred cell). The pressure of filtration system is provided with nitrogen gas. The system flow chart and photo are shown in Figure 3.4 and 3.5.

Specifications of laboratory scale dead-end membrane filtration system is illustrated in Table 3.1.

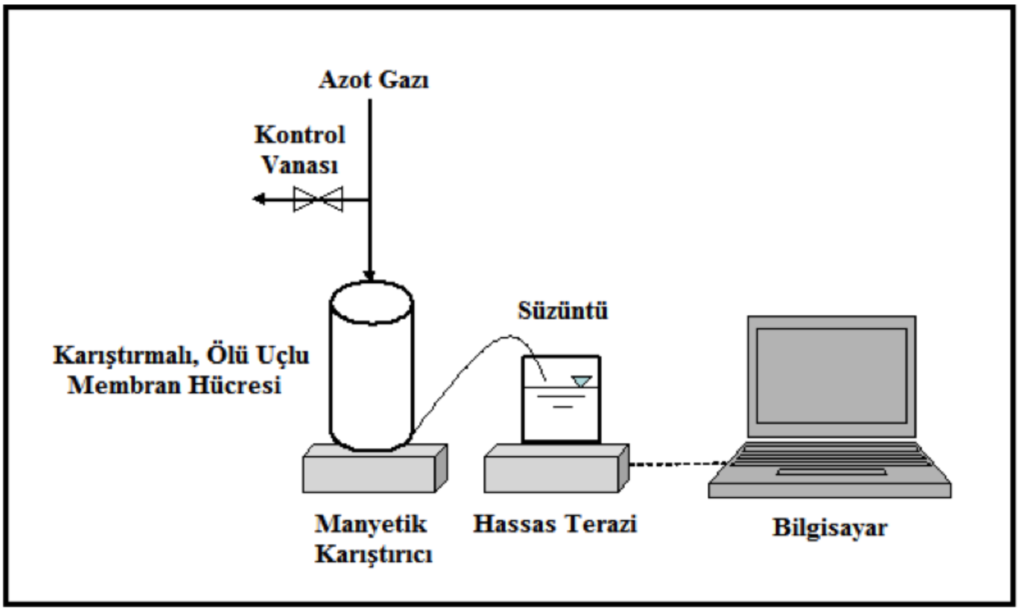

Figure 3.4 : The flow chart of laboratory-scale dead-end membrane filtration system.

Olive oil wastewater filtration was carried out under two type of membrane filters. UC010 as ultrafilter membrane with a MWCO of 10 kDa and NF90 as Nanofilter membrane with a MWCO of 200 Da. The properties of these two membrane filters are given in Table 3.2 and Table 3.3.

In this study, the working pressure of UC010 was 2 bar, while it was 30 bar for NF90.

Figure 3.5 : The laboratory-scale of dead-end membrane filtration system.

Table 3.1 : Specifications of laboratory scale dead-end membrane filtration.

Parameter	Technical specification
Membrane size (diameter)	49 mm
Activate membrane area	14.6 cm^2
Volume	300 ml
Maximum operating pressure	60 bar
Maximum operating temperature	121º C at 55 bar
pH range	adjusted based on type of membrane
Material	316L stainless steel
Cell diameter	5.1 cm
Cell length size	22.4 cm

In this experiment, two alternative approaches were tested. In the first one, chemical conditioning was introduced as the preliminary step for the sequence of *ultrafiltration* and *nanofiltration.* It should be noted that the nanofiltration process appears to be a prerequisite for a targeted COD efficiency of more than 90%, based on the peak in the soluble range reflected by the PSD analysis of the wastewater.

In the second alternative, chemical conditioning was introduced as an auxiliary step between ultrafiltration and nanofiltration.

Direct ultrafiltration and nanofiltration of the olive mill wastewater was also conducted as the control unit to set the basis of comparison for the results obtained.

Table 3.2 : Ultrafiltration membrane properties.

Type of membrane	MWCO (kDa)	Membrane Material	Water Flux (L/m^2h)	Properties
UC010	10	Polyethersulfone	>150	Hydrophilic

Table 3.3 : Flux and salt rejection data for NF membrane.

Type of membrane	Test conditions	Flux (L/m^2h)	Salt rejection (%)
NF90	70 psi, 2000 ppm $MgSO_4$	40.5	> 97
	70 psi, 2000 ppm NaCl	32.0	85- 95

3.5 Experimental Set-up and Operation of Batch Reactor

A laboratory-scale sequencing batch reactor with a net volume of 5L was selected as it is shown in the figure 3.6 with activated sludge taken from a domestic wastewater treatment plant.

The system was fed with synthetic substrate with the same characteristics of domestic wastewater, in terms of organic carbon content and COD fractions with various biodegradation characteristics. It was operated for 2 months at steady state condition and a sludge age of 8 days, to get acclimated biomass. The synthetic wastewater was prepared to include 16gr peptone, 11gr meat extract, 3gr urea, 0.7gr NaCl, 0.4gr $CaCl_2.2H_2O$, 0.2gr $MgSO_4.7H_2O$ and 2.8gr K_2HPO_4 in 1 L distilled water according to ISO 8192 (1995). It was called as peptone mixture for simplicity in the text and is rich in terms of organic acids, amino acids, peptides, vitamins and minerals. One of the main reasons of choosing peptone mixture in experiments as standard substrate for biodegradation, is it is totally biodegradable and during its OUR measurement when the profiles drops to endogenous respiration level, the COD remained in the system is just residual soluble microbial product (S_P) (Artan and Orhon, 1989).

Steady state condition was assumed when stable COD and MLVSS profiles were achieved.

The SBR system was conducted to operate with one cycle a day. During feeding period, reactor was allowed to be settled for 1hr every day and decant till volume of 1L. It received pulse feeding of 400mg/L peptone solution every day, and was aerated continuously. Temperature of the system was kept at 20±1°C. pH was maintained around 7.5 and minimum dissolved oxygen concentration of 3 mg/L was introduced to the system.

As the total COD of peptone mixture was 24700mg/L, 81 mg/L was used every day to feed 5L reactor volume. 8 days sludge age was selected therefore the amount of sludge wasted daily was 625ml.

The performance of the reactor was monitored periodically in terms of effluent soluble COD, SS, VSS and pH after reaching steady state, in order to confirm that the reactor is working properly. At steady state condition, the concentration of biomass was stabilized at 1800 mg/L as average.

Figure 3.6 : Batch reactor feeding with peptone mixture.

3.6 Respirometric Tests

Respirometric analysis included measurement the oxygen uptake rate (OUR) and recording its profile after adding substrate in the reactor. OUR measurment conducted with Ra-COMBO-1000 (Applitek CO., Nazareth, Belgium) continuous respirometer, equipped with a 2L reactor vessel with sampling interval of 1 min.

Test were performed at room teperature of 23-25^{0}C and a pH of between 7.5-8. The reactor was continuously mixed and aerated. Hach nitrification inhibitor, Formula 2533 (TM) was added to the reactor in order to inhibit nitrification during substrate degradation, and prevent oxygen consumption by nitrification. It contains 2-chloro-6 (trichloromethyl) pyridine and sodium sulfate, sustains 0.16 gr inhibitor in 300 ml sample.

The acute impact of pre-treated olive mill wastewater on biodegradation kinetics of peptone mixture was investigated. The experiments were conducted before and immediately after OMW was introduced to the system at day 0 by 5 different measurements as illustrated in table 3.4. Effluent of a sequence of utrafltration and nanofiltration after chemical conditioning was used in runs 1-4. Run 5 was conducted by raw olive oil wastewater with no pre-treatment.

Table 3.4 : Substrate concentration in respirometric tests.

Runs	**Peptone mixture** **(mg COD /L)**	**Olive oil wastewater** **(mg COD /L)**
Run 1 (control)	405	---
Run 2	405	57
Run 3	433	220
Run 4	---	57
Run 5	---	200

Respirometric tests to assess oxygen uptake rate (OUR) were conducted in 2L batch reactor started with biomass taken from SBR system at endogenous respiration stage.

A certain amount of substrate (peptone mixture and olive mill wastewater), which contain both readily and slowly biodegradable matter, was added to the system to monitor and measure the microbial oxygen uptake rate in time intervals when substrate is degraded until all substrate is consumed and the endogenous respiration is reached. All the tests were ended after reaching the endogenous respiration level.

The first OUR measurement was conducted with synthetic wastewater or peptone mixture only, as control to evaluate biodegradation characteristics of domestic sewage. In tests 2 and 3 the biodegradability of olive oil and peptone mixture with mixing ration of 15% and 50% were evaluated to determine the effect of olive oil wastewater in different concentrations on combined biological treatment. In the last measurement, olive oil wastewater with no additional peptone was applied to assess its characteristics and behavior in biodegradation.

During the experiments, soluble COD of the system was monitored. SS and VSS were measured at the beginning of each experiment before adding the substrate.

3.7 Structure of the Adopted Model

4 different respirometric tests were conducted with different ratios of OMW to domestic sewage in terms of COD. The ratios of 15% and 50% were selected for combined biological treatment.

A mechanistic model was structured to provide the kinetic information for the biodegradation of peptone and pre-treated olive mill wastewater. For this purpose, the basic template of ASM1 (Henze et al., 1987) modified for endogenous respiration (Mathieu et al., 2000; Zhou et al., 2008) was adopted with the necessary changes for additional COD fractions and biochemical processes. The proposed model involved two major parts: one related to the kinetic description of peptone degradation and the other, olive mill wastewater degradation as it is shown in table 3.5.

Based on previous results on the same synthetic substrate (Çokgor et al., 2011), peptone was considered to have three main fractions of COD; (i) the readily biodegradable COD, S_S , (ii) hydrolysable COD fractions, S_{H1} and S_{H2}, (iii) active heterotrophic biomass, X_H. Dissolved oxygen concentration S_O was also the other major components of the proposed model.

The structure of the model was based on direct microbial growth of mixed culture: Readily biodegradable COD, S_S, was used for the growth of heterotrophic biomass, X_H. The remaining hydrolysable COD fractions were transformed into readily biodegradable substrate via a dual hydrolysis process (Cokgor et al, 2009). Decay of heterotrophic biomass, X_H was defined in terms of an endogenous decay process, which generated residual particulate microbial products, X_P (Beline et al., 2007; Ropolla et al, 2009).

The second part of the adopted mechanistic model as olive mill wastewater considered to have two major COD fractions; (i) the readily biodegradable COD, S_{SOO}, (ii) hydrolysable COD fractions, S_{HOO}. Decay of this specific biomass was defined the same as the decay rate, b_H, for X_H.

The growth processes associated with X_H was defined as conventionally adopted Monod-type equations. Hydrolysis of hydrolysable COD fractions was defined with the saturation-type equations as given in the matrix format of the model structure in Table 3.5. Both endogenous decay mechanisms were described as first-degree reactions with respect to biomass concentrations.

Modeling essentially consisted of analyzing and calibrating the OUR profiles using the mechanistic model developed for this purpose. It also included a specific analysis to ensure that the parameters assessed were statistically identifiable. Kinetic and stoichiometric parameters of the adopted model were estimated using the approach defined by Insel et al. with the SIMPLEX algorithm (Insel et al, 2003)

The most frequently used simulation program, AQUASIM, was used in modeling study and evaluation based on the experimental data (Reichert et al., 1998). The UNCSIM module was used for the assessment of parameter identifiability (Brun et al., 2001). A detailed description of the assessment process is presented by Cokgor et al. (2011).

Table 3.5 : Matrix representation of adopted model structure.

Process	S_{O2}	S_{S1}	S_{SOO}	S_{H1}	S_{H2}	S_{HOO}	X_H	***Rate equation***
Growth of X_H	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$					1	$\mu_{\mathrm{H}} \frac{S_S}{K_S + S_S} X_H$
Hydrolysis of S_{H1}		-1		1				$k_{h1} \frac{S_{H1}/X_H}{K_X + S_{H1}/X_H} X_H$
Hydrolysis of S_{H2}		-1			1			$k_{h2} \frac{S_{H2}/X_H}{K_{XX} + S_{H2}/X_H} X_H$
Growth of X_H	$-\frac{1-Y_H}{Y_H}$		$-\frac{1}{Y_H}$				1	$\mu_{\mathrm{HOO}} \frac{S_{SOO}}{K_{SOO} + S_{SOO}} X_H$
Hydrolysis of S_{HOO}		-1				1		$k_{hOO} \frac{S_{HOO}/X_H}{K_{XOO} + S_{HOO}/X_H} X_H$
Decay of X_H	$-(1-f_P)$						-1	$b_H X_H$

3.8 Analytical Procedure

Samples were diluted prior to each analysis according to Standard Methods (APHA, 2005). COD of the samples were measured by open reflux titrimetric analysis according to ISO6060 methodology. For Soluble COD measurements, regular COD samples were filtered with 0.45 µm syringe filters and the filtrate was used for measurement.

Mixed liquor volatile suspended solids (MLVSS), mixed liquor suspended solids (MLSS), Total phosphorus, TKN and Total phenol were determined according to Standard Methods (APHA-AWWA-WPCF, 2005).

The AOA of olive mill wastewater was measured colorimetrically at 734 nm, with a Novespec II/Pharmica LKB colorimeter in reusable glass cell with 1cm optical path length, employing “Trolox-Equivalent Antioxidant Activity Assay” described by Re et al. (1999).

TOC was measured with TOC analyzer. The pH was measured by WTW 340i pH-meter.

4. RESULTS, CONCLUSIONS AND DISCUSSIONS

In this Chapter, results of this M.S. study, derived from the conducted experiments, analysis and observations are going to be presented.

4.1 Wastewater Conventional Characterization

Conventional environmental characterization of Olive Mill Wastewater and more specific parameters of this wastewater such as Total phenol and Anti oxidant activity (AOA) is given in Table 4.1.

As it is shown in the table, the wastewater is characterized by very high organic carbon content in terms of COD as 106500 mg/L.

All the values are in accordance with the literature. Azbar et al. (2004) indicate the COD range of olive mill wastewater as 40,000-220,000 mg/L and Total phenol as 2-80,000 mg/L. According to Dermeche et al. (2013), TOC of olive mill wastewater is 20,190-39,800 mg/L, TSS is 25,000-30,000 mg/L and VSS is 13,500-22,900 mg/L.

pH ig generally acidic and between 3 and 5.9 according to Akar T et al. (2009) and Galiatsatou P. et al. (2002). Oil and Grease is in the range of 300-23,000 mg/L as Baeta-Hall L. et al. (2005) and Cayuela ML. (2004) showed in the literature.

Table 4.1 : Conventional characterization of raw olive mill wastewater.

Parameters	Values
Total COD (mg/L)	106,500±4500
TOC (mg/L)	26,000±1100
TPh (mg/L)	1,250±35
AOA (mg/L)	27,000±800
TSS (mg/L)	29,000±1300
VSS (mg/L)	26,000±900
pH	5.27
Oil & Grease (mg/L)	4,400±160
TKN (mg/L)	940±25
Total phosphorus (mg/L)	260±8

4.2 Effect of Coagulation-Flocculation on COD Removal

The first step of experiments involved a pre-treatment stage consisting of chemical conditioning. Interpretation of chemical conditioning in this study was quite different than the traditional context of chemical settling and removal routinely implemented for sewage and industrial wastewaters. It is envisaged as part of an integrated approach, which basically changes the PSD of pollutants in a way that enhances the performance of the following membrane treatment steps.

Firstly, the olive mill wastewater was subjected to coagulation-flocculation at nine different doses of PAC in the range of 0.1-10 ml/L, in the absence of polyelectrolyte, to determine the optimum dosage of PAC.

COD values are measured and results are shown in Figure 4.1.

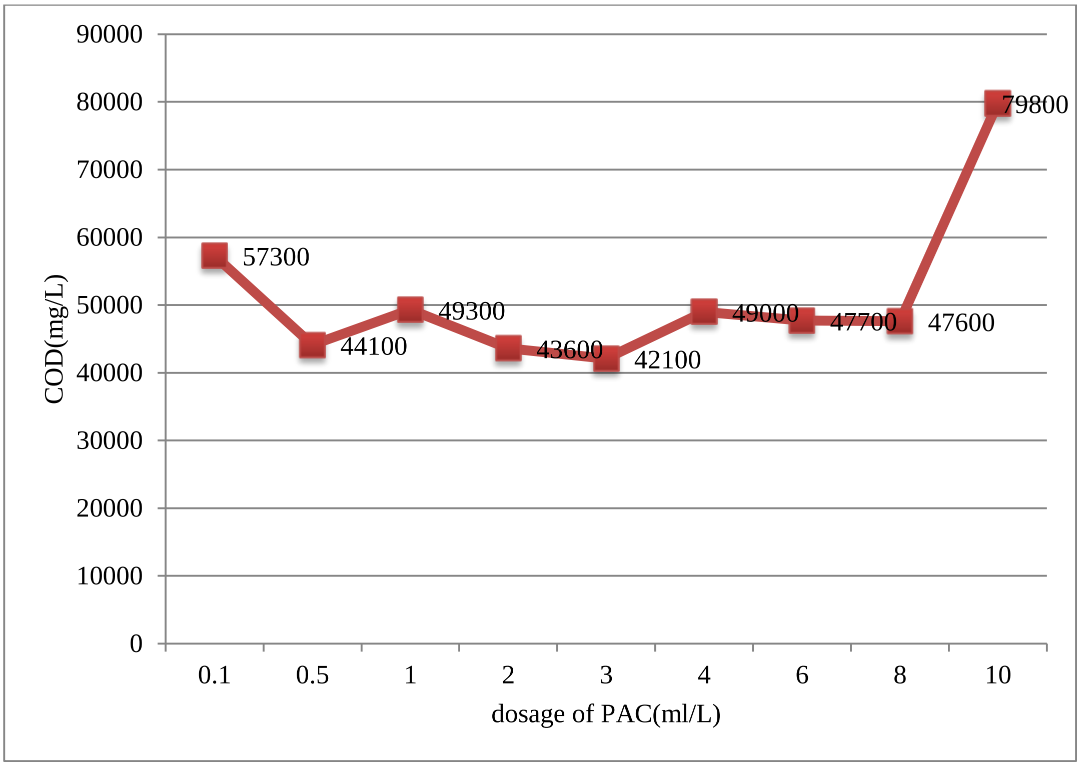

Figure 4.1 : Changes in COD of olive mill wastewater after coagulation atdifferent PAC dosages with no polyelectrolyte.

As is evident from the figure, COD profile in the supernatant after coarse filtration, which started with 57.300 mg/L at 0.1 ml PAC/L, slightly decreasing to 42.000-43.000 mg/L for the range 2.0-3.0 ml PAC/L. It is increasing again to around 80.000

mg/L and more COD removal was not observed when the PAC dose was increased up to 10 ml/L.

To select more effective type of polyelectrolyte, 0.5ml of each anionic and cationic polyelectrolyte were applied in presence of 2 and 3 ml/L PAC. The removal of COD by means of PAC and Polyelectrolyte was monitored and the results are shown in Figure 4.2.

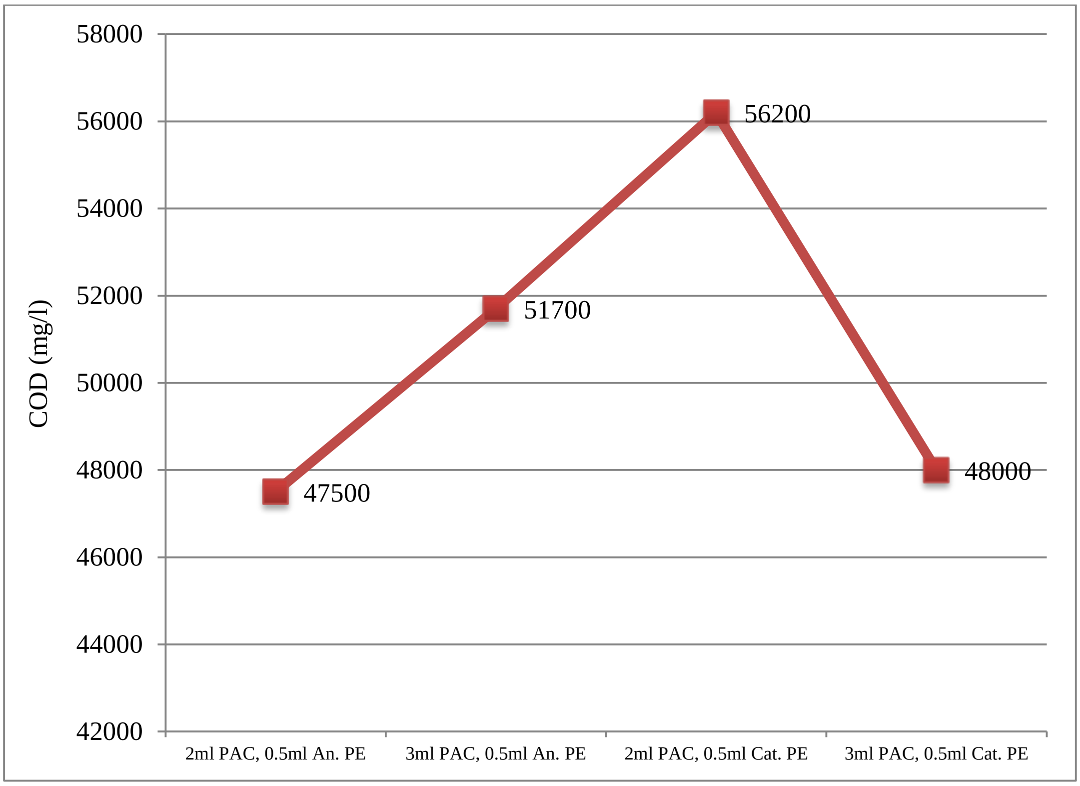

Figure 4.2 : Changes in COD of olive mill wastewater after coagulation flocculation at different dosages of PAC and presence of polyelectrolyte.

According to the Figure, highest COD removal achieved with 2ml PAC and 0.5ml/L anionic polyelectrolyte. So, the optimum PAC dosage of 2ml and anionic polyelectrolyte was selected, which allowed reaching a COD concentration of 47500mg/L (55% COD removal efficiency).

As PAC dosage was increased over 2ml/L, COD concentration of olive mill wastewater was increased consequently.

In next step, the coagulation/flocculation effect was investigated by optimum dosage of PAC (2ml/L) and different anionic polyelectrolyte doses in the range of 0.5-1.5ml for wastewater to determine the best operation dosage of polyelectrolyte. COD concentration after applying each dosage is illustrated in Fig. 4.3.

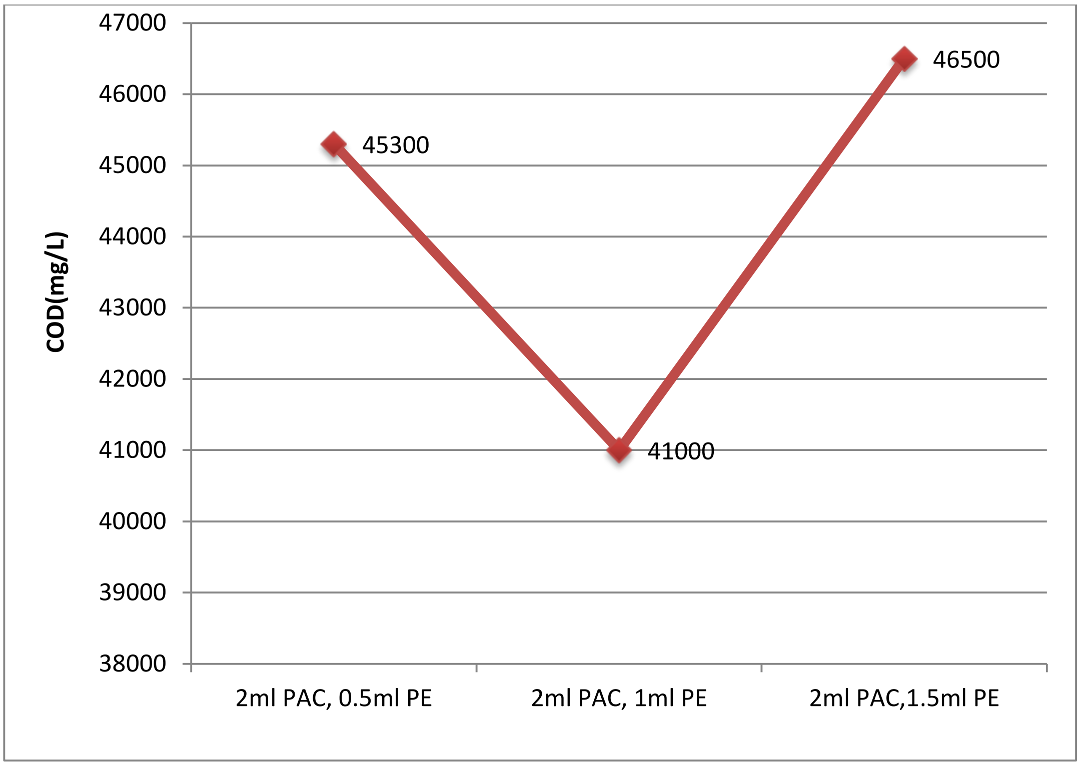

Figure 4.3 : Changes in COD of olive mill wastewater after coagulation flocculation at optimum concentration of PAC and different polyelectrolyte dosages.

The figure shows the best performance of coagulation/flocculation process at dosages of 2ml/L PAC and 1ml/L anionic PE which allowed to achieve a lowest COD of 41000mg/L (61% COD removal efficiency) after coarse filtration. Efficiency of the process was decreased by increase in polyelectrolyte dosage.

In the final Jar Test, changes in COD were monitored under optimum condition (2ml/L PAC, 1ml/L anionic polyelectrolyte) for 1L of olive mill wastewater. Final COD of 40800 mg/L was obtained after coarse filtration with % 61 removal efficiency.

It should be noted that this COD level is substantially better than the soluble COD value of 45700 mg/L obtained by filtering the raw wastewater though a 450 nm filter in accordance with the traditional approach for differentiating particulate and soluble COD fractions.

4.3 COD-based Particle Size Distribution of Olive Mill Wastewater

4.3.1 PSD before chemical conditioning

Particle size distribution is at least equally significant as the overall magnitude of a pollutant, especially if the envisaged treatment option relies on physical separation processes as in this study. PSD analysis before chemical pretreatment indicates the COD of raw wastewater based on selected size intervals and provides a fingerprint of its characteristics.

Cumulative PSD based COD fractionation of the raw olive mill wastewater is illustrated in Table 4.2.

Table 4.2 : Cumulative PSD-based COD fractionation of raw olive mill wastewater.

Separationtechnique	**Particle size (nm)**	**Cumulative COD (mg/L)**
Total		106500
Filtration		
AP40 filter	1200-1600	46100
HV filter	450	45700
Gv filter	220	43800
Ultrafiltration		
100 kDa	13	42700
30 kDa	8	38700
10 kDa	5	36000
3 kDa	3	33000
1 kDa	2	27500

As is evident from the table, the first value corresponded to total COD value of the raw wastewater and each cumulative value indicates total COD under the selected filter size. Raw olive oil wastewater has two significant fractions in terms of COD at the particle size intervals of >1600 nm and < 2 nm. First one that is bigger than 1600nm, is particulate fraction and the fraction less than 2nm, is soluble fraction. These two size intervals include colloidal COD content of olive mill wastewater.

This colloidal portion is distributed between other size intervals depends on the size of particles. As it is obvious from the table, organic carbon content of raw olive mill wastewater is more particulate nature.

Difference of the two consecutive COD values is obtained by subtracting the two related COD values and express as differential COD. So the initial COD value given in the table 4.2 corresponds to sum of soluble, colloidal, and settlable portions of the wastewater. The differential COD is outlined in Figure 4.4. PSD characterization of olive mill wastewater also provides a clear data about percent distribution of COD fractions, which represented in Figure 4.5.

Figure 4.4 and figure 4.5 provide a significant data about the variation of COD according to different size intervals. The majority of the organic matter in olive oil wastewater is particulate and bigger than 1600 nm (57%) and only 26% is soluble and smaller than 2nm. The colloidal portion between these two size intervals is 17% which is not a considerable fraction.

This profile is quite typical and agrees well with similar PSDs of COD reported in the literature for olive oil wastewaters (Arslan-Alaton et al., 2009; Dogruel et al, 2009).

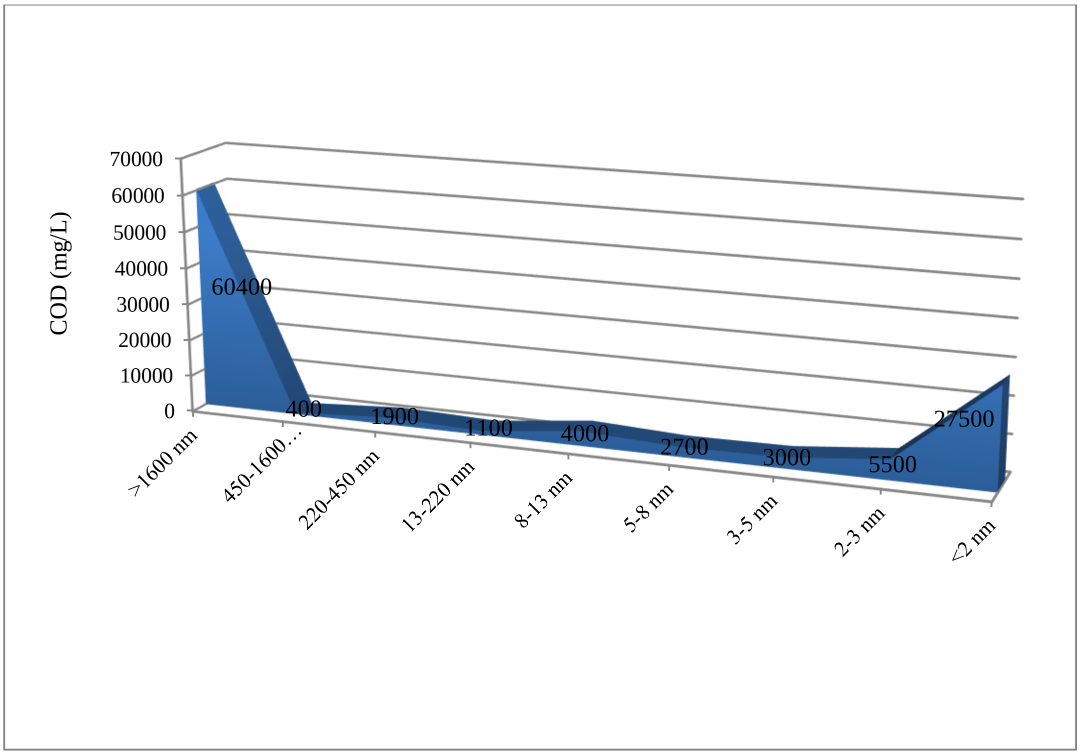

Figure 4.4 : Differential COD values of raw olive mill wastewater for selected size intervals.

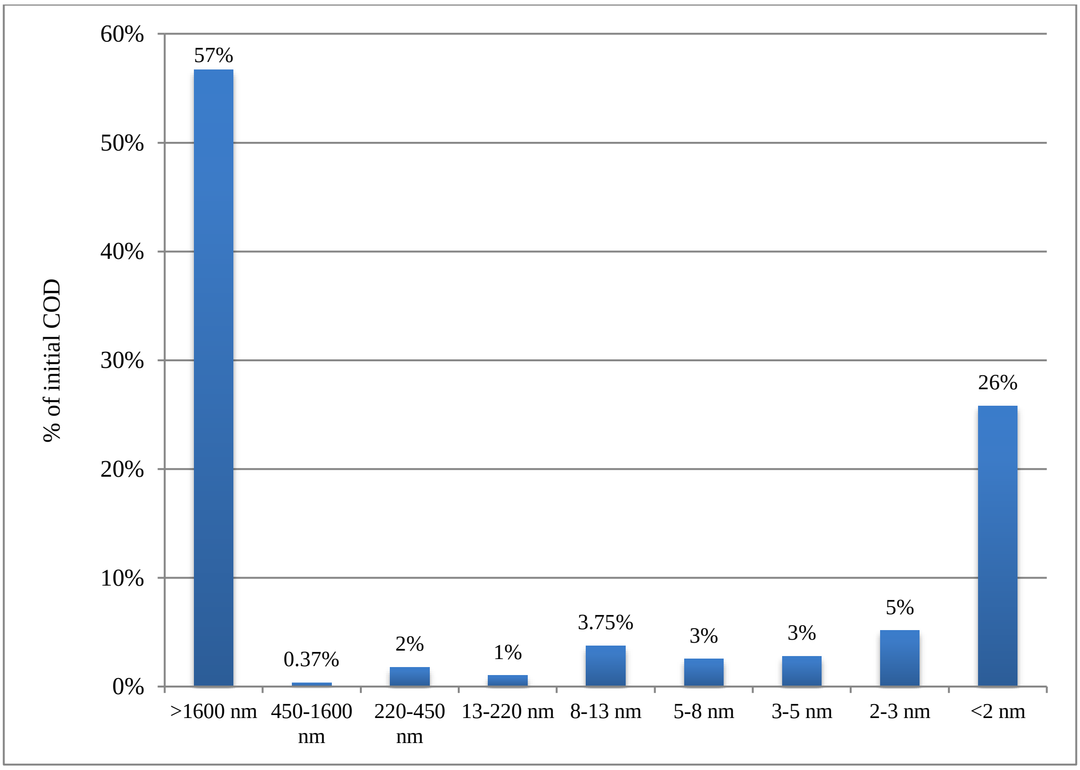

Figure 4.5 : Percent distribution of COD fractions for the raw OMW.

4.3.2 PSD after chemical conditioning

Particle Size Distribution of olive mill wastewater after chemical conditioning presents the size fractions that chemical condition provided higher performance in it.

On the other hand, it exposes the necessity of nanofiltration process as a prerequisite for a removal of remained COD after chemical conditioning, based on the peak in the soluble range reflected by the PSD analysis.

The cumulative, differential and percentage size distribution of the remaining COD after chemical conditioning is outlined in table 4.3, figure 4.6, and figure 4.7.

Results indicate the significant differences between the raw and chemically treated OMW. The chemical precipitation effectively removed the particulate portion of COD and the highest COD removal efficiency was achieved on this fraction, which appeared as the major fraction of raw wastewater.

Table 4.3 : Cumulative PSD-based COD fractionation of olive mill wastewater after chemical conditioning with PAC.

Separation technique	**Particle size (nm)**	**Cumulative COD (mg/L)**
Total		40800
Filtration		
AP40 filter	1200-1600	38400
HV filter	450	37000
Gv filter	220	37000
Ultrafiltration		
100 kDa	13	33500
30 kDa	8	29700
10 kDa	5	27200
3 kDa	3	26800
1 kDa	2	21000

After coagulation flocculation, only one COD peak remained below 2 nm, but also with a similar significant removal from 27500 mg/L COD in raw wastewater down to 21000 mg/L COD with 2.0 ml/L PAC+1.0 ml anionic PE. This level represented 51.5% of the remaining COD after chemical conditioning, which imparted a totally different COD fingerprint than that of the raw wastewater.

In the traditional understanding of the process, the potential of chemical settling is limited with the removal of particulate pollutants; in this approach particulate components are loosely differentiated by means of a single size threshold, i.e. 450 nm, routinely used for the assessment of suspended solids.

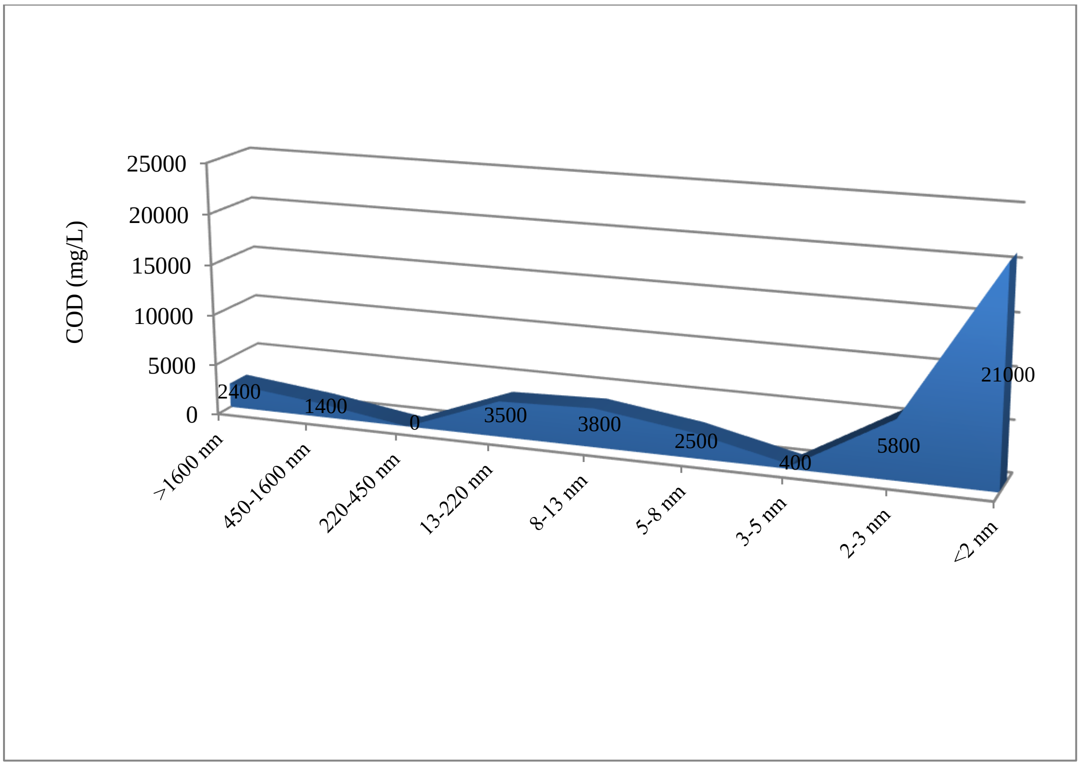

Figure 4.6 : Differential COD values of olive mill wastewater for selected size intervals after chemical conditioning with PAC.

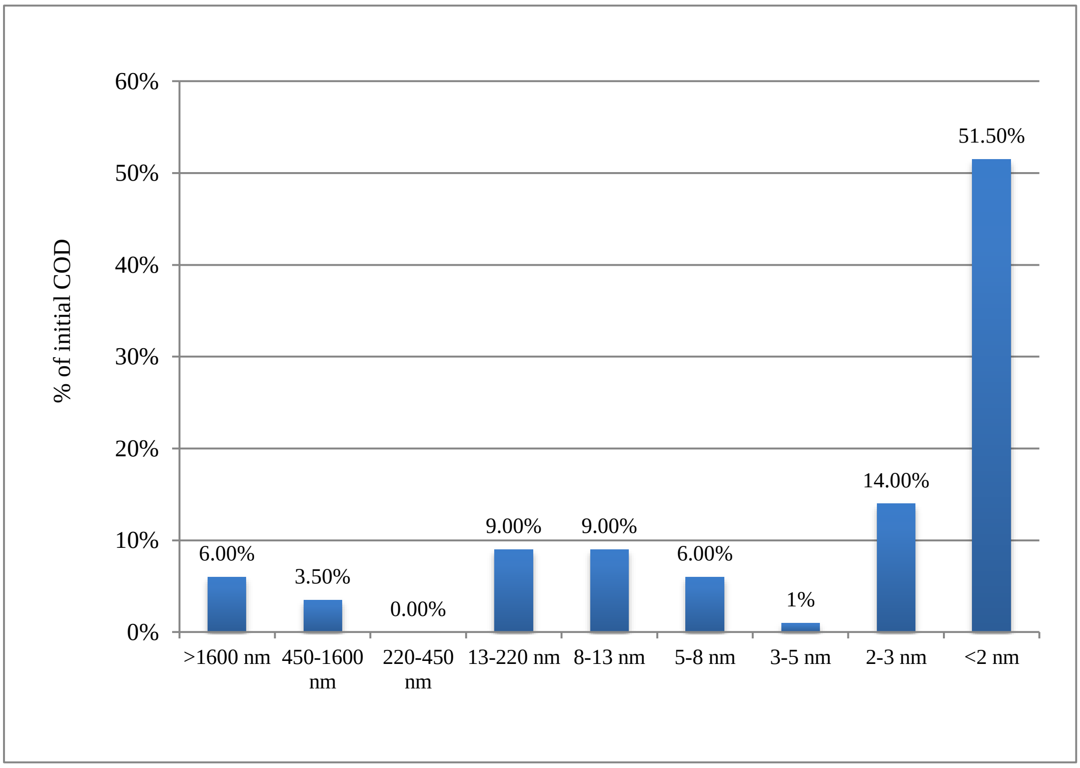

Figure 4.7 : Percent distribution of COD fractions of the olive mill wastewater after chemical conditioning with PAC.

The results of this study provided proved otherwise: First, the observed residual COD remained always below the corresponding soluble COD level in the wastewater, clearly indicating that chemical conditioning was effective, not only in removing particulate COD components but also, a part of COD fractions below 450 nm. In fact, the soluble COD fraction of around 45000mg/L in the raw wastewater was reduced down to 37000 mg/L after chemical conditioning and coarse filtration, indicating an additional COD removal of 8000 mg/L from the size range below 450 nm. Second, this was possible because chemical conditioning reshaped and restructured COD fractions in smaller sizes yielding a totally new PSD fingerprint.

This aspect is better visualized in figure 4.8, when the results of PSD tests in terms of incremental size fractions of residual COD after chemical conditioning is compared with the corresponding size fractions in raw wastewater. Results gives clear indication that major change occurred in the size range below 2 nm particularly important for membrane filtration, where the corresponding COD fraction of 27500 mg/L was reduced down to 21.000 mg/L through the joint coagulation, adsorption and entrapment effect induced of selected coagulants.

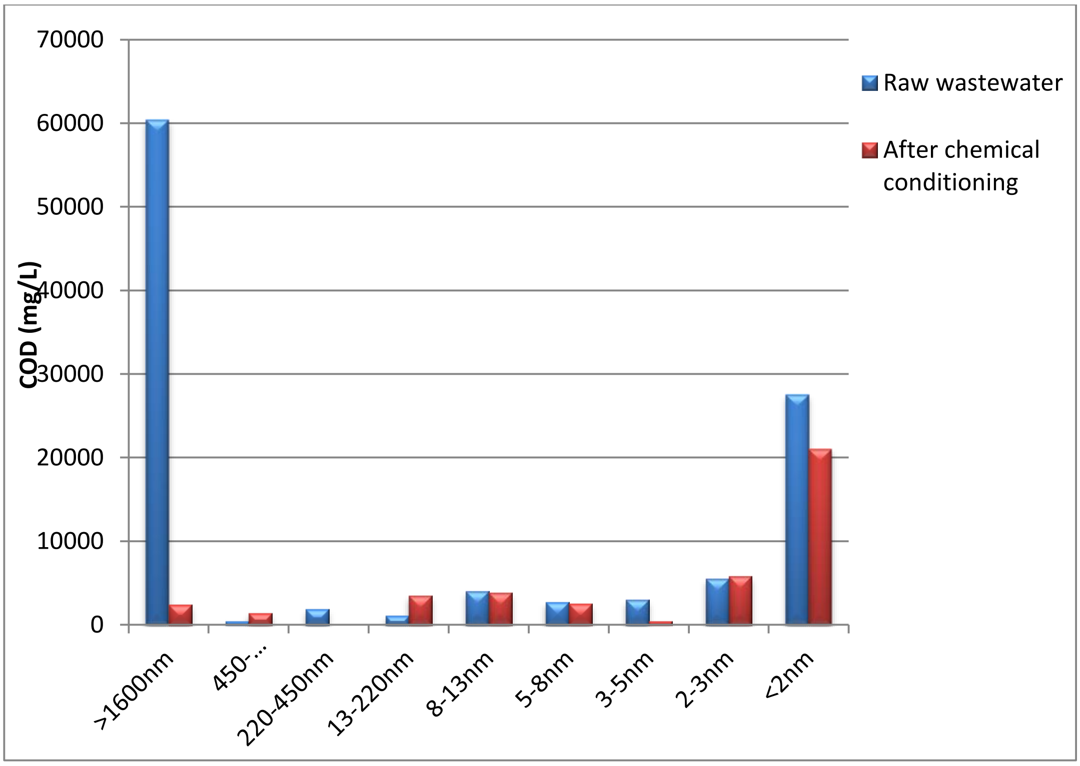

Figure 4.8 : Particle size distribution of incremental COD fractions before and after chemical conditioning.

The potential of chemical conditioning in reshaping the PSD of COD fractions in smaller size ranges was also confirmed with an additional experiment: In this test conducted as part of the PSD analysis of raw wastewater, a mixture was formed using equal aliquots from the filtrates of 13, 8 and 5nm ultrafiltration units, all well below the particulate range.

The COD of the mixture was measured as 40400 mg/L; it could be reduced to 32100 mg/l after chemical conditioning using the same dosage and coarse filtration of the supernatant. Nanofiltration by NF90 of the chemically treated sample produced a final effluent with a COD of 5700 mg/L, while direct nanofiltration of the mixture could only yield 8300 mg/. The results illustrated in figure 4.9.

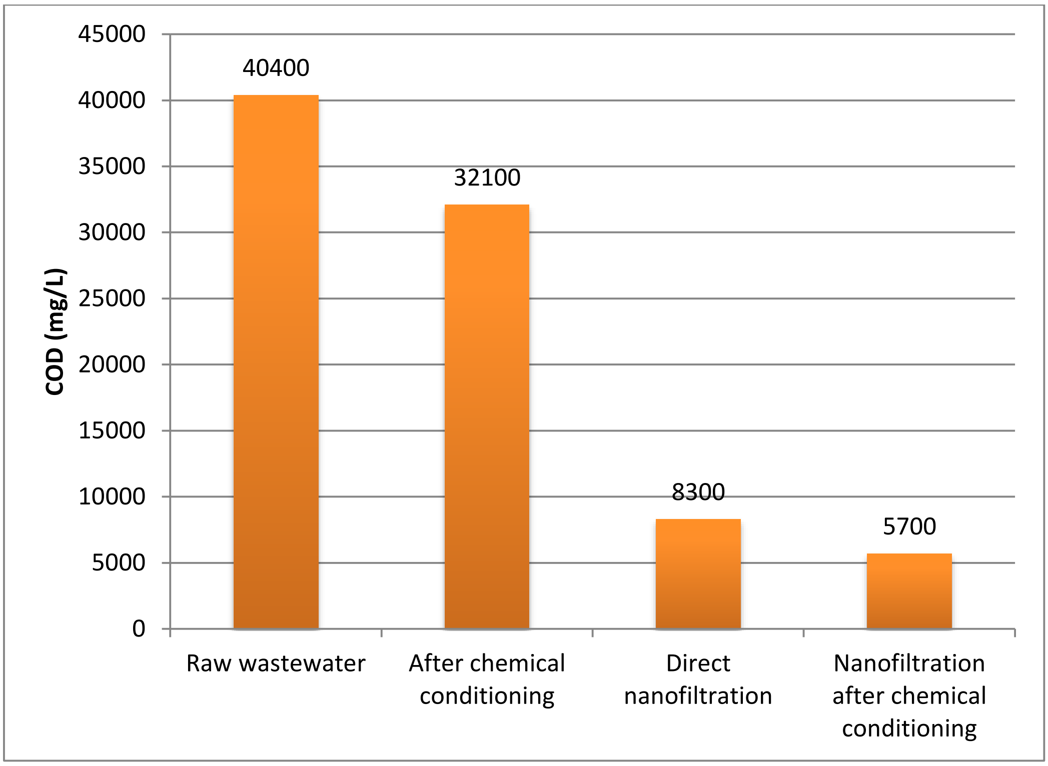

Figure 4.9 : The effect of chemical conditioning on the performance of nanofiltration through changes in PSD of COD fractions.

The difference can only be explained in terms of physical and chemical changes inflicted by chemical conditioning on COD fractions in respective size groups, especially below 2 nm.

4.4 Membrane Filtration on Olive Mill Wastewater

Related experiments were designed not only to remove organic compounds of olive oil wastewater but also to underline the merit of chemical conditioning on the performance of membrane filtration.

The experiment was started with wastewater with no chemical conditioning that served as the control, with a COD level of 51000 mg/L after coarse filtration.

Ultra filtration using UC010 was able to reduce this level down to 33300 mg/L. In comparison with that of corresponding PSD analysis which yielded cumulative COD levels of 38700 mg/L, 36000 mg/L and 33000 mg/L at 30, 10 and 3 kDa filtration respectively. The comparison indicate that the ultrafiltration basically function with an effective filtration size of around 3 kDa, due to gradual entrapment of particles on the filter.

The following nanofiltration step further reduced the COD concentration to 8300 mg/L, with an overall COD removal efficiency of %84 based on coarse filtration step and % 92 based on the initial COD content of the raw wastewater.

In the next step that was started with chemical conditioning prior to membrane filtration, the performance of coarse filter is significantly increased as the COD of the filtrate was reduced to 40800 mg/L. The positive impact of PSD also reflected on the following membrane filtration, where the effluent COD decrease to 29300 mg/L after ultrafiltration and finally to 5870 mg/L after nanofiltration steps.

The final experiment involved the sequence of ultrafiltration, chemical conditioning and nanofiltration consequently. It was started with the effluent of ultrafiltration with a COD level of 33300. Coarse filtration after chemical conditioning could still affect the PSD so that a slight COD removal could be achieved with a resulting COD concentration of 30100 mg/L. A final COD of 6200 mg/L was obtained after the final nanofiltration step.

Figure 4.10 shows the effect of chemical treatment and nanofiltration on the color of raw olive mill wastewater.

Figure 4.11 displays all the results obtained with the three process flow schemes tested with membrane filtration.

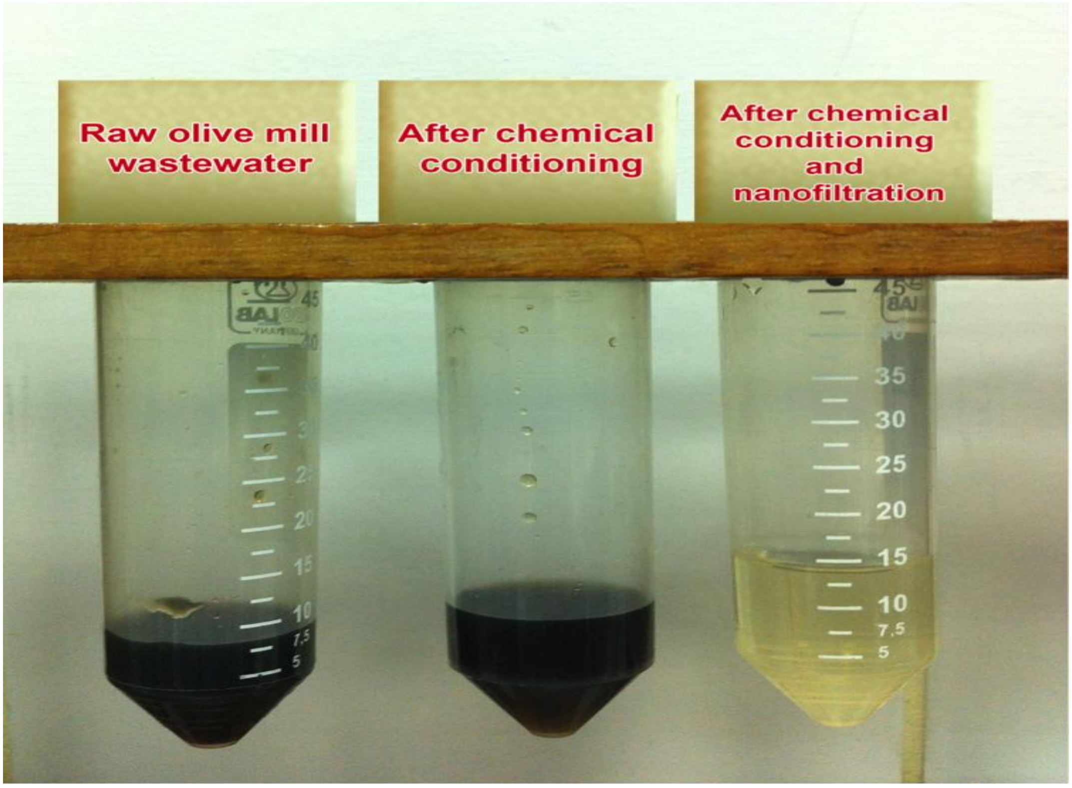

Figure 4.10 : Effect of nanofiltration on the chemically conditioned olive mill wastewater.

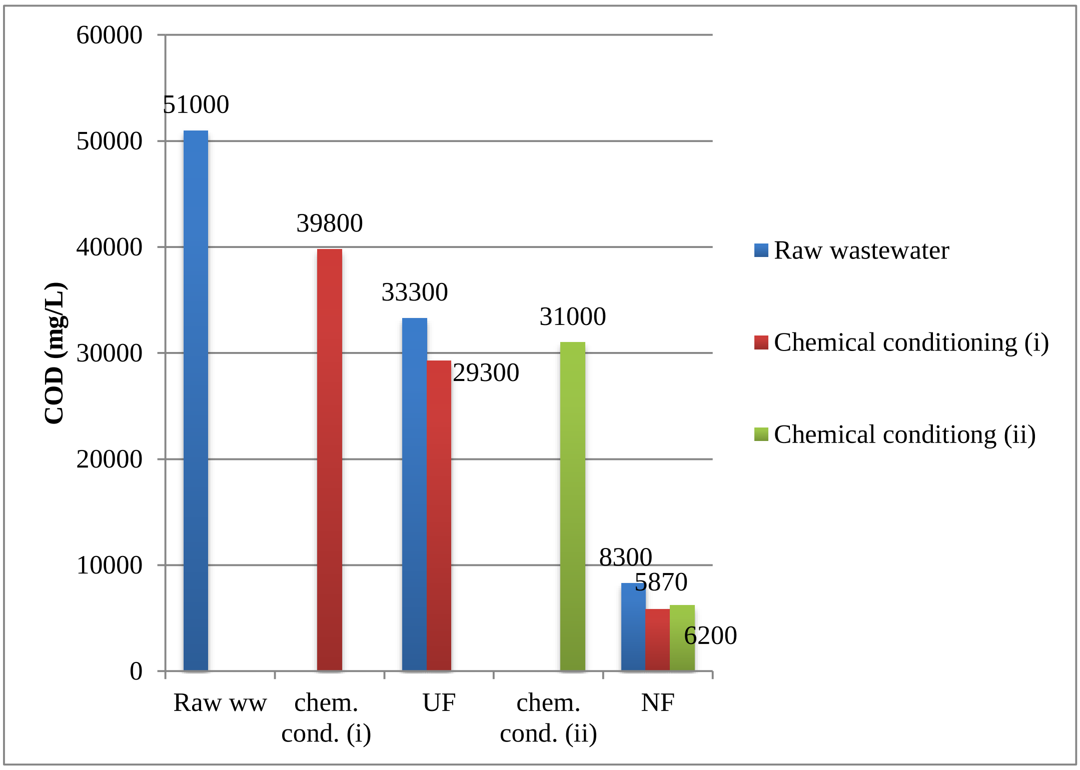

Figure 4.11 : Effect of chemical conditioning on the performance of membrane filtration.

4.5 Effect of Olive Mill Wastewater on Biodegradability of domestic wastewater

The seed was taken for respirometry analysis from the fill and draw reator which was monitored for two months at steady state condition. The influent COD was adjusted to 400mg/L COD and the average Volatile Suspended Solid concentration in the main reactor was 1800mg/L, Suspended Solid concentration 2055mg/L, and effluent soluble COD 39mg/L.

4.5.1 Experimental results

Biodegradability of the olive mill wastewater when mixed into a domestic wastewater was essentially tested on the basis of oxygen uptake rate (OUR) profiles generated in respirometric measurements, as in many similar studies (Insel et al., 2006; Pala et al., 2011). Experiments included an initial run conducted only with peptone addition, which served as the control set for all the other mixtures containing pretreated olive mill effluent in selected dilutions. The OUR profile of the control reactor (Run1) started with an initial peptone concentration of 405 mg COD/L is displayed in figure 4.12.

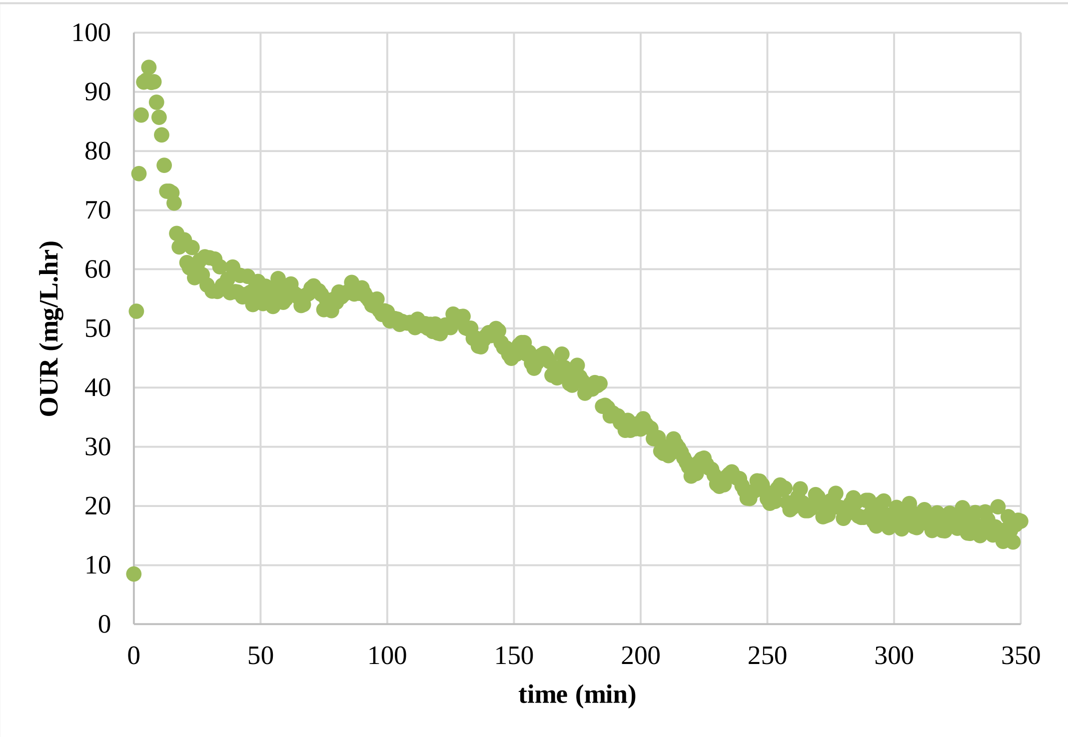

Figure 4.12 : OUR profile of the peptone mixture in the control reactor (Run 1).

It shows the typical initial peak value of slightly above 90 mg O_2/L.h, reflecting the utilization of the readily biodegradable COD fraction, followed by a gradually declining curve with different slopes associated with slowly biodegradable components in the peptone mixture. The OUR profile drops down to the initial endogenous respiration level in around 6 hours, also indicating utilization of all available biodegradable substrate in the reactor. In the other words, the peptone mixture is removing along oxygen consumption and complete consumption of it was achieved. At the end of the experiment in endogenous respiration level, the soluble COD of 31mg/L remained in the reactor indicates residual soluble microbial product (S_P), which accumulated in the system by metabolic reactions.

The soluble COD of the reactor was also monitored parallel with OUR measuremnent, with the results depicted on figure 4.13.

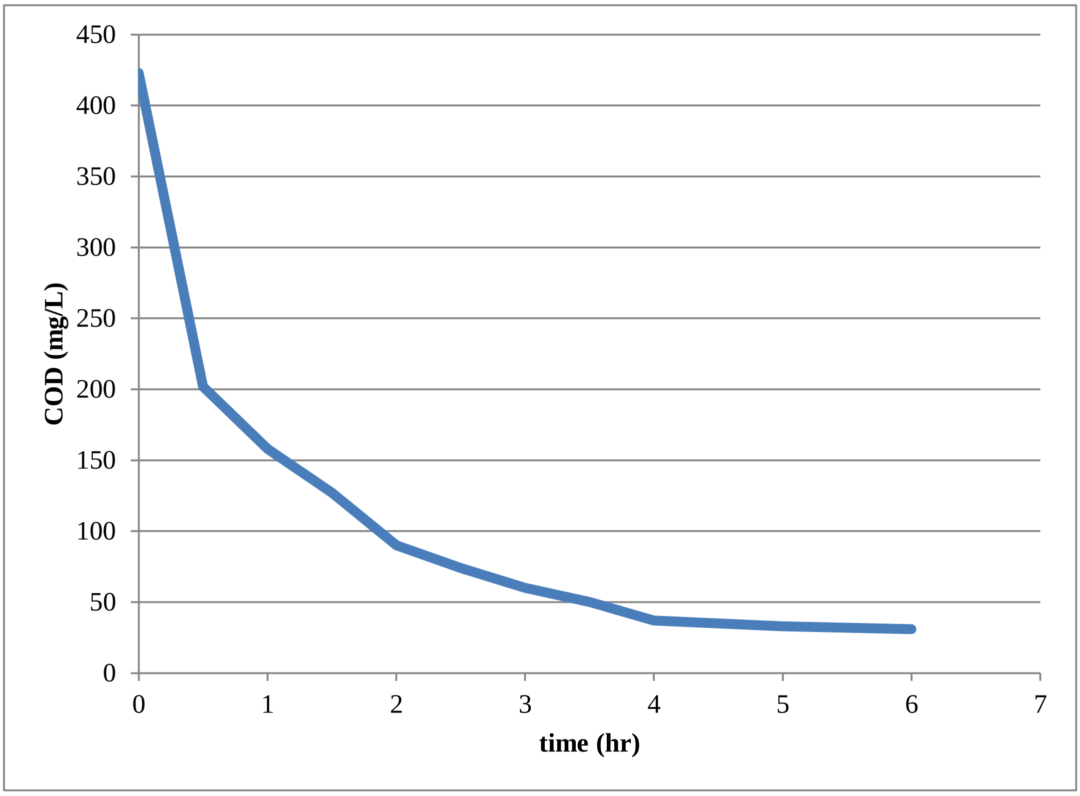

Figure 4.13 : COD profile of the peptone mixture in the control reactor (Run 1).

Next OUR profile shown in figure 4.14 belongs to two consecutive respirometric experiments: The first experiment (Run 2) was started with the same peptone concentration of 405 mg COD/L additionally including olive mill wastewater diluted to 57 mg COD/L in the respirometric vessel, which reflects mixing ratio of around

15%. At the end of the test where the OUR profile reached the endogenous respiration threshold, a second experiment (Run 4) was initiated with the same biomass seed in the reactor, this time adding only 57 mg COD/L of OMW with no peptone feeding.

It is interesting to note the following features in the recorded OUR profiles: (i) In Run 2, the initial peak was slightly higher level of around 110 mg O_2/L.h compared to the control reactor, due to additional utilization of the readily biodegradable fraction coming from the olive mill wastewater dosing. The extension of the OUR plateau after the peak was an indication for the hydrolysis and removal of the slowly biodegradable COD fraction in olive mill wastewater; (ii) No acclimatization /adjustment period was necessary for the microbial culture for the biodegradation of OMW since the total period of the test was not extended beyond 6 hours.

The maximum oxygen uptake rate for Run 4 was 65mg/L.hr, considerably less than previous measurmnet due to lower biodegradable COD concentration in the system. The OUR profiles are illustrated in figure 4.14. COD variation is showing in figure 4.15.

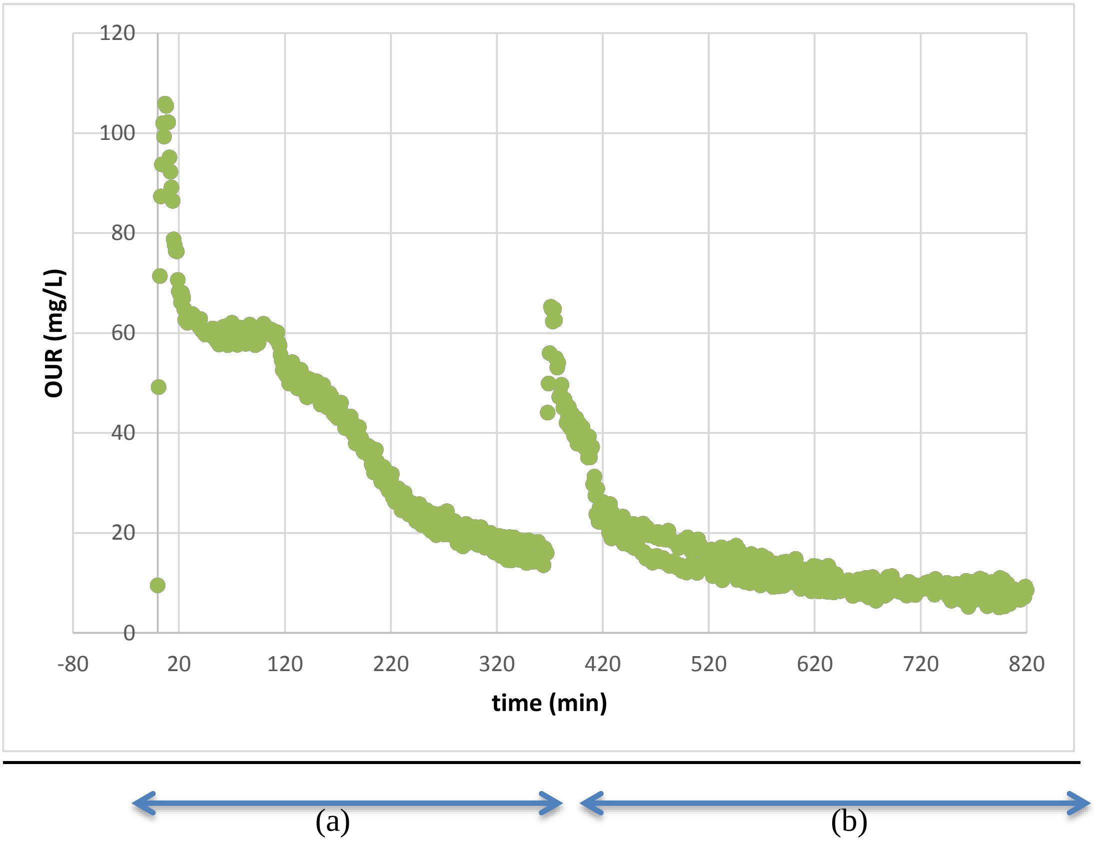

Figure 4.14 : OUR profiles of (a) peptone mixture with 57mg COD/L OMW dosing (Run 2); (b) 57mg COD/L OMW alone (Run 4).

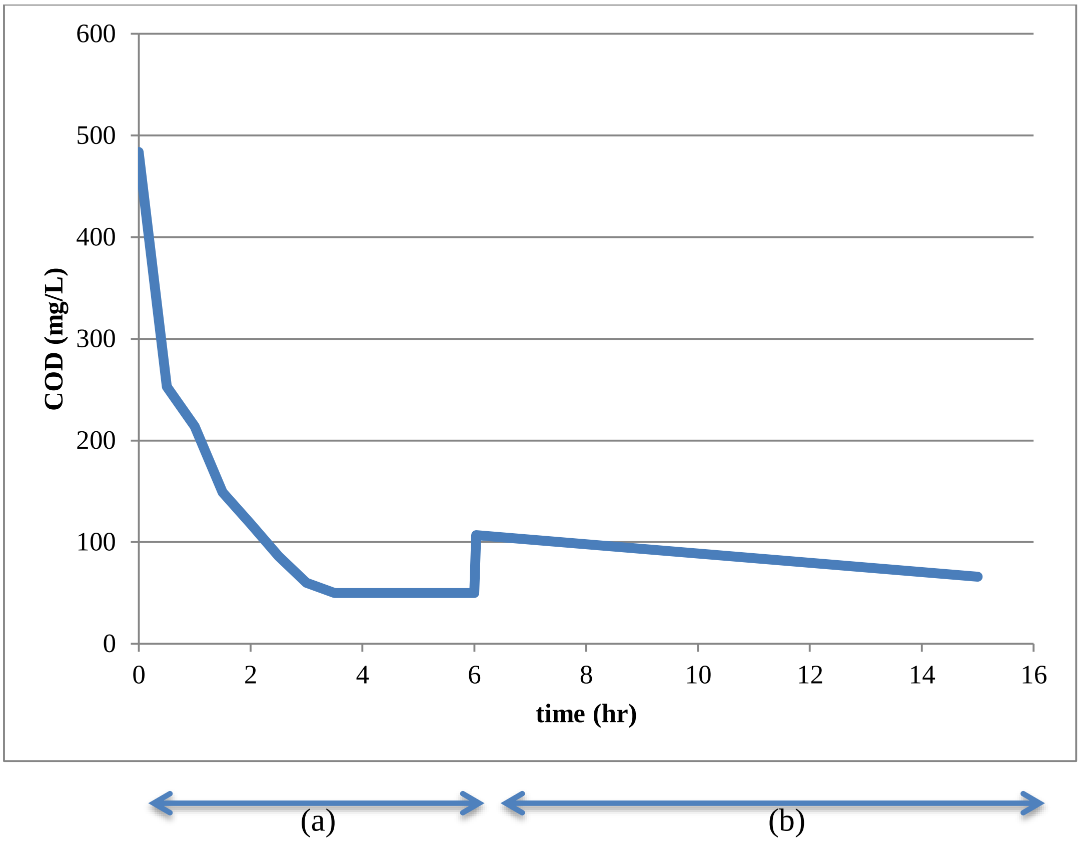

Figure 4.15 : COD profiles of (a) peptone mixture with 57mg COD/L OMW dosing (Run 2); (b) 57mg COD/L OMW alone (Run 4).

The next set of respirometric experiments were carried out with the same rationale, in a sequence of two consecutive runs, the first one (Run 3) involving 430 mg COD/L of peptone with an incremental dose of OMW diluted to 220 mg COD/L, reflcting the mixing ratio of 50%; the second run (Run 5) was similarly started in the same reactor at the end of the first experiment, including only 200 mg COD/L of *raw* olive mill wastewater. The OUR profile resulting from the first experiment basically showed the same trend with a higher peak and a more pronounced plateau, due to higher OMW dosing. It also indicated complete removal of OMW together with peptone mixture in around 7.0 hr, a period slightly longer compared with previous experiments.

The OUR peak in the following experiment was significantly reduced down to 60 mg O_2/L.h with no appreciable plateau, reflecting only partial biodegradation of raw olive mill wastewater presumably limited with simple readily biodegradable organics. This result is especially important when comparing it with the one obtained in Run 4, which only used a 57 mg COD/L dose of nanofiltration effluent. It clearly shows the merit of selected pretreatment scheme i.e. the ultrafiltration/nanofltration sequence in removing colloidal and soluble organics of recalcitrant nature, resistant to biodegradation and this way enabling co-treatment a viable option for final disposal.

Figures 4.16 and 4.17 are showing related OUR and COD profile respectively.

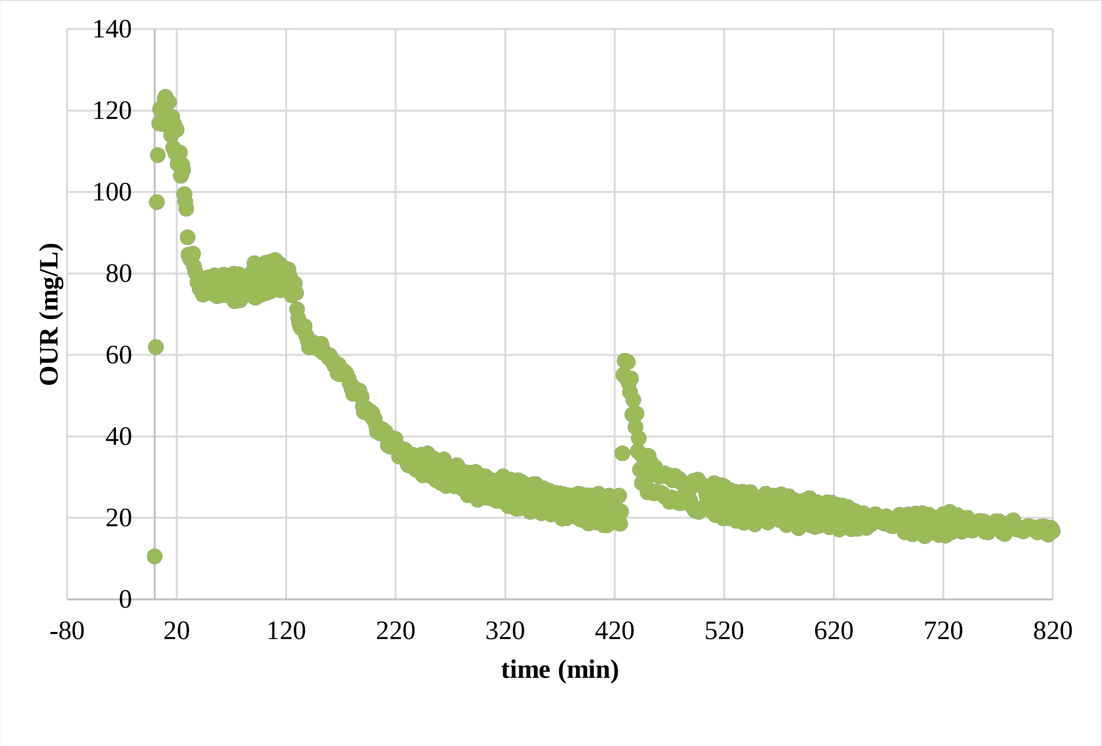

Figure 4.16 : OUR profiles of (a) peptone mixture with 220 mg COD/L of OMW dosing (Run 3); (b) 200 mg COD/L of raw olive oil wastewater (Run 5).

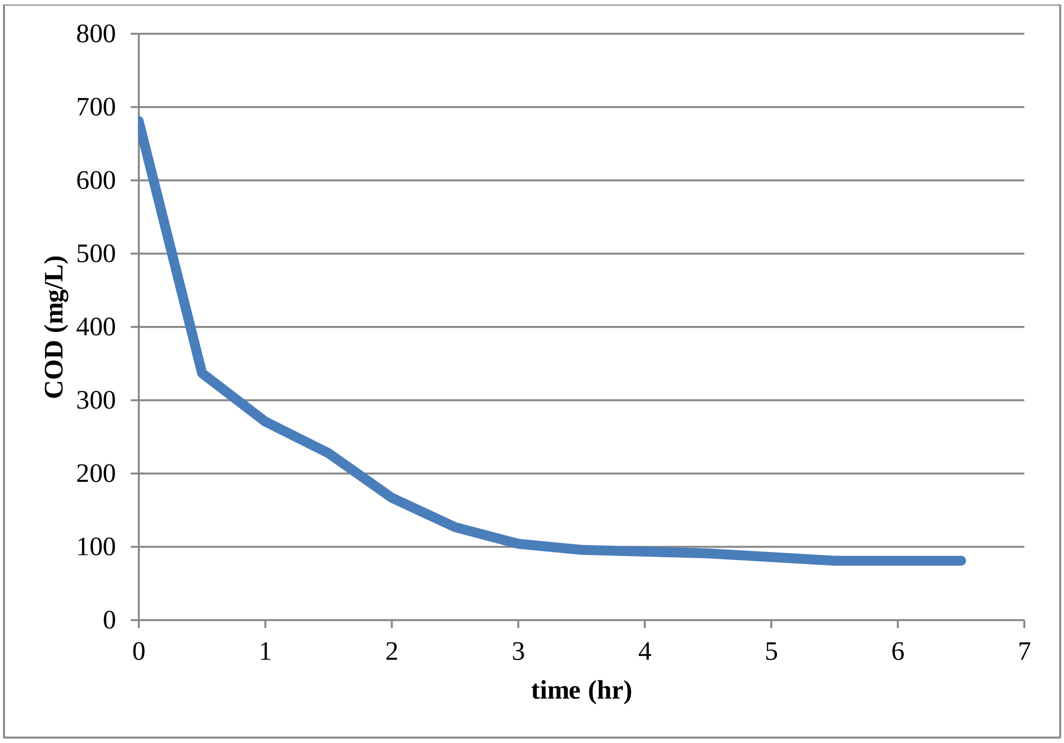

Figure 4.17 : COD profiles of peptone mixture with 220 mg COD/L of OMW dosing (Run 3).

4.5.2 Model Calibration

A fingerprint of the rates of different biochemical processes that affect oxygen consumption which achieved by OUR profile can be translated numerically by model calibration (Ciggin et al., 2012; Hocaoglu et al., 2010). Biodegradation characteristics of the peptone/OMW mixture relied on evaluating the respirometric response of the microbial culture in corresponding batch reactors testing different initial conditions; it essentially involved calibration of the selected model with the corresponding oxygen uptake rate (OUR) profiles. The results were verified with parallel COD measurements, where applicable.

The model calibration procedure assessed values of the model coefficients, which provided best fits with the experimental data as illustrated in Figures 4.15-4.18. This way, it quantified changes in the biodegradation characteristics, if any, induced by OMW dosing in terms of observable changes in the kinetics of different biochemical processes – i.e. values of kinetic coefficients - reflecting substrate utilization, as contrasted with similar results associated with the control set conducted without

OMW addition. The calibration results of model parameters are outlined in Table 4.6.

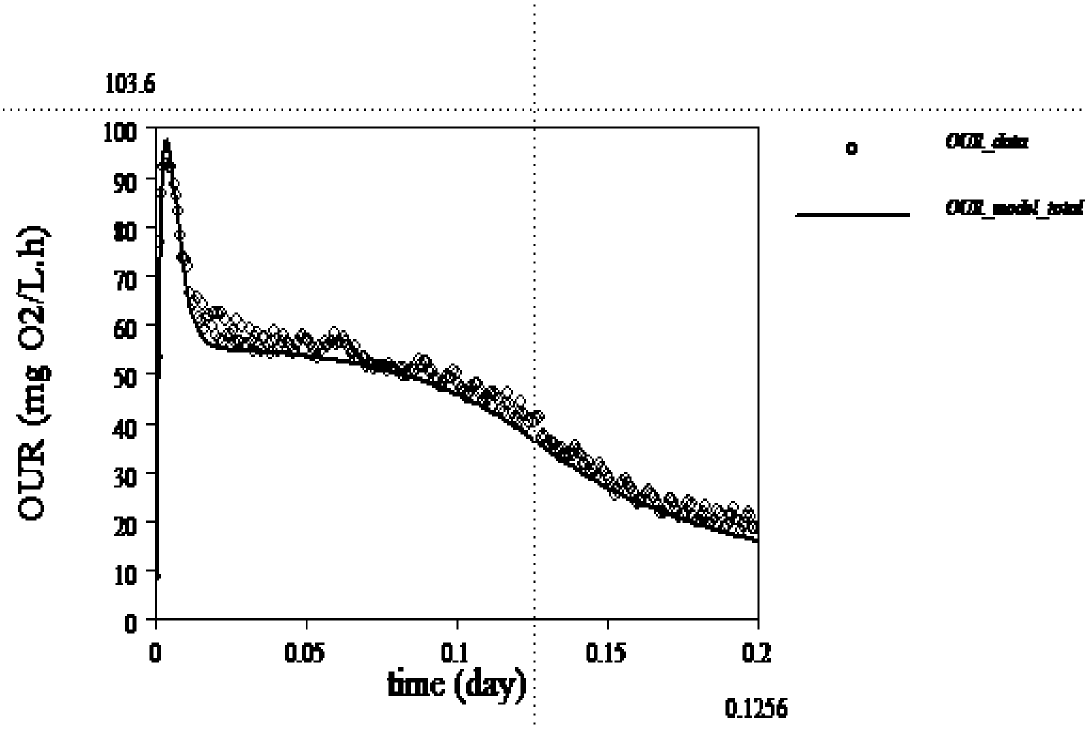

Figure 4.18 : Model calibration of OUR profile for control reactor (Run 1).

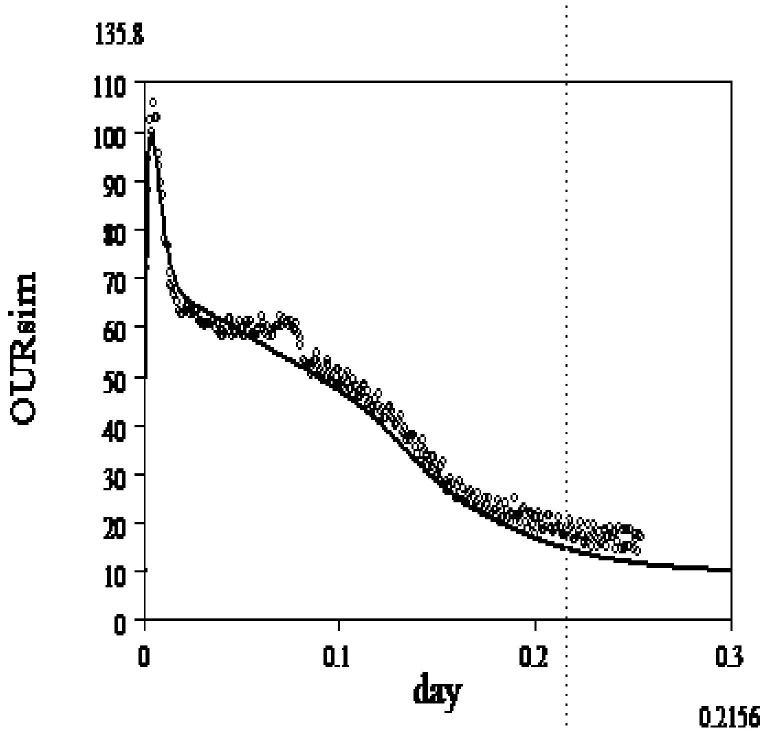

Figure 4.19 : Model calibration of OUR profile for peptone and 57 mg COD/L OMW mixture (Run 2).

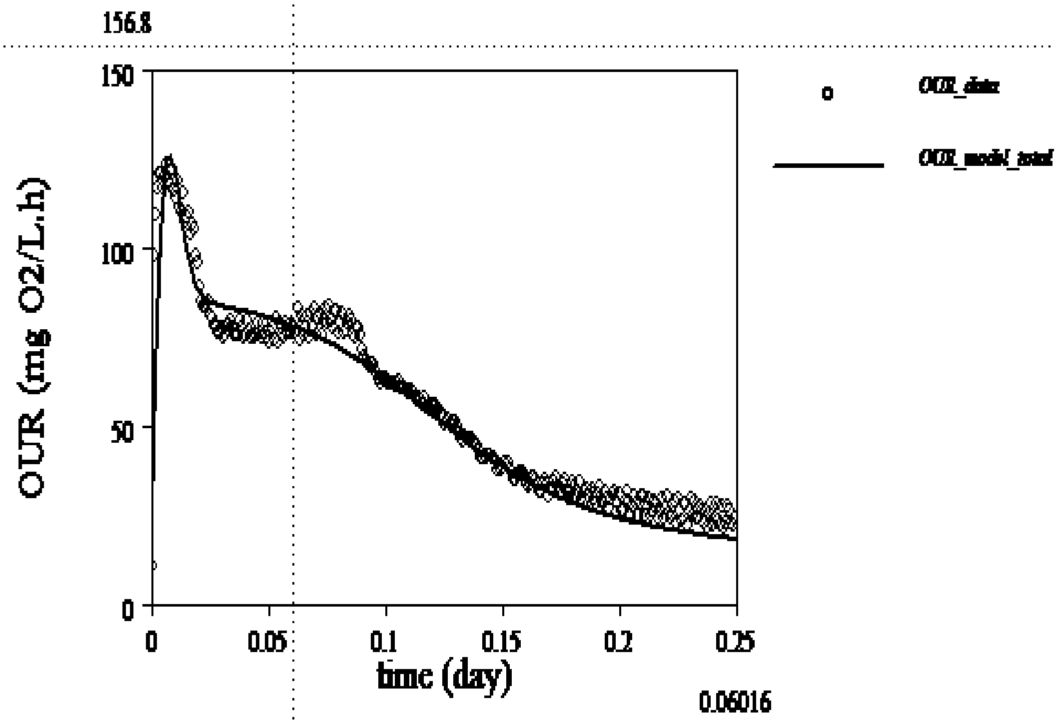

Figure 4.20 : Model calibration of OUR profile for peptone and 220 mg COD/L OMW mixture (Run 3).

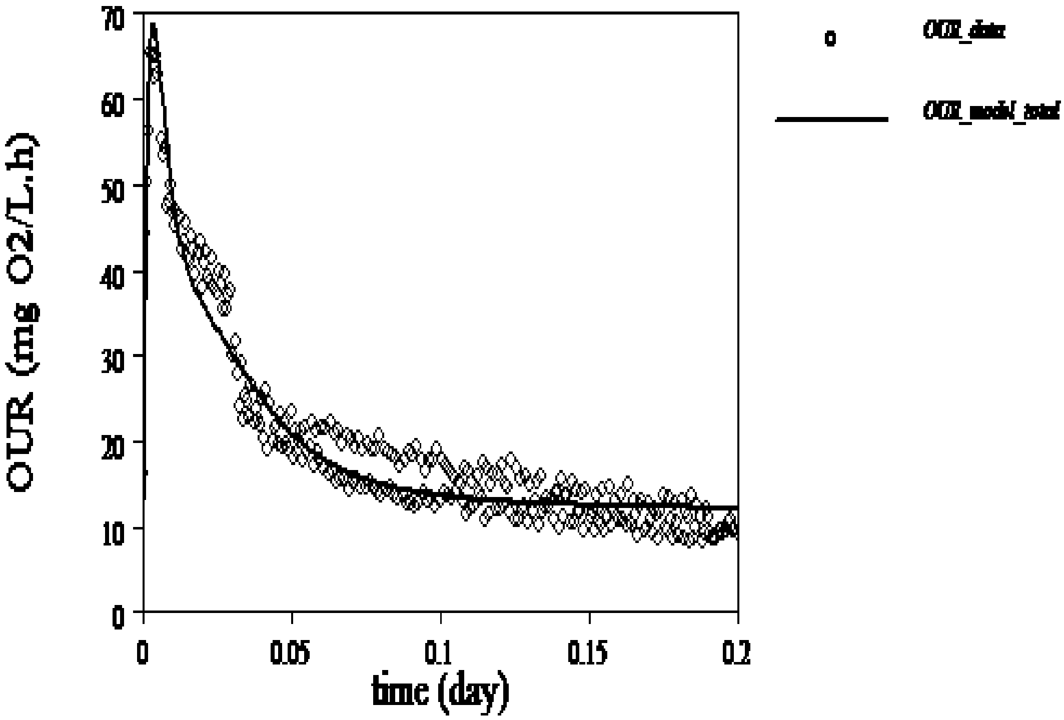

Figure 4.21 : Model calibration of OUR profile for 57 mg COD/L OMW alone (Run 4).

Table 4.4 : Assessment of model parameters via calibration of OUR profile.

Model Parameters	Symbol	Unit	Run 1 (control)	Run 2	Run 3	Run 4
Maximum growth rate on S_{S1}	$\hat{\mu}_{H1}$	1/day	7.2	7.2	7.2	-
Half saturation coefficient for S_{S1}	K_{S1}	mg COD L^{-1}	20	20	20	-
Maximum growth rate on S_{SOO}	$\hat{\mu}_{HOO}$	1/day	-	7.2	7.2	7.2
Half saturation coefficient for S_{SOO}	K_{SOO}	mg COD L^{-1}	-	46	46	46
Maximum hydrolysis rate for, S_{H1}	k_{hs}	1/day	2.8	2.8	2.8	-
Hydrolysis half saturation constant for S_{H1}	K_{XS}	g COD/g COD	0.09	0.09	0.09	-
Maximum hydrolysis rate for S_{H2}	k_{hx}	1/day	0.80	0.80	0.3	-
Hydrolysis half saturation constant for S_{H2}	K_{XX}	g COD/g COD	0.01	0.01	0.01	-
Maximum hydrolysis rate for S_{HOO}	k_{HOO}	1/day	-	1.80	1.80	1.80
Hydrolysis half saturation constant for S_{HOO}	K_{XOO}	g COD/g COD	-	0.04	0.04	0.04
Endogenous decay rate	b_H	1/day	0.24	0.24	0.24	0.24
Soluble fraction of endogenous residual	f_{ES}	-	0.05	0.05	0.05	0.05
Particulate fraction of endogenous residual	f_{EX}	-	0.15	0.15	0.15	0.15

*OO: Olive oil wastewater

Table 4.5 : Assessment of model parameters via calibration of OUR profile.

State variables	Symbol	Unit	Run 1 (control)	Run 2	Run 3	Run 4
Initial VSS concentration	X_{VSS}	mg VSS/L	890	845	1030	1030
Active biomass	X_H	mg COD/L	950	900	1100	1100
Activity	-	%	75	75	75	75
Initial biodegradable peptone mixture COD	C_{S1}	mg COD/L	405	405	433	-
Initial readily biodegradable peptone mixture COD	S_{S1}	mg COD/L	25	25	28	-
Initial readily hydrolizable peptone mixture COD	S_{H1}	mg COD/L	296	296	315	-
Initial slowly hydrolysable peptone mixture COD	S_{H2}	mg COD/L	84	84	90	-
Initial olive oil COD after nanofiltration	C_{SOO}	mg COD/L	-	57	220	57
Initial readily biodegradable olive oil COD after nanofiltration	S_{SOO}	mg COD/L	-	16	62	16
Initial readily hydrolizable olive oil COD after nanofiltration	S_{HOO}	mg COD/L	-	41	158	41

*OO: Olive oil wastewater

The peptone mixture in the control reactor was characterized by a maximum specific growth rate, $\hat{\mu}_{H1}$ of 7.2/d with a corresponding half saturation coefficient, K_{S1} of 20.0 mg/L. The dual hydrolysis concept in the model structure was justified as the calibration procedure identified two slowly biodegradable COD components, S_{H1} and S_{H2} with maximum hydrolysis rates k_{H1} and k_{H2} of 2.8/d and 0.8/d respectively. These results confirm findings on the same substrate previously reported in the literature (Pala Ozkok et al., 2011).

It is interesting to underline the merit of respirometric analysis in displaying the recalcitrant nature of row olive mill wastewater, without proper pretreatment. Significantly tampered and reduced OUR profile obtained with raw olive wastewater dosing in Run 5, may be interpreted to define only limited and partial biodegradation. In fact, basic mass balance between oxygen consumed and substrate utilized in a biochemical reaction, as discussed in detail elsewhere may be simplified into:

$$\Delta O_2 = (1 - Y_H)C_S \qquad \textbf{(4.1)}$$

where, ΔO_2 is the oxygen consumption; C_S, corresponding substrate utilization and Y_H , the yield coefficient. In Figure 4.16, the area of the OUR curve above the endogenous respiration level yields a ΔO_2 value of 16.9 mg O_2/L; Adopting theY_H value of 0.64 mg cell COD/mg COD used in model calibration, this defines C_S, i.e. the magnitude of the COD utilized in the test, as 46 mg COD/L, corresponding to only 21% of the 220 mg COD/L dose used in the experiment.

Results outlined in Tables 4.4 and 4.5 provided two significant indications: (i) OMW dosing, even at 220 mg COD/L did not exert any appreciable impact on the biodegradation of the peptone mixture: growth and hydrolysis kinetics basically remained the same, except for maximum hydrolyses rate for S_{H2} and a decrease in the level of k_{h2} from 0.8/d to 0.3/d. These changes could at worst be interpreted as slight retardation effects on the biodegradation of the peptone/OMW mixture. (ii) Full biodegradation of OMW was achieved by biomass acclimated to peptone as the only organic source when exposed to peptone/OMW mixture.

Model calibration identified two major COD fractions in OMW: the readily biodegradable fraction, S_{SO} as 28% of total COD, was utilized with any disturbance of growth kinetics; the slowly biodegradable COD fraction, S_{HO} as 72% of total COD was hydrolyzed with a maximum hydrolysis rate, k_{HO} of 1.8/d quite compatible with the range of 0.8-2.8/d defined for peptone. It is interesting to note that OMW was fully biodegraded when supplied at 57 mg COD/L to the respirometric reactor with no additional peptone dosing with the same process kinetics (Run 4).

The model indicated three different COD fractions for peptone: readily biodegradable, rapidly hydrolysable and slowly hydrolysable COD, with the concentration of 25mg/L, 294mg/L and 84mg/L, corresponding to 6%, 73% and 21% of total initial COD, respectively. Two hydrolysable fractions undergo hydrolysis to breaking down into readily biodegradable COD, then utilized by microorganisms.

The results also provide experimental support for the arguments that the peptone mixture is quite capable of simulating domestic wastewater in terms of biodegradation characteristics of different fractions obtained from different experiments as shown in table 4.6.

Table 4.6 : Kinetics of dual hydrolysis for different domestic sewage.

Kinetics	***values***	***References***
Maximum growth rate $\hat{\mu}_H$	6.0 day^{-1}	Orhon et al., 1999, Henze et al., 1995
Half saturation coefficient K_S	20 mg COD/L	
Maximum hydrolysis rate for S_{H1}, k_{hs}	3.1 day^{-1}	Ubay Cokgor, 1997
Hydrolysis half saturation constant for S_{H1}, K_{XS}	0.2 grCOD/grCOD	
Maximum hydrolysis rate for S_{H2}, k_{hx}	1.2 day^{-1}	
Hydrolysis half saturation constant for S_{H2}, K_{XX}	0.5 grCOD/grCOD	
Maximum hydrolysis rate for S_{H1}, k_{hs}	3.8 day^{-1}	Ubay Cokgor et al., 1998
Hydrolysis half saturation constant for S_{H1}, K_{XS}	0.2 grCOD/grCOD	
Maximum hydrolysis rate for S_{H2}, k_{hx}	1.9 day^{-1}	
Hydrolysis half saturation constant for S_{H2}, K_{XX}	0.18 grCOD/grCOD	

Results outlined above indicated that olive oil wastewater becomes fully biodegradable after a pretreatment scheme involving chemical conditioning and membrane filtration with an ultrafiltration/nanofiltration sequence. Biodegradation was tested in mixture with peptone, which properly simulates and approximates the biodegradation characteristics of sewage after appropriate dilution of OMW.

From a practical standpoint, the tests accounted for the seasonal nature of OMW generation and possible co-treatment during the production period; thus, they were carried out with biomass acclimated only to peptone in a fill and draw reactor and OMW exposure basically simulated a pulse addition to the system. This approach is somewhat conservative, because in reality the biomass in the joint municipal treatment system will be gradually adjusted and acclimated to OMW dosing during the seasonal generation/mixing period.

As shown in Table 4.5, model calibration also yielded parameters related to process stoichiometry, i.e. confirmed values of major state variables such as biomass, substrate, etc. It indicated that the active fraction of the biomass remained the same in all experiments with two significant implications: (i) there were no inhibitory/toxic impact of OMW dosing on the biomass, and (ii) the entire biomass participated to the utilization of peptone/OMW as organic substrate, with no appreciable metabolic adjustment problems. It also defined for OMW the same S_{SO}/S_{HO} ratio of around 40% regardless of OMW concentration in the respirometric tests. Obviously, the S_{SO} fraction included a spectrum of simple organics, which could be defined in terms of similar biodegradation properties of different wastewaters regardless of their origin, whereas the S_{HO} ratio reflected specific characteristics of OMW.

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CURRICULUM VITAE

Name Surname: Soodeh SALIMI KHALIGH

Place and Date of Birth: Tehran/Iran- 7.12.1978

E-Mail: salami@itu.edu.tr

EDUCATION:

B.Sc.: Microbiology