REMOVAL OF ENDOCRINE DISRUPTER COMPOUNDS AND TRACE ORGANICS IN MEMBRANE BIOREACTORS

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submitted by **OKAN TARIK KOMESLİ** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Göksel N. Demirer _____
Head of Department, **Environmental Engineering**

Prof. Dr. Celal Ferdi Gökçay _____
Supervisor, **Environmental Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Ülkü Yetiş _____
Environmental Engineering Dept., METU

Prof. Dr. Celal Ferdi Gökçay _____
Environmental Engineering Dept., METU

Prof. Dr. Francisco Omil _____
Chemical Engineering Dept.,
University of Santiago de Compostela, Spain

Prof. Dr. Filiz B. Dilek _____
Environmental Engineering Dept., METU

Prof. Dr. Ayşenur Uğurlu _____
Environmental Engineering Dept., Hacettepe University

Date: 19.06.2012

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : OKAN TARIK KOMESLİ

Signature :

ABSTRACT

REMOVAL OF ENDOCRINE DISRUPTER COMPOUNDS AND TRACE ORGANICS IN MEMBRANE BIOREACTORS

Komesli, Okan Tarık

Ph.D., Department of Environmental Engineering

Supervisor: Prof. Dr. Celal Ferdi Gökçay

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Endocrine disrupters and trace organic contaminants are recently recognized contaminants in wastewaters. Current concept is the multibarier approach where the contaminants are removed from the water cycle both by water and wastewater treatment facilities, as well as natural die-away. In this thesis work LC/MS/MS determination of selected EDC compounds, namely, diltiazem, progesterone, estrone, carbamazepine, benzyl butyl phthalate and acetaminophen, at ultra trace levels, have been carried out by optimizing analytical parameters. In addition, new methods were developed for their analysis in sludge samples at sub ppb levels. Following optimization and method development, occurrence of these contaminants in wastewaters and their removal in two full-scale and two pilot-scale membrane biological reactors (MBRs) was studied. Progesterone, estrone and acetaminophen were completely removed from wastewater by biodegradation. CBZ and diltiazem were not removed at all during the study. There was little effect of flux and sludge retention times on the removal of selected EDCs in these membrane plants. In SBR combined with membrane filtration, 13 different micropollutants, including Fluoxetine (FLX), Ibuprofen

(IBP), Naproxen (NPX), Diclofenac (DCF), Carbamazepine (CBZ), Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Sulfamethoxazole (SMX), Diazepam (DZP), Galaxolide (GLX), Tonalide (TON), Celestolide (CEL). CEL, GLX, TON and FLX were removed by adsorption onto the sludge while ROX, ERY, SMX, IBP and NPX were removed by biological degradation. The CBZ, DZP, TMP and DCF were not removed by biodegradation or adsorption. Whereas, following the addition of powdered activated carbon, all these compounds were removed entirely from the wastewater stream by accumulating in sludge.

Keywords: Endocrine Disrupter Compounds, Membrane Bioreactor, removal, optimization, wastewater

ÖZ

ENDOKRİN BOZUCULARIN VE İZ ORGANİK BİLEŞİKLERİN MEMBRAN BİYOREAKTÖRLERDE GİDERİMİ

Komesli, Okan Tarık
Doktora, Çevre Mühendisliği Bölümü
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Endokrin bozucular ve iz organikler son zamanlarda atıksularda dikkat çeken kirleticilerdir. Şimdiki anlayış bunların çoklu engellerle önlenmesidir. Bu yaklaşıma göre su döngüsü içersinde maddelerin su ve atıksu arıtma safhalarında uzaklaştırılması veya kendiliklerinden yok olmaları beklenmektedir. Bu tez çalışmasının ilk bölümünde seçilen maddelerin, (diltiazem, progesterone, estrone, carbamazepine, benzil butilfitalat ve acetaminophen), ölçüm optimizasyonu yapılmıştır. Daha sonra seçilen hormon bozucu maddelerin sıvıda ve çamurda analizleri için metotlar geliştirilmiştir. Geliştirilen metotların deteksiyon limitleri sıvılar için 0.14 ile 0.26 ng/L arasında çamur numuneleri için ise 0.24 ile 0.78 µg/kg arasındadır. Optimizasyon ve metot geliştirme yapıldıktan sonra belirlenen hormon bozucu maddelerin atıksuda bulunması ve iki adet gerçek ölçekli ve iki adette pilot ölçekli membran biyoreatörlerde arıtılması incelenmiştir. Çalışmalarda incelenen progesterone, estrone ve acetaminophen arıtım sırasında tamamen biyolojik olarak arıtılmıştır. Diltiazem ve CBZ membran biyoreaktörler ile arıtılamamıştır. Çalışma sırasında ayrıca akının ve çamur yaşının etkisi

incelenmiş olup çok fazla bir etkisi görülmemiştir. Çalışmanın İspanya ayağını oluşturan kısımda ardışık kesikli reaktöre entegre edilmiş membran filtrasyonu ile seçilen 13 farklı maddenin Fluoxetine (FLX), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), Carbamazepine (CBZ), Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Sulfamethoxazole (SMX), Diazepam (DZP), Galaxolide (GLX), Tonalide (TON), Celestolide (CEL), arıtımı incelenmiştir. Çalışma sırasında CEL, GLX, TON ve FLX maddeleri çamura adsorbe olarak atıksudan uzaklaştırılmıştır. ROX, ERY, SMX, IBP ve NPX maddeleri ise biyolojik olarak parçalanmışlardır. CBZ, DZP, TMP ve DCF ne biyolojik olarak ne de adsorbe olarak arıtımı yapılamamıştır. Kesikli reaktör içerisine toz aktif karbon katılarak arıtım verimi artırılmış olup membran filtrasyonu sonrasında bu maddeler ölçülebilir değerlerin altında kalmıştır.

Anahtar kelimeler: hormon bozucu maddeler, membran biyoreaktör, arıtım, optimizasyon, atıksu

To my father, my mother and my wife.

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ABBREVATION LIST

AF	Ammonium format
BBP	Butyl Benzyl Phthalate
BNR	Biologic Nutrient Removal
CAS	Conventional Activates Sludge
CBZ	Carbamazepine
CE	Collusion Energy
CEL	Celestolide
COD	Chemical Oxygen Demand
DCF	Diclofenac
DCMS	Dichloromethylsilane
DO	Dissolved Oxygen
DZP	Diazepam
EDCs	Endocrine Disrupter Compounds
ERY	Erythromycin
ESI	Electrospray Ionization
FA	formic acid
FLX	Fluoxetine
FV	Fragmantor Voltage
GC/MS	Gas Chromatography Mass Spectrometer
GLX	Galaxolide
H	Henry coefficient
HPLC	High Pressure Liquid Chromatography
HRT	Hydraulic Retention Time
IBP	Ibuprofen
K_{biol}	Pseudo first order degradation constant
K_d	Sludge-water distribution coefficient
K_{ow}	Octanol-water partition coefficient
LC/MS/MS	Liquid Chromatography tandem Mass Spectrometer

LOD	Limit of Detection
LOQ	Limit of Quantification
MBR	Membran Bioreactor
MW	Molecular Weight
NPX	Naproxen
PAC	Powdered Activated Carbon
PES	Polyether sulfones
PI	Product Ion
PP	polypropylene
PPCPs	Pharmaceuticals and Personal Care Products
ppm	part per million
ppb	part per billion
ROX	Roxithromycin
SBR	Sequencing Biological Reactor
SMX	Sulfamethoxazole
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SRT	Sludge Retention Time
STW	Sewage Treatment Work
T	Temperature
SVI	Sludge Volumetric Index
TMP	Trimethoprim
TSS	Total Suspended Solid
TON	Tonalide
VRM	Vacuum Rotation Membrane
VSS	Volatile Suspended Solid
WWTP	Wastewater Treatment Plant
YES	Yeast Estrogen Screen

CHAPTER 1

INTRODUCTION

1.1. General

Water is one of the most vital element for life and is essential for men's existence. Paradoxically, municipal and industrial wastewaters generated as a result of human activity for his own wealth and prosperity; can yet jeopardize his sole existence by polluting the water resources. Most of the treated and non-treated wastewaters are disposed into rivers and other water sources thereby introducing pollutants into the vital element. In the 20^{th} century, gross organic pollution expressed by the generic parameters such as COD and BOD has been centre of focus. Variety of treatment processes have since been developed to deal with this form of pollution and are now under control. In the early 21^{st} century trace organics having impact on the hormones and which are normally present at sub ppm (part per million) levels in the effluents and surface waters, has now been recognized as a form of pollution having long term effects on the biota and man. With the growing industrialization and modernization and in order to facilitate everyday life and save lives through human and veterinary medicine, modern chemistry has introduced numerous compounds into the waters. Additionally, with the rising life standards personal care products have entered into everyday life of men and women. All these compounds, some way or other, find their ways into the wastewaters and surface waters. Many industrial effluents also discharge these compounds into the environment through their discharges. Hazard due to trace organics have long been a suspect but technology was not sufficient to resolve them in waters. However, recent advances in analytical chemistry make detection as low as 10^{-9} g/L possible in waters.

It is now possible to analyze extremely trace quantities of the newly recognized contaminants, including pharmaceuticals, synthetic fragrances, detergents, disinfectants, plasticizers, preservatives, synthetic and natural hormones; which are collectively termed under the generic name Endocrine Disrupter Compounds (EDCs). The name comes from the mimic effects of these compounds on the natural hormones that make up the endocrine system. The EDCs are a wide range of contaminants which are detectable in wastewater treatment plant effluents (WWTP) and surface waters around the world. From the accumulating studies it is now clearly understood that conventional treatment may not be very effective in the removal of these substances in wastewater. Some of these chemicals can penetrate through the standard wastewater treatment systems and pollute the receiving waters which are ultimately consumed by humans. Although there are no set standards yet for these compounds, their treatment before disposal is mandatory to preserve public and environment health. Research is now underway to develop technologies that are capable of removing these contaminants from effluents. Advanced treatment technologies, such as membrane bioreactors; which is a form of activated sludge process coupled to membrane filtration, are being explored to determine their effectiveness in the treatment of EDCs.

1.2. Scope of the Study

The scope of the research herein is the following;

- Optimization of analysis of selected EDCs by LC/MS/MS
- Develop new methodology for the determination of selected EDCs in liquid and solid samples

- Determine treatability of some selected common EDCs in two full-scale MBR plants in Turkey
- Determine effect of membrane flux and SRT on the removal of selected EDCs in MBRs

- Removal of selected EDCs and Pharmaceuticals and Personal Care Products (PPCPs) in a novel SBR+membrane filtration system, the SeMPAC process.

- Complete Characterization of the SeMPAC Process in terms of conventional pollutant removal.

1.3. Description of the Chapters

In Chapter 1, the general view of the Endocrine Disrupter Compounds (EDCs), and scope of the study is presented.

In Chapter 2, updated background information in the literature related to research on EDCs removal is presented. The main subjects in this chapter are wastewater reuse, membrane bioreactors, description of endocrine disrupter compounds, sources, methods of analysis, removal mechanisms and fates during treatment, effect on wildlife and humans, and information on selected endocrine disrupters.

In Chapter 3, the Materials and Methods used during the study are described. Essential information on four different membrane bioreactor plants studied during this work is given. Information on the LC/MS/MS instrument used and techniques developed for the instrumental analysis are presented. The analytical methods used in Spain Studies for PPCPs determinations are presented.

In Chapter 4, the optimization of analysis of selected EDCs (diltiazem, progesterone, estrone, carbamazepine and acetaminophen) are described. The newly developed method for the simultaneous determination of selected EDCs in liquid samples after solid-phase extraction and the ultrasound-aided sequential extraction method for the determination of these compounds in sludge samples by LC/MS/MS are given in this chapter. The occurrence and removal of selected EDCs in two full-scale MBR plants are presented. Effect of flux and SRT on Clear-Box; and the membrane and activated carbon effects on removals of EDCs in SBR+membrane are also described in this chapter. Removal mechanisms of selected compounds in four different MBR treatment plants, including accumulation, biodegradation are presented.

In Chapter 5, the results of the research are concluded.

CHAPTER 2

LITERATURE REVIEW

2.1. Wastewater Reuse

Around 70% of the earth's surface is covered by waters, 1% is accessible as fresh water resource. Yet clean water resources have been fast decreasing due to population growth, industrial development, falling groundwater levels. Wastewaters generated from human activities pollute clean water resources when they are directly disposed into the natural water sources. The ninety 90% of the freshwater are being used for the purpose of irrigation and industrial usage, while just 10% is used for drinking water (Scharnagl et al., 2000). Currently, more than 1 billion people can not find potable water and this is expected to increase to 2.5 billion in the year 2025 (Scharnagl et al., 2000; Howell, 2004). Around 80% of the world's population has no access to proper sanitation and short of water security (Gilbert, 2010). Due to unsafe drinking waters and insufficient sanitation, 88% of the waterborne diseases are reported to have originated from such poor quality waters. In order to protect the ecosystem and humans health, wastewaters are being treated to an acceptable quality since 200 years back.

The negative effects on the clean water resources have led to scarcity of clean waters in the world, whereupon scientists shifted the focus to find new water resources. One newly developed resource is desalination of sea or ocean waters by reverse osmosis. However, application of reverse osmosis is restricted in the world; not only due to high investment and maintenance costs but also to the requirement of closeness to the sea. An alternative resource for fresh water may be the treated wastewaters, which may be applicable wherever human settlements

exist. Thus, treatment with an aim of reuse has gained more importance lately. The first application of reuse was started by the U.S. farmers by using treated wastewaters in crop production and irrigation (Okun 2000; Comerton 2002). In addition to crop irrigation, treated wastewaters can be re-used for many other purposes including toilet flushing, industry, car washing, recharging of groundwater etc. (Asano and Cotruvo, 2005; Wintgens et al., 2002; Rosenbulum, 1999; Balannec, 2002; Koyuncu et al., 2000).

Wastewaters should be treated to acceptable levels for reuse in order to meet the pathogenic and fecal indicator microorganism standards to safeguard the public health. The set standards for water reuse are evolving in time as new scientific evidence accumulates. The reuse standard applicable to wastewaters in Turkey is the "Turkish Water Pollution Control Regulation, Technical Aspects Bulletin", published in 4th September 1991, as summarized in Table 2.1.

Table 2.1. Turkish Reuse Standards in Crop Irrigation (SKKY, 1991)

	Irrigation Water Class				
Quality Criteria	**Class I (Very Good)**	**Class II (good)**	**Class III (usable)**	**Class IV (usable with caution)**	**Class V (unusable)**
$EC_{25}x10^6$	0-250	250-750	750-2000	2000-3000	> 3000
Variable Sodium Percentage (% Na)	< 20	20-40	40-60	60-80	> 80
Sodium Adsorbtion Ratio (SAR)	< 10	10-18	18-26	> 26	
Sodium carbonate residue (RSC) meq/L mg/L	 > 1.25 < 66	 1.25-2.5 66-133	 > 2.5 > 133		
Cl (meq/L) (mg/L)	0-4 0-142	4-7 142-249	7-12 249-426	12-20 426-710	> 20 > 710
$SO_4^=$ (meq/L) (mg/L)	0-4 0-192	4-7 192-336	7-12 336-575	12-20 575-960	> 20 > 960
Total Salt (mg/L)	0-175	175-525	525-1400	1400-2100	> 2100
Boron (mg/L)	0-0.5	0.5-1.12	1.12-2.0	> 2.0	-
NO_3^- or NH_4^+ (mg/L)	0-5	5-10	10-30	30-50	> 50
Fecal coliform (1/100 ml)	0-2	2-20	20-100	100-1000	> 1000
BOD_5 (mg/L)	0-25	25-50	50-100	100-200	> 200
SS (mg/L)	20	30	45	60	> 100
pH	6,6-8,5	6,5-8,5	6,5-8,5	6,5-9	6<or>9
Temperature (^oC)	30	30	35	40	> 40

As seen in this table, the stipulated standards are rather strict and difficult to achieve with conventional treatment systems and require advanced treatment technology. The membrane bioreactor (MBR) process is currently the leading

technology to fulfill these standards and reuse of wastewaters both in irrigation and household usage (Komesli et al., 2007).

2.2. Membrane Bioreactors (MBRs)

The MBR process is a combination of biological treatment and membrane separation combined in a single process to achieve the same effect as with the activated sludge process (Komesli et al., 2007; Gunder, 2004; Manem and Sanderson, 1996). Membranes have been used as filtration devices in water and wastewater treatment since early 1960s (Yoon, 2003; Visvanathan et al., 2000). The first reported study to couple an activated sludge process with membrane filtration to replace the secondary clarifier, dates back to 1967 by Smith (Brindle and Stephenson, 1996). In this first application, membrane filtration was separated from the activated sludge tank and named as external membrane bioreactor. In this configuration, mixed liquor in the aeration tank was forced through the filter at high pressure. The high energy consumption was a great handicap against the development of MBR technology. Yamomoto et al., (1989) submerged the membrane filter into the activated sludge tank in order to overcome high energy consumption without compromising in water quality. The new configuration was called as internal MBR. This development in membrane bioreactor technology broadened use of MBRs in the world wide due to good effluent quality (Gagliardo et al., 2001; Lawrence et al., 2002), lower plant footprint owing to high MLSS concentrations and very long sludge retention times that can be maintained in the aeration tank (Cote et al., 1997), and by overcoming settling problems (Buisson et al.,1998; Cicek et al.,1998). A 7 log removal of coliforms without chemical addition (Komesli et al., 2007) and removal of over 99% of suspended solids, and organic matter could be achieved in this process. There are already a number of wastewater reuse applications in Turkey as given in Table 2.2.

Table 2.2. Some full scale MBR application

No	Type of membrane	Type of wastewater	Flowrate (m^3/d)	Location	Membrane Company	Ref.
1	Flat sheet, ultrafiltration	domestic	200	METU, Turkey	Huber, VRM	Komesli et al., 2007
2	Flat sheet, ultrafiltration	municipal	360	Jebel Ali, Dubai	Huber, VRM	Huber, 2012
3	Flat sheet, ultrafiltration	municipal	1500	Konacık, Bodrum	Kubato	Muz et al., 2012
4	Flat sheet, ultrafiltration	municipal	12900	Swanage, UK	Kubato	Judd, 2006
5	Flat sheet, ultrafiltration	municipal	290	Vienna	Norit	Judd, 2006
6	Hollow fiber	municipal	630	Simmerath Germany	Koch, Puron	Judd, 2006
7	Hollow fiber	Industrial	2000	Sobelgra, Belgium	Koch, Puron	Judd, 2006
8	Hollow fiber	Municipal	48000	Kaarst, Germany	Zenon	Judd, 2006
9	Hollow fiber	Municipal	42000	Brescia, Italy	Zenon	Judd, 2006
10	Hollow fiber	Food	150	Ontario, USA	Zenon	Judd, 2006

Effluent quality obtained in these plants is very high considering classical pollution parameters. However, recently upon development of sophisticated analytical equipment a new genera of pollutants, termed micropollutants or endocrine disrupter compounds, have been recognized as having ill effects on the biota and humans. Although there have not been standards set for these

micropollutants yet, their presence in the environment, effects on the biota and their removal mechanisms are under investigation. The current concept in combating these pollutants in the environment is the ‘multiple barrier’ approach. This is a combination of eradicating such micropollutants in wastewaters and potable waters, as well as in surface waters. MBRs have since grown anticipation towards the removal of these compounds in wastewaters while providing excellent quality reuse waters.

2.3. Endocrine Disrupter Compounds (EDCs)

Following urbanization and industrialization, there has been over 100000 chemicals produced during 1930s (Snyder et al., 2003). These chemicals have been eventually disposed into the rivers, lakes and seas through sewers with or without treatment. Since it first published in 1930s, for over 70 years, it was known that natural and synthetic hormones had effects on the endocrine system (Snyder et al., 2003). After their appearance in the environment during 1950s, observations on the wildlife indicated that population of fish, birds, reptiles and mammals were decreasing (Bowden, 2009; Fossi and Marsili 2003; Colborn, 1996). These observations were the first step-stone of the increasing concern over the effects of these chemicals on the biota. Following these observations, Stumm-Zollinger and Fair, 1965, documented the presence of estrogens in the environment (Stumm-Zollinger and Fair, 1965, Snyder and Benonti, 2010). At the time scientists had been researching for over 30 years on some pesticides (DDT, DDE, Lindane), polycyclic aromatic hydrocarbon (PAHs) and dioxins (Birkett and Lester, 2002). However, concern over these compounds in waters and wastewaters had not grown until 1990s until realizing their effects on living organisms (Snyder and Benonti, 2010; Desbrow et al. 1998; Routledge et al. 1998).

In 1994 some sexual abnormalities in fish living near wastewater treatment plant outfalls were noticed (Purdon et. al.1994). In that particular study male fish

were observed to produce the female yolk precursor protein, vitellogin, as a result of exposure to 17 B-Estradiol. Research also showed that male fish population decreased sharply where wastewater disposal to rivers took place. The public awareness arose after the book *Our Stolen Future* by Rachel Carson was published in 1996. The book captured scientific interest of most scientists in the field and initiated many research projects.

These micro-compounds mimic hormones and block receptor cites thereby disrupting normal functioning of the endocrine system. In other words, they change the production, action, secretion, and elimination of endogenous hormones when come across in the environment. The schematic of endocrine disruption is given in Figure 2.1.

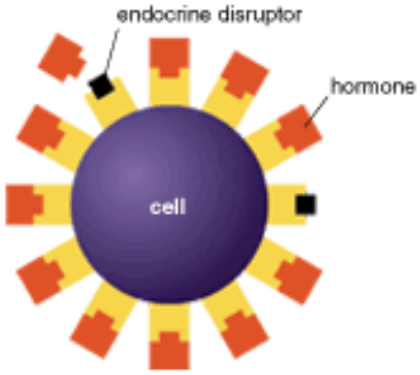

Figure 2.1 Schematic diagram of hormone blockage with endocrine disrupters

As seen in this figure, micro-pollutants are blocking the receptor instead of hormones binding to the cite. This way, these compounds can disrupt development, reproduction, immune function, behavior, and all other life functions mediated by hormones. Owing to their disrupting effect on the endocrine system, they are called Endocrine Disrupter Compounds (EDCs) and their first definition was made by Kavlock et. al., 1996 as;

"exogenous agents that interfere with the production, release, transport, metabolisms, binding action or elimination of the natural hormones in the body responsible for the maintenance of the homeostasis and regulation of developmental processes".

This was the first description of EDCs in the literature. However, this definition was too wide and included too many chemicals; therefore, The U.S. Environmental Protection Agency (USEPA) re-described EDCs as:

"*an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior"* (EPA,1997).

Although the best known reference to this class of compounds is ' endocrine disrupter compounds', yet there are synonyms used, such as emerging contaminants, hormonally active agents, endocrine-active agents, endocrine modulating substances (AWWA, 2005). Basing on this description, many research have been initiated on the effect of EDCs on estrogens, androgens and thyroid. Domain of endocrine disrupters have since been classified into several major groups. These are;

a. *Estrogenic* compounds that mimic or block natural estrogen
b. *Androgenic* compounds mimic or bloc natural testosterone
c. *Thyroidal* compounds with direct or indirect impacts on the thyroid

Although EDCs are divided into three classes, the sub-classes and the effects of each compound falling into these classes have not been strictly categorized. There are more than 87000 known chemicals which may considered within EDCs; including natural hormones, synthetic hormones and their metabolites non-steroidal, synthetic compounds, plasticizer, flame retardants,

surfactants, and pesticides, pharmaceutical and personal care products (PPCPs) (Caliman and Gavrilescu, 2009). However, some do not consider pharmaceuticals, personal care products, pesticides, industrial chemicals, phtytoestrogens within EDCs and some do think that they may well be categorized under a sub-group of EDCs. For example, United Kingdom Institute of Environment and Health tentatively assigned 966 compounds as potential EDCs. Yet there are neither set standards nor any standard methods for the determination of EDCs. Moreover, due to the large number of compounds where each having some kind of hormone disrupting properties, it is not possible to set different standard for each and every compound.

In this study, removal of six different EDCs including natural hormones, phthalate and pharmaceuticals were investigated in this Turkish leg of the study. In addition, thirteen different pharmaceuticals and personal care products were investigated in the Spanish leg.

2.3.1 Information on Selected EDCs in Turkey

Some of the most frequently occurring EDC compounds, including pharmaceuticals, diltiazem, carbamazepine (CBZ), acetaminophen, a phthalate ester, Benzyl Butyl Phthalate (BBP), and natural hormones, progesterone and estrone, in the wastewater were chosen for study at the Turkish leg of the study. These are tabulated in Table 2.3.

Table 2.3. Selected EDCs studied in the Turkish leg of the study

Compound	**MW**	**Solubility in water (mg/L)**	**Log K_{ow}**	**Henry's law constant**	**pKa**	**References**
Estrone	270.4	12.42	3.43	$3.8*10^{-10}$	10.4	Birkett and Lester, 2002
Carbamazepine	236.27	17.7	2.3-2.5	$1.1*10^{-10}$	13.9	Suarez et al.2008
Progesterone	314.47	-	-	-	-	-
BBP	312.37	3.8	4.84	-	3.1	ECHA ,2008
Acetoaminiphen	151.17	12.78 ($1.4*10^{4}$)	0.46	-	9.38	AWWA, 2005
Diltiazem	414.53	465	2,79	-	8,43	AWWA, 2005

As seen in this table, estrone, progesterone and acetaminophen are highly biodegradable compounds by microorganisms. However, diltiazem, CBZ and BBP are not biodegradable and tend to be sorbed by the sludge.

2.3.1.1 Natural Hormones (Progesterone, Estrone)

Two different natural hormones were investigated in this study. Estrone was the first one, which is one of the three common forms of the natural hormone, estrogen, in the body. The others are estradiol and estriol. Although**,** estrone is considered a weaker form of estrogen, it is the major source of estrogen released by females who have undergone menopause. (Varney et. al., 2004). This natural hormone is discharged by humans and animals through urine and feces as non-active conjugates of sulfuric and glucuronic acid (Holdbrook et. al., 2002.). The maximum concentration in wastewater was found about 180 ng/L. (Komori et al. , 2004)

Progesterone is the other hormone investigated in this study. Previously researche have focused on only estrogens due to their abundance in the environment. However, steroid hormones, such as progesterone became important after determining these comounds in 4.3% of 139 United States streams (Barron et. al., 2006). Although this is a natural hormone, it can be synthesized by both males and females. It is mainly involved in the female menstrual cycle and is abundantly released during pregnancy until birth. The hormone is released in the urine, which is the primary source for wastewaters. The maximum concentration of progesterone in wastewater was reported as 0.199 μg/L, and the avarage was 0.11 μg/L (Kolpin et al., 2002). The removal of this compound in convetional activated sludge process was about 80% (Pauwels et. al., 2008)

2.3.1.2 Phthalates

Esters of phthalates are one of the most important classes of EDCs, which are ubiquitous in the environment including sediments, natural waters, soils and aquatic organisms (Chatterjee and Karlovsky, 2010, Giam et al. 1984; Staples et al. 1997), and the drinking water, air, and food. They are man-made products. Among phthalate esters, butyl benzyl phthalate (BBP) is one of the most important environmental contaminant which is widely used in vinyl tiles and in PVC as plasticizer. Therefore, municipal and industrial wastewaters are the most significant source of this compound since municipal wastewater contains high concentrations, in the mg/L range, through runoffs and discharges from households (Vikelse et al. 1998). Repotedly, it has negative health effect mostly on male species, including decrease sperm number, toxicity to testes, prostate, and seminal vesicle (Ema et al. 2003; Ema and Miyawaki 2002; Moral et al. 2007; Swan et al., 2005). Researche has since focused on this compound (Bornehag et al. 2004; Liu and Chen 2006; Nakai et al. 1999). Due to its low solubility in water (Bauer and Herrmann 1997), the compound concentrates in sludge; ranging between 12 to 1,250 mg/kg (Staples et al. 1997).

2.3.1.3 Pharmaceuticals in Turkey

Removal of three pharmaceuticals, carbamazepine, diltiazem and acetaminophen were investigated in this study.

Carbamazepine is an anti-epileptic agent used for epilepsy treatment (Nentwig et. al., 2004), and treatment of depression (Kudoh et al. 1998). The compound is a very important endocrine disrupter due to its frequency of occurrence and high concentration in sewage. It was detected in municipal sewage in Europe and North America (Birkett and Lester, 2002; Heberer 2002). This compound enters the water cycle via untreated or inadequately treated wastewaters discharged to rivers, lakes and sea. Researcher indicated that its

concentration in rivers and streams may be quite high, reaching up to 2.1 μg/L (Ferrari et al. 2003).

Diltiazem is another pharmaceutical which is considering as an EDC. It is used mostly for the treatment of hypertension. It is also used as a preventive medication for migraine. This compound is disposed by the body through the urine and may be detected in wastewaters and sediments at concentrations such as 0,016μg/L and 1,48 μg/kg, respectively (Stackelberg et. al., 2007).

The last compound studied was the acetaminophen, which is a widely used analgesic and antipyretic drug all over the world (Gusseme et. al., 2011). It is also used in the cure of cold and flu. This compound was recognized as one of 95 wastewater contaminants chosen by the National Reconnaissance, completed in 1999-2000 in 139 U.S. streams (Kolpin et al., 2002). Over 90% of acetaminophen may be removed by conventional activated sludge systems (Gomez et al., 2007a).

2.3.2 Information on Selected EDCs in Spain

Fluoxetine (FLX), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), Carbamazepine (CBZ), Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Sulfamethozole (SMX), Diazepam (DZP); personal care products, Galaxolide (GLX), Tonalide (TON), Celestolide (CEL) were investigated in Spain side of the studies. The information about the compounds was given Table 2.4.

Table 2.4. Selected EDCs used in the study (Spain)

Compound	**MW**	**Solubility in water (mg/L)**	**Log K_{ow}**	**Henry's law constant ($atm \cdot m^3 \cdot mol^{-1}$)**	**pKa**	**K_{biol} ($L \cdot g^{-1}SS \cdot d^{-1}$)**
CEL	244	0,22	5,4-6,6	$7,3* 10^{-1}$	-	-
GLX	258	1,8	5,9,-6,3	$1,3* 10^{-4}$	-	<0,03
TON	258	1,2	4,6-6,4	$5,3* 10^{-3}$	-	<0,02
IBP	206	21	3,5-4,5	$1,5* 10^{-7}$	4,9-5,7	9-35
DCF	296	2,4	4,5-4,8	$1,9* 10^{-10}$	4,0-4,5	<0,1
DZP	285	50	2,5-3,0	$1,5* 10^{-7}$	3,3-3,4	<0,03
ERY	734	1,4	2,5-3,0	$2,2* 10^{-27}$	8,9	0,5-1,0
ROX	837	0,02	2,1-2,8	$1,0* 10^{-24}$	9,2	<0,3
TRM	290	400	0,9-1,4	$9,8* 10^{-13}$	6,6-7,2	-
SMX	253	610	4,5-4,8	$2.6*10^{-11}$	5,6-6,0	
NPX	230	16	3,2	$1,4* 10^{-8}$	4,2	0,4-1,9
FLX	346	60	4,0	$3,6* 10^{-6}$	10,1	-
CBZ	296	17,7	2,3-2,5	$1,1* 10^{-7}$	7,0-13,9	<0,01

2.3.2.1 Pharmaceuticals

There are different classes for the pharmaceuticals including antibiotics, anti-inflammatories, anti-depressants, anti-epileptics and tranquilizers. The first group is antibiotics used for the kill or slow- down of the growth of bacteria. These compounds are mainly used for the medicine of humans and animals. During the study, removal of Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Sulfamethoxazole (SMX) were investigated. ERY is macrolide antibiotics and mainly used for people who have ROX is a derived form of ERY and used to treat respiratory tract, urinary and soft tissue infections. Moreover, it is also used for the treatment of male-pattern hair loss. Like ROX, TRM is used for the treatment of urinary infections. SMX is the last antibiotic most often used as part of a synergistic combination with TMP. ibuprofen, naproxen and diclofenac are non-steoridal Anti-Inflammatories Drugs used to reduce inflammation. Fluoxetine is an anti-depressants and used for the treatment of major depression. The last compound is diazepam as a Tranquilizers used for the treatment of anxiety, depression and insomnia. The selected pharmaceuticals are the most used compounds and mostly detected in wastewater influents.

2.3.2.2 Personal Care Products

Personal Care Products is a wide range of group substances including, perfumes, colognes, cotton mouthwash, shampoo, fragrances, toothpaste etc. Synthetic musk is one type of personal care product. This synthetic musk is divided into three major classes, aromatic nitro musks, polycyclic musk compounds and macrocyclic musk compounds. CEL, GLX and TON are polycyclic musk compounds containing more than one ring in its molecular structure. From the studies, polycyclic musks may break down the body's defenses against other toxic exposures, and these chemicals are considered as endocrine disrupter compounds (Luckenbach et al., 2005).

2.4. Effects of EDCs Wildlife and Human

At the beginning of the 1960s, Stumm-Zollinger and Fair reported incomplete removal of steroids during treatment of wastewaters (Chang et al., 2009). However, due to extremely low concentrations in the wastewater influents and effluents, it was impossible to analyze these trace contaminants in these years. In order to find a link between exposure to EDCs and the human and environment health much research has since been undertaken. It was rather difficult to draw such links with those days' technology, therefore, EDCs did not attract much attention (Aherne et al, 1985) until the publications on the effects of ethynylestradiol on fish appearing in the literature (Purdom et al., 1994). Since then many research results have been published on the effects on fish and aquatic life. Yet, these reports had limited significance without the knowledge on the levels of EDCs present in the environment. Following the advances in analytical chemistry, the levels of these compounds in environmental samples are better known and it is now possible to predict impacts on the environment (Hohenblum et al., 2004; Kim et al., 2007). Therefore, occurrence of EDCs in the environment and in effluents is becoming more important in the present day and age (Lacey et al., 2011; Crane et al., 2006; Fent et al. 2006; Santos et al., 2006).

Reportedly the most important adverse effects in the environment is associated with the fishes (Nagler 2001, Ternes et al. 1999) based on toxicological tests (Campbell et al., 2006). Toxicity of EDCs were observed in the laboratory with several compounds.In a study Joss et al. 2004, natural hormones, estradiol and estrone, and the artificial hormone, ethinylestradiol, were held responsible for significant endocrine-disrupting effects seen in an aquatic environment. Hong et al. (2007), showed that Diclofenac, whose concentrations was just around 1 μg/L, has been found to cause vitellogenin induction in male fish. Moreover, declining effect in vulture population in India was attributed to this compound. Butyl benzyl phthalate (BBP), which is a common EDC originating from phthalate esters, reportedly caused developmental and testicular toxicity as well as malformations

and embryonic deaths in mice and in rats (Ema et al. 2002; Gray et al. 2000; Piersma et al. 2000). Moreover, it is reported that BBP possesses significant reproductive toxicity as it causes sperm production decrease and alters sexual development in newborn child, and induces genomic changes in rat mammary gland (ECHA, 2008). Dallinga et al. (2002) demonstrated the relationship between lower sperm counts and high concentrations polychlorinated biphenyls (PCB), which are also classed as EDCs. The musk Fragrances, which are absorbed through human skin and are proved carcinogenic in a rodent bioassay (Bolong et al., 2009).

As concluded from the above studies, many parts of wild-life and human are affected from the chemicals considering as EDCs. This affected parts include brain, immune system, cardiovascular system, lungs, mammary glands, liver, kidneys, reproductive tract (ovaries, testes, uterus, prostrate), adipose tissue, and bones (Chang et al., 2007; Muller, 2004). Results of exposure from EDCs are reduced fertility, spontaneous abortion, skewed sex ratios, male and female reproductive tract abnormalities, precocious puberty, polycystic ovary syndrome, neurobehavioral disorders, impaired immune function and a wide variety of cancers (McKinlay et al. 2008).

2.5. Methods for Analyzing of EDCs

Accurate analysis of EDCs is very important due to their very low concentrations which puts great pressure on the analyst. For example since the sun light can disrupt the structure of these compounds clean amber glasses should be used during sample collection. Another important aspect is the sampling time and procedure. Since their concentrations are very low, composite samples should be taken in order to overcome variations in time. Once samples are collected they should immediately be transferred to the laboratory at 4 ^{o}C and extraction should start as early as possible to overcome biological degradation.

Bio-analytical techniques are important tools under development for monitoring certain EDCs. These techniques employ a biological end point. The simplest methods are receptor binding assays and cellular bioassays that have rapid response and high sensitivities at relativity low costs (Routledge and Sumpter, 1997; Snyder *et al.*, 2003). In these techniques, the estrogenic activity of an environmental matrix may be assessed by measuring positive response of a bioassay without the need for identifying the individual estrogenic contaminants. Within these bioassay techniques the yeast estrogen screen (YES) and MCF-7 breast cancer cell assay (E-Screen) are the most common ones (Soto et al., 1995, Holbrook, 2003). Similar to detection by analytical instruments, this method too employs a prior liquid-liquid or solid phase extraction procedure before analysis. The concentrated extract is then introduced to the bioassay medium and the estrogenic activity of the concentrated sample is then quantified. The YES method is the most commonly used bioassay for wastewater applications although it has certain limitations in quantifying estrogenic activity.

The main technique of quantification of EDCs in wastewaters and natural waters is by using instrumental analyses. Since there are numerous endocrine disrupting compounds, it is not possible to analyze all by a single analysis technique. Therefore, relatively limited number of compounds may be analyzed routinely with a single analytical method. With the advent of analytical chemistry, 10^{-9} g/L can now be detected in environmental samples by chromatographic and mass-based detection methods. However, before applying instrumental mass-based analytical methods, extraction steps including pre-cleaning pre-concentration should be applied to prepare and concentrate samples for detection by mass-based chromatography (Liu et al., 2004). Samples should be extracted as soon as possible to overcome biodegradation and accumulation on the bottle wall. Conventional extraction techniques, liquid/liquid extraction, soxhlet and steam distillation are not effective due to high amount of solvent and time consumption. Therefore new extraction methods, such as solid phase microextraction (SPME) and solid phase extraction (SPE) have been developed.

Although SPME developed by Pavliszyn et al., (1990) could be used for the extraction of EDCs from the water and wastewater, it is not effective in the presence of high amount of organics in the sample. In addition, reproducibility is another big problem for application of this method.

SPE is the most common technique for the pre-cleaning and pre-concentration of the samples. During the SPE method, cartridge or disk containing appropriate sorbent binds the target compounds. Before passing the samples through the cartridges, cartridges need to be pre-conditioned using ultrapure water and solvent. Pre-filtered Liquid samples are passed through 0,45 μm glass-fiber filters to overcome impurities before passing through the spe cartridges. Then, a stream of nitrogen or air is used to dry the cartridge sorbents. At the time of analysis, compounds held on the sorbent are eluted by a small amount of solvent. Next, solvent is evaporated by nitrogen gas to a small volume for injection into the instrument. Variety of cartridges exist in the market for sample enrichment, however the most commonly used brand is OASIS HLB.

Following SPE pre-concentration, Gas Chromatography combined with Mass Spectrometry (Regal et al., 2009; Honour 2006; Rhijn et al. 2006) and/or liquid chromatography with mass spectrometric (MS) detector (Ternes *et al.*, 1998; Snyder *et al*., 1999; Heberer and Dumnbier, 2000) has been used. However, for GC applications, derivatization following extraction is often required. By this way it should be possible to detect lower than 1 μg/L EDCs (Bruchet *et. al.,* 2002) in environmental samples. However, derivatization step is time consuming and complicated and the derivatives produce are often unstable over time. For these reasons, LC/MS/MS has gained importance in analysis of EDCs at very low concentrations. Since derivatization is not needed in LC/MS/MS, it provides a rapid and easier method for the detection of EDCs. In spite of these advantages LC/MS and LC/MS/MS systems are high priced instruments and are not supported by compound libraries (Thomaidis et. al., 2007). The analyses of EDCs

in water and wastewater samples employ both instruments. The information of analyses of EDCs are summarized in Table 2.5.

Table 2.5. Information of analyses of EDCs

Compounds	**Instrument**	**Extraction**	**Limit of detection (ng/L)**	**Reference**
Diltiazem	LC-MS/MS	SPE	5	Choi et al., 2008
	GC/MS	SPME	18.9	Carballa et al 2004
	GC/MS	LLE	10	Soliman, 2003
Progesterone	GC/MS	SPE	2	Esperanza et al., 2007
	GC/MS	LLE	10	Soliman, 2003
	GC/MS-MS	SPE	2	Belfroid et al. 1999
Estrone	LC-MS/MS	SPE	15	Gentili et al. 2002
	LC-MS/MS	SPE	1.2	Komori et al. 2004
	GC/MS	SPE	1	Esperanza et al., 2007
	GC/MS	SPME	0.5	Carballa et al 2004
	GC/MS	LLE	10	Soliman, 2003
CBZ	GC/MS	SPE	30	Gomez et al.,2007
	LC-MS/MS	-	5	Radjenovic et al., 2009
	GC/MS	SPME	22.2	Carballa et al 2004
	LC-MS/MS	-	23	Radjenovic et al., 2009
Acetaminophen	GC/MS	SPE	32	Gomez et al.,2007
	LC-MS/MS	SPE	5	Choi et al., 2008
CEL	GC/MS	SPE	30	Bester, 2004
	GC/MS	SPME		Garicano et al., 2003
GLX	GC/MS	SPME	1.2	Carballa et al 2004
TON	GC/MS	SPE	3	Bester, 2004
	GC/MS	SPME		Garicano et al., 2003
	GC/MS	SPME	1.8	Carballa et al 2004
DZP	GC/MS	LLE	25	Soliman, 2003

Table 2.5. Information of analyses of EDCs (cont'd)

Compounds	**Instrument**	**Extraction**	**Limit of detection (ng/L)**	**Reference**
NPX	LC-MS/MS GC/MS	- SPME	20 6.7	Radjenovic et al., 2009 Carballa et al 2004.
DCF	GC/MS LC-MS/MS GC/MS	SPE - SPME	100 29 16.7	Gomez et al.,2007 Radjenovic et al., 2009 Carballa et al 2004
ERY	LC-MS/MS GC/MS	- SPME	4 6.7	Radjenovic et al., 2009 Carballa et al 2004
ROX	LC-MS/MS	SPE	1.2	Serrano et al., 2011
SMX	LC-MS/MS	-	0,5	Radjenovic et al., 2009
TMP	LC-MS/MS LC-MS/MS	- SPE	1.7 10	Radjenovic et al., 2009 Choi et al., 2008
FLX	LC-MS/MS	-	10	Radjenovic et al., 2009

2.6. Removal of EDCs in Wastewaters

Recent developments in analytical chemistry and observations of negative effects of some micro-pollutants on the wild-life, research has now focused on the EDCs removal during treatment. Desbrow et al. (1998), and Song et al. (2009) noted that sewage treatment work (STW) effluent are the major source of pollution by EDCs in the ecosystem due largely to the fact that STWs are not able to reduce these compounds to levels lower than the known effective concentrations for fish. As such, the most pressing issue is to identify the effective treatment methods which can remove these compounds from wastewaters. Alternatively, identification of non-degradable or poorly degradable EDCS in STWs should place a pressure on the pharmaceutical industry to replace those with the degradable ones.

Treatment of EDCs have been studied in conventional activated sludge (CAS) and biological nutrient removing (BNR) activated sludge systems, which are currently the most established treatment processes in the world. The removal of EDCs is dictated by the physicochemical characteristics of these compounds. Their treatment is mainly by two mechanisms; biodegradation and adsorption by sludge. In the absence of degradation in the anaerobic digesters; crops receiving treated sludge as soil conditioner may be adversely affected and may accumulate these and pass them to the food cycle. The studies on removal of EDCs in WWTPs are summarized in Table 2.6.

Table 2.6. Studies on removal of EDCs in WWTPs

Compounds	Location of Study	Removal %	Type of treatment	Reference
Diltiazem	TanCheon, Korea (FS)	-	CAS	Choi et al., 2008
	JungRang, Korea (FS)	-	CAS	Choi et al., 2008
	NanJi, Korea (FS)	-	CAS	Choi et al., 2008
	SeoNam, Korea (FS)	-	CAS	Choi et al., 2008
Progesterone	USA (PS)	99	CAS	Esperanza et al., 2007
Estrone	Kristianstad WWTP, South Sweden (FS)	78	CAS+Chemical treatment	Zorita et al. 2009
	U.K.	88	CAS	Ternes et al. 1999
	USA	64	CAS	Snyder, 2002
	WWTPs, Korea	87,1	CAS+BNR+UV	Behera et al., 2011
	USA (PS)	99,9	CAS	Esperanza et al., 2007
CBZ	Netherlands	9	CAS	Heberer 2002
	Austria	-	CAS	Clara et al., 2005
	Terrassa, Spain (FS)	-	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	-	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	-	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	9,5	CAS	Rosal et al., 2010
	WWTPs, Korea	23,1	CAS+BNR+UV	Behera et al., 2011

Table 2.6. Studies on removal of EDCs in WWTPs (cont'd)

Compounds	Location of Study	Removal %	Type of treatment	Reference
CBZ	TanCheon, Korea (FS)	50	CAS	Choi et al., 2008
	JungRang, Korea (FS)	50	CAS	Choi et al., 2008
	NanJi, Korea (FS)	-	CAS	Choi et al., 2008
	SeoNam, Korea (FS)	50	CAS	Choi et al., 2008
	Galicia, Spain (PS)	99	SBR (+AC)+Membrane	Serrano et al., 2011
Acetaminophen	Belgium (LS)	99,9	MBR	Gusseme et al.,2011
	Spain (FS)	99	CAS	Gomez et al.,2007
	Terrassa, Spain (FS)	99	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	99,9	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	99,9	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	100	CAS	Rosal et al., 2010
	WWTPs, Korea (FS)	99,9	CAS+UV	Behera et al., 2011
	TanCheon, Korea (FS)	99	CAS	Choi et al., 2008
	JungRang, Korea (FS)	99	CAS	Choi et al., 2008
	NanJi, Korea (FS)	99	CAS	Choi et al., 2008
	SeoNam, Korea (FS)	99	CAS	Choi et al., 200
	Dortmund, Germany (FS)	64	CAS	Bester, 2004
	De Bilt, Netherlands (FS)	32	CAS	Garicano et al., 2003
CEL	Nieuwegein Netherlands (FS)	42	CAS	Garicano et al., 2003
	Overvecht Netherlands (FS)	12	CAS	Garicano et al., 2003
	Zeist Netherlands (FS)	57	CAS	Garicano et al., 2003

Table 2.6. Studies on removal of EDCs in WWTPs (cont'd)

CEL	Overvecht Netherlands (FS)	12	CAS	Garicano et al., 2003
	Zeist Netherlands (FS)	57	CAS	Garicano et al., 2003
	Galicia, Spain (FS)	82	CAS	Carballa et al. 2007
	U.K.	39-94	CAS	Kanda et al.,2003
GLX	Switzerland	75	CAS	Kupper et al.,2004
	Germany	61-72	Selector+ CAS	Fahlenkamp et al., 2004
	Dortmund, Germany (FS)	63	CAS	Bester, 2004.
	Austria (FS)	80	CAS	Clara et al.,2004
	Germany	71-82	Selector+ CAS	Fahlenkamp et al., 2004
TON	De Bilt, Netherlands (FS)	15	CAS	Garicano et al., 2003
	Nieuwegein Netherlands (FS)	54	CAS	Garicano et al., 2003
	Overvecht Netherlands (FS)	22	CAS	Garicano et al., 2003
	Zeist Netherlands (FS)	56	CAS	Garicano et al., 2003
	Galicia, Spain (FS)	83	CAS	Carballa et al. 2007
DZP	Germany (FS)	68	CAS	Rodrigues 2003
	Austria (FS)	0-25	Selector+ CAS	Krauzinger et al., 2004
	Kristianstad WWTP, South Sweden (FS)	99	CAS+ Chemical	Zorita et al. 2009
IBP	Almeria Spain (FS)	99	CAS	Gomez et al.,2007
	Terrassa, Spain (FS)	99	CAS	Radjenovic et al., 2009

Table 2.6. Studies on removal of EDCs in WWTPs (cont'd)

IBP	Terrassa, Spain (PS)	99	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	95	CAS	Rosal et al., 2010
	WWTPs, Korea (FS)	98.2	CAS+BNR+UV	Behera et al., 2011
	Galicia, Spain (FS)	63-67		Carballa et al 2004
NPX	Kristianstad WWTP, South Sweden(FS)	94	CAS+ Chemical treatment	Zorita et al. 2009
	Terrassa, Spain (FS)	72	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	91	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	91	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	61	CAS	Rosal et al., 2010
	WWTPs, Korea	95.7	CAS+BNR+UV	Behera et al., 2011
	Galicia, Spain (FS)	46-50		Carballa et al 2004
DCF	Kristianstad WWTP, South Sweden(FS)	-105	CAS+Chemical Treatment	Zorita et al. 2009
	Almeria, Spain (FS)	59	CAS	Gomez et al.,2007
	Terrassa, Spain (FS)	22	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	65	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	63	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	5	CAS	Rosal et al., 2010
	WWTPs, Korea	81.4	CAS	Behera et al., 2011

Table 2.6. Studies on removal of EDCs in WWTPs (cont'd)

	Terrassa, Spain (FS)	35	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	43	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	25	Hollow fiber MBR	Radjenovic et al., 2009
ERY	Alcala´de Henares, Spain (FS)	4,3	CAS	Rosal et al., 2010
	Galicia, Spain (PS)	99	SBR (+AC)+Membrane	Serrano et al.,2011
ROX	Galicia, Spain (PS)	99	SBR (+AC)+Membrane	Serrano et al.,2011
	Terrassa, Spain (FS)	73	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	80	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	78	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	17,3	CAS	Rosal et al., 2010
SMX	WWTPs, Korea	51.9	CAS+BNR+UV	Behera et al., 2011
	TanCheon, Korea (FS)	43-83	CAS	Choi et al., 2008
	JungRang, Korea (FS)	58-91	CAS	Choi et al., 2008
	NanJi, Korea (FS)	33-80	CAS	Choi et al., 2008
	SeoNam, Korea (FS)	16-71	CAS	Choi et al., 2008

Table 2.6. Studies on removal of EDCs in WWTPs (cont'd)

TMP	Terrassa, Spain (FS)	40	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	66	Flat sheet-MBR	Radjenovic et al., 2009
	Terrassa, Spain (PS)	47	Hollow fiber MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	5,4	CAS	Rosal et al., 2010
	WWTPs, Korea	69	CAS+BNR+UV	Behera et al., 2011
	TanCheon, Korea (FS)	35-99	CAS	Choi et al., 2008
	JungRang, Korea (FS)	76-99	CAS	Choi et al., 2008
	NanJi, Korea (FS)	0-99	CAS	Choi et al., 2008
	SeoNam, Korea (FS)	0-75	CAS	Choi et al., 2008
	Galicia, Spain (PS)	99	SBR (+AC)+Membrane	Serrano et al.,2011
FLX	Kristianstad WWTP, South Sweden(FS)	>90%	CAS+ Chemical treatment	Zorita et al. 2009
	Terrassa, Spain (FS)	33	CAS	Radjenovic et al., 2009
	Terrassa, Spain (PS)	98	Flat sheet-MBR	Radjenovic et al., 2009
	Alcala´de Henares, Spain (FS)	62	Hollow fiber MBR	Rosal et al., 2010
	Galicia, Spain (PS)	99	SBR (+AC)+Membrane	Serrano et al.,2011

As seen in this table, removal of a particular compound may be different in different studies. These may be explained by the operating parameters of the particular treatment plants, such as sludge age or hydraulic residence times (Clara et al., 2005), and environmental conditions such as temperature and light intensity (Andreozzi et al., 2003) employed in a particular plant. In addition to these parameters, concentration of the compounds, seasonal variations are reported to effect the removal efficiencies (Castiglioni et al.,2006; Clara et al., 2005b; Vieno et al., 2007). Carballa et al., (2005) has reported that conventional activated sludge process was not effective in removing PPCPs. Higher temperature supported higher PPCPs removals, ranging from 10 to 40 % (Carballa et al., 2005).

In addition to the conventional systems, much research have been carried out to improve EDCs removal in WWTPs by including additional units to the treatment trains (Batt et al., 2007; Nakada et al., 2007; Ternes et al., 2003), such as UV and activated carbon. Advanced treatment technologies including ultrafiltration and membrane bioreactors have been investigated for the removal of EDCs. However, conclusive evidence does not exist to show any effects on EDCs removal by UV (Drewes et. al. 2002; Ternes et. al. 2002). A 90% removal of pharmaceuticals has been reported with a chlorine dose of 1 mg/L and 40 min contact time (Adams at. al.2002). However, addition of chlorine did not entirely remove EDCs that was present in the wastewater but converted them into chlorinated by-products (Moriyame et. al. 2004).

Micro and Ultrafiltration, which are used to improve treatment, provide physical barrier capable of high EDC removals, but removals are dependent upon compound structures (size, polarity) and membrane properties. This type of filtration units can remove particles containing EDCs (Capangpangan et al. 1996); but micro and ultrafiltration is not effective on removal of dissolved EDCs. Conversely, nanofiltration is generally able to achieve a good effluent quality where EDC removal is achieved at a higher percentage than those obtained in

micro and ultra filtrations. Disadvantage of the process is the high cost of operation due to huge pressure drop across the membranes.

Activated carbon is very effective for the removal of organic compounds, mainly the non polar compounds (Ying et al., 2004). It is also used to remove many different pesticides, pharmaceuticals, and EDCs (Sacher et al., 2000; West, 2000).Moreover, powdered activated carbon appears to be the most effective adsorbent especially for those substances which are refractory organic, non-biodegradable compounds (Schafer et al., 2003). In addition, it was proven that PAC was more effective for the removal of EDCs than coagulation (Bodzek and Dudziak, 2006). In the recent years MBR plants coupled with activated carbon filters were studied. The post granular activated carbon application accounted for further 50% reduction in EDCs (Bodzek and Dudziak, 2006).

Reverse osmosis (RO) is most effective removal method for EDCs since cut off point is less than 0,01 nm, where even mineral ions are removed at this pore sizes. The removal percentage of EDCs by RO is variable between 77- 99% for different membranes, but it is mostly over 90% (Schafer et al. 2003). However, investment and maintenance costs are very high for RO treatment of wastewaters.

The MBR is considered forefront of wastewater treatment and reuse technology due to its many advantages over CAS process (Spring, 2002). Moreover, re-use standards can be met by MBR technologies (Howell, 2004; Visvanatan, 2000). MBRs are mainly used to obtain high effluent quality and to remove suspended solids and pathogens completely from wastewaters since solid liquid separation is excellent in microfiltration or ultrafiltration. The main difference between MBR and CAS is the final separation step; where MBR uses microfiltration or ultrafiltration to separate solid phase from the liquid while CAS depends on gravity settling for phase separation. In MBR, longer SRTs and higher sludge concentrations may be maintained in the aeration tank as compared to the

CAS system. Permeate leaving the MBR system is usually sterile. However, like the other treatment processes, MBR plants are not design to remove EDCs from wastewaters. Removal of EDCs by MBR systems have been thoroughly studied (Hai et al., 2011, Cirja et al., 2008, Weiss and Reemtsma 2008; Clara et al., 2005). Studies have shown that MBRs can remove some of the hormones and pharmaceuticals by up to 99% (Kim et al.2007). They showed that their MBR system could 99% remove the natural hormones including, estriol, testosterone, and certain pharmaceuticals e.g., acetaminophen, ibuprofen, and caffeine. However, erythromycin, trimethoprim, naproxen, diclofenac, and carbamazepine were not studied by Kim et al., 2007.

In a different study, MBR was found capable of achieving greater EDCs removal, as compared to CAS, because of better solids removal during filtration and the very long SRTs employed (Wintgens et al.,2002). However, the ability to remove EDCs in MBRs has not been adequately quantified according to Holbrook et al. 2002. Kimura et al. (2005) found that MBRs exhibited much better removal regarding ketoprofen and naproxen compared to conventional treatment systems.

CHAPTER 3

MATERIALS AND METHODS

3.1. Introduction

In this chapter, four flat sheet membrane bioreactor (MBR) used in this study are described. Although all the plants are flat sheet, each has different configuration and treatment capacity. After description of the MBR plants, the sampling and conservation of the selected compounds is given.

The general parameters measured in influent, sludge and effluent during the studies in Turkey and in Spain are explained in this chapter. These parameters include total suspended solid, volatile suspended solid, oxygen, pH, ammonia, turbidity, nitrite, nitrate and chemical oxygen demand. Standard methods were used during these experiments.

Information of chemicals used during the studies and selected micropollutants is also described in this chapter. Extraction and pre-concentration methods for selected Endocrine Disrupter Compounds (EDCs) from wastewater to be employed are presented. Lastly, analytical methods used for the measurement of selected EDCs are described. Information of analysis in Turkey and Spain given separately since different methods and equipments were used during the study.

3.2. Description of MBR Systems Studied

In this study, four different MBR plants were used to see the removal efficiency of the selected EDCs. Two were full-scale plants and two were pilot

scale. Reasons for using pilot MBRs in the study were several. For example changing operational parameters in a pilot-scale is very easy compared to a full scale. Moreover spiking of selected EDCs for a controlled study is only possible in a pilot-scale rector when these compounds are under limit of detection. Pilot MBR was most appropriate to study effects of activated carbon on adsorption of these compounds.

First of the two full-scale MBR plants was a flat sheet type Vacuum Rotation Membrane (VRM, HUBER) plant, installed at METU with about 150 m^3/d capacity. The second full-scale MBR plant was located in Southern part of Turkey, Bodrum- Konacık, and is a flat sheet membrane bioreactor (Kubota) with a capacity of about 1200 m^3/d. The first pilot-scale plant was also a flat sheet MBR plant (Membrane Clear Box, Huber), which could handle a flow rate of about 1 m^3/d, placed near to the VRM plant and shared the same influent as with VRM. The last plant was a pilot-scale sequential batch reactor (SBR) coupled with a membrane unit, having a capacity of 0,03 m^3/d, located in Santiago de Compostela, Spain.

3.2.1. Full Scale VRM (Vacuum Ratation Membrane) Plant

The schematic diagram of the VRM membrane bioreactor located in Middle East Technical University Campus is given below in Figure 3.1 (Komesli et al.,2007).

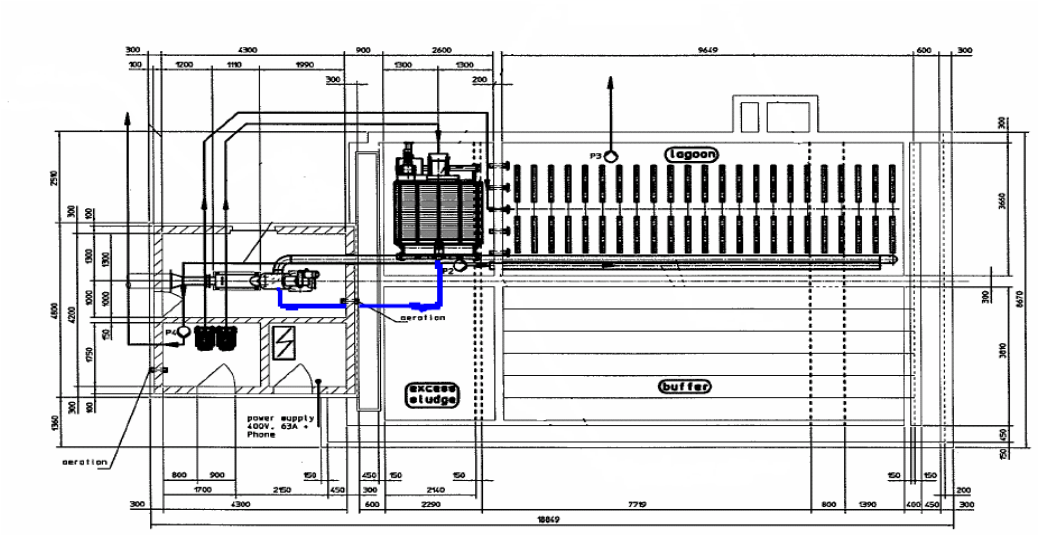

Figure 3.1 Schematic diagram of Full scale VRM-Plant, METU, Turkey.

As seen in Figure 3.1, the plant consists of two tanks and the peripheral equipments. A partitioning wall between the two tanks separates the two tanks. However, the two tanks are connected by five orifices, each controlled manually. The wet volume of the first tank is 85 m^3, and is used for aeration of the biological sludge. The second tank is about 23 m^3 in volume and is used to house the VRM unit.

Wastewater coming from METU academic village and dormitories of ODTUKENT was used in the study. Wastewater from departments and laboratories was not handled. Wastewater from dormitories and academic village was first collected in a 10m^3 holding tank and then pumped to the both treatment plants which were placed about 15 m higher and 50 m away from the storage tank. A 4 cm coarse screen is located at the inlet to the tank. Before entrance of wastewater to the treatment plants, a screw type fine screen with 3 mm openings, Rotomat Ro9 produced by Huber, separates small particles from the wastewater to protect the membranes. The greater particles can not pass through the screen and the screen was cleaned periodically by automation. The fine screen is the only equipment which was not operated by the main control panel. After passing the screen, wastewater enters the aeration tank to contact with the activated sludge.

The aeration tank was aerated by Magnum type 26 tubular membrane diffusers. The tubular diffusers are connected to Rietschle SAH 275/ 5,5 kW blower.

A submerged pump pumps out excess sludge from the aeration tank. The Sludge Retention Time (SRT) of the plant is adjusted by this way. The capacity of the pump is 13 L/sec and the amount of sludge disposed is set by a timer. Temperature and dissolved oxygen concentration were being measured in this tank by using a Jumo dTrans O_2-01 type of oxygen-meter.

Following the aeration tank wastewater passes through the submerged orifices into the VRM chamber and filtered through the flat sheet submerged membranes rotating on a cylindrical membrane holder. On the VRM module 720 membrane sheets exist, which are consist of a trapezoidal polypropylene (PP) base plate with welded on Polyether sulfones (PES) membranes. The total membrane area is 540 m^3. The ultrafiltration membranes have nominal pore sizes of 0.038 μm. Figure 3.2. shows the VRM unit.

Figure 3.2 Vacuum Rotation Membrane Unit (Komesli et al., 2007)

Naming of the VRM (Vacuum Rotation Membrane) module is after rotational movement employed during its operation. The reason for rotation is to reduce the amount of course aeration needed for cross flow over the membrane surfaces. The rotation speed was 2.5 rpm. A Rietschle SAH 275/ 5,5 kW blower is connected to the bottom of this unit to give coarse aeration through diffusers for creating cross-flow and cleaning of plate surfaces. A recirculation pump, identical to the sludge wastage pump, exists at the bottom of the filter chamber to balance sludge concentration between membrane and aeration tanks. The complete plant is controlled via a control panel located in the control room. Picture of the VRM plant is shown in Figure 3.3.

Figure 3.3 The Full-Scale MBR Unit at METU Campus, Turkey.

The VRM module operates batch wise in 5 min cycles. The permeate pump operates for 4 mins. and is off for one minute, at every cycle. Liquid levels of both tans are controlled with level sensors, Vegawell 72. The level sensors are connected to the control panel. The level sensor in the aeration tank is connected to a submerged pump inside the initial storage tank. When the level of liquid drops below a set point in the aeration tank wastewater storage pump operates and

raw wastewater is pumped to the plant. Conversely when the level goes beyond the upper set point pump is deactivated. The second level sensor placed in the VRM chamber sends information to the permeate pump. When the level in the VRM tank is lower than the set point, suction by the permeate pump stops to protect the membrane plates from exposure to air.

Information regarding METU-VRM plant is given in Table 3.1.

Table 3.1 Properties of the METU-VRM Plant

Aeration tank volume	85 m^3
Membrane Unit Volume	23 m^3
Membrane Type	plate and frame
Total Membrane Area	540 m^2
Membrane Material	polyethersulfones (PES)
Nominal Pore Size	0.038 μm
Hydraulic Retention Time (HRT)	15-24 h
Sludge Retention Time (SRT)	10 days
Flux	8.3-13 L/h-m^2
Recirculation Ratio	3.0

As can be seen in Table 3.1, HRT and flux settings were changed deliberately to observe effects of these parameters onto the removal of selected EDCs.

3.2.2. Pilot Scale Flat Type Membrane Bioreactor: Clear Box Plant

A pilot-scale, plate-type biomembrane unit, so called Clear Box, MCB, is the second plant located close to the VRM plant. This plant was also used was used to follow EDCs removal. The plant flow-diagram is given in Figure 3.4.

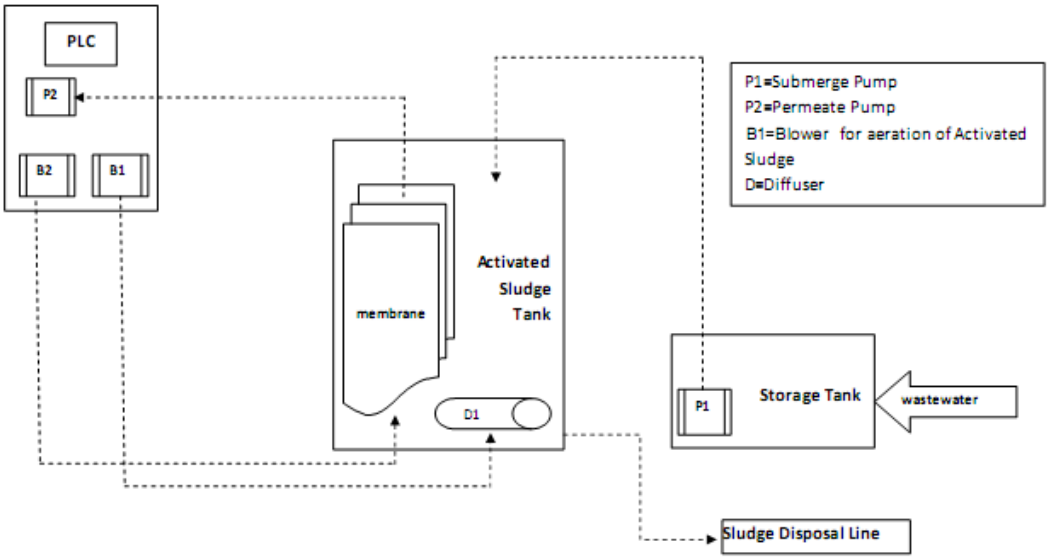

Figure 3.4 Flow Diagram of Clear-Box Membrane Bioreactor

Clear-Box MBR plant is composed of three parts, as seen in Figure 3.4. The first part is the storage tank of the incoming wastewater. Filtered wastewater through the tubular screen is shared between the two plants. After screening of wastewater through Rotomat Ro9, wastewater is channeled to the Clear-Box storage tank. This tank, shown in Figure 3.5, was produced from steel and had about 350 L capacity.

Figure 3.5 Storage tank for wastewater

As clearly seen from Figure 3.5, wastewater was transferred from the storage tank to the MBR tank by means of a submerged pump. In order to prevent overflowing of wastewater over the storage and aeration tanks, two level sensors were immersed in these tanks and were connected in parallel to the delivery pump. A level sensor coupled to the pump was used to protect the pump against dry running.

The second part of the pilot plant is the MBR aeration tank. The volume of this tank was about 750 L and the membrane module was directly submerged in this tank. Identical membranes in quality, as with the full scale VRM unit, were used on this plant with the exception that membranes were on a solid pedestal and were not rotating. The pore size of this membrane was again 0.038μm and membrane material was PES. The total membrane area was 3 m^2 and daily flow handled by the unit was between 600 and 1600 L (Figure 3.6).

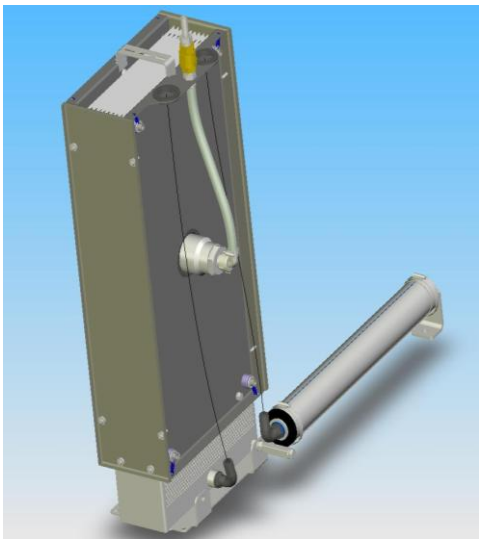

Figure 3.6 Membrane Clear Box (MCB) System

As it is seen in the Figure 3.6, permeate was sucked from the middle of the module by a vacuum pump. Membrane plates were being held inside a steel box to protect them. In addition, a cross flow was maintained by employed course aeration from the bottom of the module to create turbulence. The aeration tank was equipped with a membrane-type diffuser, as seen in Figure 3.6. The aeration tank of the MBR plant is shown in Figure 3.7.

Figure 3.7 Clear-Box MBR Plant

As seen in Figure 3.7, another level sensor is employed in this tank to protect the membrane module against drying. When the level decreases under the set level, it signals to the permeate pump to stop. Additionally, a level sensor connected to the submerged pump inside the storage tank, is activated when the level of liquid drops to a minimum value in the aeration tank. Excess sludge is manually discharged from the aeration tank.

The third part is the control panel of the plant, as seen in Figure 3.8.

Figure 3.8 Control panel of the Clearbox- MBR

The MBR plant control panel contains separate blowers for aeration and cross flow, a suction pump, flowmeter, and a pressure gauge for measuring transmembrane pressure. The flow rate was adjustable from the control unit. Operating cycle was 210 sec suction and 30 sec relaxation without suction. The upper blower shown in Figure 3.8 is used for aeration and the other was used for course aeration of the membrane module. The properties of all the pilot plant is summarized in Table 3.2.

Table 3.2 Properties of the Clear-Box Pilot Plant

Storage tank volume	350 L
MBR tank volume	750 L
Membrane Type	plate and frame
Total Membrane Area	3 m^2
Membrane Material	polyethersulfones (PES)
Nominal Pore Size	0.038 μm
Hydraulic Retention Time (HRT)	10-24 h
Sludge Retention Time (SRT)	10-25 days
Flux	8.3-13 L/h-m^2

As seen in Table 3.2, properties of the Clear-Box system were close to the VRM Plant. The SRT was changed between 10 to 25 days to see its effects on the removal of selected EDCs.

3.2.2.1 Sample Collection from VRM and Clear-Box MBR Plants

During some part of the study, 24 hour composite samples from influent and effluent of pilot scale MBR were collected by a pump and stored in a refrigerator. VRM Composite effluent samples, before and after UV treatment, was collected for 24 hours in the same manner, as seen in Figure 3.9.

Figure 3.9 Collection of composite sample

Small peristaltic pumps, 13mL/min, connected to a timer were used to get the samples. Influent was collected from just after the fine screen and effluents were collected from the sampling taps.

Due to extremely low concentrations of some selected EDCs, which were falling below the limit of detection, these could not be measured at all in the wastewater. Therefore, analyses of some selected EDCs directly from the wastewater were not possible. A concentrated EDC solution had to be spiked to the storage tank of Clear-Box, so as to bring these to detectable concentrations. Spiked samples were drawn from inlet and outlet of the plant, as well as the aeration tank of the Clear-Box MBR unit. No spiking was carried out for the VRM Plant. After collecting samples, these were transferred to the laboratory in glass amber bottles, in an ice-box to prevent any decomposition.

3.2.3. Konacık, Bodrum MBR Wastewater Treatment Plant

The third plant studied was a full-scale flat-sheet type membrane bioreactor located in Konacık-Bodrum, Turkey. This wastewater treatment plant has been operated by the Konacık Municipality since 2007. The composition of wastewater was almost totally municipal and did not include any industry. Total flow of influent was about 1100 m^3/day. The flow diagram of the treatment plant is given in Figure 3.10.

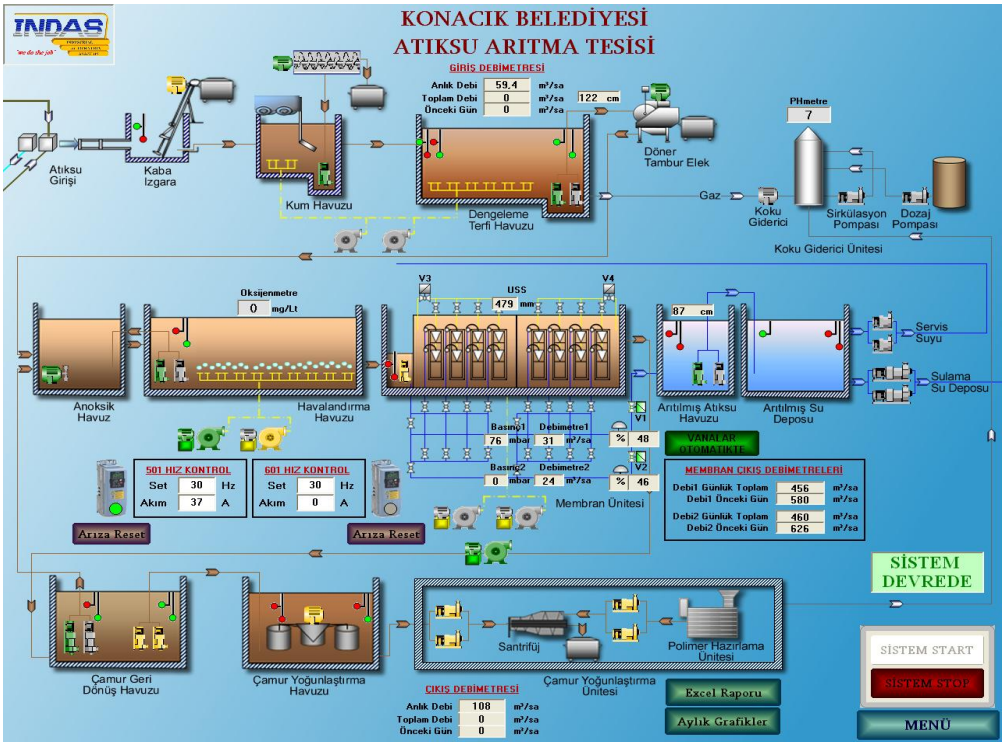

Figure 3.10 Flow Diagram of Konacık MBR Wastewater Treatment Plant

As seen in Figure 3.10, four tanks exist in the system. These are equalization, anoxic, aerobic and membrane tanks. At the entrance of the plant, a coarse screen and a grit chamber are located. An equalization tank of about 115 m^3 volume is used to balance shock loads during treatment. Wastewater passes through fine screens to protect the membrane. A pre-anoxic tank exists before the aeration tank in order to remove nitrogen from the wastewater. The volume of the

anoxic tank is 180 m^3. The calculated HRT for the anoxic zone is about 4 hours. Volume of the aerobic tank is 600 m^3. The nominal HRT of this tank is. The last tank in the treatment train is the membrane chamber. This is consist of two basins with a total volume of 128 m^3. Flat sheet membranes of Kubota make, having a total surface area of 2560 m^2 and 0.04 μm pore size, was used to separate solids from the treated. The view of aeration tank of the plant is given in Figure 3.11.

Figure 3.11 The view from Konacık MBR Wastewater Treatment Plant

Konacık MBR Wastewater Treatment Plant is being controlled automatically by a control panel. The properties of the treatment plant are given in Table 3.3.

Table 3.3 Properties of Konacık-Bodrum MBR Plant

Equalization Tank	115 m^3
Anoxic Tank	180 m^3
Aerobic Tank	600 m^3
Membrane Chamber	64*2 = 128 m^3
Membrane Type	plate and frame
Total Membrane Area	2560 m^2
Nominal Pore Size	0.04 μm
Hydraulic Retention Time (HRT)	16-20 h
Sludge Retention Time (SRT)	25 days
Flux	18 L/h-m^2

All the analysis, except for EDCs, was carried out in the Laboratories of Konacık Municipality.

3.2.4. SBR+Membrane Pilot Scale Plant, Spain

The last membrane process studied in this thesis work was a sequencing batch reactor (SBR) combined with an external submerged membrane. This unit was in University of Santiago de Compostela, Spain. SBR is a modified version of the conventional activated sludge process, designed to operate under semi steady-state conditions. This system is operated in a batch mode where aeration and settling is achieved in the same tank, though different in time. Following aeration, settling period is initiated. The settled supernatant is then transferred to the membrane chamber for polishing purpose.

A flat sheet type membrane was used in this system. The schematic diagram of the system is given in Figure 3.12.

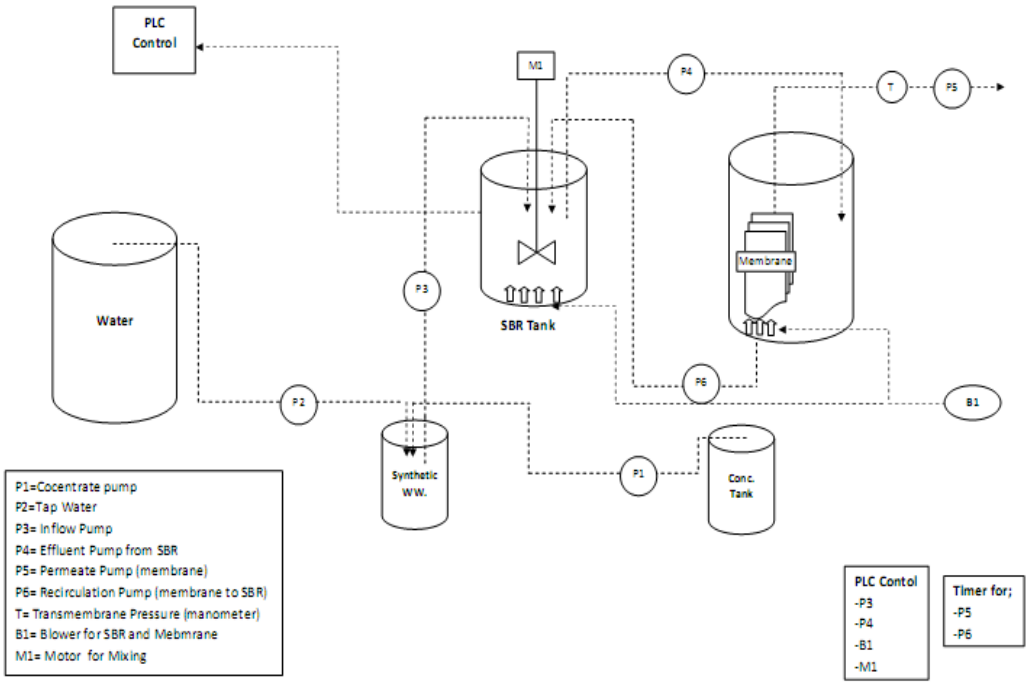

Figure 3.12 Flow Diagram of SBR+MBR Wastewater Treatment Plant

As seen in Figure 3.12, the system was composed of three parts. The first part was the synthetic wastewater preparation tank. The second part was the SBR tank and the last part was the membrane unit chamber.

The first tank of the system contains preparation equipments for the synthetic wastewater feed, 30 L daily. Three tanks and two pumps exist for the preparation of synthetic wastewater. The first tank was the concentrate tank which included organics, nitrogen, phosphorus, and other nutrients and selected endocrine disrupting compounds. The composition of one liter concentrated influent water is given in Table 3.4.

Table 3.4 Concentration of compounds in concentrated wastewater

Compound	**C (g/L)**
$CH_3COONa.3H_20$	22.86
NH_4Cl	3.34
KH_2PO_4	0.39
$NaHCO_3$	4.45

Additionally, 1 mL trace element was added to each liter of concentrated wastewater. The complete composition trace elements are given in Table 3.5.

Table 3.5 Concentration of trace elements in concentrated wastewater

Compound	**C (g/L)**
$FeCl.6H_2O$	1.5
H_3BO_3	0.15
$CuSO_4.5H_2O$	0.03
KI	0.03
$ZuSO_4.7H_2O$	0.12
$CoCl_2.6H_2O$	0.15
$MnCl_2.4H_2O$	0.12

Following the preparation of the concentrated wastewater, these were placed in a 20 L tank. A peristaltic pump of Masterflex make, P1, was used to transfer concentrated water into the mixing tank. The flowrate of P1was fixed and could be regulated by a timer to give 1,87 mL/min. The second tank was tap water tank and the volume of this tank was about 200 L. A same type of peristaltic pump with an adjustable flow rate was used to transfer tap water into the mixing tank. The flow rate of this pump was set as 19 mL/min, and was operating continuously. The last tank was the mixing tank where concentrated and tap water were mixed together. The peristaltic pump, connected to a PLC, was used to transfer the synthetic wastewater form this tank to the SBR tank. Flow rate of the

pumped water was 535 ml/min and transferred 7.5 liter of synthetic wastewater into the SBR tank at each cycle.

The SBR tank, constituting the second part of the system, was being controlled by the PLC unit. The SBR was operating on 4 cycle per day. Cycle time was 6 hours with anoxic, aerobic, react, settling/decanting and withdraw phases. The time schedule for the SBR unit is given in Table 3.6.

Table 3.6 Time schedule for SBR operation.

Process Cycle	**Time (min)**
Inflow Pumping	14
Mixing	70
Aeration	210
Settling	42
Withdraw	14

After transferring wastewater to the SBR tank, the mixing motor with a speed of 60 rpm was started to mix the activated sludge and wastewater during the anoxic cycle. This period occurs without aeration to create an anoxic environment for denitrifies in order to remove nitrogen in the system. Mix phase continued for 70 minutes during each cycle. Upon completion of this period, aeration was turned on for 210 min. During aeration fine bubbles were used to aerate the mixed liquor. The stirrer also continued mixing the contents in the SBR tank. Nitrification occurred during aerated phase. This period was followed by the settling stage, which took 42 mins. Aeration discontinued during settling and solids separated from the liquid at this phase.

This system was somewhat differed from conventional SBR; since in conventional SBR, the MLSS concentration is usually about 3-4 g/L and settling period runs between 5-10 minutes with a clear supernatant due to excellent solids separation. However, in the present system settling time was too long compared to

the conventional, owing to high sludge concentration of about 8-9 g/L. Moreover, settling time was hardly enough to separate such a high concentration of sludge from the supernatant. Complete separation of supernatant from the sludge was achieved in the membrane chamber. The volume of transferred supernatant also included the recirculation volume of sludge from the membrane chamber during the period. The properties of SBR+Membrane plant are given in Table 3.7.

Table 3.7 Properties of the SBR+Membrane Plant, Spain

SBR Tank	30 L
Membrane Chamber	18 L
Membrane Type	plate and frame
Total Membrane Area	0,2 m^2
Nominal Pore Size	0.4 μm
Hydraulic Retention Time (HRT)	24 h
Sludge Retention Time (SRT)	infinite
Flux	6.25 L/h-m^2

The third part of the reactor was the membrane chamber. In this chamber, 2 flat sheet membrane plates, bought from Kubota, and having a total surface area of 0,2 m^2, was being used. The membrane, having 0.4 μm pore size, is classified as microfiltration membrane. The flow rate through the membrane unit was adjustable by a timer. The suction period during operation cycle of the membrane unit was 7,5 min followed by a 1,25 min relaxation without suction. A continuous course aeration was used for creating a cross flow and to clean membrane surfaces. Suction was created by a Masterflex type of adjustable peristaltic pump, P5. A flux rate of about 6,25 L/m^2-h could be maintained. At the bottom of the membrane chamber, another peristaltic pump, P6, was connected to the SBR tank to recycle sludge. Recycle ratio was about 0,5Q. The SBR+ Membrane pilot plant used in Spain studies is shown in Figure 3.13.

Figure 3.13 The view of SBR + Membrane Unit

3.3. Analytical Methods

The analytical part of the study is divided into two parts. The first part being those applied in Turkey and the second one in Spain.

3.3.1. Analysis of Classical Pollution Parameters in Turkey

The analyses of the following parameters were performed after the start up of both MBR Plants at METU. The analysis of the Konacık-MBR was carried out by Konacık Municipality Laboratory (certificated from TURK-AK) using Standards Methods (APHA, 2001). Two replicates were analyzed for all the parameters.

3.3.1.1 Turbidity

Turbidity in VRM and Clear-Box MBR systems in METU Campus, were measured using Hach 2100 N model turbidimeter. Gelex type turbidity standard, ranging between 0 to 1000 NTU (Cat No. 22955-04) was used to calibrate the turbidimeter for influent analysis and Gelex turbidity standard solution was used for the ranges between 0 to 1 NTU (Cat No. 22955-01), for effluents.

3.3.1.2 Chemical Oxygen Demand (COD)

The COD is the oxidation of all organic compounds with a strong oxidation agent (potassium dichromate) under acidic conditions. During the study, colorimetric HACH Method (5220 D) was used for determination of COD in influent, supernatant of sludge and effluent of both plants in METU campus. The Hach COD kit (Cat No.LCK314) was used for the high range (100 to 2000 mg/L) and (Cat No.LCK315) were used for the low range, 15 to 150 mg/L, analysis. The 2 mL influent samples were placed in test tubes and digested in an electrical heating block for about 120 min at 160 ^{0}C. For the supernatant and effluent samples, the same procedure but this time using a low range COD kit were used. Calorimetric measurements were carried out in a DR5000 dedicated spectrophotometer. The Hach DR5000 spectrophotometer reads directly from the barcode of reagent kits and sets itself to the particular analysis by arranging the calibration curve and wavelength setting.

3.3.1.3 pH

A Hach sension 156 type of pH-meter was used to measure the pH in the influent and effluent samples from the MBR plants at METU Campus. Hach-buffer solution, pH 4 and 9, were used to calibrate the instrument.

3.3.1.4 Temperature and Dissolved Oxygen

In the VRM plant, temperature and dissolved oxygen were measured automatically, in situ, by a Jumo dTrans O_2-01 dissolved oxygen and temperature-meter. The probe of the instrument was submerged in the aeration tank and was continuously monitoring the DO in the tank. The signals from the oxygen meter were being sent continuously to the plc unit on the control panel and every ten minutes to a data-logger. Oxygen and temperature in the Clear-Box MBR plant was being measured manually by using a Yellow Springs model oxygen meter. Prior to analysis temperature of the liquid was measured and oxygen meter was set to this temperature before reading the oxygen.

3.3.1.5 Salinity and Conductivity

Salinity and conductivity were measured by A YSI 33 model conductivity and salinity-meter.

3.3.1.6 Total Suspended Solid (TSS)

Total Solid Suspended measurements in the aeration tank of VRM, filter chamber of VRM, and aeration tank of Clear-Box were performed according to Standard Methods (2540B) (APHA, 2001). Pre-cleaned empty ceramic dishes were weighted first. Then, 50 mL of sample was put inside the dish and evaporated to dryness at 103-105 ^{o}C, in 8-10 hours. Dryed samples were cooled in a dessicator and re-weighed. Difference between the weights of the dried sample and the empty dish were determined and TSS concentration was calculated as follows:

$$TSS = \frac{(M_2 - M_1)}{mL\ of\ sample * 1000\ mL}$$

M_2= mass of MLSS after 105 ^{o}C (g) M_1=mass of filter (g)

3.3.1.7 Mixed Liquor Volatile Suspended Solids (MLVSS)

The MLVSS measurements were performed according to the Standard Methods (2540E) (APHA, 2001). In this method, the dried dishes were placed in a 550 ^{o}C owen for 30 min. Difference between 105 ^{o}C and 550 ^{o}C weights gave the VSS concentration.

3.3.2 Determination of Selected EDCs in Turkey

3.3.2.1 Chemicals and Reagents

Analytical reagent grade chemicals were used throughout this study. LC-MS-grade methanol and acetonitrile, GC-grade toluene and acetone were purchased from Merck (Darmstadt, Germany). Dichloromethylsilane (DCMS) used as silylation reagent was obtained from Aldrich (Milwaukee, WI, USA). Glass-fiber prefilters (0.7 μm pore size, 47 mm diameter) were obtained from PAL Life Sciences (Mexico). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, USA). Formic acid (Merck) and ammonia (Merck) were used in the preparation of mobile phases. All of the chemicals used throughout this study were of analytical reagent grade. The selected EDCs, diltiazem (>99%), and progesterone (>99%) were obtained from Sigma, Benzyl Butyl Phthalate (BBP) (>98%) was obtained from Aldrich, carbamazepine (Cbz) (>99%), acetaminophen (>99%) and estrone (>99%) were supplied by Sigma-Aldrich.

3.3.2.2 Stock Solutions of EDCs

Stock solutions of these compounds were prepared in 1000 ppm stock solutions by first dissolving a known amount in methanol and bringing up to 1 Liter mark by distilled water. They were used in the calibration curve preparation and for spiking influents to Clear-box.

3.3.2.3 Glassware

To overcome contamination and sorption on to the glassware, all the glassware used for EDCs analysis was separated from the other glassware used in the laboratory. In addition, due to the high hydrophobicity of EDCs, all glassware was coated with silane to overcome adsorption problems on to the glass wall. Firstly, dichloromethyl silane (DCMS) prepared in toluene, 10% (v/v) was used for rinsing the glassware. Next, all the glassware were rinsed three times with toluene, followed by three times rinse with acetone. Finally glassware was heated to 150 °C for at least 12 h for fixation of the silylation reagent onto the glass wall. In order to conserve wastewater samples against biological breakdown, solid sodium azide (0.01%, w/v) was added into the bottles.

3.3.2.4 Filtration of Samples

Prior to SPE cartridge application of samples, pre-filtration was undertaken by vacuum filtering samples through glass-fiber prefilters (0.7-µm-pore size, 47 mm diameter), obtained from PAL Life Sciences (Mexico).

3.3.2.5 Solid Phase Extraction (SPE) Apparatus

In liquid samples, concentrations of EDCs are normally too low and below the limit of quantification (LOQ). Moreover, other impurities which would affect chromatographic analysis need to be removed. A method is required not only to remove impurities but also to concentrate selected compounds. Solid phase extraction (SPE) is a powerful method for solving these problems. In this method, one or more components to be analyzed are transferred from the aqueous phase to a more stationary solid phase. Then hydrophobic solvents are used to elute sorbed materials. In this study, influent and supernatant of VRM, Clear-Box and Konacık MBR were first filtered through ordinary filter paper to remove coarse particles and to prevent clogging of glass fiber filters.

For applications, sample volumes of 500-1500mL, chosen according to anticipated compound concentrations, were filtered through glass fiber filters with pore sizes of 0.7 μm. Subsequently, pH of filtrate was adjusted to 7 for highest recovery by the cartridges. Determination of optimum pH 7 was studied as given in Chapter 4. After pH adjustment, filtered samples were loaded to SPE cartridges under vacuum. Oasis HLB SPE Waters, cartridges were used for SPE applications. A 3 mL 60 mg and 6 mL 200 mg, cartridges were pre-conditioned by passing 10 mL methanol and then 10 mL ultra-distilled water. Filtered samples were then passed through cartridge columns at the rate of 10mL/min, under vacuum; followed by drying under vacuum for 15 min. By this way EDCs in the samples were sorbed by the cartridges as shown in Figure 3.14.

Figure 3.14 Suction apparatus for EDCs

After EDCs were absorbed by OASIS HLB SPE, cartridges were eluted by passing 25 mL methanol. Then, stream of nitrogen gas or 42^{o}C temperature was used until the complete evaporation of methanol. In order to match the matrices of both the samples and the calibration standards, compounds were taken into 1 mL methanol and ultra-distilled water mixture (25% methanol v/v). Finally, samples were analyzed by Liquid Chromotography Tandem Mass Spectrometer.

3.3.2.6 Liquid Chromotography Tandem Mass Spectrometer (LC-ESI-MS/MS)

The analysis of EDCs in water and wastewater is difficult due to very low concentrations. In addition, devices used for analysis of EDCs should be accurate and highly sensitive. Initially, gas chromatography with mass spectrometric detector (GC/MS) was intended to be used. However, the selected EDCs were polar and there was a need for derivatization of them before analyzing in GC/MS. Moreover, structural change obtained by derivatization was seen to degrade rapidly making detection difficult and unreliable. Therefore a Liquid Chromatography equipped with tandem mass spectrometer became instrument of choice. This is the most widely used technique for the analysis of endocrine disrupting compounds. During the study Agilent 1200 type HPLC and 6410 type quadropole MS detector consisting of autosampler, degasser, and binary pump equipped with electrospray ionization interface (ESI) were used for the analyses. The ESI was used for ionization of the sample for the measurement of compounds by MS. The tandem mass HPLC used is shown in Figure 3.15.

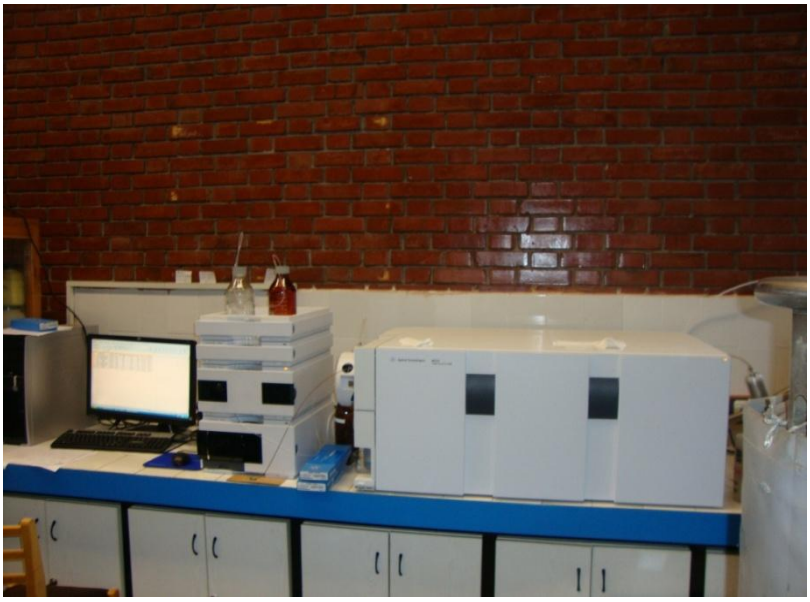

Figure 3.15 LC-ESI-MS system for detection of selected EDCs.

As can be seen in Figure 3.15, there were two mobile phases sitting on the top of the HPLC. These mobile phases, A and B, were used for carrying the sample to the ionizer. In phase A, 5 mμ ammonium format; and formic acid to make 0.1% were added in one liter of ultra-pure Milipore-Q water. In phase B, 0.1% formic acid and 5 mμ ammonium format were added to one liter of methanol. The mobile phase program is given in Table 3.8.

Table 3.8 Experimental separation parameters for HPLC

Parameter	**HPLC**
Mobile Phase	a) 0-0.3 min
	90% Mobile Phase A
	10% Mobile Phase B
	b) 0.3-1.0 min
	90-5.0% of Mobile Phase A
	10-95% of Mobile Phase B
	c) 1-5 min
	5% of Mobile Phase A
	95% of Mobile Phase B
	d) 5-5.1 min
	5-90% of Mobile Phase A
	95-10% of Mobile Phase B
	e) 5.1-10 min
	90% of Mobile Phase A
	10% of Mobile Phase B

The HPLC/MS/MS equipment has its own program for operation and optimization of the parameters. At the beginning of the study, fragmantor voltage (FV), product ions and collusion energies (CE) were optimized for each compound. Column was disconnected from the HPLC-ESI-MS/MS to decrease the analysis time. Two options, positive, $[M+H]^+$, and negative, $[M-H]^-$, ions were

tested during the optimization. First, the liquid sample was introduced through the capillary needle in the nebulizer. In the nebulizer, drying gas caused solvent evaporation and ionization. Then, excess surface charge density increased until natural repulsion between the ions from the droplets (Herbert, 2002). Leaving the nebulizer, sample enters the tandem mass spectrometer which is also named as triple- quadropole MS/MS. The name refers to three separate mass quadropoles in the detector. In the first quadropole (Q1), fragmantor voltage is applied to produce the precursor ions. Then, ions enter the second quadropole (Q2) where applied energy into the collision cell produces product ions. Finally, third quadropole (Q3) scans the mass range for providing mass spectrum of the product ions. As a result of optimization efforts, all the parameters of analysis, mobile phase, columns, injection volume and flowrate, were optimized to obtain high signal to noise ratios (S/N) for every compound. During optimization routine, one parameter was optimized while others were kept constant. After finding the most favorable parameters, the method was applied to the samples to determine selected EDCs in wastewater samples. The optimization studies are given in Chapter 4.

3.3.3 Analysis of Classical Pollution Parameters in Spain

The following general parameters were analyzed during the SBR+ Membrane reactor studies in Spain. Liquid samples included influent, effluent of SBR, and permeate of membrane. Solid samples included activated sludge in SBR tank, membrane chamber and recycle line during the study. Similar to the analysis done in Turkey, Standards Methods (APHA, 2001) were used during these analyses.

3.3.3.1 Chemical Oxygen Demand (COD)

The COD analysis was carried out according to Standard (APHA, 2001, Method # 5220C). Prior to analysis, influent and effluent of SBR, and permeate of

the membrane were filtered through nitrocellulose-fiber filters (Whatman, GFC) with a pore size of 0.45 μm.

3.3.3.2 Ammonium-Nitrogen (NH_4^+-N)

The colorimetric method (Wheatherburn, 1967) was used for determination of ammonia- nitrogen (NH_4-N) in samples. NH_4-N was determined by using Shimadzu UV-1603, UV Visible Spectrophotometer. In order to measure the NH_4-N, phenol-nitroprusiate solution and hypochlorite solution prepared previously were added into the sample. The preparation of the solutions is given below.

Preparation of Solutions

Phenol-nitroprusiate: 15 g phenol and 0.05 g sodium nitroprusiate were dissolved in 250 mL of buffer solution. The buffer solution was prepared by dissolving 30 g $Na_3PO_4.12H_2O$, 30 g $Na_3C_6H_5O_7.2H_2O$ and 3 g EDTA in 1000 mL distilled water and adjusted to pH 12.

Hypochloride solution: 15 mL of commercial bleach was mixed with 1 N 200 mL NaOH and filled up to 500 mL with distilled water.

After preparation of the solutions, 1 mL and 1.5 mL of Phenol-nitroprusiate and Hypochloride solutions were added to 2.5 mL of sample, respectively, and waited for 45 min. at room temperature. The color developed was measured in spectrometer at 635 nm. The calibration curve drawn between 0-1 mg NH_4-N/L is shown in (Figure 3.16).

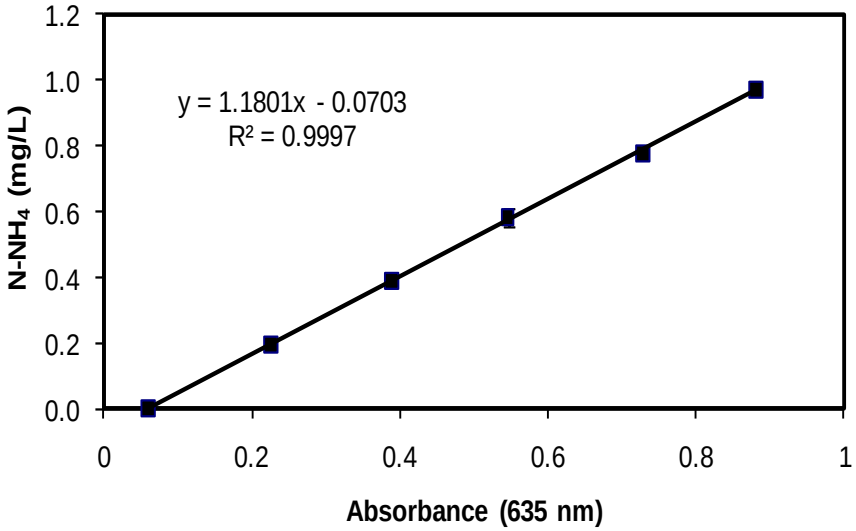

Figure 3.16 Calibration curve for ammonium-N determination.

3.3.3.3 Nitrite-Nitrogen (NO_2^--N)

Nitrite is the first oxidation form of NH_4^+. In order to determine NO_2-N^- in the sample, Method No 4500- NO_2-B^- described in Standard Methods for the Examination of Water and Wastewater was used.

The calibration curve is presented in Figure 3.17.

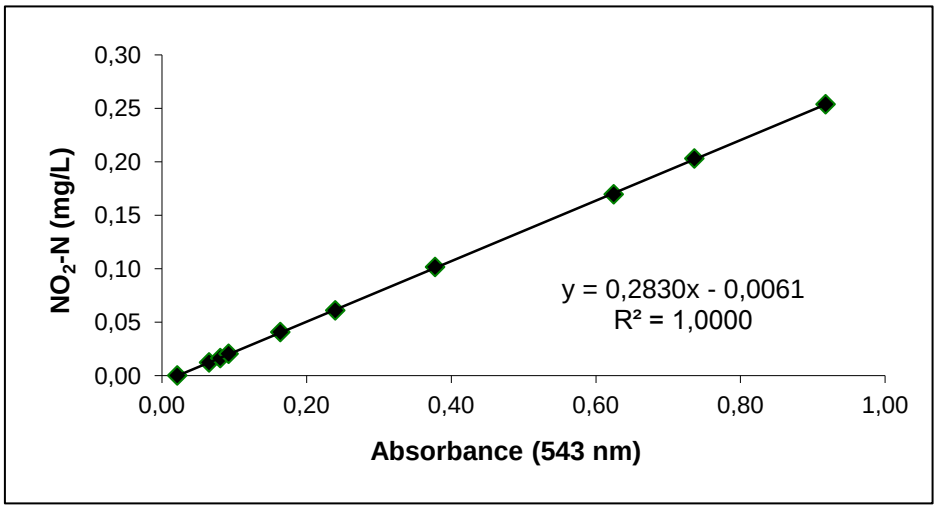

Figure 3.17 Calibration curve for nitrite concentration determination.

3.3.3.4 Nitrate-Nitrogen (NO_3^--N)

NO_3^- -N in water samples were measured according to Standart Methods Method No 4500-NO_3^-B; at 220 and 275 nm. At 220 nm NO_3^-- N ions dissolved organics absorb light at this wavelength. Whereas at 275 nm, only measure dissolved organics are etected. The difference between the two wavelengths gives NO_3^-- N concentration in the sample. During the analysis, 0.1 mL 1N of HCl was added into 5 mL of sample. Then it was measured directly in the device. The measuring range was 0-4 mg/L NO_3^- -N; where necessary, sample was diluted. The calibration curve is given in Figure 3.18.

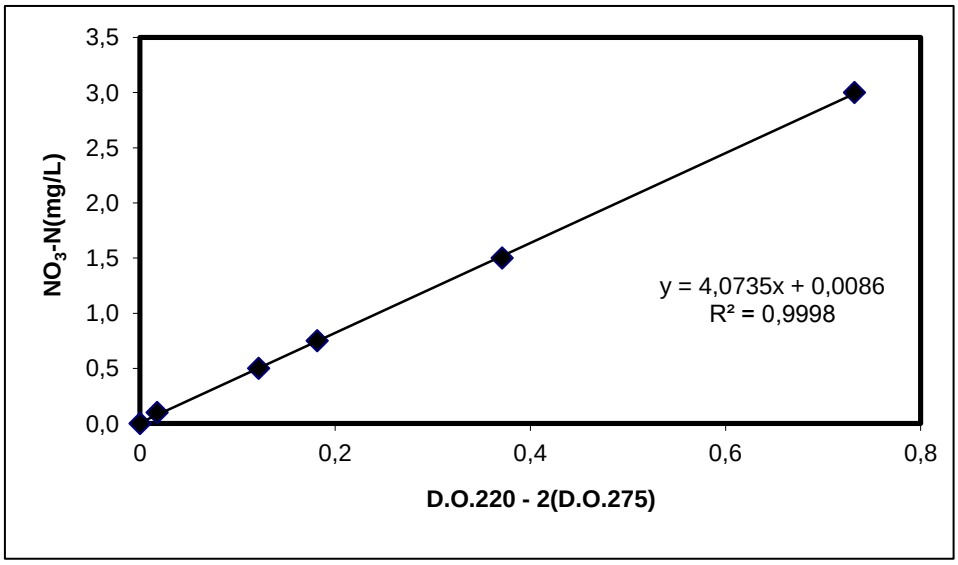

Figure 3.18 Calibration curve for nitrate concentration determination.

3.3.3.5 Phosphate (PO_4^{-3})

Prior to the analyses, samples were filtered through 0.45 μm membrane filters (Millipore). A Waters Capillary Ion Analyzer (CIA) was used for the PO_4^{-3}-P analysis. Sodium sulphate (0.01 M) was used as electrolyte (Vilas-Cruz et al., 1994). In addition, CIA-Pak™ OFM Anion BT Waters electro-osmotic modifier, 50 mL L^{-1}, (Ewing et al., 1989) was also added into the samples. The sample was

forced to migrate through melting silica covered with poliamida capillary, 60 cm long and with 45 μm internal diameter, kept at 25 °C by the application of an electric current. In this equipment, a hydrostatic injection, 10 cm height for 30 seconds, and an indirect detection, UV, 254 nm, 240 kV, 16-22 μA, was used. Five calibration points in the range between 3-100 mg L^{-1} was drawn daily for quantification of the samples (Figure 3.19.).

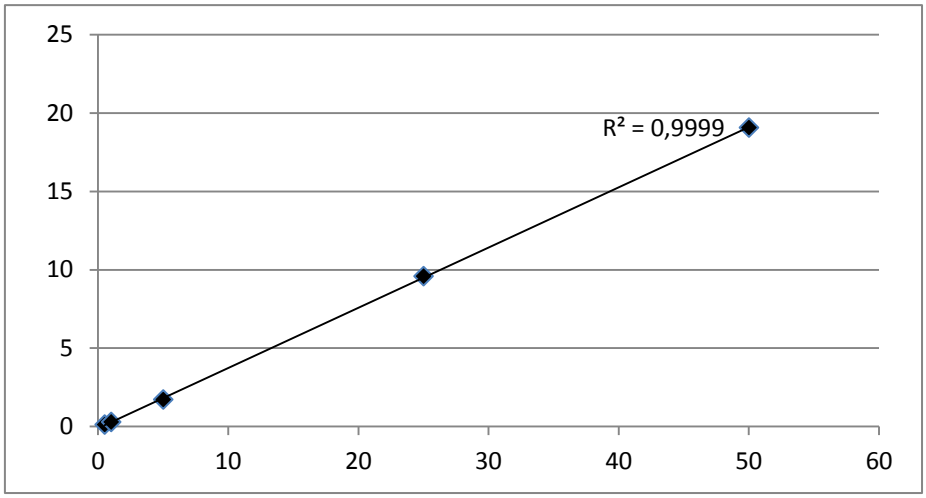

Figure 3.19 Calibration curve for phosphorus concentration determination.

3.3.3.6 pH

A Crison Instruments S.A. 52-03 type pH meter was used to measure the pH of the samples. The sensitivity of the instrument was ±1 mV, corresponding to 0.01 pH units. The probe was calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

3.3.3.7 Dissolved Oxygen (DO)

A dissolved oxygen probe (AQUALITYC, model OXI-921) connected to a DO meter (M-Design Instruments TM-3659) was used to control DO concentration in the reactor.

3.3.3.8 Temperature

The same equipment as for the DO measurements was used to measure the Temperature.

3.3.3.9 Total Suspended Solid (TSS)

Total Suspended Solids measurements in the SBR tank, membrane chamber and the recycle line were performed according to Standard Methods (2540B) (APHA, 2001). The 20 mL samples were filtered through dried and weighed glassfiber filters (Whatman, GF/C, 4.7 cm of diameter, 1.2 μm of pore size); then filters were dried at 103-105 °C for 2 hours and weighed.

3.3.3.10 Volatile Suspended Solid (VSS)

The VSS measurements were performed according to the Standard Methods (2540E) (APHA, 2001). After determination of TSS, glass fiber filters were burned in 550 ^{o}C oven for 30 mins, cooled and weighed. The difference between TSS and ash weights gave the VSS concentration.

3.3.3.11 Sludge Volumetric Index (SVI)

The SVI is the volume, in mL, occupied by a gram of dry sludge after 30 minutes of settling. Concentration of the sludge sample in the SBR tank was kept around 2.5 g/L. The reason for choosing 2.5 g/L is that common SVI method is not sensitive above 5 g/L. A well-mixed mixed liquor sample was placed into a 1 L measuring cylinder. The volume of the settled sludge was recorded after 30 minutes. In order to observe the settling rate of the zone, volume of the settled sludge was measured and noted every 1 minute up to 6 minutes, then every 5 minutes until for 30 minute. The SVI 2.5 was calculated as below.

$$SVI = \frac{V_{30} * 1000}{MLSS} \quad \text{Eq-3.1}$$

V_{30}= ml of the sludge after 30 minutes (mL L^{-1})

After given the information about the general parameter analyses, the information about the properties, extraction and analytical procedure of selected micropollutants in Spain were given below.

3.3.4 Determination of Selected Endocrine Disrupter Compounds in Spain

In this section, chemicals and reagents used during the analysis, and information about selected 13 different EDCs, including pharmaceutical and personal care products (PPCPs), are described. Preparation and Solid Phase Extraction (SPE) of liquid and solid samples, liquid chromatography tandem mass spectrometer (LC-MS/MS) and Gas chromatography mass spectrometer (GC/MS) procedures are also explained in this sub-section.

3.3.4.1 Chemicals and Reagents in Spain

Analytical reagent grade chemicals were used throughout all of this study. HPLC-grade methanol, acetone, acetonitrile and Ethyl acetate were taken from Promechem (LGC), J.T. Baker and Parreac. Glass-fiber prefilters (0.7 and 0.45 μm pore size, 47 mm diameter) were obtained from Millipore (No: AP4004705, APFC04700). Milli-Q water purification system (Millipore, USA) was used to obtain ultrapure water during the analysis. Formic acid (Merck) and ammonia (Merck) were used in the preparation of mobile phase. All of the PPCPs and hormones used throughout this study were analytical reagent grade. The selected pharmaceuticals, Fluoxetine (FLX), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), Carbamazepine (CBZ), Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Diazepam (DZP); personal care products, Galaxolide

(GLX), Tonalide (TON), Celestolide (CEL) were obtained from Sigma. The properties of the selected EDCs were given in the following section.

3.3.4.2 Stock Solutions of EDCs

Stock solutions of these compounds were prepared in different concentrations by dissolving in methanol and acetone. The amount of concentration, solvent, feed concentration, recovery, limit of detection and limit of quantification of each compound was given in Table 3.9.

Table 3.9 Stock concentration of each compound

Compound	**Stock Conc. (ppm)**	**Solvent**	**C_{feed} (µg/L)**
GLX	20000,00	Acetone	40
TON	20000,00	Acetone	40
CEL	19922,00	Acetone	40
CBZ	4986,67	Acetone	20
DZP	5000,00	Acetone	20
FXT	2500,00	Methanol	20
IBP	5000,00	Methanol	10
NPX	5000,00	Methanol	10
DCF	5000,00	Methanol	10
SFM	5000,00	Methanol	10
ROX	5000,00	Methanol	10
TRM	5000,00	Methanol	10
ERY	5000,00	Methanol	10

After preparation of stock solutions, these were spiked into the synthetic wastewater at different amount. Spiking concentration was determined with reference to the concentration in wastewaters.

3.3.4.3 Glassware

To overcome contamination, all the glassware used for EDCs analysis was separated from the other glassware used in the laboratory. After analysis of the samples, all the glassware was washed with detergent without phosphorus, ethanol and distilled water.

3.3.4.4 Sampling

During the study liquid and solid samples were taken from different points. Liquid samples were taken from influent, supernatant of SBR and permeate of membrane. Solid samples were taken from SBR tank, membrane chamber, recycle line. Moreover, sludge from the surface of membrane was taken when there was a sludge layer on it. After taking the liquid samples, they were stored in 4 ^{o}C and were analyzed within 24 hours. The sludge samples were stored at – 20 ^{o}C for maximum one week prior to analyses. Samples were prepared before analyses with GC/MS/MS or LC/MS/MS as follows:

3.3.4.5 Preparation of Samples

Before the analysis of EDCs, samples needs to prepare to the analysis. The steps of preparing samples were divided into two parts as liquid and solid.

3.3.4.5.1 Preparation of Liquid Samples

Some of the compounds were measured by GC/MS and some of them were measured by LC/MS/MS. Therefore, each sample was prepared for not only GC/MS analyzes but also for LC/MS/MS analyzes. Firstly, in order to clear solid materials and impurities from the samples, 250 mL samples from influent, effluent of SBR and permeate of membrane were filtered through glass fiber filters having 0,45 μm pore size. Then, pH of samples to be analyzed by GC/MS

was adjusted to 2.5. pH of samples were not adjusted for LC/MS/MS analyses. Following filtration and pH adjustment of the samples, solid phase extraction (SPE) was carried out. Before start of extraction for GC/MS, surrogate was added to the samples.

3.3.4.5.2 Preparation of Solid Samples

During the study in Spain, sludge samples from SBR tank, membrane chamber, recycle line and surface of membrane plate were taken for analysis to determine their removals of the selected compounds. The sludge samples, were then put into -20 ^{o}C fridge before lyophilization using Labconco Dry Freezer. Dry solids samples were weighed and placed in bottles. 4 mL methanol was put on top and mixture was vortexed for 1 minute. Homogenized samples were treated with ultrasound for 15 minutes to extract the compounds into methanol. After completion of ultrasound treatment, samples were centrifuged to separate methanol faction from solids. Separated methanol fraction is placed into a 250 mL aluminum bottle. The same procedure was repeated for 4 and 2 mL methanol and 2+2 mL of acetone. Following this step, prepared samples were put in a rotary evaporator produced by Butci R205 After evaporation,the volume of the rest sample was about 2 mL. Then sample was filtered through glass fiber filter and put in a 200 mL flask. Volume was brought up to 200 mL mark with distilled water. Then all the samples were stored at 4 ^{o}C. Since compounds were being analyzed in LC/MS/MS and GC/MS, they were divided into two parts, 100 mL each. The pH was adjusted to 2,5 for samples to be analyzed by GC/MS. Since there could be some solid particles in the samples, glass fiber was put at the entrance of the cartridge. Then solid phase extraction was applied to them.

3.3.4.6 Solid Phase Extraction

After preparation of the samples, the SPE was used for pre-concentration of the compounds. Oasis HLB 3 CC SPE cartridges were pre-conditioned by different procedures for GC/MS and LC/MS/MS analyzes as given in Table 3.10.

Table 3.10 Preconditioning of the cartridge for LC/MS/MS and GC/MS

LC/MS/MS Analyses	**GC/MS Analyses**
3 mL Methyl *tert*-butyl ether	3 mL Ethyl acetate
3 mL MeOH	3 mL MeOH
3 mL distilled water	3 mL distilled water (pH=2)

Following pre-conditioning of the cartridges, filtered samples were passed through SPE cartridges at a flow rate of 10 mL/min under vacuum. For high throughput a Phenomenex, USA vacuum manifold with 24-orifices was used as shown in Figure 3.20.

Figure 3.20 The Solid Phase Extraction (SPE) Manifold used for extraction of different samples.

After passing the entire sample through the cartridges, 10 mL distilled water at pH=2 for GC/MS analyses, and 10 mL distilled water for LC/MS/MS analyses were passed through the cartridges. After solid phase extraction, cartridges were dried under nitrogen stream for 45 and 60 minutes for GC/MS and LC/MS/MS analyses, respectively.

Following nitrogen drying, 3 mL Ethyl-acetate was used to elute the cartridges for GC/MS. In addition, 1,5 mL of a mixture of methanol: Methyl tert-butyl ether (10:90) followed by 1.5 mL of methanol was used to elute the cartridges for analyses in LC-MS-MS instrument in the positive ESI mode.

After elution step, LC/MS/MS was used for analyzing ERY, FLX, ROX, SMX and TMP; and GC/MS was used for analyses of CEL, GLX, TON, CBZ, DZP, IBP NPX and DCF.

3.3.4.7 Liquid Chromotograpy Tandem Mass Spectrometer

During the study, four antibiotics ERY, ROX, SMX and TMP and one anti-depressant, FLX, was measured by Agilent API 4000 G1312A type HPLC equipped with a binary pump and an autosampler and coupled by triple quadruple Mass Spectrometer produced by Applied Biosystems, Foster City, CA. Phenomenex Sinergy 4u Max-RP 80A (250 mm x4.6 mm x 4 μm) column was used for the separation of species of interest. A 0.1% formic acid in ultra pure H_2O for Mobile Phase A and 0.1% formic acid in liquid chromatography gradient CH_3OH for Mobile Phase B were used as binary gradient. Positive mode was used for the separation of the compounds. Separation parameters for HPLC are given in Table 3.11.

Table 3.11 The HPLC parameters optimized for the separation of analytes.

Parameter	**HPLC**
Column	Phenomenex Sinergy 4u Max-RP 80A (250 mm x4.6 mm x 4 μm)
Mobile Phase	a) 0-3.5 min 95% of 0.1% Formic Acid in ultra pure H_2O (Mobile Phase A) 5% of 0.1% Formic Acid in CH_3OH (Mobile Phase B) b) 3.5-10 min 90-20.0% of Mobile Phase A 5-80% of Mobile Phase B c) 10-13 min 20% of Mobile Phase A 80% of Mobile Phase B d) 13-21 min 0% of Mobile Phase A 100% of Mobile Phase B e) 21-30 min 0-95% of Mobile Phase A 100-5% of Mobile Phase B
Flow Rate, mL/min	0.7
Injection volume, μL	5.0

The above optimization parameters were used for the separation of compounds in LC/MS/MS. Optimization results of the compounds were given below.

Table 3.12 Optimization results of the compounds

	Compound	MW	Q1 (Quant.)	Q3 (Qual.)	Polarity	Retention Time
1	ERY	734.4	158.3	576.3	Positive	13.3
2	ROX	837.37	679.3	158.2	Positive	13.9
3	SMX	254	156	92.2	Positive	13.4
4	TMP	291.1	260.9	230	Positive	10.7
5	FLX	310.1	44.1	148.2	Positive	13.6

In addition to this information, limit of quantification and recoveries of the compounds were in Table 3.13.

Table 3.13 Limit of Quantifications and recoveries for compounds analyzed by LC/MS/MS

	Compound	LOQ (ng/L)	Recovery	R^2
1	ERY	1.2	83.3	99.89
2	ROX	1.2	76.6	99.87
3	SMX	-	-	99.88
4	TMP	6	98.4	99.88
5	FLX	1.2	68	99.83

After analyses with LC/MS/MS, concentrations of compounds were calculated for each compound by dividing by the recovery percentage.

3.3.4.8 Gas Chromotograpy Mass Spectrometer

In order to analyze CEL, GLX, TON, CBZ, DZP, IBP NPX and DCF, Varian Saturn 2100 type ion trap mass spectrometer consisting of CP8400 automatic injector attached to Varian CP 3900 gas chromatography was used. CP-

Sil 8 CB-MS low bleed (30m x 0.25mm x 0.25μm) was used as a column for the separation of the compounds. During the analyses, ultra-pure helium with 1 mL/min flowrate was used as carrier gas. The information about the operation conditions for GC/MS was given in Table 3.14

Table 3.14 Operating conditions of GC/MS detection.

parameters	
Injector	
Splitless time	1 min
Inyector temperature	250 °C
Gas flow (He)	1 mL min^{-1}
Injector volume	1 μL
Solvent	Ethylacetate
GC temperatures	
Initial temperature	70 °C
Inicial time	2 min
1^{st} ramp	25 °C min^{-1}
Final temperature	150 °C
2^{nd} ramp	3 °C min^{-1}
Final temperature	180 °C
3^{rd} ramp	8 °C min^{-1}
3^{rd} Temperature	--280 °C
Time Duration	15 min
Total Time	42.7 min (last compound:36.0 min)
MS parameters	
Solvent Delay	10 min.
Filament current	10 μA
Ionization	100 eV
Transfer line Temperature	280 °C
Acquisition rate	1 s/scan
Mass acquisition mode	full scan 50-550 amu

The operation conditions for GC/MS are given above. Retention times, qualitative and quantitative ions are given in Table 3.15, recovery and R^2 of each compound are given in Table 3.16.

Table 3.15 Optimization results of the compounds

	Compound	MW	Q1 (Quant.)	Q3 (Qual.)	Retention Time
1	CEL	244	229	173,244	14.0
2	GLX	258	243	213	17.1
3	TON	258	243	159,187	17.3
4	IBP	206	263	75,161	16.9
5	NPX	230	287	185,75	23.9
6	CBZ	236	193+293	250	26.1
7	DZP	285	256:258+283:286		26.0
8	DCF	296	352+354+356		

Table 3.16 Limit of Quantifications and recoveries for compounds analyzed by GC/MS

	Compound	LOQ(ng/L)	Recovery	R^2
1	CEL	2	63.5	99.63
2	GLX	2	71.2	99.90
3	TON	2	67.8	99.39
4	IBP	30	97.3	99.86
5	NPX	30	86.5	99.63
6	CBZ	480	79.2	99.65
7	DZP	240	84.7	99.04
8	DCF	120	81.8	99.30

All the information for analyses of the selected compounds in Spain was given above. The calibration curves of the compounds were given in Appandix A.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. EDC ANALYSES RESULTS

4.1.1 Optimization of LC/MS/MS for the Selected EDCs

At the beginning of the study, fragmantor voltage (FV), product ions and collusion energies (CE) for each compound were optimized. Column was disconnected from the HPLC-ESI-MS/MS to decrease the analysis time. Two options, positive and negative ions, were tested during the optimization. Parameters were kept constant in the optimization of ES-MS/MS system; nebulizer pressure 50 psi, emv 400 V, drying gas (N_2) temperature and volume 350 °C, 11.0 L/min, injection volume 30 µL, flow rate 0.2 mL/min, drew speed was 200 µL/min. An example for the optimization of diltiazem is given in the sub-section 4.1.1.1 Lastly, optimization results for the rest of the selected EDCs are given.

4.1.1.1 Optimization of the Diltiazem

Diltiazem was the first compound optimized. Negative and positive ions were tested to find the optimum ion. The peak heights for the respective ions were found as $2{,}2*10^5$ and $8{,}5*10^7$ as seen in Figure 4.1.1 and 4.1.2 during the optimization.

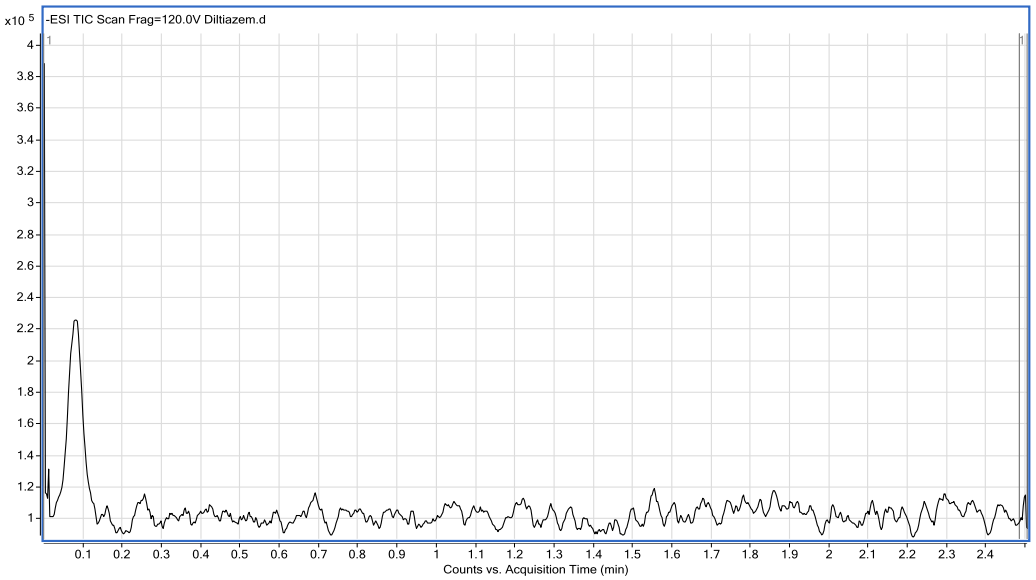

Figure 4.1.1 Negative ion scan for Diltiazem

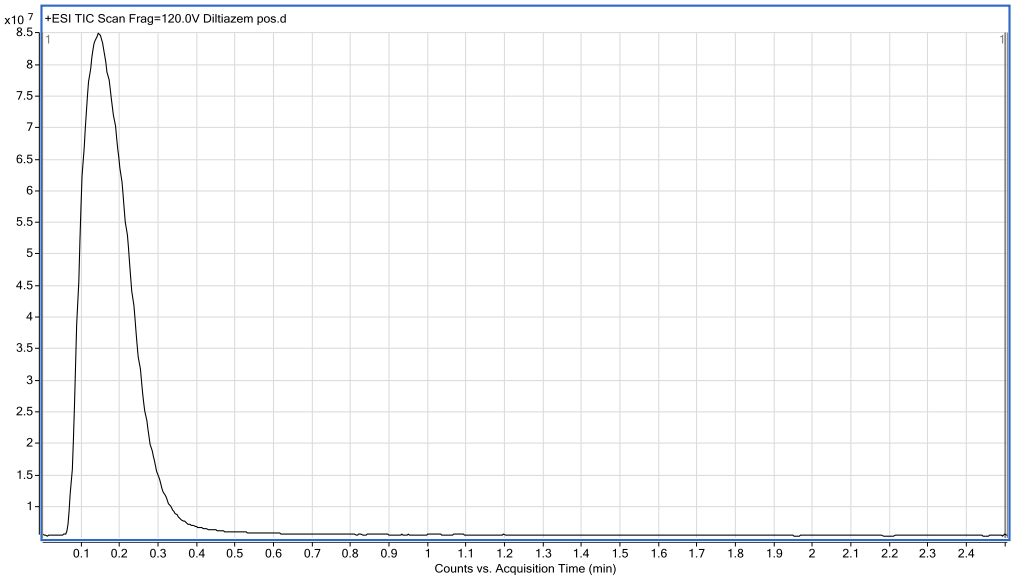

Figure 4.1.2 Positive ion scan for Diltiazem

Therefore positive ion was chosen for diltiazem. After determining positive ion, different fragmantor voltages (FV) (accelerating voltage) were used to maximize the MH^+ ion transmission; and minimize collision induced dissociation (CID), as seen in Figure 4.1.3.

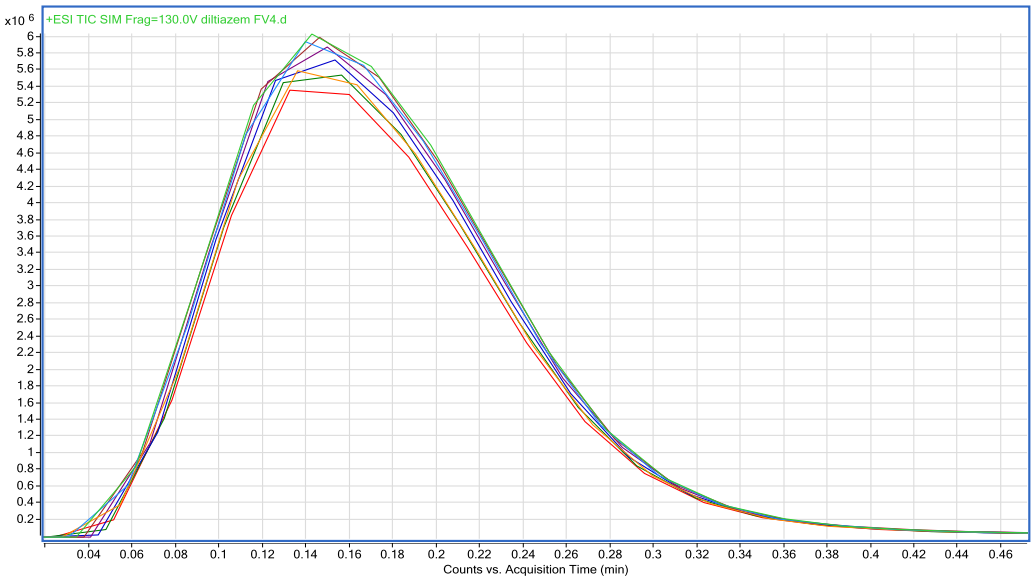

Figure 4.1.3 Optimization of Fragmentor Voltage for Diltiazem

As it is seen from Figure 4.1.3., different FVs were tried for diltiazem, from 70 to 150, and the highest peak was observed at 130 volt. After the determination of FV, the product ions of the diltiazem were found by applying different collosion energies (CE) as seen in Figure 4.1.4.

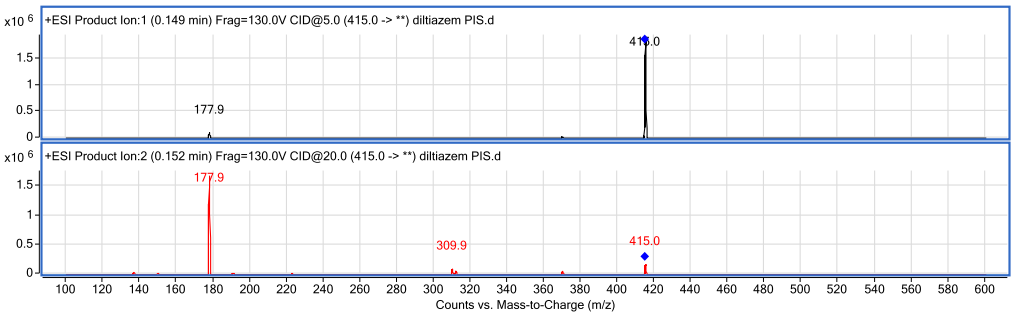

Figure 4.1.4 Product ions for diltiazem

The 5 and 20 volts were chosen as CE to find two product ions for diltiazem, whose moleculer weight is 415, as seen in Figure 4.1.4. The upper chromotogram was for CE 5 volts and the lower was for CE 20 volts. When t CE was chosen as 5 volts, only 177.9 m/z count (mass/charge) was observed as the principal product ion, as seen in Figure 4.1.4. When CE was adjusted to 20 volts,

a peak at 309.9 m/z count was also Identified. During the analyses of the samples, the higher peak , 177.9 m/z, was used for quantification, and 309.9 m/z, was used for qualification.

Following determination of the product ions for diltiazem, different collosion energies, ranging from 12 to 28, were applied to find the maximum product ion signal. The highest signal was observed at 24 volts, as seen in Figure 4.1.5.

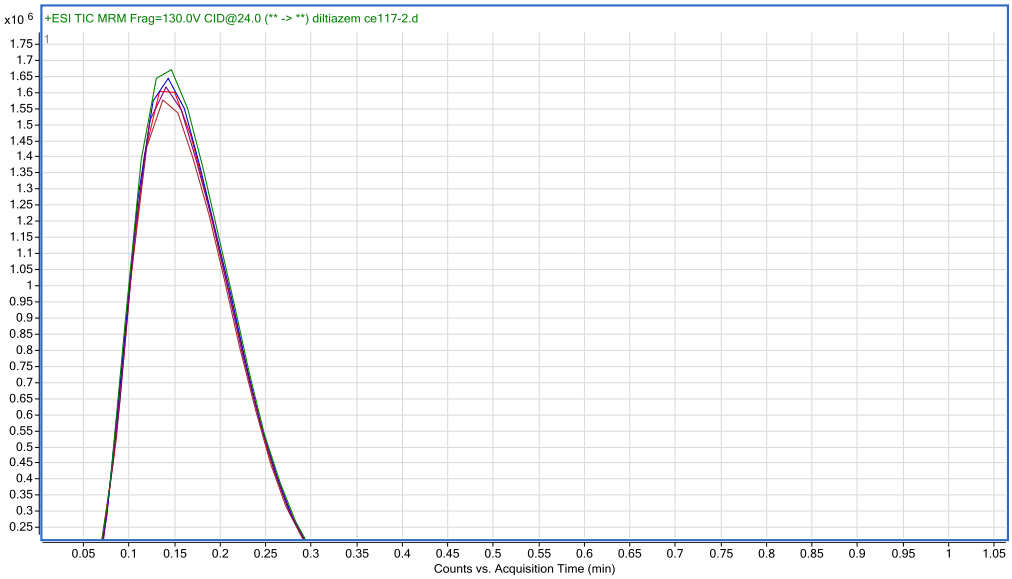

Figure 4.1.5 Optimization of Collosion Energy for Diltiazem

The same procedure was applied for the optimization of the other compounds of interest and the respective optimized parameters are tabulated in Table 4.1.1

Table 4.1.1 Optimization results for selected EDCs

Compound	**MW**	**Q1**	**Q3 (Quant)**	**Q3 (Qual)**	**FV**	**CE**	**Polarity**
Diltiazem	414.5	415	177.9	309.9	130	24-30	Positive
Progesterone	314.5	315	109	97	120	30-23	Positive
BBP	312.4	313	91	148.9	70	20-9	Positive
Estrone	270.4	271	253	159	110	9-20	Positive
Cbz	236.3	237	194	192	120	18-22	Positive
Acetoaminiphen	151.2	152	110	93.1	90	14-22	Positive

The other optimization figures were given in Appendix-A.

4.1.1.2 Mobile Phase Optimization

In the mobile phase optimization, different mobile phases were applied to find the best separation conditions. During the optimization, one parameter was optimized while others were kept constant. After finding the optimized parameters, these were applied to all the samples to determine selected EDCs in wastewater samples.

The peak areas of signals were used in the optimization. Four different mobile phase systems were composed by mixing two channels, A and B, for the preparation of mobile phases given in Table 4.2. In channel A, ultra distilled water, as the aqueous solvent, containing 0.1% formic acid (FA); and in channel

B, HPLC grade methanol, as an organic solvent, containing 0.1% formic, was used. The formic acid was used to improve the chromatographic peak shape and to provide a source of protons in reverse phase LC/MS. Ammonium format (AF) was added to the solvents as a buffer. The optimum chromatographic separation parameters were employed to obtain not only good separation but also stable signals.

Table 4.1.2 Mobil phase program used in the study.

No	Mobile Phase A	Mobile Phase B
1	0.10% FA in Ultra-pure water	0.10% FA in CH_3OH
3	0.10% FA + 2.0 mM AF in Ultra-pure water	0.10% FA + 2.0 mM AF in CH_3OH
4	0.10% FA + 5.0 mM AF in Ultra-pure water	0.10% FA + 5.0 mM AF in CH_3OH
5	0.10% FA + 10.0 mM AF in Ultra-pure water	0.10% FA + 10.0 mM AF in CH_3OH

When 0.1% FA without any buffer was added to the mobile phases, very good separation was observed for all the compounds. However, the peak heights were not very high as seen in Figure 4.1.6.

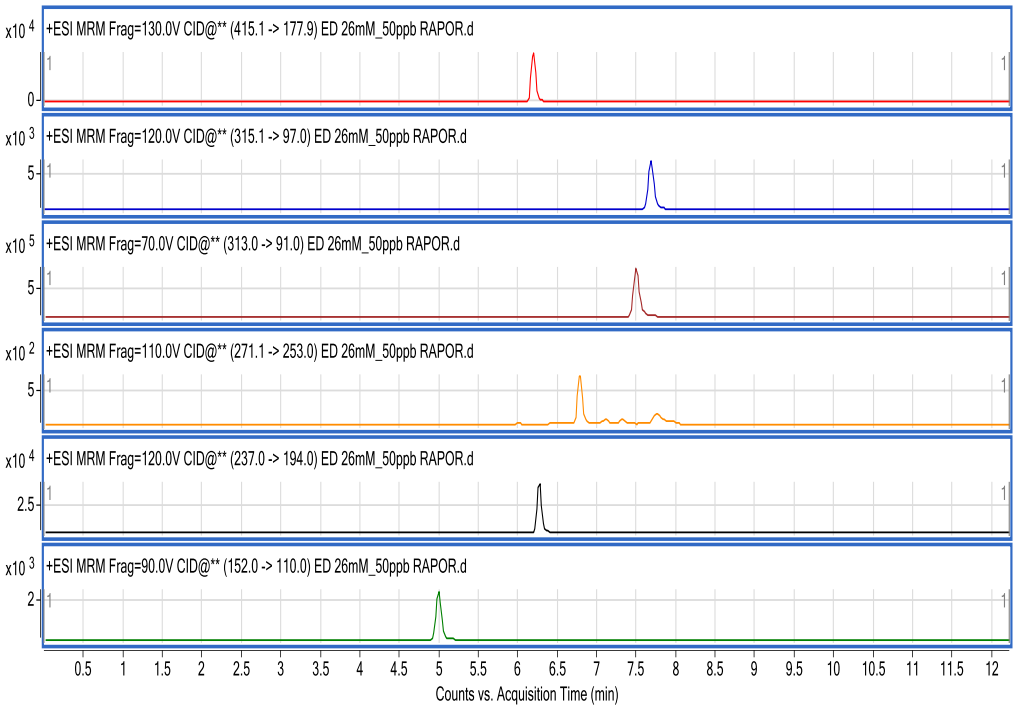

Figure 4.1.6 Mobil phase just with 0.1% FA

Therefore, In order to increase the peak heights, 2 mM FA as buffer was added to each solvent. Although, peak heights of diltiazem and carbamazepine were increased, the peak shapes for the other compounds deteriorated, as can be seen in Figure 4.1.7.

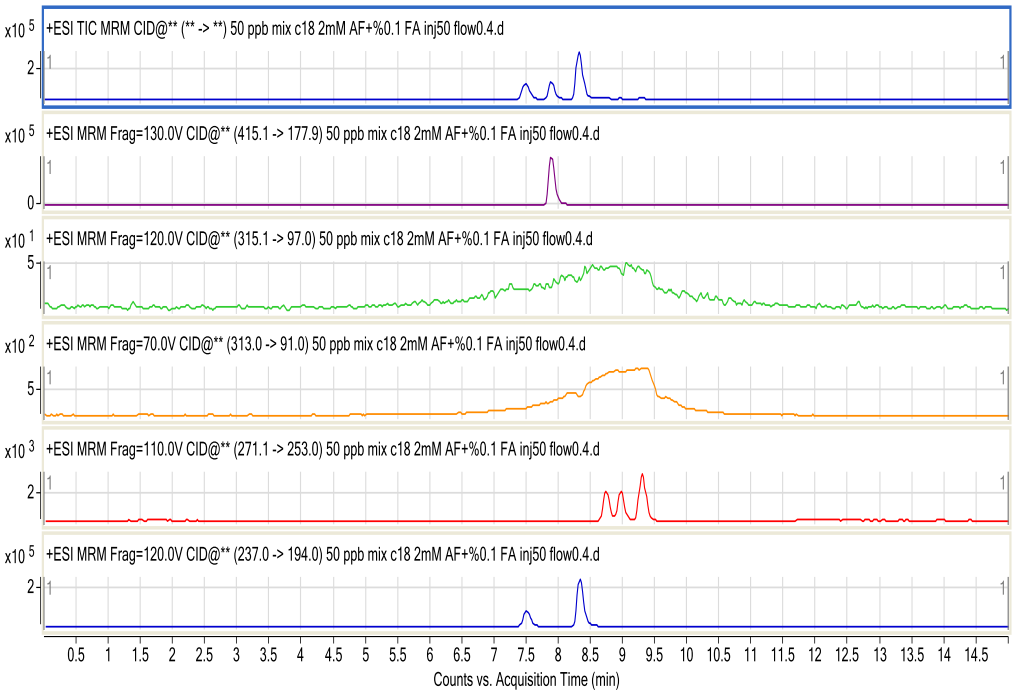

Figure 4.1.7 Mobil phase as 0.10% FA + 2.0 mM AF

After observing signal deterioration with 2.0 mM AF addition to the mobile phases, 5.0 mM AF was added to increase peak heights and shapes and for good separation of the compounds. The chromatogram for mobile phase with 0.10% FA + 5.0 mM AF are given in Figure 4.1.8.

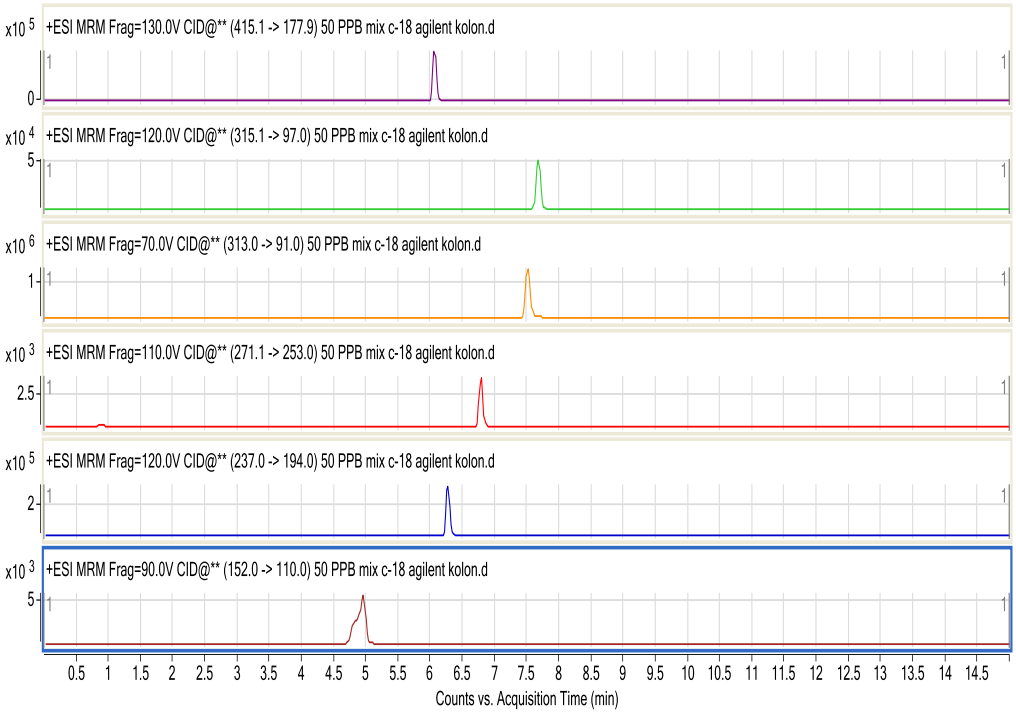

Figure 4.1.8 Mobil phase as 0.10% FA + 5.0 mM AF

As seen in Figure 4.1.8, separationof all the peaks were satisfactory. In addition, peaks were very high, compared to the other mobil phases. There was only peak broadining in the case of acetaminophen. In order to understand the peak shapes and separation changes, 10 mM AF was added to each solvent as seen in Figure 4.1.9.

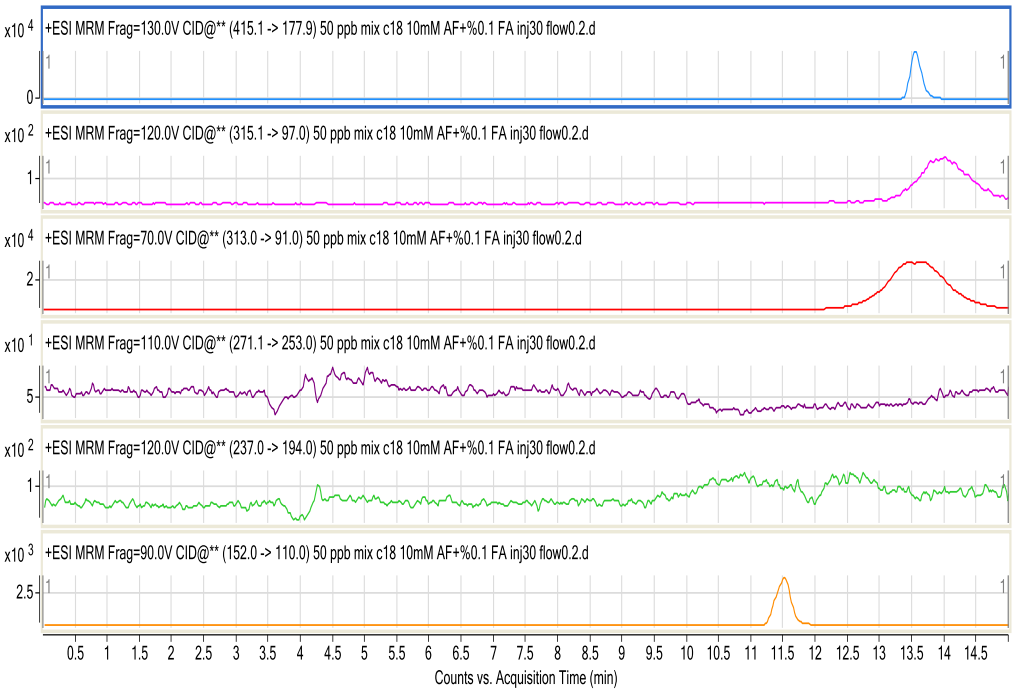

Figure 4.1.9 Mobil phase as 0.10% FA + 10.0 mM AF

When 10.0 mM AF was added to the mobile phases, peak symmetries for all the peaks were more or less destroyed. Moreover, retention time was over 12 minutes.

Amongst all the mobile phase systems given in Table 4.1.2, number 3 was selected as the best one. All the analytes of interest could be retained in the column and good separation of all the species could be achieved with this mobile phase. No significant changes were observed in the analyte retention times (less than 1.0%) after several injection of the mixed standard solution.

4.1.1.3 Column Optimization

Different columns were tried to find the best separation conditions. Five different columns were tested during column optimization. Before using each column, mobile phase was passed through the column for about 30 minutes for conditioning of the column. During the optimization, 25.0 µg/L mixed standard

solution was used. Peak heights, peaks symmetries and differences in the retention times of analytes were taken into consideration in this optimization. First Nucleodur C-18 column was tested and the chromatogram for selected EDCs was given in Figure 4.1.10.

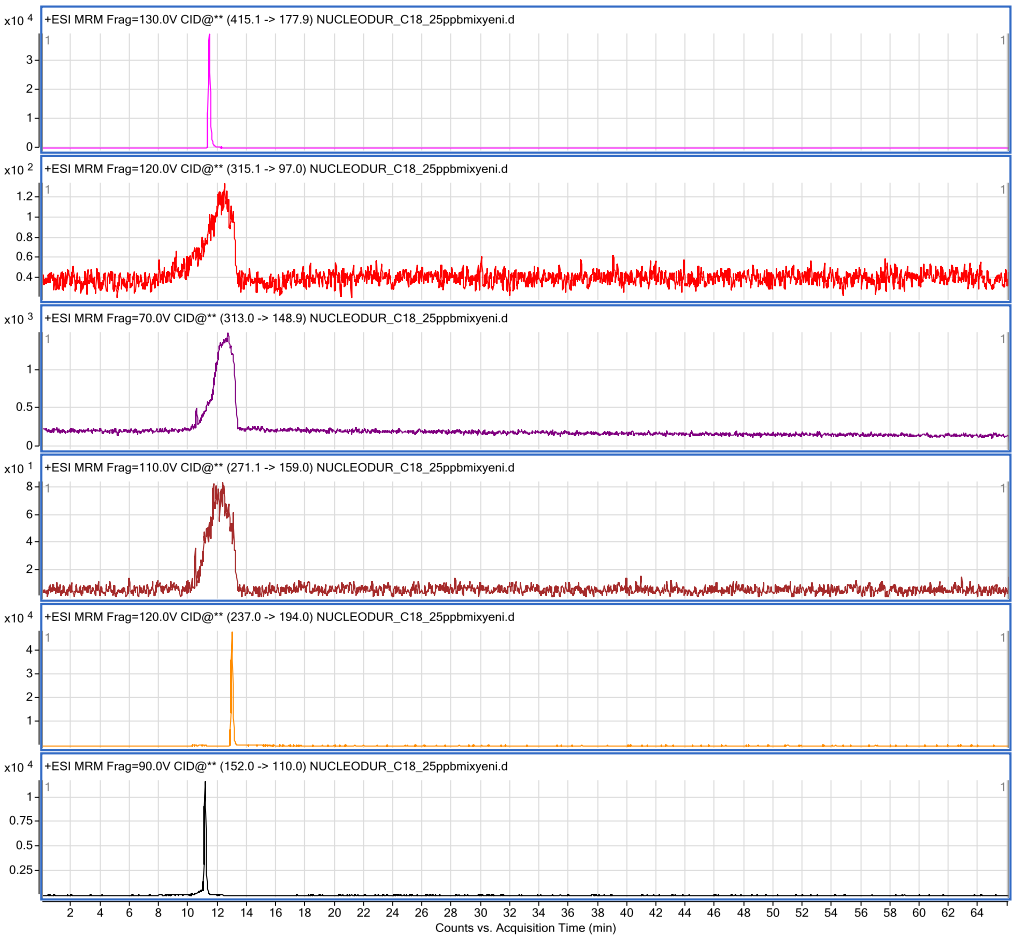

Figure 4.1.10 Optimization of Nucleodur C-18 column for selected EDCs analysis

As it can be seen from Fig 4.1.10, retention times for all the compounds were identical at around 12 mins, without any separation. Moreover progesterone, BBP and estrone could not be measured with this column.

The second column was Dionex C-18 (150 mm×4,6 mm, 3 µm). The chromatogram for Dionex C-18 is given in Figure 4.1.11.

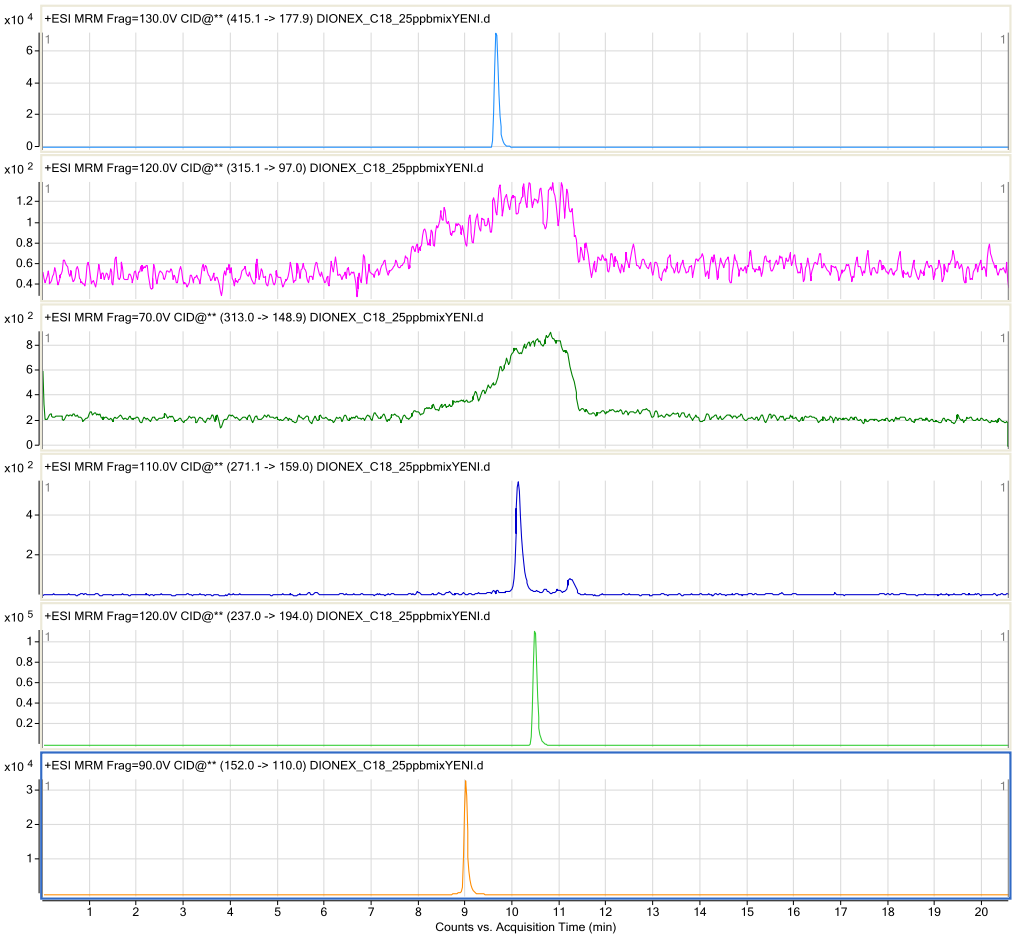

Figure 4.1.11 Optimization of Dionex C-18 column for selected EDCs analysis

The retention times for the compounds were between 9 and 11 min. Although separation for diltiazem, carbamazepine and acetaminophen were very good, other compounds came almost at the same retention time. Furthermore, progesterone and BBP could not be measured at all and the estrone peak was not very high, as seen in Figure 4.1.11.

Altima C-8 (150 mm×4,6 mm, xx µm) was the third column tested for column optimization and chromatogram for the selected compounds is given in Figure 4.1.12.

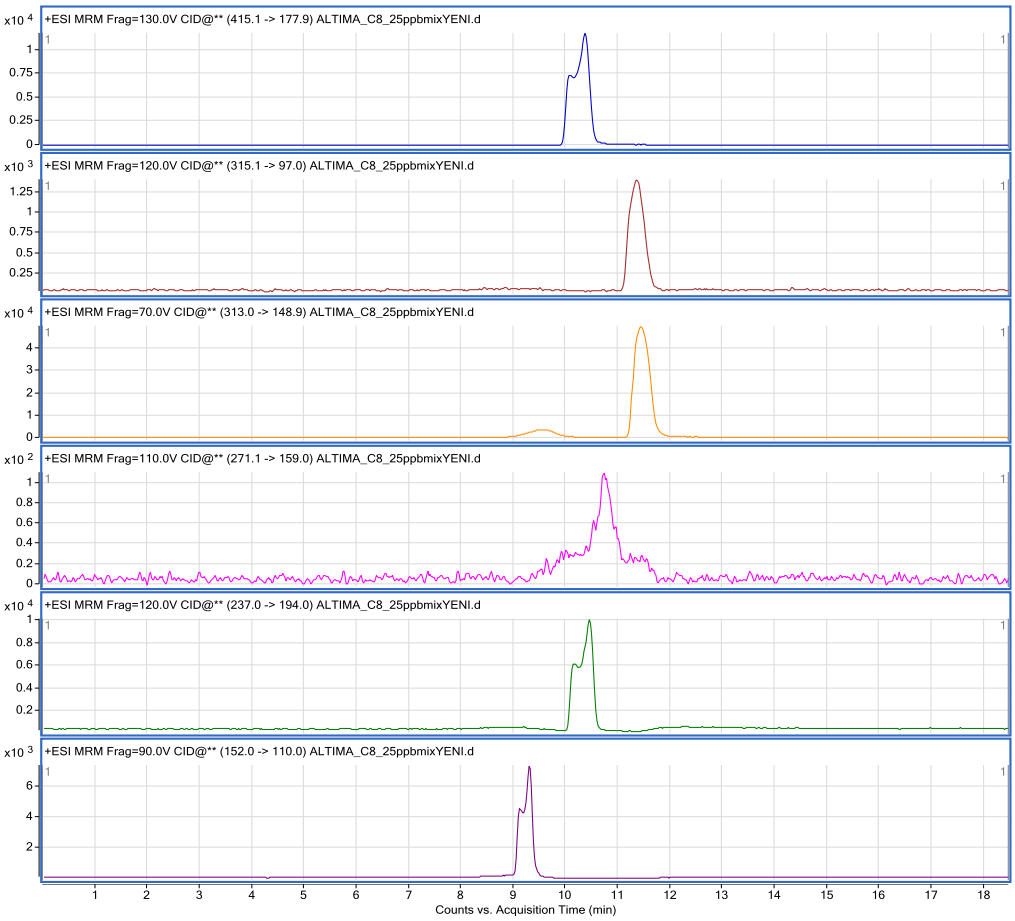

Figure 4.1.12 Optimization of Altima C-8 column for selected EDCs analysis

Although only estrone could not be measured with this column, the other peak heights were low and not good as seen in Figure 4.1.12. Moreover, peak shapes of all the species were broadening.

To find the best separation and highest of peaks, Zorbax C-18 (75 mm×3 mm, 3.5 µm) was utilized. However, separation period was about 12 min. In addition, progesterone and BBP could not be measured, as seen in Figure 4.1.13.

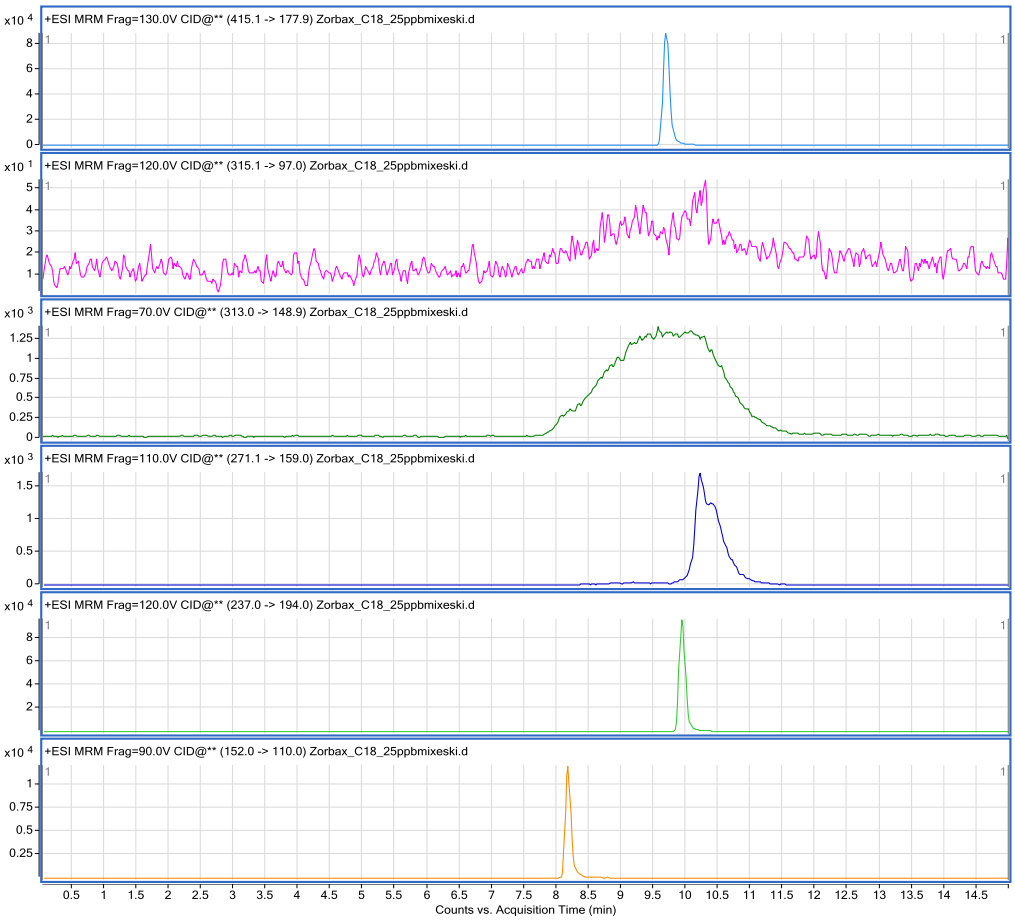

Figure 4.1.13 Optimization of Zorbax C-18 column for selected EDCs analysis

The last column tested for the optimization was Zorbax C-8 (100 mm×3 mm, 3 μm). Separation period was 9 minutes as seen in Figure 4.1.14.

Figure 4.1.14 Optimization of Zorbax C-8 column for selected EDCs analysis

As it can clearly be seen from Figure 4.1.14, with Zorbax C-8 column, all of the species could be separated from each other successfully, and peak shapes of all the species were symmetric. Moreover, resolution for all the analytes was obtained successfully. Therefore, Zorbax C-8 column was selected to be used during the analyses of selected EDCs in all samples.

4.1.1.4 Injection Volume Optimization

Injection volume was the other parameter optimized in this study. In order to find the optimum value of injection volume, seven different injection volumes from 5 to 40 µL were tested. The chromatogram for the injection volume of 5 µL is given in Figure 4.1.15.

Figure 4.1.15 Chromotogram for 5 µL injection volume

As can be seen in Figure 4.1.15, although shapes of the peaks were symetrical, still they were not highly resolved. Therefore, the injection volumes was increased to 10 µL, as shown in Figure 4.1.16.

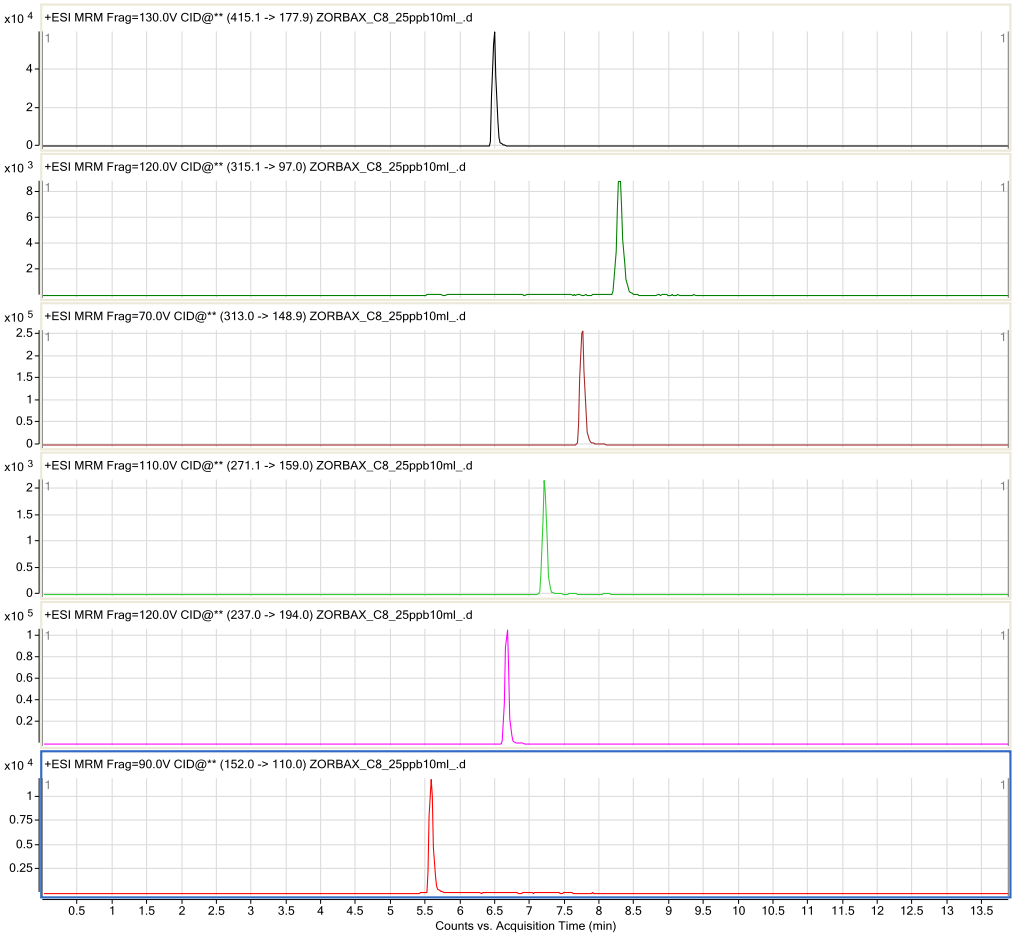

Figure 4.1.16 Thwe Chromotograms for 10 µL injection volume

When the volume of the injection was increased to 10 µL, the peak heights doubled. However, this was still not considered enough so the injection volume was further increased to 15 µL as given in Figure 4.1.17.

Figure 4.1.17 Chromotogram for 15 µL injection volume

As seen in Figure 4.1.17, when the volume of injection was increased, all the peak heights have also increased without any detoration of the peak shapes. In order to find the maximum peak heights with symetric peaks injection volume was increased to 20 µL and the chromatogram in Figure 4.1.18 was obtained.

Figure 4.1.18 Chromotograms for 20 µL injection volume

All the peaks were high without any peak broadening. The retention time was about 9 minutes with good separation. In order to determine the optimum volume, 25 µL was tested as seen in Figure 4.1.19.

Figure 4.1. 19 Chromotograms for 25 µL injection volume

Although very little increase in peak hight could be observed for carbamazepine and acetaminophen at 25 µL injection volume, the peak of progesterone was broken in two. As can be seen in Figure 4.1.20, the injection volume was increased to 30 µL to show clearly that increased volume above 20 µL has a negative effect on the chromatogram shapes, hence on sensitivity of the analysis of these compounds

Figure 4.1.20 Chromotograms for 30 µL injection volume

When the injection volume was 30 µL, and 40 µL the peak heights started to decrease as seen in Figure 4.1.20 and 4.1.21. Therefore, 20 µL of injection volume was selected as the optimum one by considering the separation power of the species and the peak shapes. The flow rate of the mobile phase was set as 40 µL during injection volume analyses.

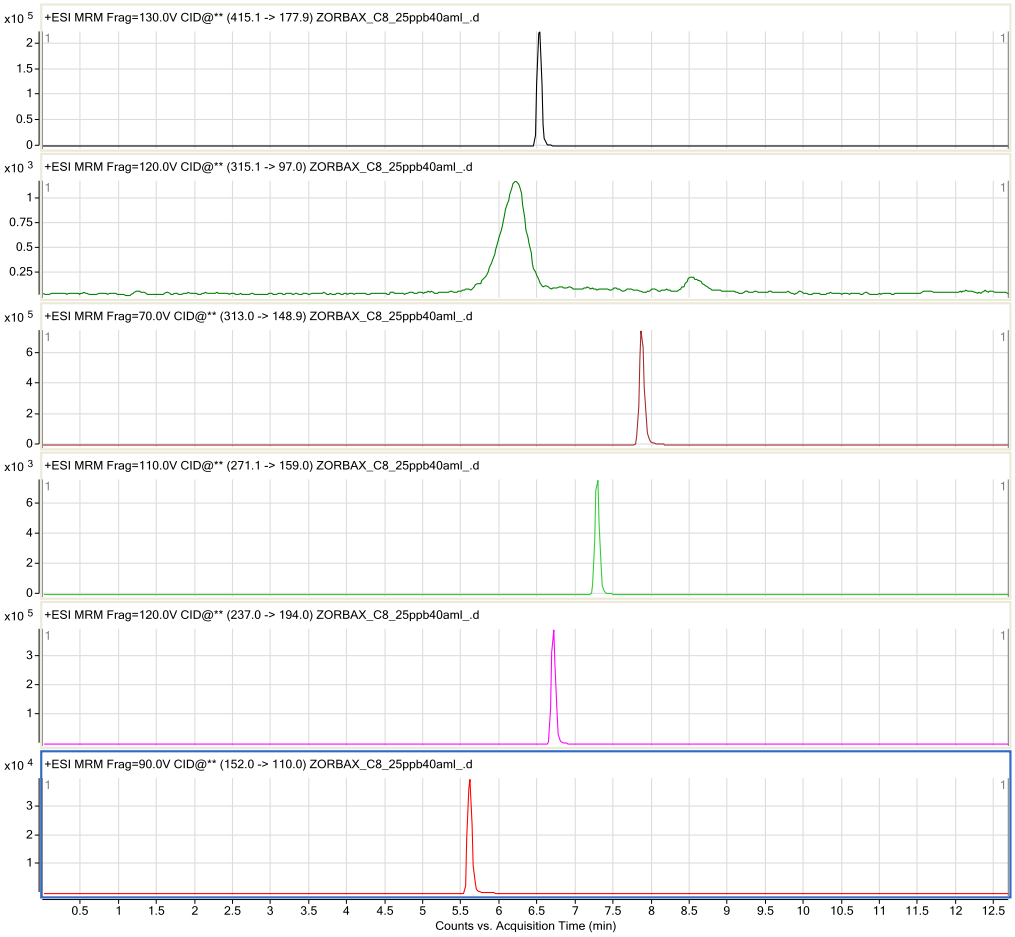

Figure 4.1.21 Chromotogram for 40 µL injection volume

After injection volume optimization, the flowrate of mobile phase optimization was done.

4.1.1.5 Flow Rate Optimization

Flow rate of mobile phase was also optimized during the study. 0.10, 0.20, 0.30, 0.40 and 0.50 mL/min were tried as mobile phase flow rates. In the case of 0.1 mL/min of flow rate, there was no signal observed for any of the analytes with the exception of acetaminophen as seen in Figure 4.1.22.

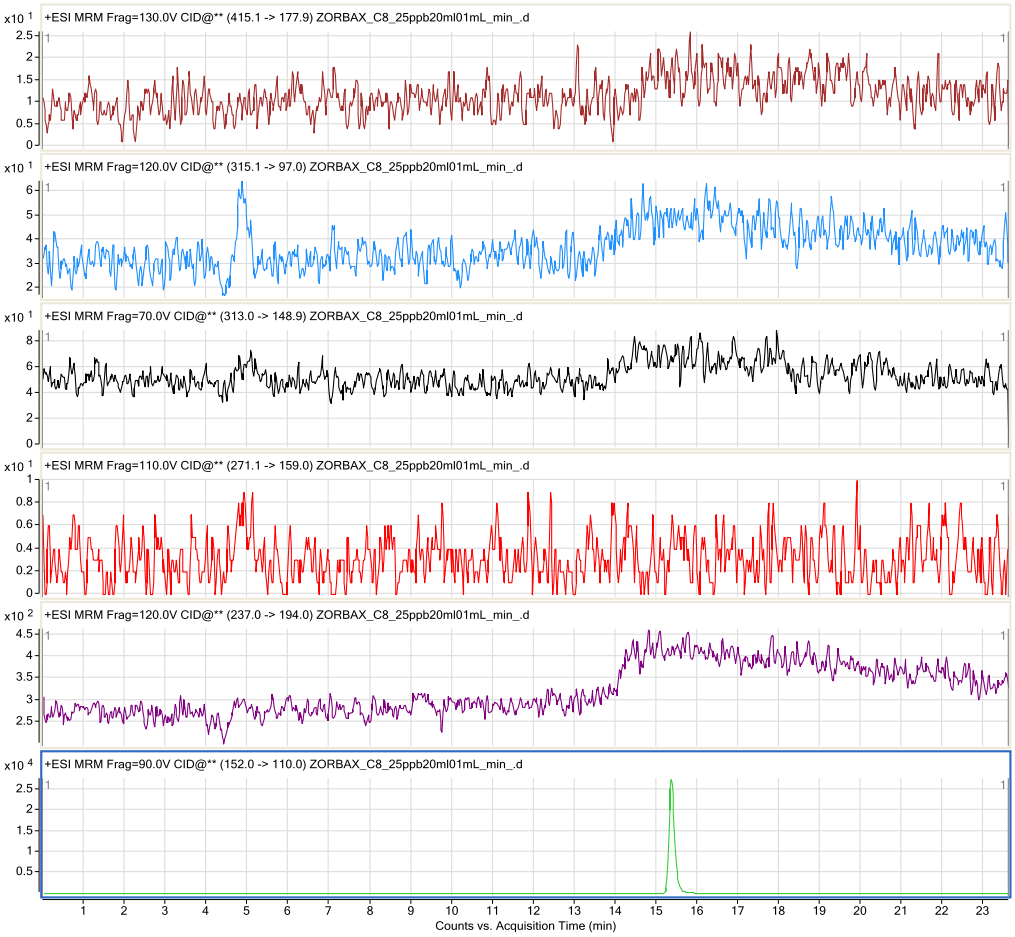

Figure 4.1.22 Chromotogram for 0,1 mL/min flowrate

When the flow rate of the mobile phase was increased to 0.2 mL/min, similar observations for progesterone, BBP and estrone were obtained as seen in Figure 4.1.23

Figure 4.1.23 Chromotograms for 0,2 mL/min flowrate

Using 0.3 mL/min flow rate, signals of progesterone and BBP were poor considering peak shapes and peak symmetry as seen in Figure 4.1.24.

Figure 4.1.24 Chromotogram for 0,3 mL/min flowrate

When the flow rate was adjusted to 0.4 mL/min, peak broadening was observed for progesterone, BBP and estrone as seen in Figure 4.1.25.

Figure 4.1.25 Chromotogram for 0,4 mL/min flowrate

In order to eliminate or minimize peak broadening, flow rate was increased to 0.5 mL/min as seen in Figure 4.1.26.

Figure 4.1.26 Chromotogram for 0,5 mL/min flowrate

By using this flow rate, broadening was eliminated and sufficient resolution for all the analytes was obtained. Hence, 0.5 mL/min was selected as the optimum flow rate for the mobile phase.

4.1.1.6 Column Recovery

Recovery of the analytes from the analytical column was also investigated during the study. The aim was to determine the retaining effect of column on analytes during the analyses of the compounds in samples. First a mixed standard solution containing 100.0 ng/mL of diltiazem, progesterone, BBP, estrone, CBZ and acetaminophen was injected to the HPLC column under optimum conditions. Eluent was collected throughout the gradient elution for 10 mins. Then, the same

experiment was also performed using no column. A mixed standard containing 100.0 ng/mL of diltiazem, progesterone, BBP, estrone, CBZ and acetaminophen was injected to HPLC using the same loop and eluent which were collected before the column. Both collected solutions were aspirated to ES-MS/MS system and the results are given in Table 4.1.3.

Table 4.1.3 Column recovery results for EDCs (N=3).

Name	%Recovery
Diltiazem	101.6 ± 3.7
Progesterone	98.3 ± 4.7
BBP	98.3 ± 2.9
Estrone	97.2 ± 4.1
Cbz	102.2 ± 2.7
Acetaminophen	106.0 ± 7.6

As can be seen in Table 4.1.3, there was no significant difference in the signals of solutions obtained with and without using column. Recovery for all species from the analytical column used for the separation of all the analytes was found to be very close to 100%. This meant that recovery from the column was high enough for quantitative measurements of analytes of interest. Evidently there was no loss in the column, and analytes were completely eluted from the column under the optimum conditions.

4.1.1.7. Cartridge Recovery

Although different brands of cartridges have available for pre-cleaning and pre-concentrating of the samples, Waters Oasis HLB cartridges were used in this study. Solid phase extraction procedure for this cartridge was mentioned in the Materials and Methods section. This product disables undesired outcomes of sample extraction like silanol activity, breakthrough of polar compounds and can

perform well at a pH range of 1-14. Although this wide range of working pH, it was considered to make a pH optimization and find recoveries at different pH values. To do so, extraction procedure described before was applied to 50 ppb of standard mix was spiked wastewater samples of which pH's were brought to 2, 2.5, 3, 3.5, 4, 4.5, 5, 7, 8, 9 and 10. Then, it was filtered from 0.7 glass fiber filter before passed it to cartridges. After the analysis of these samples with LC/MS-MS (ESI), recoveries were calculated and given in Table 4.1.4.

Table 4.1.4 Recovery data of pH optimization. (**%**)

Diltiazem	**Progesterone**	**BBP**	**Estrone**	**Acetaminophen**	***pH***
68.50±0.06	100.00±0.02	90.35±1.78	100	-3.53±0.45	***2***
71.70±0.06	99.02±0.02	86.90±1.71	100	-20.30±2.62	***2,5***
84.02±0.08	100.00±0.02	87.70±1.73	100	-0.49±0.01	***3***
36.30±0.03	100.00±0.02	87.70±1.73	100	2.67±0.34	***3,5***
71.70±0.01	100.00±0.02	85.30±1.68	85,06	3.67±0.47	***4***
99.80±0.09	100.00±0.02	88.30±1.74	81,8	1.94±0.25	***4,5***
99.70±0.09	99.90±0.02	90.40±1.78	100	8.02±1.03	***5***
99.60±0.09	99.80±0.02	92.30±1.82	100	30.0±3.87	***7***
99.20±0.09	98.70±0.02	86.90±1.71	87,9	-1.49±0.19	***8***
99.20±0.09	99.50±0.02	81.10±1.60	100	31.60±4.07	***9***
0	99.30±0.02	71.20±1.40	86,2	15.10±1.94	***10***

It was seen from the Table 4.1.4, the highest recovery was achieved as pH 7. Therefore, after filtration of the samples, pH was arranged as 7 by added H_2SO_4 to all samples.

4.1.1.8. Calibration Curves and Analytical Figures of Merit

Calibration curves for selected EDCs for drawn during the analyses of the samples. During drawing of calibration curve, concentration versus peak areas was used. Calibration curves for each EDCs was given below.

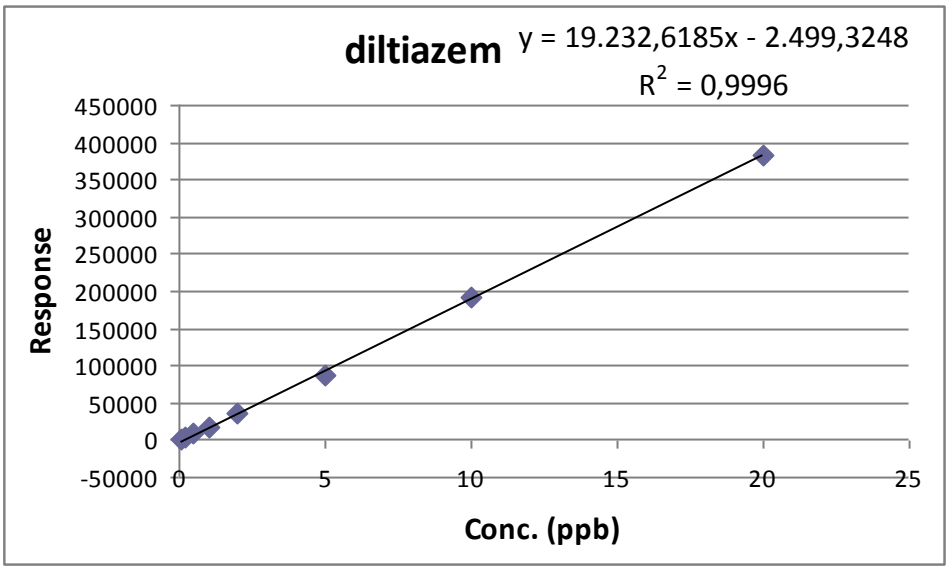

Figure 4.1.27 Calibration Curve for diltiazem

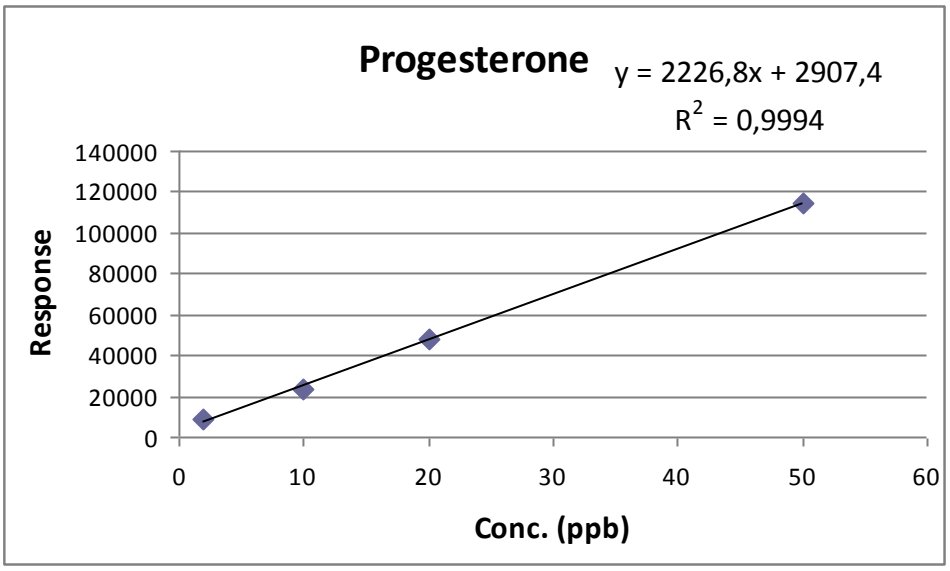

Figure 4.1.28 Calibration Curve for progesterone

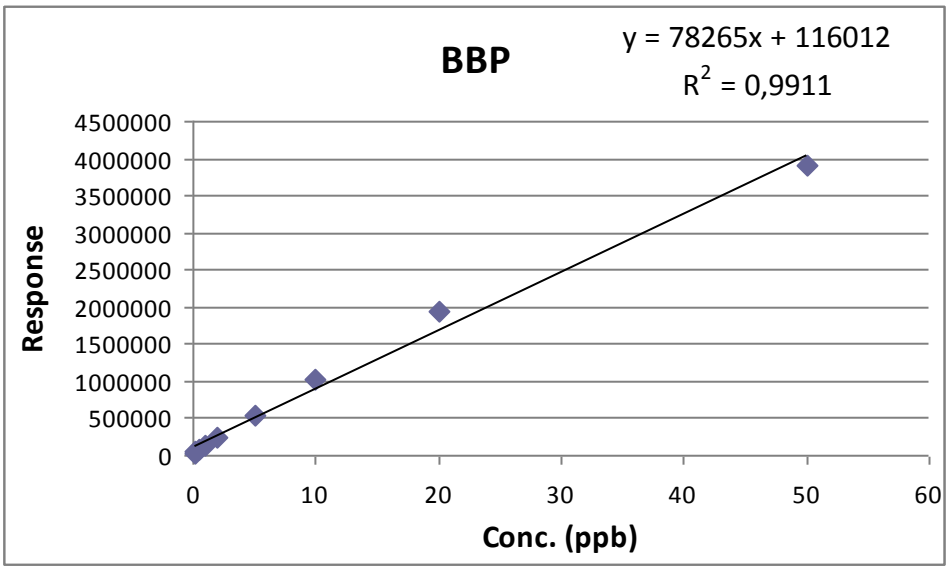

Figure 4.1.29 Calibration Curve for BBP

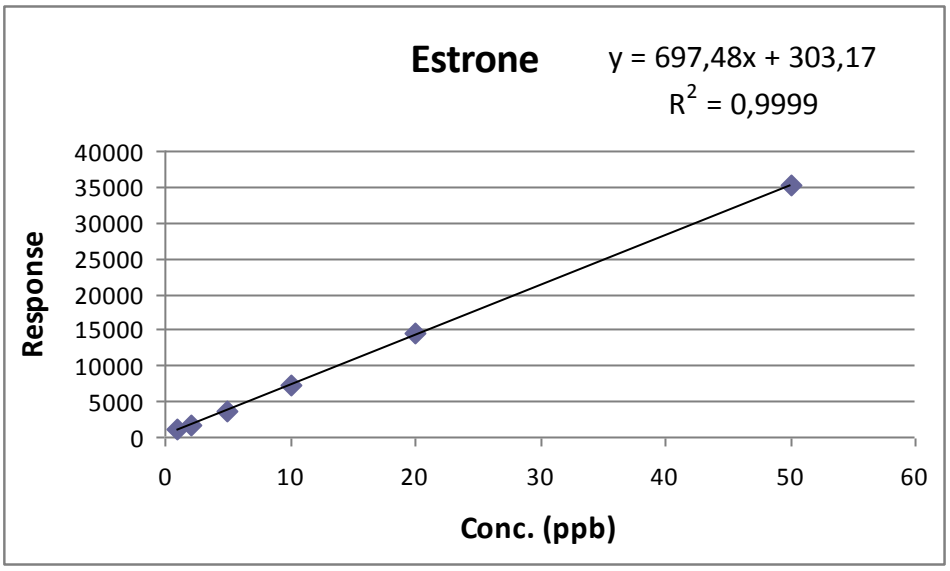

Figure 4.1.30 Calibration Curve for estrone

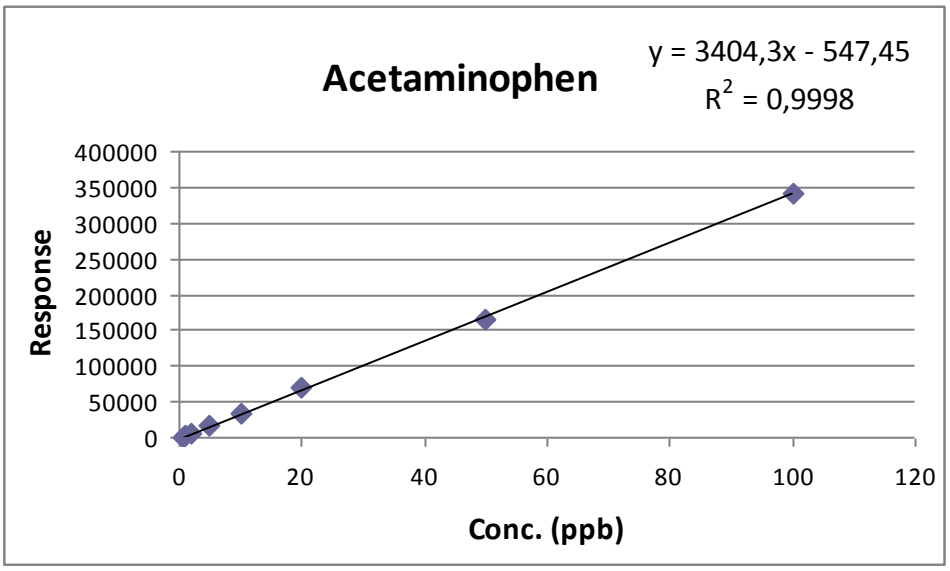

Figure 4.1.31 Calibration Curve for acetaminophen

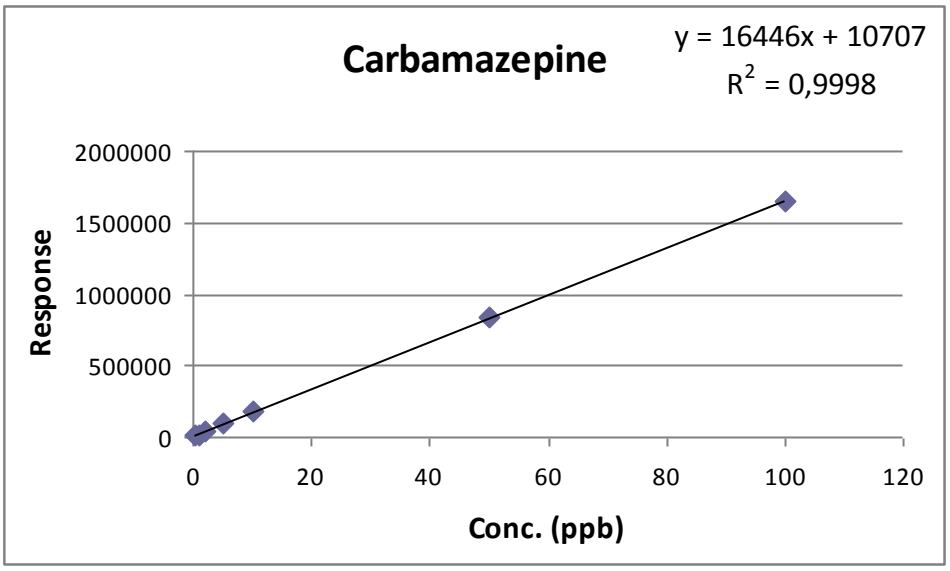

Figure 4.1.32 Calibration Curve for carbamazepine

After plotting all the calibration curves, whose R^2 values for each species were found to be at least 0.99, Limit of Detection (LOD) and Limit of Quantification (LOQ) values were calculated. For the calculation of LOD and LOQ values, peak areas of 0.25 µg/L mixed standard solution was used. For this purpose, mixed standard was analyzed ten times. The reproducibility of the chromatographic method was also determined. No significant changes were observed in the retention times (less than 1.0%) of analytes. Following formulas were used to calculate LOD and LOQ values.

LOD= 3xStandard Deviation of 0.25 µg/L mixed standard solution /Slope

LOQ= 10xStandard Deviation of 0.25 µg/L mixed standard solution /Slope

The analytical figures of merit for the HPLC-ES-MS/MS system are given in Table 4.1.5.

Table 4.1.5 Analytical figures of merit.

Analaytes	Equation (y=mx+n)	Linear Range (µg/L)	R^2	LOD (µg/L)	LOQ (µg/L)
Diltiazem	y = 19234x -2499	0.25 – 20.0	0.9996	0.13	0.43
Progesterone	y = 2226,8x + 2907,4	0.25 - 50.0	0.9994	0.12	0.40
BBP	y = 78265x + 116012	0.10 – 50.0	0.9911	0.04	0.13
Estrone	y = 697,48+303,17	0.25 – 50.0	0.9998	0.13	0.43
CBZ	y = 16446x -547,45	0.25 - 100.0	0.9998	0.12	0.40
Acetaminophen	y = 3404,3x + 1406.8	0.10 – 100.0	0.9998	0.05	0.17

After determination of LOD and LOQ for each compound, the influent and effluent concentrations were determined.

4.1.2. Optimization and Analyses of Selected EDCs in Sludge Samples

In order to determine the selected EDC concentrations in sludge samples a new procedure was developed. In this method, all the selected compounds were extracted from the sludge samples simultaneously. Prior to the analyses, type of solvent and time of ultrasound extraction were optimized. At the beginning, sludge put in a centrifuge, 3500 rpm 5 min, to settle down the sludge and to get rid of the supernatant. Then, sludge samples were dried at 105^{o}C and washed to eliminate any background interference. A 0.5 g dried sample was spiked by adding 1.0 mL of EDC mixture containing 20 ng/mL of each compound, so as to bind the compounds to the dried sludge samples. Methanol was then evaporated. Next samples were homogenized by grinding and put into a 250 mL Erlenmeyer flask. In order to compare the effectiveness in extraction, dichloromethane (DCM) and methanol were used as extracting solvents. A 100 mL methanol and 100 mL DCM were placed into each erlenmayer flask. Prepared samples were placed into an ultrasonic bath for 30 mins for extraction. At the end of 30 mins, samples were centrifuged at 3400 rpm for 10 min to eliminate suspended solids and aliquots

were collected. This procedure was repeated three times and a 300 mL of solution was obtained at the end. The 300 mL of each solvent was evaporated at 40 ^{o}C, leaving EDCs on the glassware. Glassware was washed with 3.0 mL of 75% methanol-ultra de-ionized water (v/v) and the wash solvent was collected. Then, samples were analyzed by HPLC-ESI/MS/MS and The Total Ion Chromatographs (TIC) of methanol versus DCM was given in Figure 4.1.33 for comparison.

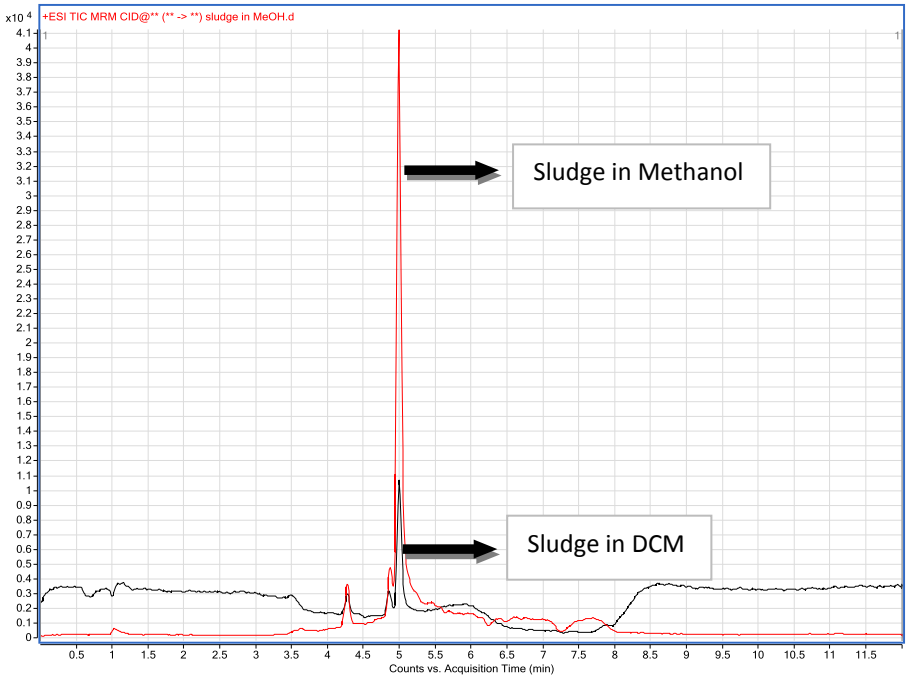

Figure 4.1.33 Comparison of TIC for methanol and DCM

As can be seen in Figure 4.1.33, the TIC obtained for methanol was much higher than TIC for DCM. After determination of the solvent type; number of replicates needed for maximum extraction recovery was. It was found that most of the analytes were extracted in the first 100 mL of solvent; only small amounts could be extracted in the second and third extractions, as shown in Figure 4.1.34.

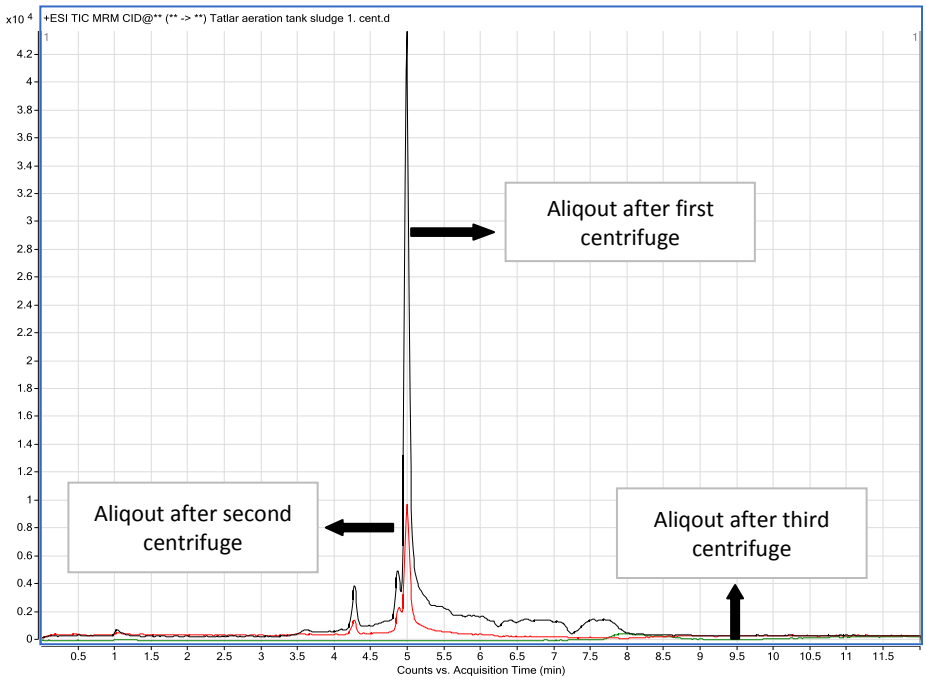

Figure 4.1.34 The TIC of EDCs in all three aliquots after centrifugation

As can clearly be seen in Figure 4.1.34, after third extraction, there was no analyte left to be detected in the sample. For this reason, three times ultrasound extraction of sludge samples were chosen as optimum to be on the safe side. During this extraction procedure, also 3 different procedures, as given in Table 4.1.6, were applied to complete the optimization of extraction.

Table 4.1.6 Analytical figures of merit.

	Time for ultrasound (min)	**Amount of Methanol (mL)**
Procedure 1	30	100 (3 times)
Procedure 2	45	100 (3 times)
Procedure 3	30	50 (6 times)

The procedure given in Fig. 4.2.1 was followed to optimize extractions and TICs were obtained in triplicates to judge the most efficient combination, as given in Figure 4.1.35.

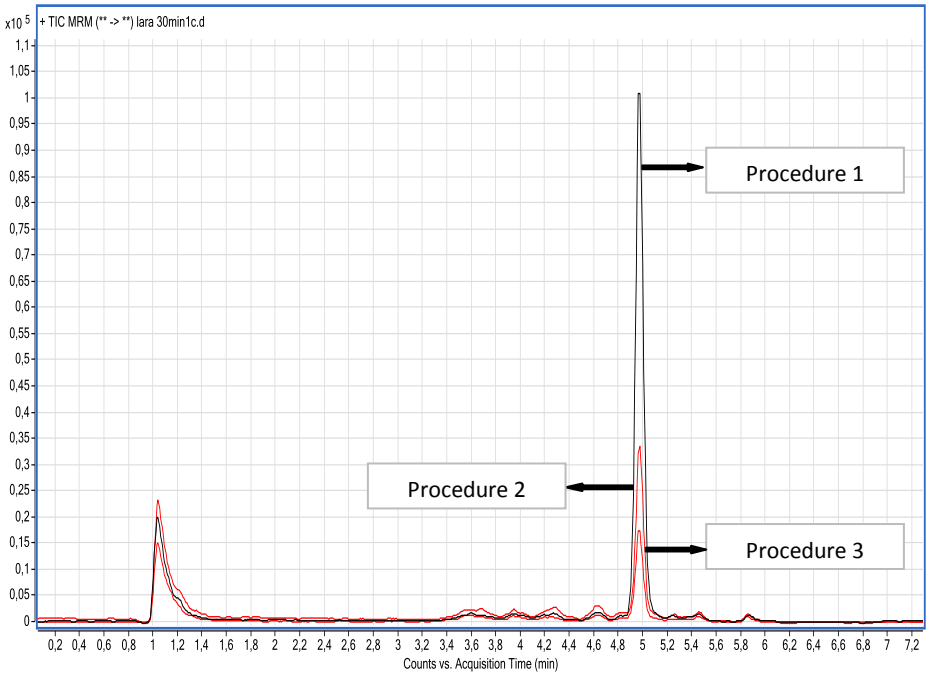

Figure 4.1.35 The TIC of analytes in different extraction procedures

From the TIC analyses the highest peak was determined by Procedure-2 which was 3 times 100 mL methanol with 30 min. ultrasound extractions. This completed sludge extraction optimization. In addition, standard addition was applied to make sure that there was no matrix interference to the analysis. During standard addition, mixed standard solution at different concentrations was prepared and added to the sludge samples. Results obtained from standard addition and from direct calibration plots were compared and it was observed that slopes of both plots were not significantly different from each other. Consequently, direct calibration was used during the study.

From the calibration cure of the study, the R^2 values obtained in linear calibration plots were at least 0.99 for each species. The mixed standard solution

containing 0.25 μg/L of each analyte was analyzed ten times for LOD and LOQ calculations. Reproducibility of retention times of signals was also determined. The % relative standard deviation, RSDs, of retention times were found lower than 0.8% for all the analytes tested in recovery experiments. From the calculations of extraction recoveries of the analytes for sludge, higher than 90% recovery were achieved for all the analytes as given in Table 4.1.7.

Table 4.1.7 Analytical figures of merit for sludge.

Analaytes	Equation (y=mx+n)	Linear Range (µg/L)	LOQ for sample (µg/kg)	Recovery (%)
Diltiazem	y = 14265x + 2526.8	0.50-20	2,6	96.2 ± 0.3
Progesterone	y = 7433.2x + 2662.2	0.10-100	2,4	97.5 ± 1.1
BBP	y = 52969x + 17875	0.10-20	0,8	93.0 ± 0.3
Estrone	y = 749.71x – 166.28	0.50-100	2,5	97.2 ± 0.6
Cbz	y = 49852x + 1519.5	0.5-50	2,4	95.3 ± 0.4

4.2. TREATMENT STUDIES

4.2.1. Treatment of Selected Endocrine Disrupter Compounds by Vacuum Rotation Membrane (VRM) Bioreactor

The first treatment plant used for the investigated of the removal of selected EDCs was Vacuum Rotation Membrane (VRM) bioreactor. At the beginning of the study, the SRT was arranged as 10 days to understand the effect of SRT on the removal of selected EDCs. The wastewater was passed through the fine screen, 3 mm openings, and then activated sludge tank was fed with the wastewater. The MLSS concentration in the aeration tank was about 4.5 g/L. The MLSS concentration in membrane chamber was about 7.5 g/L since solid liquid separation was done in this chamber. In addition, after membrane filtration UV disinfection was applied. Since the same influent was being used in the Clear-Box MBR system, the same fluxes, 13, 16, 20, 23 and 26 L/m^2-h, were applied to both. However, at higher fluxes an error has occurred in the VRM system and higher fluxes could not be tried. During the study, four different liquid samples were taken including influent, liquid of activated sludge after settling (supernatant), after membrane filtration (before UV) and after UV filtration. The pH in the influent was 7.5 and in the effluent it was 7.4-7.7. Moreover, the DO in the aeration tank was around 2 mg/L. Temperature in the aeration tank was always over 15 ^oC. The influent turbidity was between 91 and 144 NTU and in the effluent it was always less than 1. The influent and effluent conductivity was always between 1250 and 1450 μmho/cm. The influent turbidity was between 91 and 144 NTU and in the effluent it was always less than 1. The influent and effluent conductivity was always between 1250 and 1450 μmho/cm. As can be seen in Figure 4.2.1, over 90% COD removal was achieved.

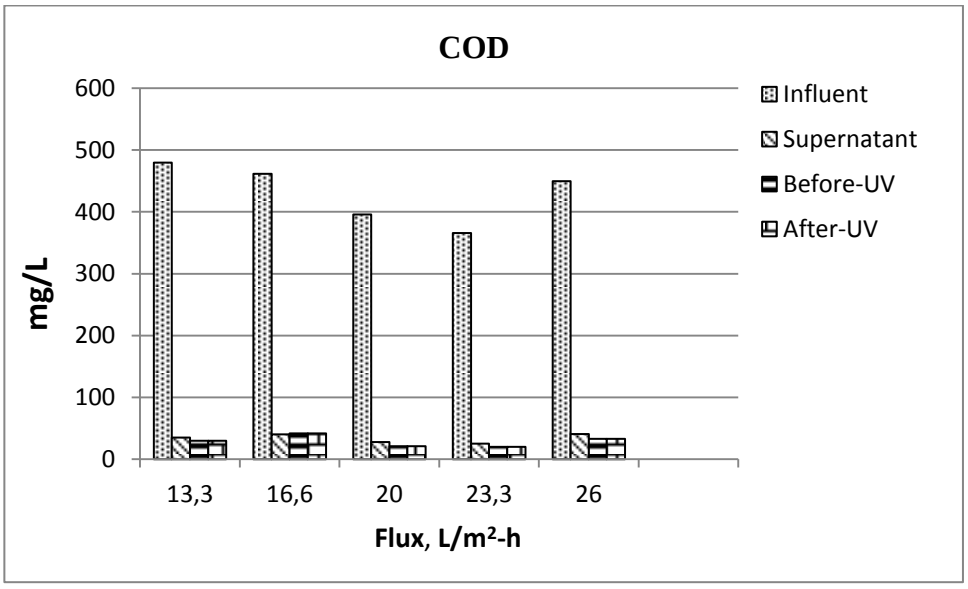

Figure 4.2.1 COD concentration in the influent, supernatant and before and after UV for VRM Plant when Θ_c=10 days

As can be seen from Figure 4.2.1, COD concentration in the supernatant was same as that after membrane filtration. After steady-state conditions were reached, composite samples, at 4 ^{o}C, were collected from influent, membrane effluent (before UV) and after UV disinfection point. Grab sample from the aeration tank were also taken. Samples were immediately transferred to the laboratory for analysis. In addition, in flux experiments flux rate was momentarily changed to the desired settling without affecting HRT of the system.

Diltiazem removal in VRM plant:

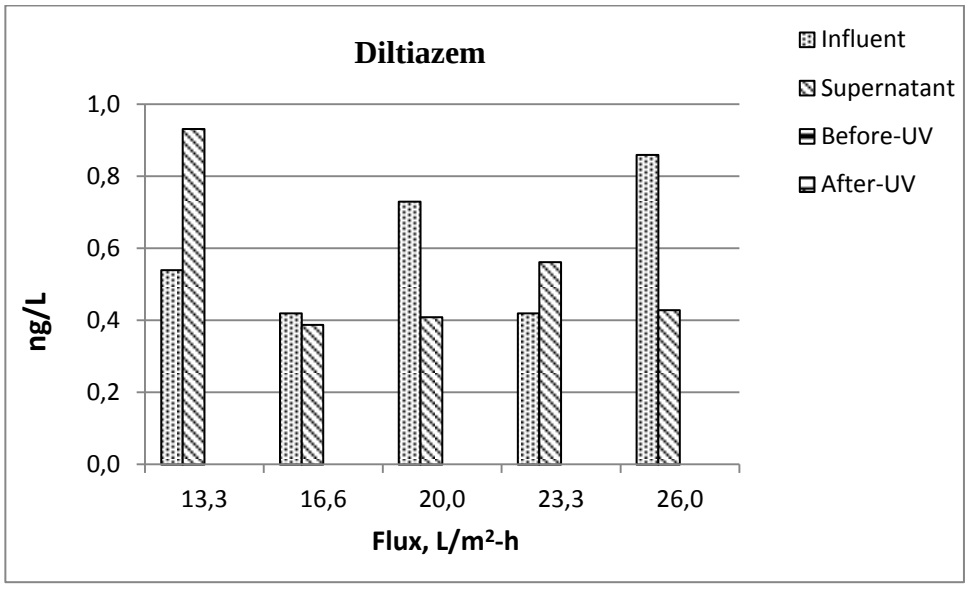

Figure 4.2.2 Diltiazem concentrations at different fluxes in influent, supernatant, before and after UV in VRM Plant when Θ_c=10 days

As seen in Figure 4.2.2, influent and aeration tank supernatant concentrations of diltiazem were not far apart for all the fluxes tested. The slight difference observed between influent and supernatants at some fluxes may be due to an experimental artifact observable at such trace levels. After membrane filtration, diltiazem concentration was under the limit of detection. Diltiazem was not detected in the sludge samples too.

The second compound investigated in VRM plant was progesterone. The flux results are given in Figure 4.2.3.

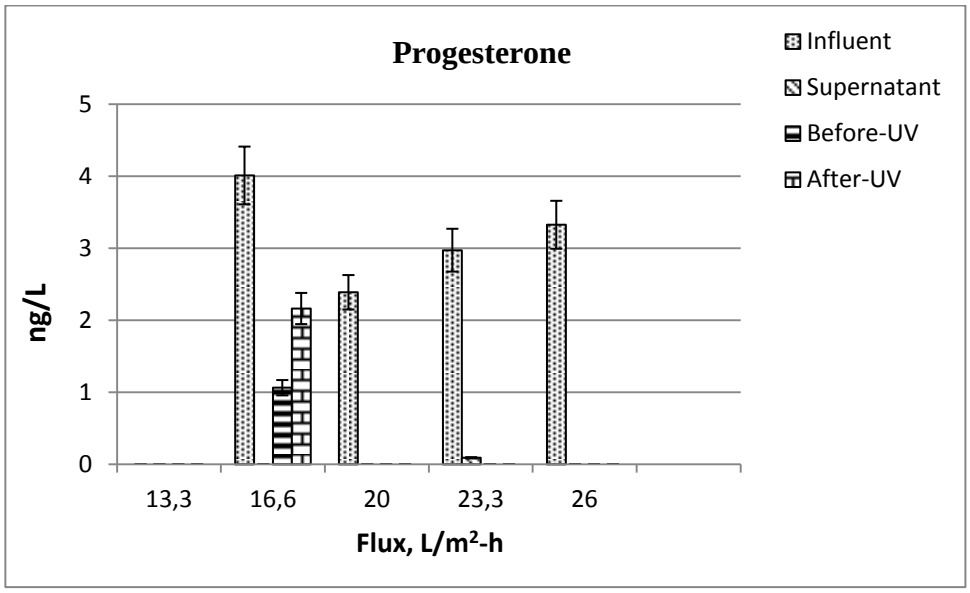

Figure 4.2.3 Progesterone concentrations in influent, supernatant, before and after UV in VRM Plant when Θ_c=10 days

As can be seen in Figure 4.2.3, concentration of progesterone was under the limit of detection in supernatant, permeate and after UV disinfection. Observations at flux 16,6 L/m^2-h is obviously erroneous due to trace level analysis. This results clearly show that progesterone was removed biologically and no noticeable effect of permeate flux exists. Progesterone in sludge was also below detection limit.

Another natural hormone studied was estrone. The estrone removal in VRM at differing membrane fluxes is given in Fig. 4.2.4 when SRT was 10 days.

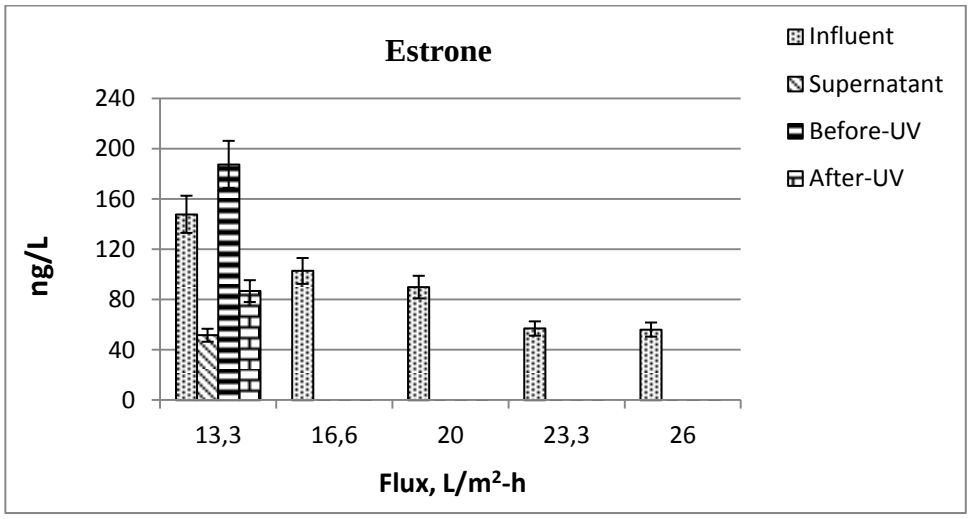

Figure 4.2.4 Estrone concentration in the influent, supernatant, before and after UV in VRM Plant when Θ_c=10 days

As seen from Fig. 4.2.4 estrone concentration was under limit of detection except for when flux was 13,3 L/m^2-h which is obviously erroneous due to trace level analysis. Estrone could not be detected in sludge samples.

CBZ was another compound investigated during the study. The removal efficiency in VRM for Θ_c=10 days is given in Fig. 4.2.5.

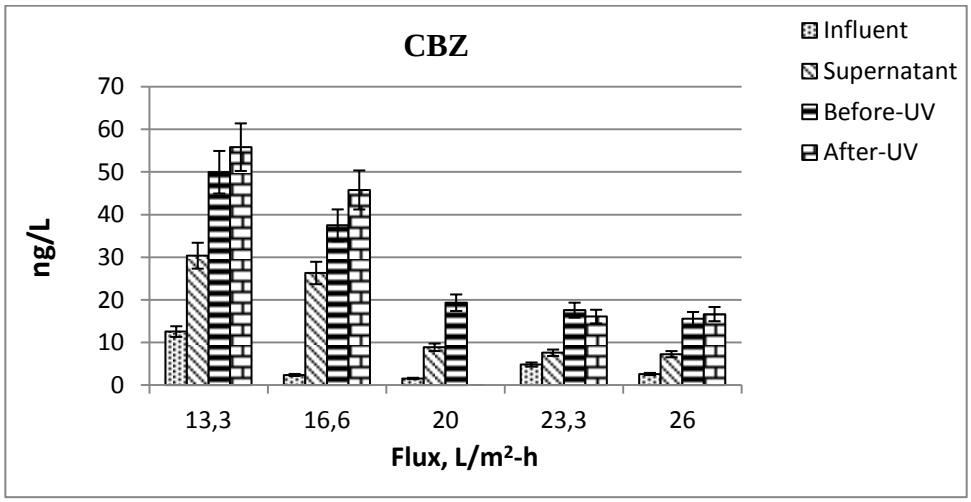

Figure 4.2.5 CBZ concentration in the influent, supernatant, before and after UV in VRM Plant when Θ_c=10 days

The CBZ concentration in the influent was less than those in the supernatant and permeate samples. This is obviously due to an artifact arising from the background wastewater matrix. Evidently high organics content in the influent obscured the compound from detection. Whereas, after wastewater going through biological treatment, matrix organics have been removed thus making the compound available to detection. The CBZ concentration in the supernatant was also less than that in the permeate before and after UV disinfection. This was systematically so; indicating that CBZ concentrates on the membrane surface and released during suction. The difference between supernatant and the permeate was clearly due to presence of membrane. It can be deduced that CBZ is not removed by the full scale membrane plants when SRT was 10 days and UV has no effect. CBZ concentration in sludge was under limit of detection.

The last compound investigated during this study was acetaminophen; whose results are summarized in Figure 4.2.6.

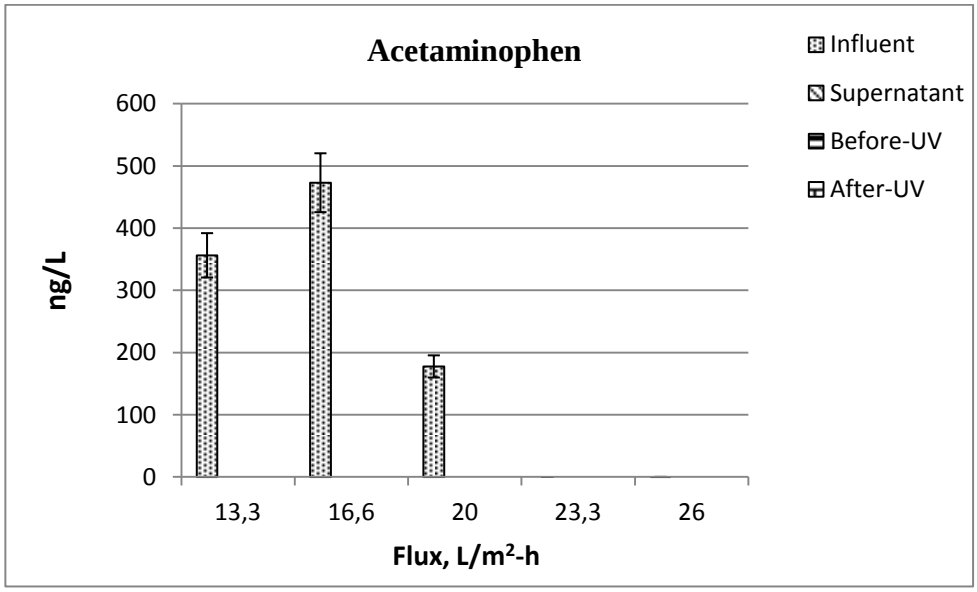

Figure 4.2.6 Acetaminophen concentration in the influent, supernatant, before and after UV in VRM Plant when Θ_c=10 days

As seen in Figure 4.2.6, acetaminophen could not be detected in permeates or supernatants, indicating that it is completely biodegraded. Variations in the influent concentration were evidently due to experimental error occurring at trace determinations. Sludge did not contain acetaminophen.

4.2.2. Treatment of Selected Endocrine Disrupter Compounds by Clear-Box Membrane Bioreactor with Different Sludge Retention Times

The second treatment plant used for the study of EDCs removal was the Clear-Box pilot unit, produced by HUBER A.G. This plant was composed of 3 m^2 plate type membrane unit submerged in an activated sludge tank. Same wastewater was shared between VRM and Clear-Box units. After passing the fine screen; wastewater was collected in a storage tank to be dosed into the Clear-Box unit. In order to see the effect of operating parameters on the removal of selected EDCs, different solid retention times (SRT), 10, 15, 20 and 25 days, were maintained at different fluxes.

4.2.2.1. SRT=10 days for Clear-Box MBR Plant

The first SRT studied was 10 days. After reaching steady-state, MLVSS was maintained at 8 g/L in the plant. Permeate flux was adjusted to 13, 16, 20, 23, 26 and 30 L/m^2-h, sequentially for the experiments.

Experiments were carried out in replicates at the same flowrate in two successive days. The 24 hours influent composite samples were collected after the fine screen. Sludge samples were also collected during the experiments. In order to see the level of ongoing treatment in Clear-Box, general pollution parameters were first analyzed. During the study, oxygen concentration in the activated sludge chamber was always over 1 mg/L and close to 2 mg/L. During the study, samples were taken in the influent, liquid of activated sludge after settling, supernatant, and permeate of membrane. The pH in influent and effluent was

around 7.5 and 8. The turbidity in the influent was between 95 and 140 NTU and in the permeate was below 1 NTU. Fecal coliform was not detected in the permeate of membrane. Influent COD concentration was between 350 and 550 mg/L and permeate of membrane COD was between 12 and 40 mg/L. The COD concentrations at different fluxes are given in Figure 4.2.7.

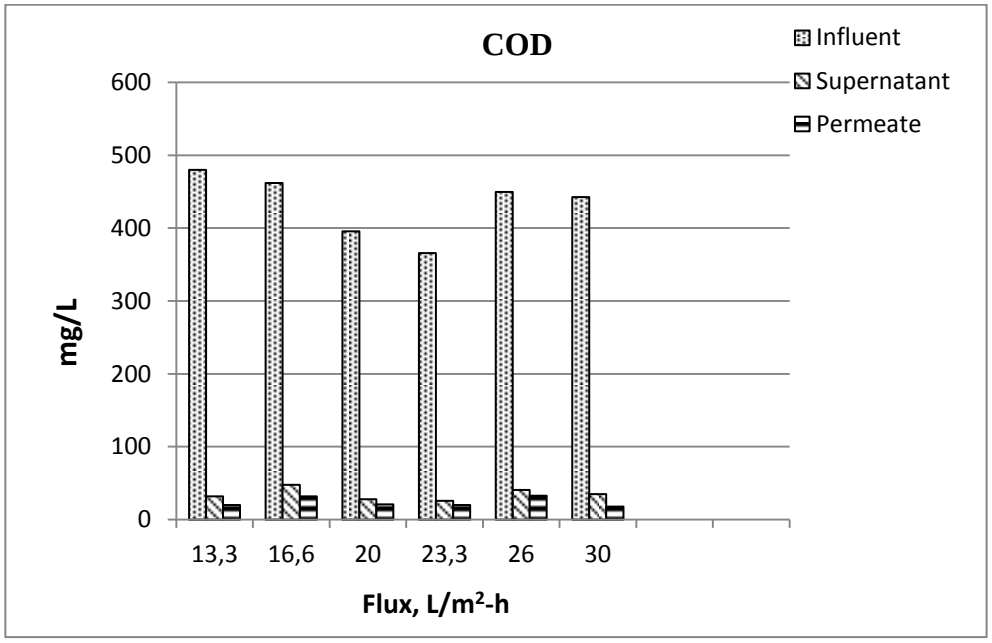

Figure 4.2.7 COD concentration in the influent, supernatant and permeate for Θ_c=10 days.

As seen in Figure 4.2.7 over 90% of COD was being removed when SRT was 10 days. Once steady-state was established removal of selected EDCs in Clear-box membrane unit was investigated by using the real wastewater without any spike of the compounds.

In flux experiments flux rate was momentarily changed to the desired settling without affecting HRT of the system. Occurrence and removal of diltiazem in the Clear Box MBR system was investigated for Θ_c=10 days. The results are given below:

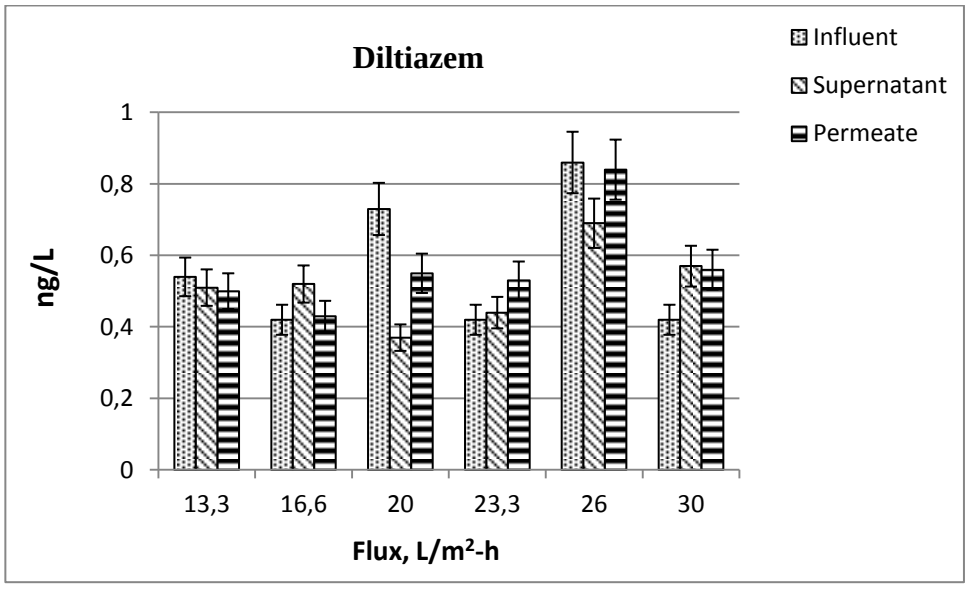

Figure 4.2.8 Diltiazem concentration in the influent, supernatant and permeate of membrane when Θ_c=10 days

It is clear from Figure 4.2.8 that, diltiazem could not be removed by the activated sludge process nor by additional membrane separation. Influent was variable between 0.42 and 0.86 ng/L. The supernatant concentration, which represents an activated sludge effluent, was almost the same as with the influent. There was barely noticeable differences in the membrane permeate, as compared to supernatant, particularly at higher fluxes. At fluxes between 13 and 16 L/m^2-h the influent, supernatant and membrane permeate concentrations were almost the same. However, when the flux was 23 and 26 L/m^2-h, the diltiazem concentration in permeate was higher than diltiazem concentration in the influent. This could be explained by sludge deposited on the surface of membrane releasing the compound at higher flux. However this result contradicts those obtained in full scale VRM, where diltiazem was found completely biodegraded in VRM. Since both plants utilize identical biomass for treatment and share a common feed wastewater; this suggests a scaling-up effect.

Progesterone was the second compound investigated during the study. Since this is a natural hormone excreted by the pregnant women, the

concentration was very low and sometimes not detectable in the influent of the Clear-Box MBR plant. The influent concentration of progesterone was between 0.29 and 4.01 ng/L and between 0.12 and 0.70 ng/L in the supernatant. It was not detectable in the membrane permeates, as can be seen in Figure 4.2.9. Absence of any detectable amount in sludge suggested complete biodegradation.

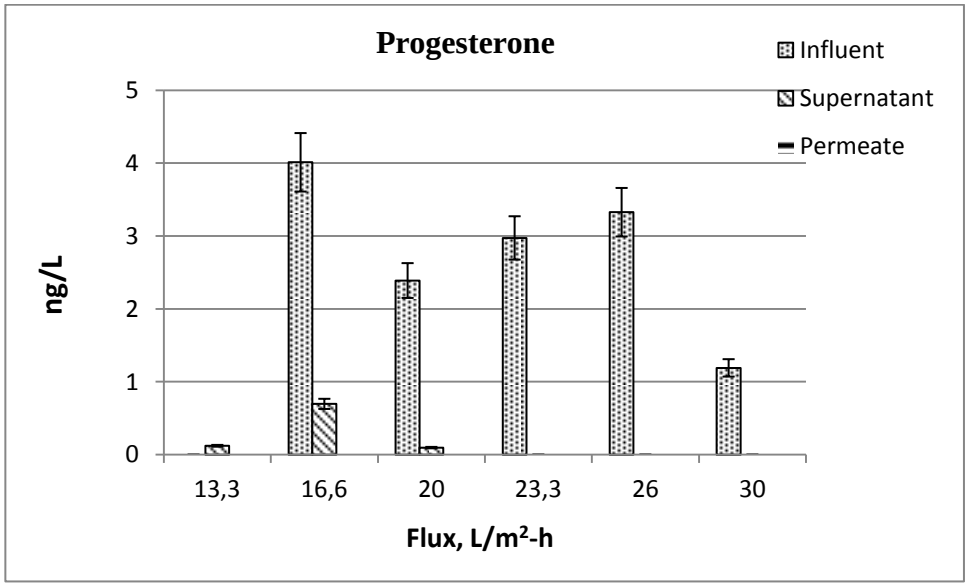

Figure 4.2.9 Progesterone concentration in the influent, supernatant and permeate of membrane when Θ_c=10 days

BBP, widely used in vinyl tiles and in PVC as a plasticizer, was not detected in the influent wastewater when Θ_c was 10 days during the study.

Estrone, which is a weaker form of estrogen, whose main source is women who have undergone through menopause, was another compound investigated during the study. It was detected between 52 to 810 ng/L in influent wastewater samples during the study. The results are summarizes in Figure 4.2.10.

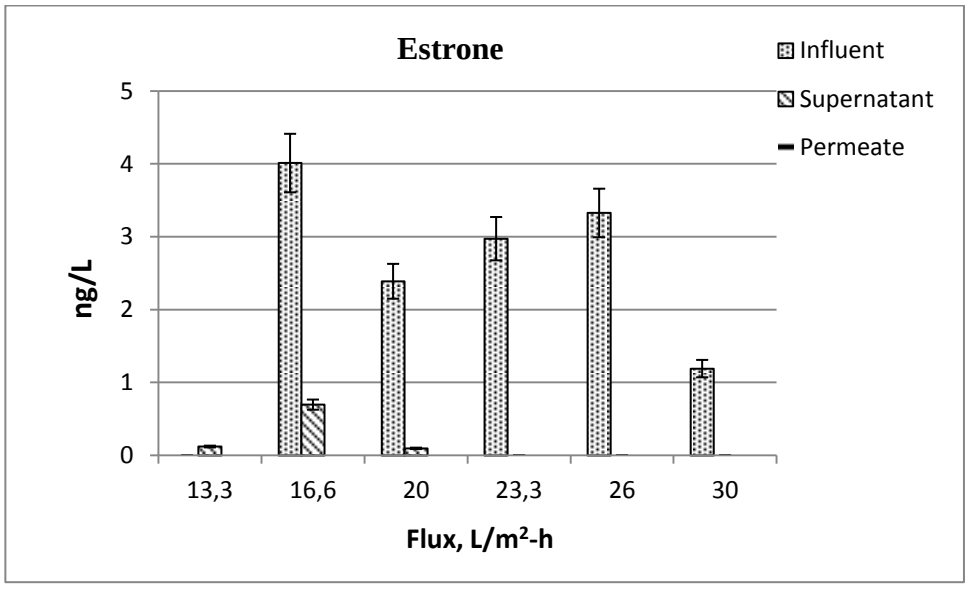

Figure 4.2.10 Estrone concentration in the influent, supernatant and permeate of membrane when Θ_c=10 days

Estrone was close to limit of detection in the influent and was not detected in the supernatants and permeates. Although, the log k_d value for estrone was medium, e.i. 2.4-2.9 (Suarez et al., 2008), it was not deposited in the sludge. Moreover, k_{biol} of estrone is between 200 and 300 (L g^{-1} SS day^{-1}) (Suarez et al., 2008), all the estrone was biodegraded in the system.

Carbamazepine, which is used widely as an anti-epileptic agent for newly diagnosed cases of epilepsy, and for treatment of depression, was another selected EDC investigated during the study. The measured concentration in the influent, supernatant and permeate of membrane are given in Figure 4.2.11.

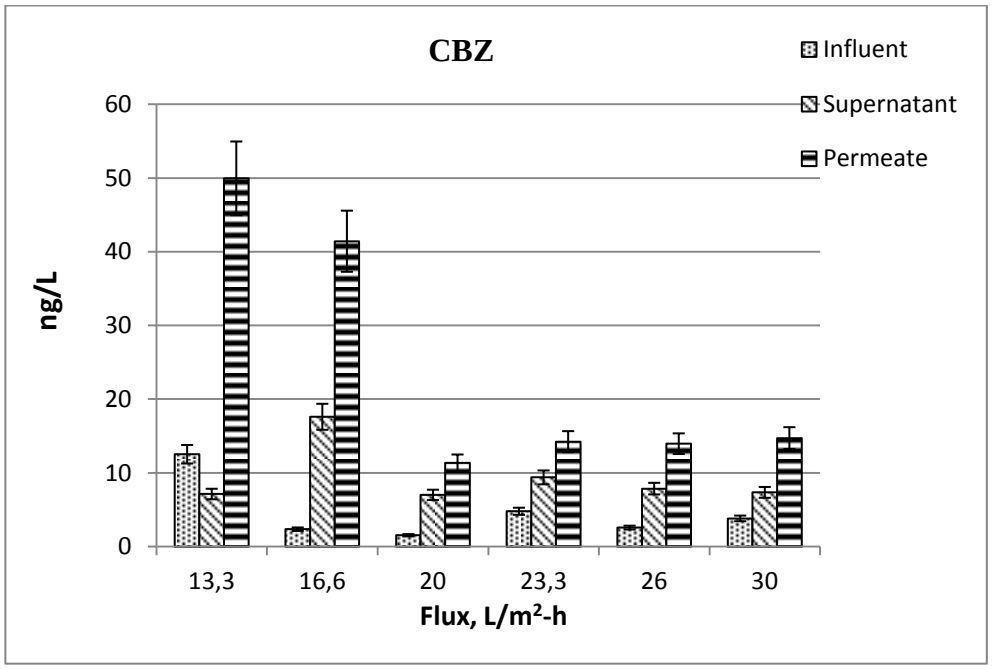

Figure 4.2.11 CBZ concentration in the influent, supernatant and permeate of membrane when Θ_c=10 days

The log k_d and k_{biol} for CBZ are both very low (Suarez et al., 2008), meaning that this compound is neither biodegradable nor removed by sorption onto the sludge. In fact it could not be detected in sludge. It is evident from Figure 4.2.11 that this compound is concentrated on the membrane and permeate effluent concentrations were higher than the supernatants. This finding is consistent with the previous findings with VRM (Section 4.2.1, Figure 4.2.5). In the case of lower levels observed in permeates at higher fluxes suggest that this compound, which is adsorbed onto the membrane, was diluted by the increased flow rate of the passing fluid at higher fluxes. Moreover, influent concentration was lower than permeate or supernatant levels in Figure 4.2.11. This is clearly due to analysis artifact, where compound was masked for detection by the background organics in the influent but un-masked upon treatment.

The last compound studied was acetaminophen which is widely used as fever-reducer and pain killer. The concentration of this compound at different fluxes for Θ_c=10 days is given in Figure 4.2.12.

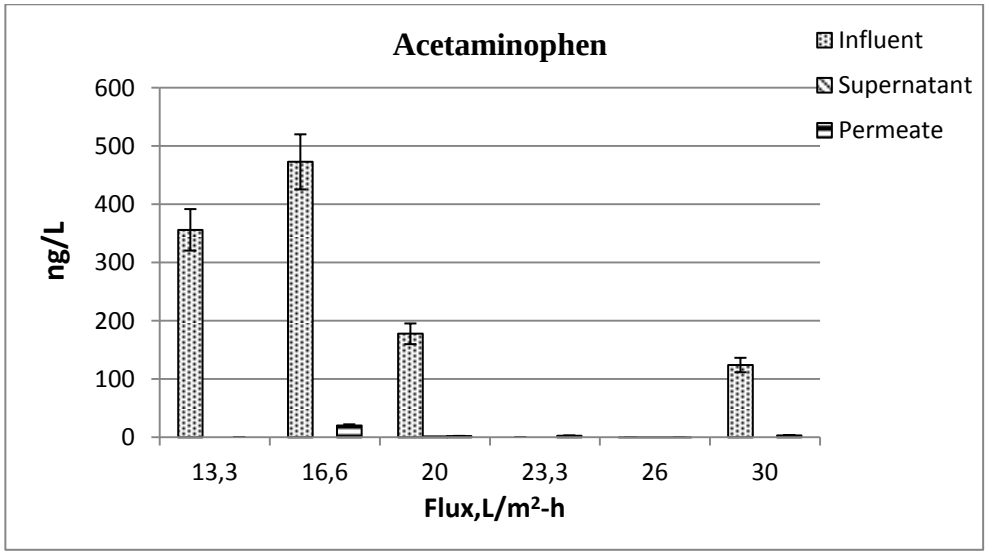

Figure 4.2.12 Acetaminophen concentration in the influent, supernatant and permeate of membrane when Θ_c=10 days

Acetaminophen concentration in the wastewater influent was between 100–480 ng/L. Although, biodegradation and sludge-water partition coefficients are quite low for this compound (Jones 2002), it was almost entirely removed by the activated sludge process, as indicated by its absence in supernatants. However, consequent upon membrane filtration it was detected between 2.1 to 20.2 ng/L in the permeate. Around 95% removal was achieved after membrane filtration. Its detection in permeates suggest concentration of this compound by the membranes thereby making it unavailable to microbial degradation.

4.2.2.2 SRT=15 days for Clear-Box MBR Plant with EDC spiked influent.

The SRT was adjusted to 15 days in order to see the effect of SRT on removal of selected EDCs. In order to attain steady-state conditions Clear Box MBR was operated for over 30 days, 2 SRTs, before experimentation. The MLSS

concentration stabilized at 7.5 g/L at the end of this period. The dissolved oxygen concentration was 2 mg/L. During these experiments EDCs were spiked into the wastewater to overcome fluctuation in concentrations and for ease of detection. Following 5 HRTs; samples were collected at differing fluxes and corresponding COD values are given in Figure 4.2.13. In addition, in flux experiments flux rate was momentarily changed to the desired settling without affecting HRT of the system. As can be seen from this figure over 90% COD removal could be achieved.

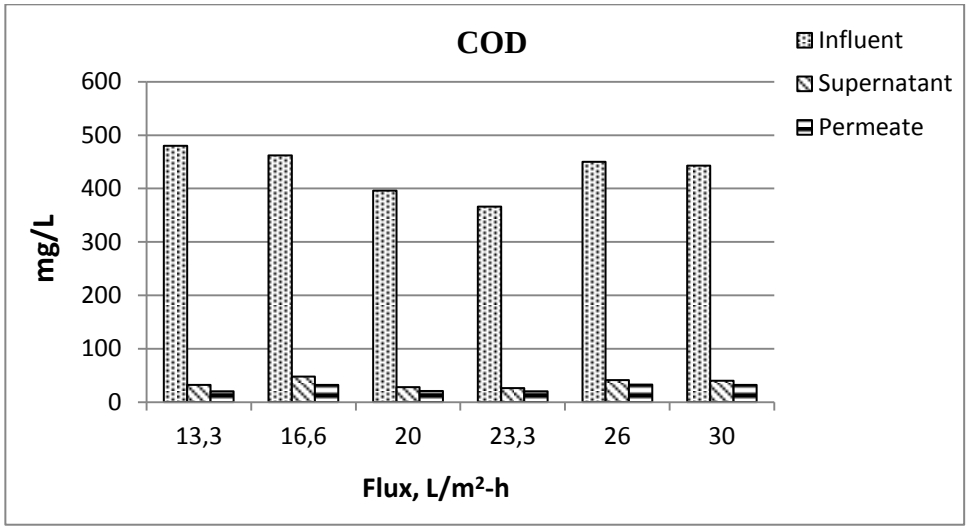

Figure 4.2.13 COD concentration in the influent, supernatant and permeate for Θ_c=15 days.

In order to analyze selected EDCs, 100 mL samples from the influent, supernatant and effluent were passed through the SPE cartridge. Then, samples were eluted with 25 mL methanol. In order to skip the drying step, 75 mL distilled water was added to the eluates to obtain 25% (v/v) MeOH- H_2O solution. Finally, these were analyzed in LC/MS/MS.

During the analyses, two replicate samples were collected at every flux studied, and the supernatants.

The first compound studied in this context was Diltiazem. Its determined concentration in the influent, supernatant and membrane permeates are given in Figure 4.2.14.

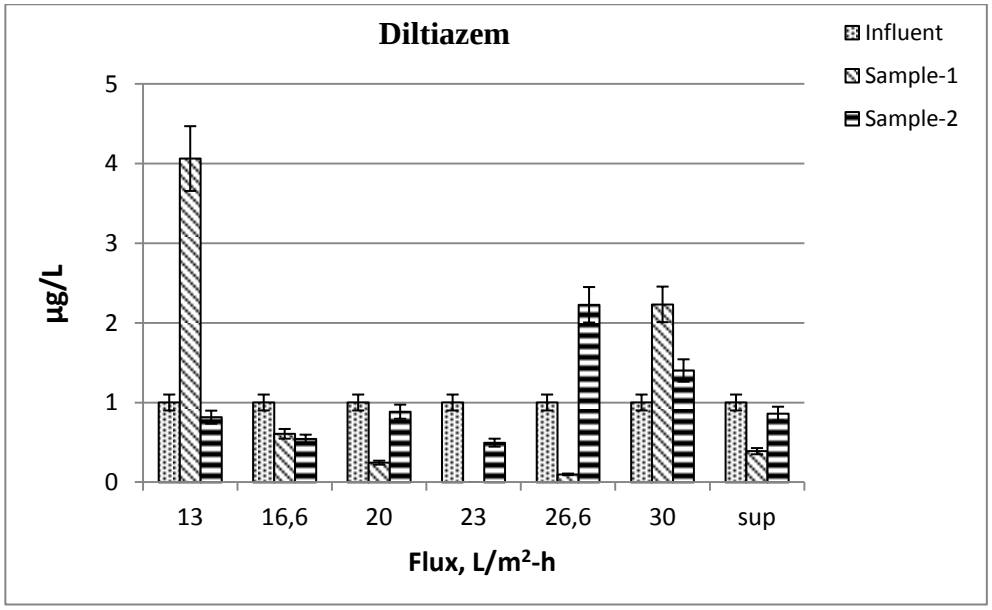

Figure 4.2.14 Diltiazem concentration in the influent, supernatant and permeate of membrane when Θ_c=15 days

As can be seen from Figure 4.2.14 that influent concentrations were steady at around 1 ppb while effluents were variable. Even two effluent samples at the same flux were markedly different. This behavior was attributed to the membrane effect and to the somewhat dynamic state of suction cycles. Evidently, compounds were enriched on the membrane surface due to adsorption and were released by the changing flux during suction cycle. Also state of the biofilm deposition, or so termed concentration polarization, may account for this outcome. At the start of a suction cycle membranes are exposed, whereupon biofilm gradually deposits over the membrane surfaces due to suction; followed by suction stop and sweeping of biomass with the course air bubbles during relaxation. However, it is seen from Fig. 4.2.14 that diltiazem was not removed at significant amount in this membrane treatment system, at 15 days SRT.

The second compound tested was progesterone. Influent concentration of progesterone was about 3.7 μg/L and the effluent concentrations detected in the replicate effluent samples are given in Figure 4.2.15.

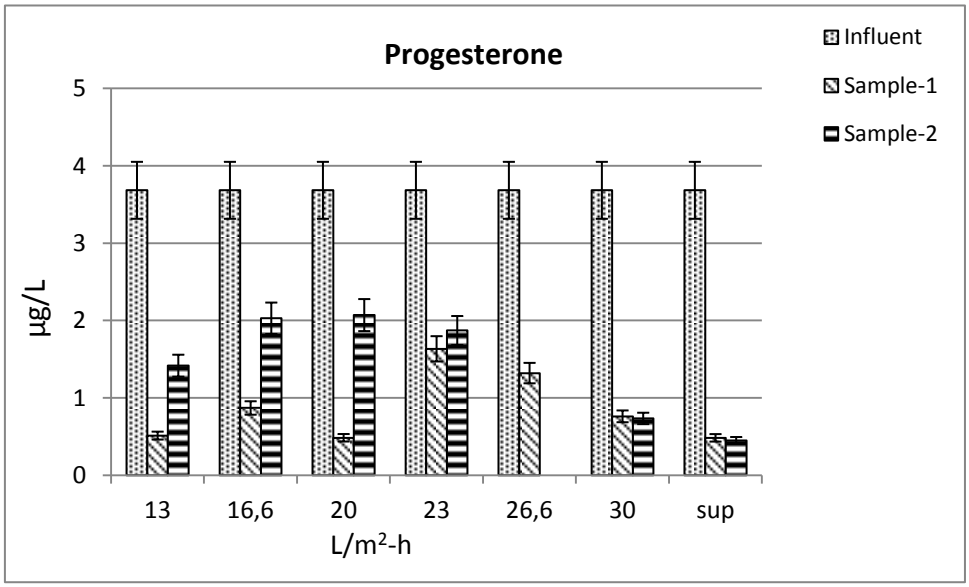

Figure 4.2.15 Progesterone concentration in the influent, supernatant and permeate of membrane when Θ_c=15 days

As seen in Figure 4.2.15., variability between effluent replicate samples was still observable. For example when the flux was 13 L/m^2-h, removal in the first sample was over 86%, while it was 60% in the second replicate. However, this fluctuation was not observed in the supernatant samples. It could also be deduced from Figure 4.4.9, that removal of progesterone somewhat decreased with the increasing fluxes. The highest removal was observed in the supernatant samples.

Another compound investigated during the study was estrone. Its removal is given in Figure 4.2.16.

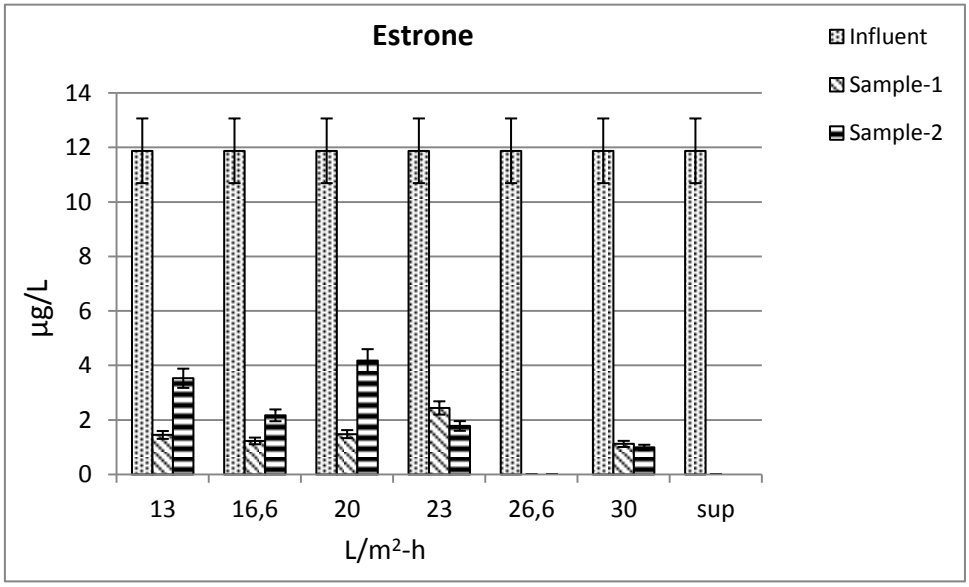

Figure 4.2.16 Estrone concentration in the influent, supernatant and permeate of membrane when Θ_c=15 days

As seen in Figure 4.2.16, influent estrone concentration was 11.8 µg/L. Higher removals of estrone were obtained at the higher fluxes. There were up to 50% difference between replica samples, as observed earlier with the other EDCs tested. For example, when the flux was 13 L/m^2-h, 87 and 70% removals were observed in two replicate samples. Estrone concentration observed in the supernatant was below the limit of detection.

The other compound studied in Clear-BOX MBR system at 15 days Θ_c was CBZ.

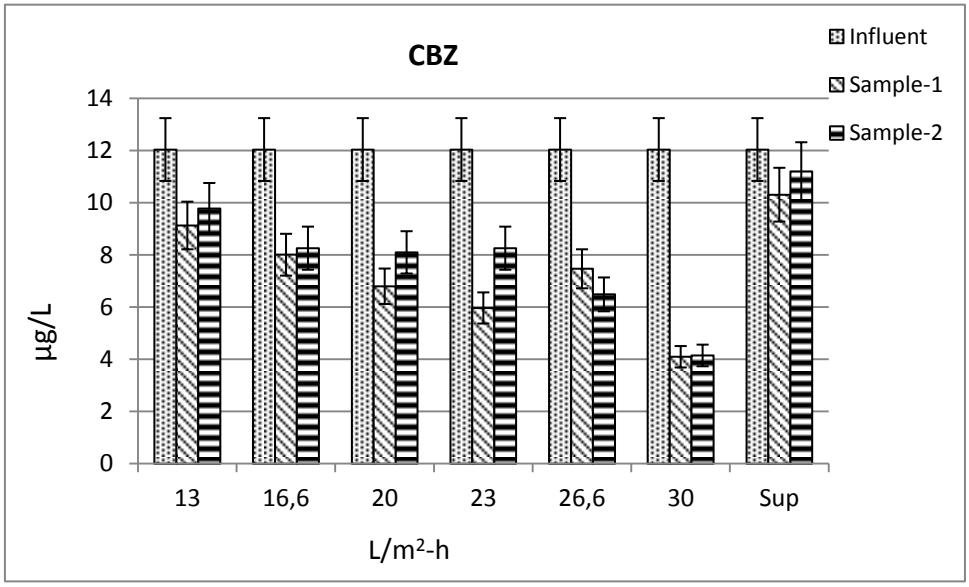

Figure 4.2.17 CBZ concentration in the influent, supernatant and permeate of membrane when Θ_c=15 days

The concentrations of CBZ detected in the influent, supernatant and permeate at different fluxes are given in Figure 4.2.17. There was not great difference between the concentration of CBZ in the influent and the supernatants. It is understood that CBZ is not removed by biological degradation. However, it was clearly seen that there was a membrane effect on the CBZ removal. Contrary to the other EDCs tested there were not large fluctuations between the replica samples and CBZ removal increased at increasing fluxes. The highest removal was about %60, observed when the flux was 30 L/m^2-h.

The last compound studied in this series was acetaminophen.

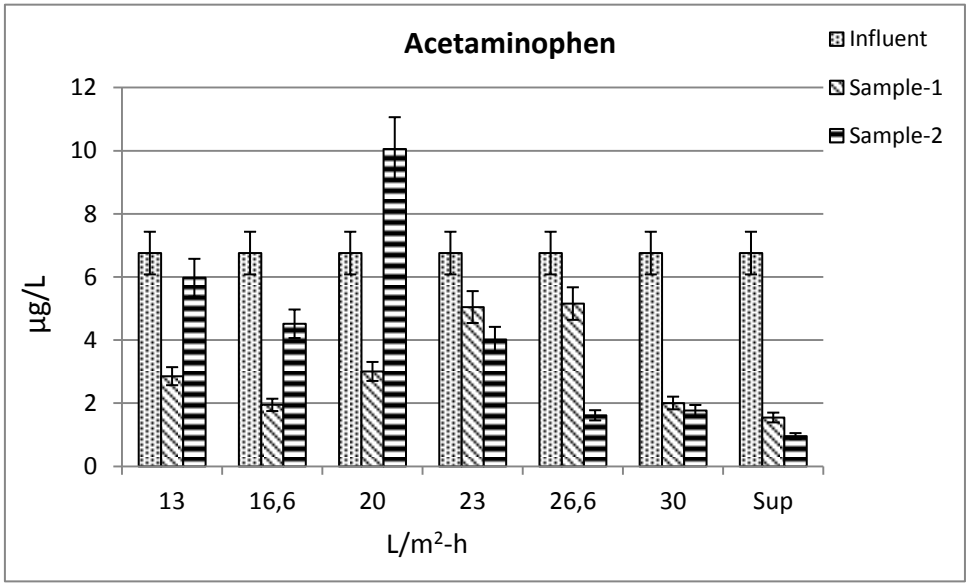

Figure 4.2.18 Acetaminophen concentration in the influent, supernatant and permeate of membrane when Θ_c=15 days

As seen in Figure 4.2.18, influent acetaminophen concentration was 6.5 µg/L. Then, the flow rate was changed to see the effect of flux on the removal of acetaminophen. Except for flux at 30 L/m^2-h; there were huge differences in the detected acetaminophen concentrations in the replicate samples. The highest removal of acetaminophen after membrane filtration was 76% and the lowest was 30%. However, the acetaminophen concentration in the supernatant was lower than 1,5 µg/L and lower membrane permeates. This is indicative of the compound being enriched by the membranes due to adsorption and not being available to microorganisms for biodegradation.

4.2.2.3. SRT=20 days for Clear-Box MBR Plant

The next SRT tested was 20 days. In order to reach steady-state conditions, Clear-Box MBR plant was operated for about 40 days, for twice the period of the selected SRT. The MLSS concentration was steady 7.5 g/L. In flux experiments flux rate was momentarily changed to the desired settling without affecting HRT

of the system. The COD removals observed at this SRT are given in Figure 4.2.19.

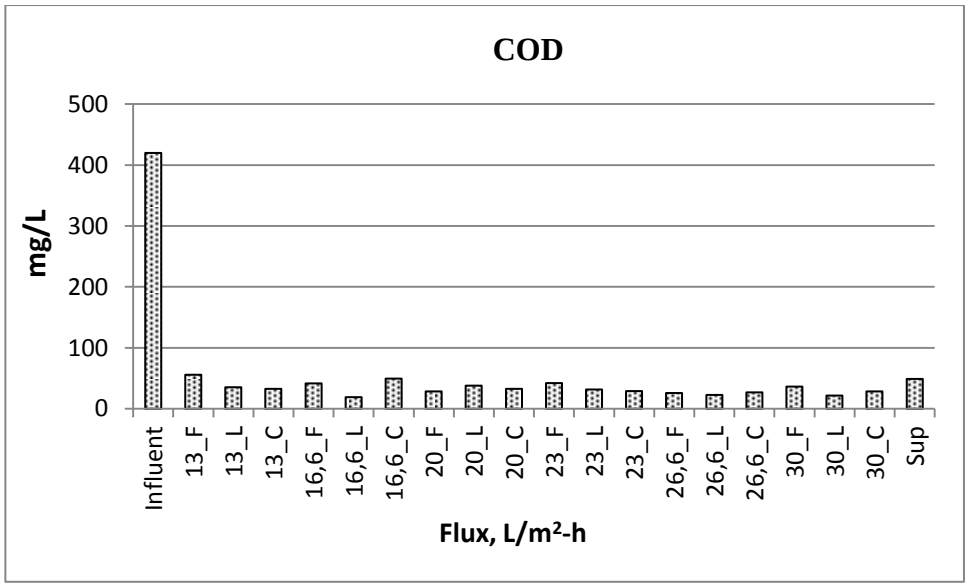

Figure 4.2.19 COD concentration in the influent, supernatant and permeate for Θ_c=20 days.

As seen from Figure 4.2.19, COD removals were over 90% at steady-state. In order to understand the effect of bio-film building on the membrane plate surfaces, or concentration polarization, on the removals; different samples were taken at different periods of suction cycle. The first sample was taken in the first minute of suction (between 0-1 min) designated with *"F"* in this figure. The second sample was taken at the last minute of suction (between 3.5-4.5 min); designated with *"L"*. Lastly, in order to average a complete suction cycle, a sample was accumulated during the course of a complete suction cycle (0-4.5 min); designated with *"C",* at all fluxes.

The first compound studied at 20 days SRTs was diltiazem.

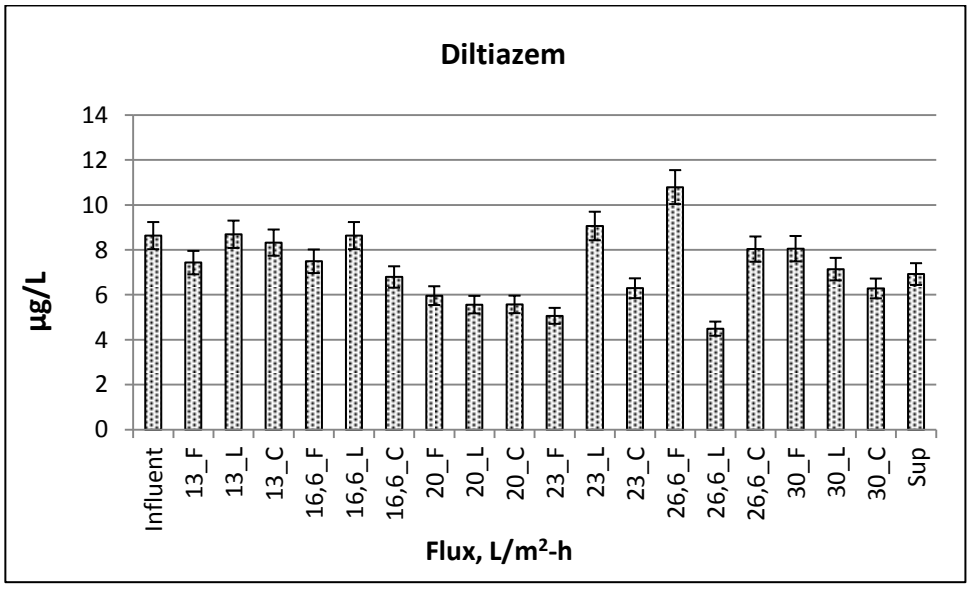

Figure 4.2.20 Diltiazem concentration in the influent, supernatant and permeate of membrane when Θ_c=20 days

As seen in Figure 4.2.20, the influent concentration of diltiazem was 8.5 µg/L. There were noticeable differences in permeate concentrations at different periods of suction. Generally, permeate diltiazem concentration in the first minute of suction was lower than the last minute. In case of 4.5 min composite samples, the permeate concentration was almost at the same level as with the influent at 13 L/m^2-h but decreased up to 20 l/m^2-h and then increased again at higher fluxes Unlike the previous SRTs, sharp differences between replicate samples was not noticeable.

The second compound studied was progesterone.

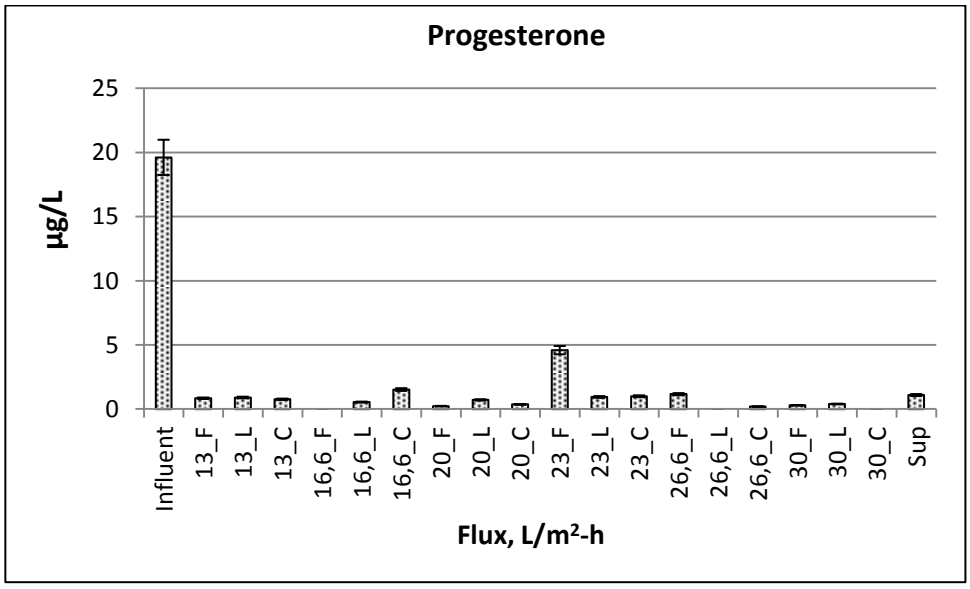

Figure 4.2.21 Progesterone concentration in the influent, supernatant and permeate of membrane when Θ_c=20 days

As seen in Figure 4.2.21, influent concentration of progesterone was 19.5 µg/L and over 95% removal was achieved at all fluxes. There was no membrane effect on progesterone removal, as compared with the supernatant. Compared to the shorter SRTs, removal at 20days SRT was higher.

The other compound was estrone but it could not be detected both in the influent and effluents, therefore could not be studied here.

CBZ was studied at 20 days SRT.

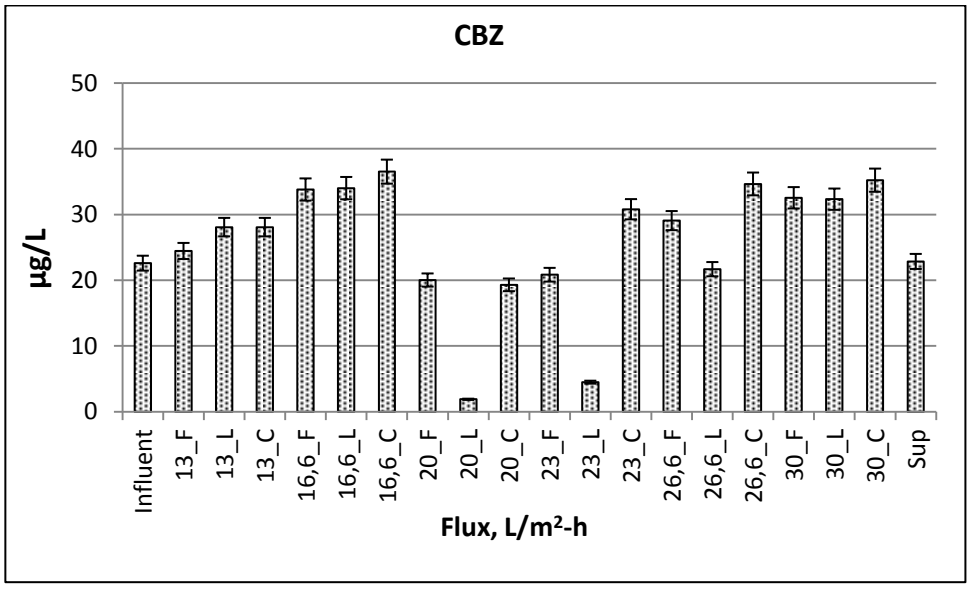

Figure 4.2.22 CBZ concentration in the influent, supernatant and permeate of membrane when Θ_c=20 days

As seen in Figure 4.2.22, the influent concentration was lower than permeate levels at lower fluxes. The supernatant concentration was identical to the influent, indicating no biological degradation. The higher than influent concentrations observed in the permeates could be explained by the membrane effect. As can be deduced from 20 and 23 L/m^2-h fluxes, a huge difference could be seen between the sample taken at the first and last minute of sampling.

The last compound investigated when SRT 20 days was acetaminophen. The removals of acetaminophen are given in Figure 4.2.23.

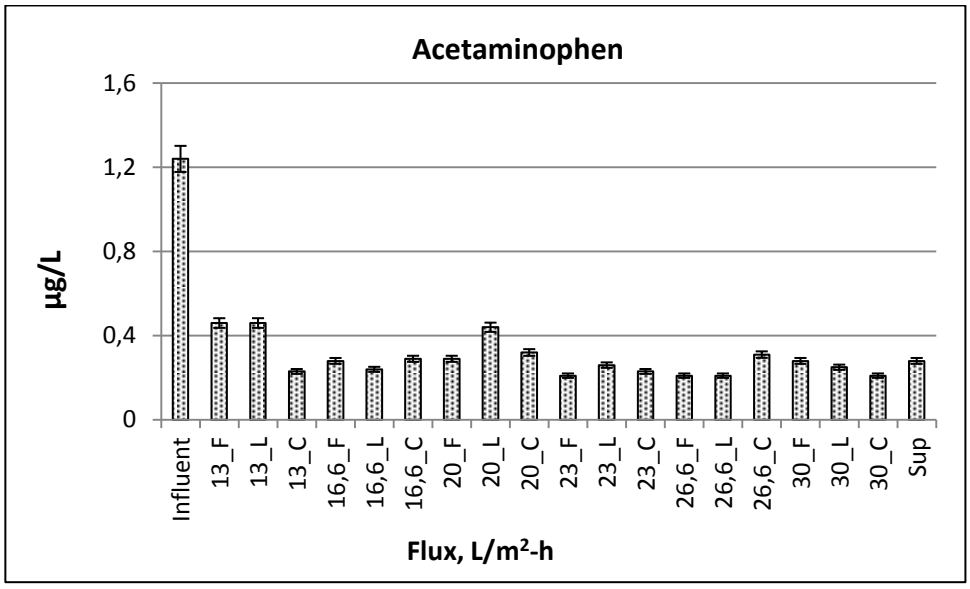

Figure 4.2.23 Acetaminophen concentration in the influent, supernatant and permeate of membrane when Θ_c=20 days

From this figure it can be seen that there was no appreciable difference between permeate and supernatant concentrations and the compound was removed over 80 % by biodegradation.

4.2.2.4. SRT=25 days for Clear-Box MBR Plant

The last SRT studied in Clear-Box experiments was 25 days. In order to reach steady-state conditions, Clear-Box MBR plant was operated for about 50 days, for twice the period of the selected SRT. The sludge concentration was around 6.5 g/L at steady-state. The DO concentration was measured as 2 mg/L. As in previous SRT studied, suction was 4.5 min accompanied with 45 sec relaxation without vacuum. In flux experiments flux rate was momentarily changed to the desired settling without affecting HRT of the system. The effluent COD concentrations measured at steady-state was given in Figure 4.2.24.

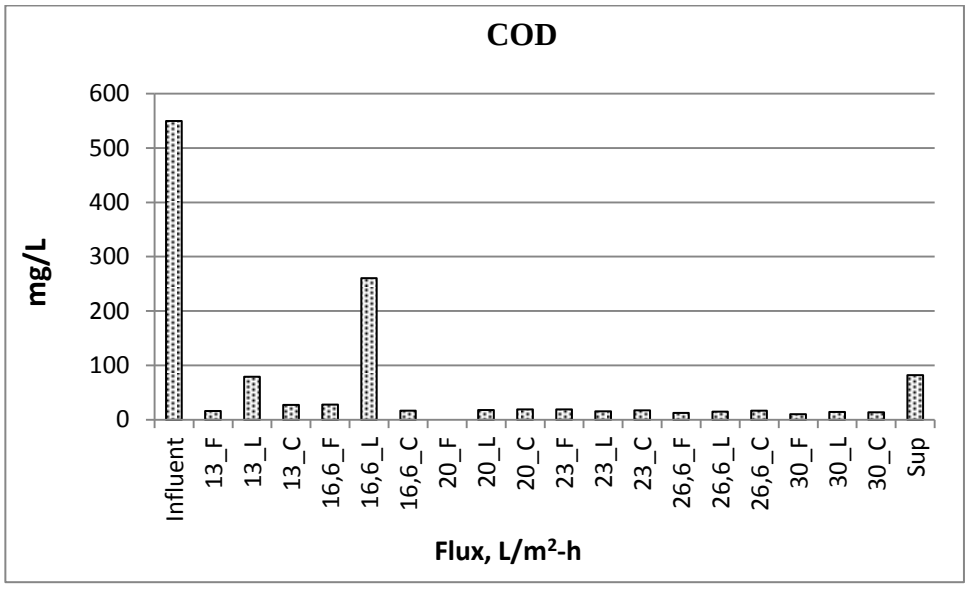

Figure 4.2.24 COD concentration in the influent, supernatant and permeate for Θ_c=25 days.

As seen in Figure 4.2.24, the COD concentration observed in the last minute of suction was higher than that observed in the first minute of suction at lower fluxes. However composite sample CODs were still low and supernatant COD was higher than the composite samples.

Following the steady-state, the investigated compounds were spiked to the storage tank starting from 4 days before the experiments. Diltiazem was the first compound to be investigated in the Clear-Box MBR plant when Θ_c was 25 days.

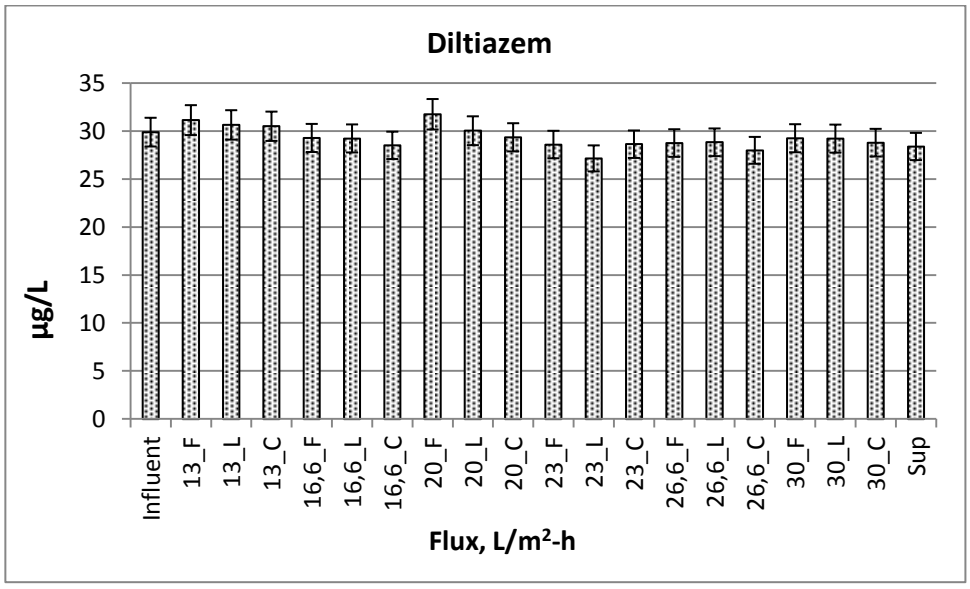

Figure 4.2.25 Diltiazem concentration in the influent, supernatant and permeate of membrane when Θ_c=25 days

As seen in Figure 4.2.25 diltiazem was not removed at all in any flux tested when Θ_c was 25 days. Fluctuation of effluent concentrations at different suction, as was observed in the shorter sludge ages could not be observed. This could be explained by the high diltiazem concentration present in the influent. Yet, concentration of diltiazem in the first minute sample was slightly higher than that in the last minute sample. This may be attributed to the bio-film build up during suction.

The second compound studied was progesterone

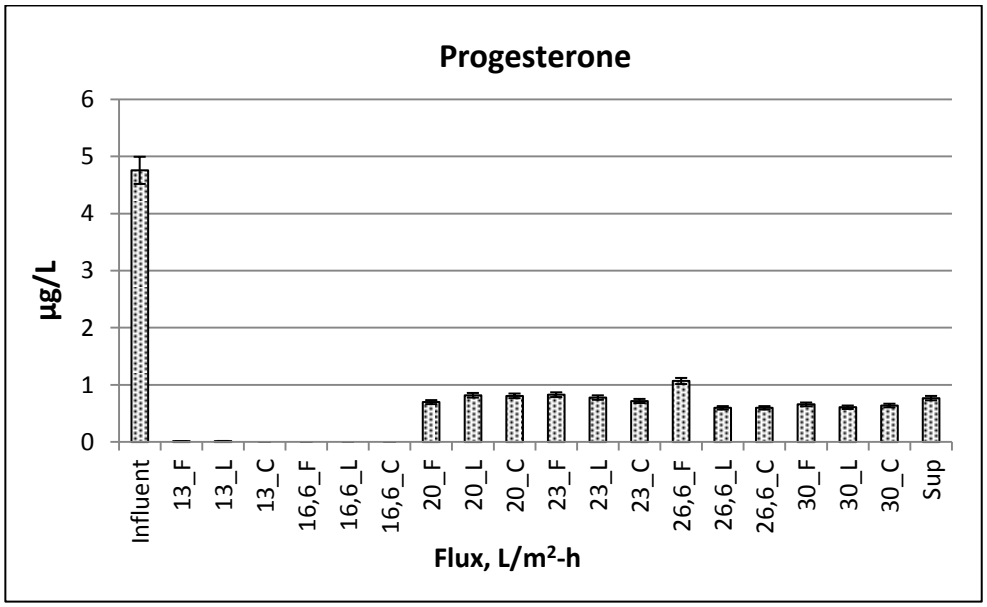

Figure 4.2.26 Progesterone concentration in the influent, supernatant and permeate of membrane when Θ_c=25 days

As seen from Figure 4.2.26, influent progesterone concentration was 5 µg/L and when flux was 13 and 16.6 /m^2-h, removal efficiency was over 99%. However, when flux increased the removal efficiency decreased to about 75%. at and above 20 L/m^2-h. Removal was slightly lower (75-80%) in the first minute samples, than the last minute samples (85%).

The next compound studied in Clear-Box MBR was Estrone at 25 days SRT. Influent concentration of estrone was 11 µg/L but it was below limit of detection in permeate and supernatant. Therefore, results are not plotted.

CBZ was the another compound investigated. The results were given below.

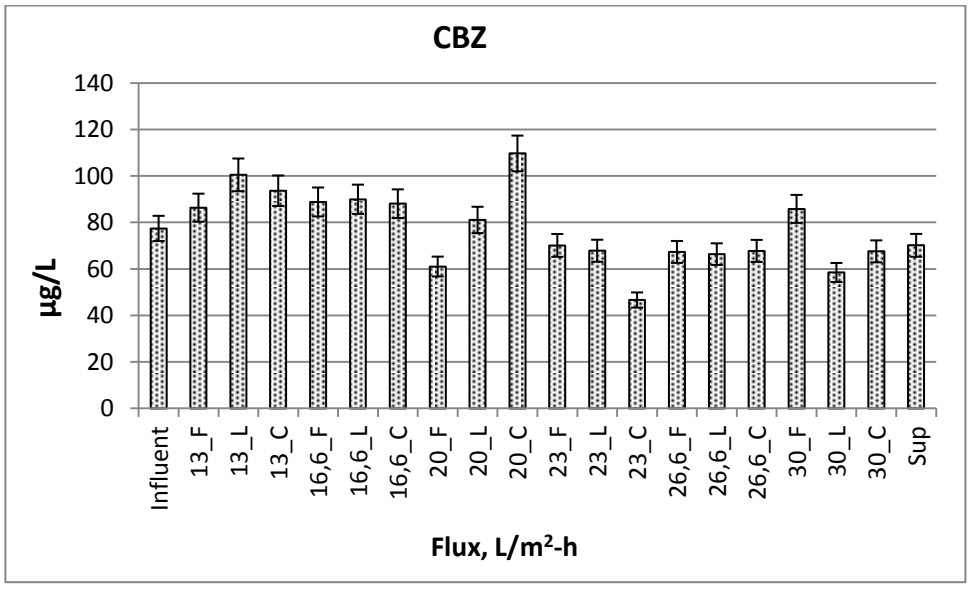

Figure 4.2.27 CBZ concentration in the influent, supernatant and permeate of membrane when Θ_c=25 days

As seen in Figure 4.2.27, influent concentration of CBZ was 80 µg/L. The CBZ was not biodegraded by the microorganisms, and was not removed by sorption on to the sludge either. When the flux was low, effluent CBZ concentration was higher than the influent. When flux was increased to 26.6 and 30, 10 to 15% removal was observed, presumably due to sorption by the membrane. In addition, CBZ concentration in the supernatant was less than CBZ concentration in the permeate of membrane when flux was not high.

The last compound investigated during the study was acetaminophen. The influent concentration was 14 µg/L. It was under limit of detection hence results are not plotted here.

During the study, four different SRT was used for investigation of the effect of SRT on the removal of selected compounds. The removal of selected compounds with different SRTs was given in Figure 4.2.28.

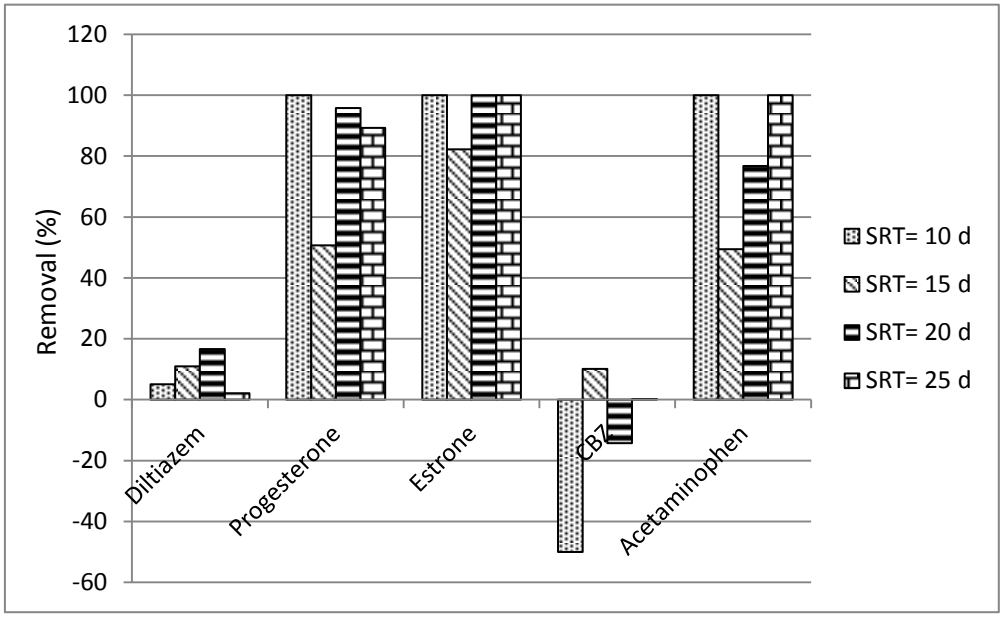

Figure 4.2.28 Removal efficiencies of the Selected EDCs with different SRTs

As was seen in the above figure, the removal of diltiazem was increased when SRT was increased. Although the influent concentration of diltiazem was very low when SRT was 10 days, the removal was about 5%. When the SRT was increased to 15 and 20 days the removal increased for 11 and 16.7%. However, the minimum removal of diltiazem was observed when SRT was 25 days. During this period the removal of diltiazem was about 2%. It was clearly seen from the Figure 4.2.28, progesterone and estrone was completely removed when SRT was 10 days. When SRT was 15 days the removal efficiencies of both natural hormones decreased. However, the removal percentage increased, over 95% for progesterone and 99% for estrone, when SRT was increased. There was not any removal observed in CBZ with different SRTs. The last compound was acetaminophen investigated the effect of SRT on the removal of this compound. Since influent concentration was very low when SRT was 10 days, over 99% removal was observed. However, after spike of these compounds to the influent the removal efficiency decreased for 46%. When the SRT was increased to 20 days the removal percentage increased to 76%. The highest removal was achieved

when SRT was 25 days. Although influent concentration of acetaminophen was 14 μg/L, the effluent concentration was under limit of detection.

In addition to the effects of SRT on the removal of selected compounds, effect of the suction time on the removal of compounds was also investigated. The removal efficiency of diltiazem was higher in the first minute when the flux was low. However, when the flux increased the removal percentage was higher in the last minute. There were not any big differences for the other compounds when compared to the vacuum time.

4.2.3. Occurrence and Removal of the Selected Endocrine Disrupter Compunds in Konacık Membrane Bioreactor

The third treatment plant studied for the removal of selected EDCs was Konacık Membrane Bioreactor. This is a domestic wastewater treatment plant whose plate type membranes are produced by Kubato. Since it is a touristic place, there is no industrial input to the influent. The total membrane surface area of the plant is 2560 m^2 and daily flow handled is about 1250 m^3. The hydraulic retention time (HRT) within the plant was about 16 h at the time of sampling. The Sludge retention time was determined as 25 days during the operation. The MLSS concentration in the aeration tank was about 11-12 g/L and the transmembrane pressure (TMP) was between -40 and -200 mbar during the operation. Since the treatment plant was operated by Konacık Municipality, only removal efficiencies of the selected EDCs were investigated at the set operation conditions by the works. All analyses, except for EDCs, were carried out by Konacık Municipality lab according to standard methods. The general operational characteristics of the plant are given in Table 4.2.1.

Table 4.2.1 Removal efficiency of the Konacık MBR WW Treatment plant

Parameter	Influent (mg/L)	Effluent (mg/L)	Removal (%)
COD	340	< 25	>90
Suspended Solids	185	<10	>95
BOD_5	220	< 25	>90
Total nitrogen	65	< 15	>75

As seen from this table removal of BOD_5 and COD were over 90%. Removal of total nitrogen was over 75% due to the anoxic zone present in the treatment train.

Removal efficiency of the selected EDCs by this plant was determined by analyzing composite samples obtained from influent and effluent of the plant. Grab samples were taken from the activated sludge tank and transferred swiftly to the METU laboratory for analysis. A 250-500 mL influent and 1000 mL effluent were filtered through OASIS HLB cartridges to concentrate and clean up.

Diltiazem was the first compound analyzed in this series. It was almost detected in all the samples from Konacık MBR. The measured concentrations ranged from 0.26 to 21 ng/L for influent and 0.25 to 22.5 ng/L for effluent, as summarized in Figure 4.2.29.

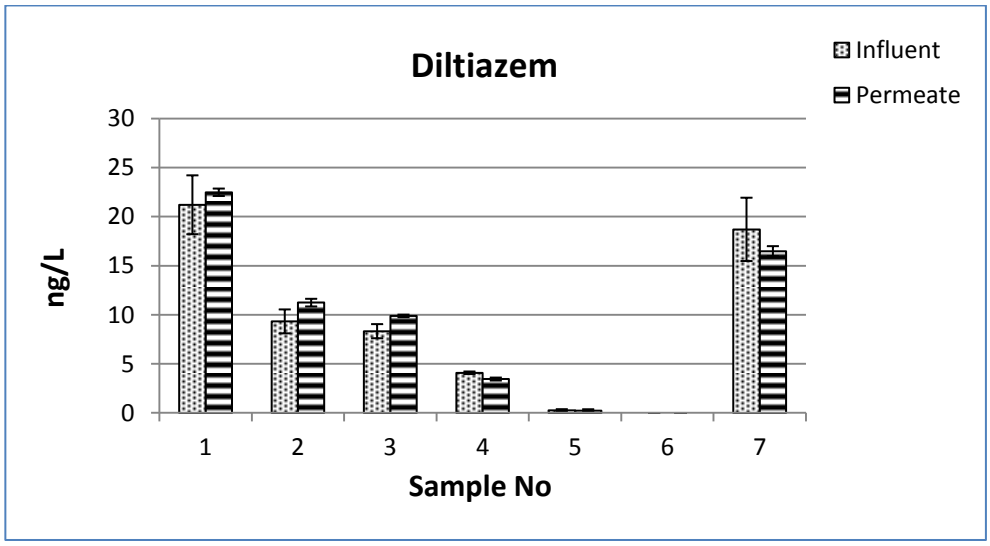

Figure 4.2.29 Influent and effluent concentrations of diltiazem in Konacık MBR Plant

As seen in Figure 4.2.29, influent concentrations of diltiazem were almost identical to the effluents, indicating non-removal of this compound in the treatment plant. Occasionally effluent concentrations were slightly higher than the influents. This may be attributed to the 10^3 times concentration of the samples.

Diltiazem concentration in the sludge samples was also analyzed but these were under limit of detection; hence were not plotted in the figure.

The second compound investigated in Konacık MBR was progesterone. Progesterone, which is a streoid hormon, is involved in the female menstrual cycle, and discharged during pregnancy until birth. As was mentioned in the literatuar survey, it was detected in 4.3% of 139 United States streams (Barron, 2006). The main sources of this compound in the environment is wastewaters. Progesterone is excreeted through urine. Progesterone could not be detected in the influents and effulens of Konacık MBR Plant. It could not be detected in the sludge samples too.

The third compound analyzed in Konacık MBR plant was BBP. BBP concentration was always under limit of detection in both influent and effluent samples. Similarly estrone, a natural hormone, could not be detected in any of the samples; nor in sludge. This is evidently owing to the high bio-degradation rate of this compound.

CBZ, which is a medication used for the treatment of epilepsy was investigated in Konacık samples. Its removal in this plant is given in Figure 4.2.30.

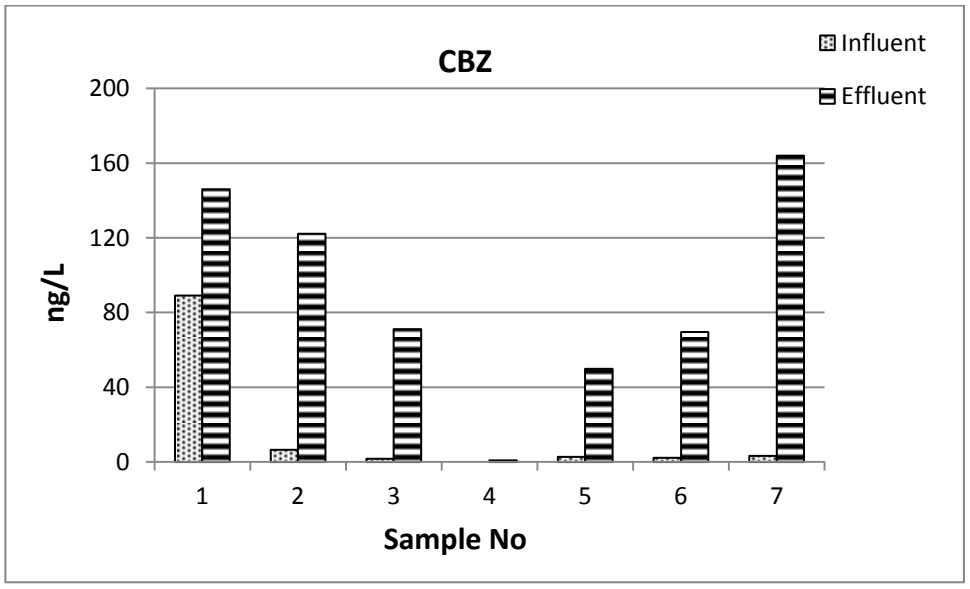

Figure 4.2.30 Influent and effluent concentrations of CBZ in Konacık MBR Plant

As seen in Figure 4.2.30, influent CBZ concetrations were consistently below effluent the concetrations. This observation was attributed to an experimental artifact. Where, high organic backround matrix present in the influents must have masked the actual compound during spe concentration step by competetively binding to the open cites on the adsorption surfaces of OASIS cartridge leaving little room for CBZ to bind. Conversely, when background organics are removed biologically, more room remains in the effluents for adsorption on to the cartridege. Thus compounds are effectively concntrated. An

attempt was made to clean the background chemicals by passing influents through flourisil cartridges before SPE concentration, but did not improve the analysis.

The last compound studied in Konacık MBR plant was acetaminophen. Acetaminophen was detected in all the influent samples but not in permeates, as shown in Figure 4.2.31. influent concentration was between 115 and 3500 ng/L and effluent concentration was almost under the LOQ.

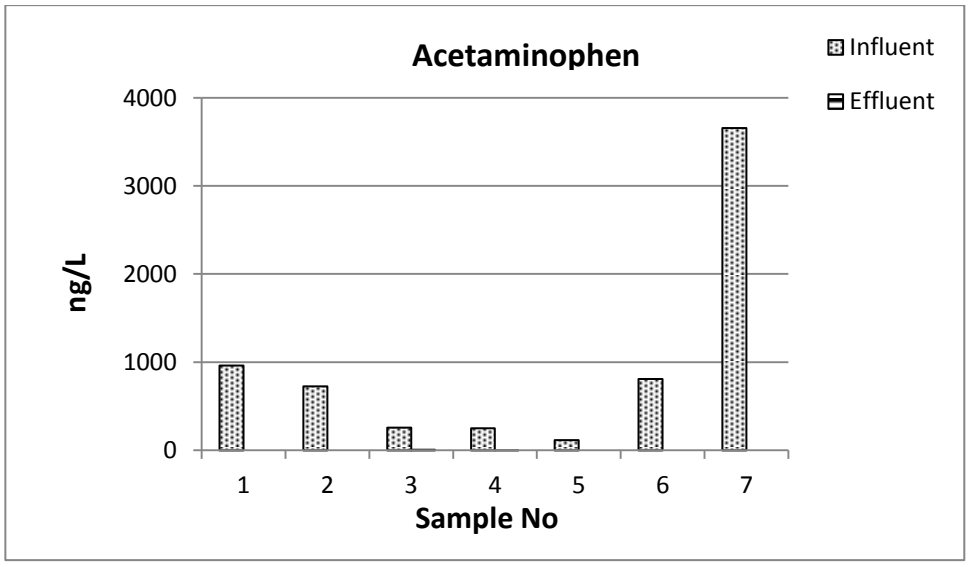

Figure 4.2.31 Influent and effluent concentrations of acetaminophen in Konacık MBR Plant

Acetaminophen concetration in the sludge samples are given in Figure 4.2.32.

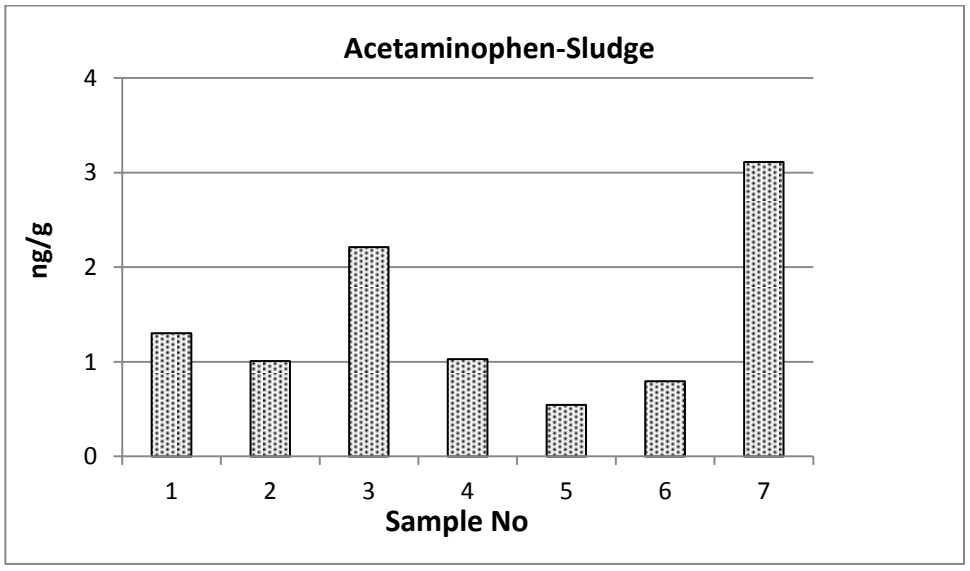

Figure 4.2.32 Concentrations of acetaminophen in sludge samples in Konacık MBR Plant

As was seen in Figure 4.2.31, acetaminophen concentration in the sludge samples was very low; suggesting that acetaminophen was removed mainly by biological action.

4.2.4. Treatment of Selected Endocrine Disrupter Compounds by Sequencing Batch Reactor Combined with Membrane Separation

The last treatment application for selected EDCs removal was the sequencing batch reactor combined with membrane separation. Before the start-up of the SBR+membrane system, timers of all the pumps in the system were adjusted. Next, system was started-up using clean water for leak control and cycle adjustment. Actual operation has started with a transfer of activated sludge from ***Calo e o Milladoiro*** plant and having MLSS of about 2.5 g/L. After settling the sludge and discarding the supernatant, MLSS was brought to 8-8.5 g/L. System was started using synthetic wastewater on 10th October 2011 and operated over 140 days. This period was divided into three periods. In the first period, which was 32 days, treatment of classical pollution parameters by the SBR+membrane

process was investigated. In the second period, which lasted for 76 days, selected endocrine disrupter compounds, EDCs, were spiked to the synthetic wastewater in order to determine removal of EDCs along with the pollution parameters. Third and the last period was 33 days, where 1 g powdered activated carbon (PAC) was added to each liter activated sludge in the SBR tank, to increase removal of the selected EDCs. This system was called SeMPAC process, patented by Prof. Omil and his team. During the study, sludge retention time (SRT) was equal to the period of operation, which was 140 days. No excess sludge was disposed during this time.

4.2.4.1 Analyses of General Pollution Parameters during the Study

During the study MLSS, MLVSS, DO, temperature and pH in the SBR and membrane chamber were measured two times a week. The COD, NH^{+}_{4}-N, NO^{-}_{2}-N, NO^{-}_{3}-N and PO^{2-}_{4}-P were analyzed in the influent, supernatant of SBR after sludge has settled and the membrane permeate.

The MLSS and MLVSS concentrations in SBR and membrane chamber are given in Figure 4.2.33 and 4.2.34.

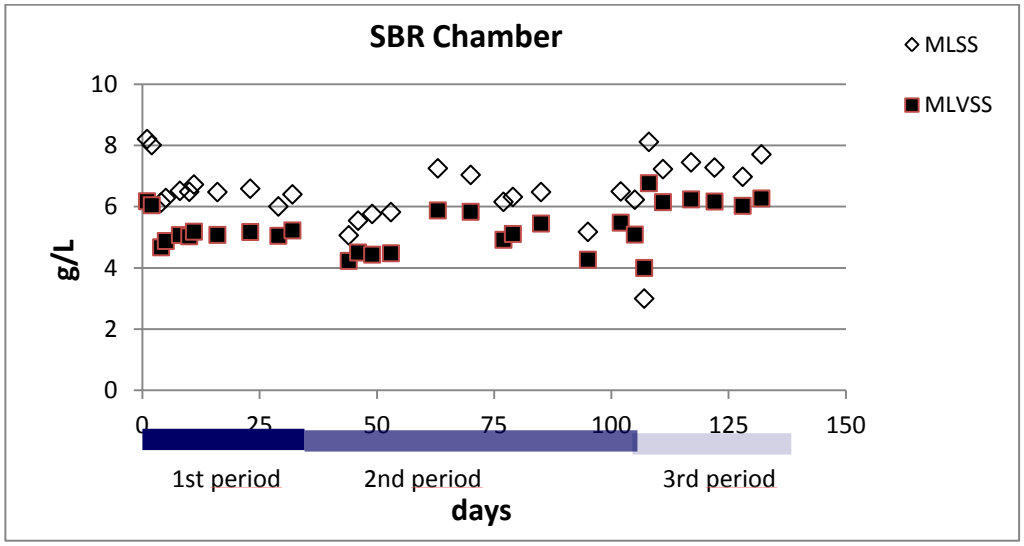

Figure 4.2.33 MLSS and MLVSS concetrations in SBR chamber

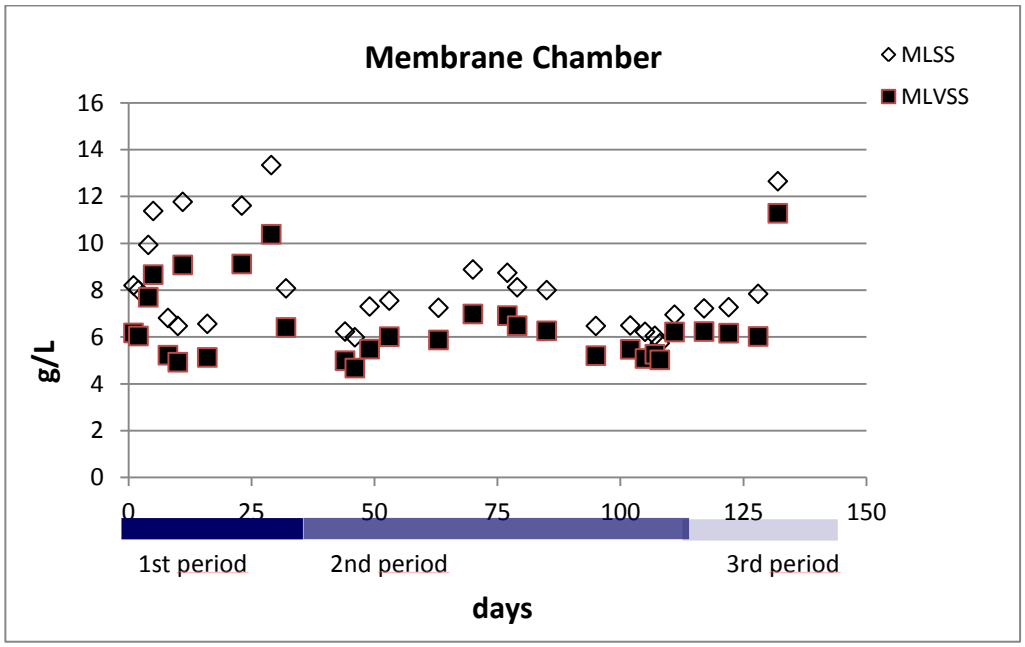

Figure 4.2.34 MLSS and MLVSS concetration in membrane chamber

As seen from Figures 4.2.33 and 4.2.34, the MLSS and VSS concentrations were almost identical without any increases during the 1st and 2nd periods. The MLSS concentration increased very little after addition of 1 g/L powdered activated carbon to the system.

As can be seen in Figure 4.2.34, MLSS and VSS concentrations in the membrane chamber increased from initial 6 g/L to 14 g/L. This is explained by the continuous suction of the vacuum pump connected to the membrane during this period, which progressively increased biomass concentration there. In other words, each day had four cycles and at the end of the cycle supernatant transferred to the membrane chamber. This supernatant diluted the activated sludge in the membrane chamber. After transfer of supernatant from the SBR tank to the membrane chamber, MLSS concentration in the SBR and membran chambers were almost the same about 6 g/L. However, the suction from the membranes was continuous so the sludge concentration in the chamber increased during the period over 14 g/L. This MLSS concentration difference explained for this reason.

Temperature, dissolved oxygen and pH in the SBR and membrane chamber were measured continuously during the study. Temperature was almost constant at 19-20 ^{o}C in both chambers. The pH in the SBR chamber was between 8,3 and 8,7; and 8,6 in the membrane chamber. The dissolved oxygen concentration in the membrane chamber was 7 mg/L but changing in the SBR chamber in accord with the changing zones during cycles. Oxygen concentration in the aerobic zone was about 2 mg/L.

The COD, NH_4^+-N, NO_2^--N and NO_3^--N and PO_4^{2-}-P in the influent, SBR supernatant and membrane permeate are given in Figure 4.2.35.

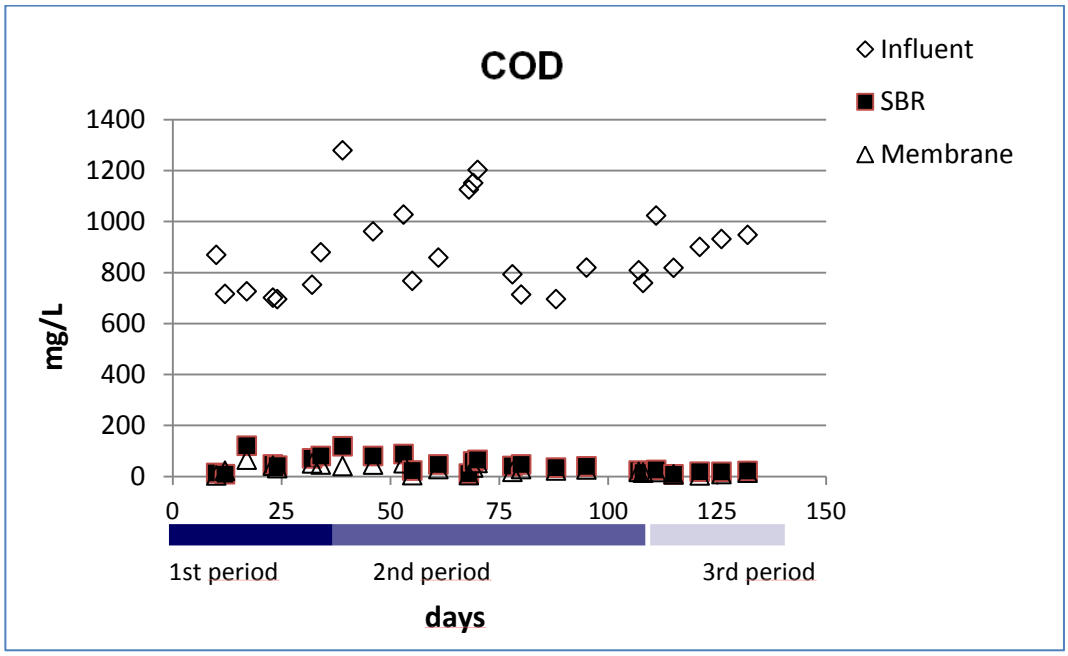

Figure 4.2.35 COD concentration in the influent, SBR supernatant and membrane permeates

As seen in Figure 4.2.35, influent COD was around 700 mg/L during the first period. The COD removal in SBR supernatant, and membrane permeate were both over 90% during the first part of the study. The COD concentration in membrane permeate was slightly lower than the SBR supernatant as there was additional aeration in the membrane chamber. Since spiked PPCPs were prepared by dissolving them in methanol and acetone, over 100mg/L of COD increased in

the influent. Although there was higher COD was coming to the reactor during the second period, still same COD removal was achieved as with the first period. In the last period over 98% of COD removal was affected by addition of PAC.

The NH_4^+-N, NO_2^--N and NO_3^--N concentrations observed in influent, SBR supernatant and membrane permeates are presented in Figure 4.2.36. Influent NH^+_4-N concentration was between 60 to 85 mg/L and it was almost completely oxidized in SBR. At the end of 210 min. aerobic zone it was around 5 mg/L and 3 mg/L in the membrane permeate. The NO_2^--N concentration was always less than 0.2 mg/L in all the samples and could not be plotted in a figure.

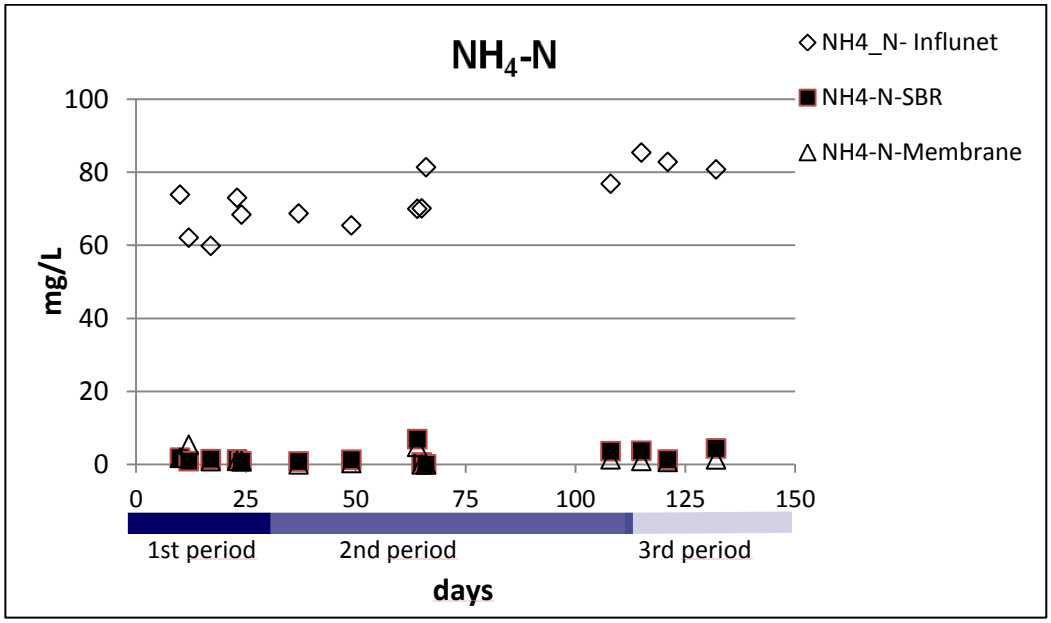

Figure 4.2.36 NH_4-N concentration in the influent and in the effluent of SBR and membrane chambers

The NO_3^--N concentration in the influent, SBR supernatant and membrane permeate are given in Figure 4.2.37.

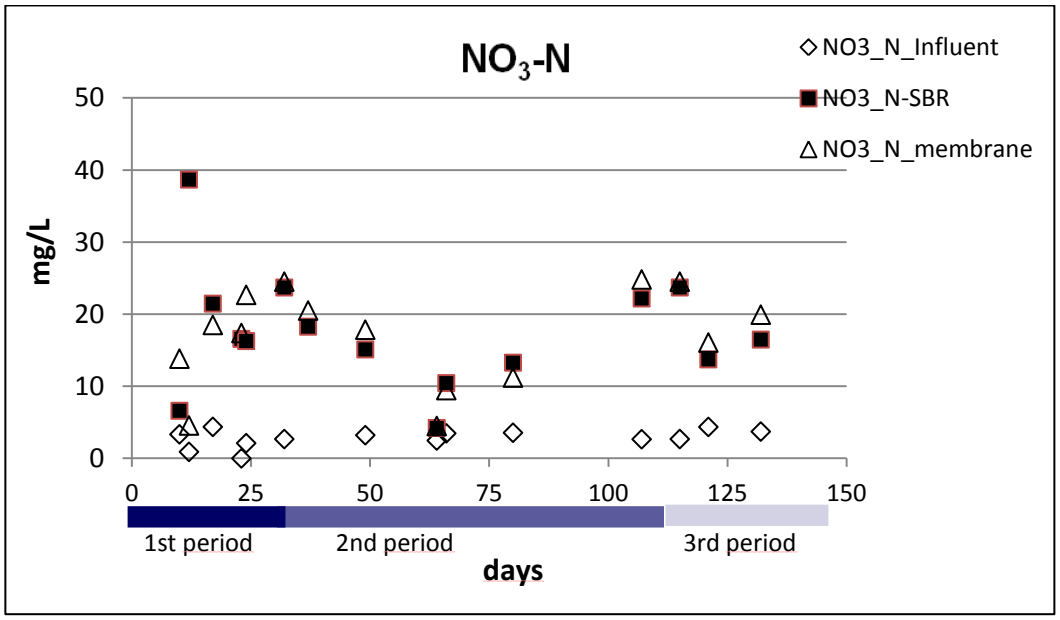

Figure 4.2.37 NO_3-N concentration in the influent and in the effluent of SBR and membrane chambers

As seen in Figure 4.2.37, influent NO_3^--N concentration was below 5 mg/L; but increased to around 20 mg/L in SBR supernatant and membrane permeate as a result of NH_4^+-N oxidation. From the Figure 4.2.36 and 4.2.37, it is clearly seen that almost all of the NH_4-N was converted to NO_3-N and about 70% of nitrate was denitrified.

As seen in Figure 4.2.38, influent concentration of PO_4^{2-}-P was between 2 and 8 mg/L and PO_4^{2-}-P concentration in the SBR supernatant and membrane permeate were almost zero.

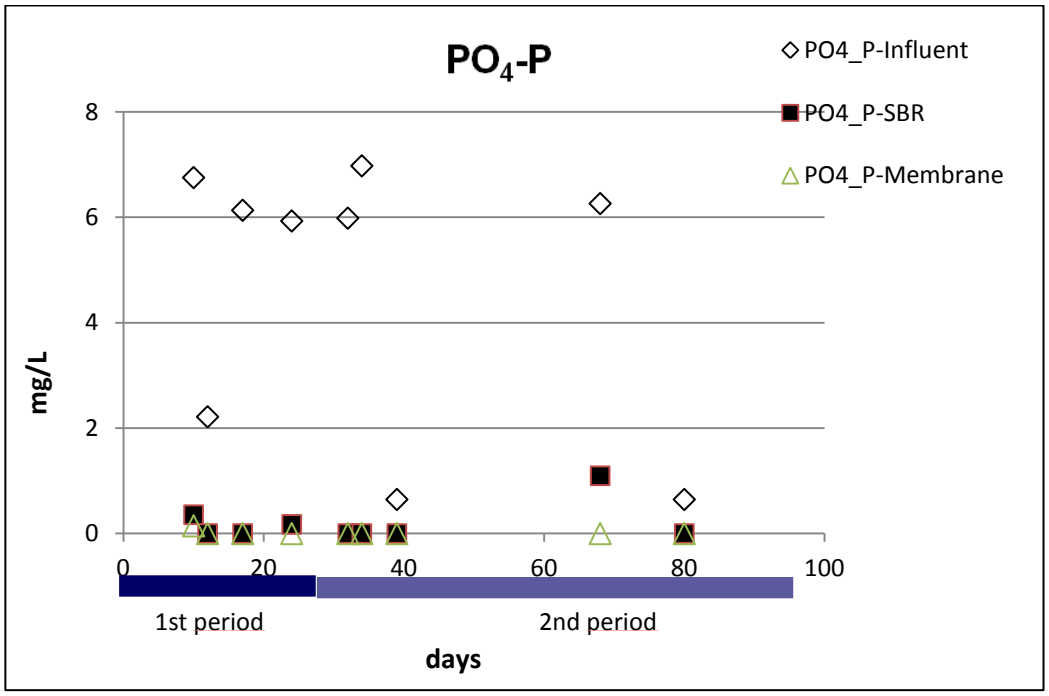

Figure 4.2.38 PO_4^{2-}-P concentration in the influent effluent from SBR and membrane chambers

$SVI_{2.5}$ was also measured and it was found 80 mL L^{-1}. It means that when sludge was arranged 2.5 g/L, it was good setleable.

In addition to the removals of general pollution parameters in SBR+membrane system, these parameters were also measured at every cycle to analyze the treatment profiles.

4.2.4.2 Effect of Different Zone on Removal of General Parameters

In order to understand the changes of the conventional parameters in different zone, samples were taken each phase of the cycle. For conventional parameters, 10 different points were selected.

The first parameter studied was COD during the cycles. This is summarized in Figure 4.2.39.

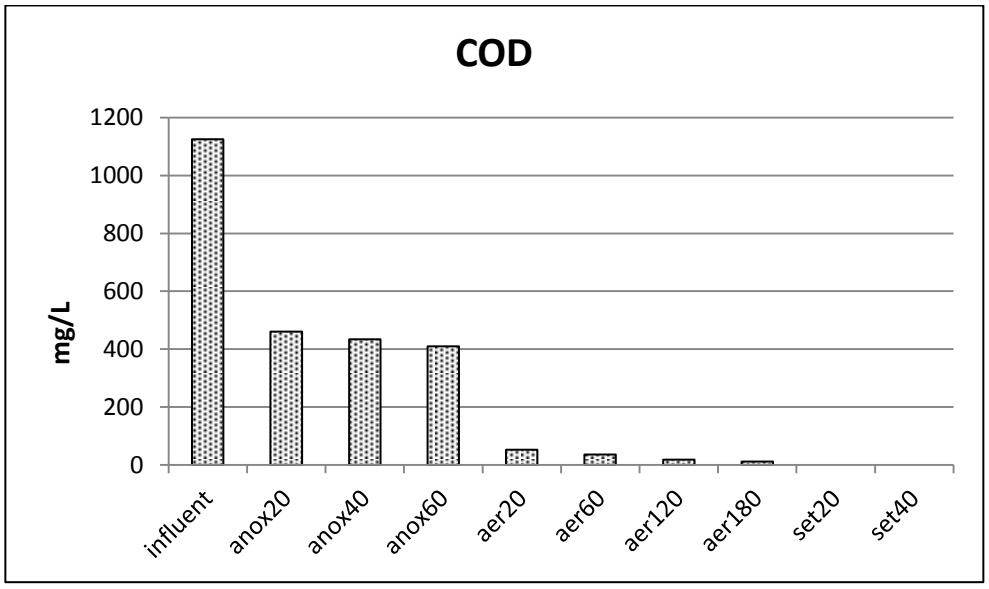

Figure 4.2.39 COD profile during ona cycle

As seen in Figure 4.2.39, influent COD concentration was close to 1200 mg/L. The SBR tank volume is about 30 L and each cycle 7,5 L of wastewater was treated. Therefore, the exchange ratio of the SBR tank was 7,5/30= 25%. After transferring the synthetic wastewater into the system, COD concentration decreased to around 400 mg/L due to this dilution. During the anoxic zone there was very little removal of COD. At the end of the anoxic zone, aerobic zone started and all the organics were consumed very fast due to high MLSS concentration. At the end of this cycle COD concentration was almost zero.

The second parameter profiled was NH_4^+-N. Influent NH_4^+-N was about 70 mg/L. After feeding the SBR tank with synthetic wastewater, NH_4^+-N concentration decreased to 20 mg/L du to exchange volume. In the anoxic zone there was no removal. NH_4^+-N decreased to below 10 mg/L in the aerobic zone, as summarized in Figure 4.2.40.

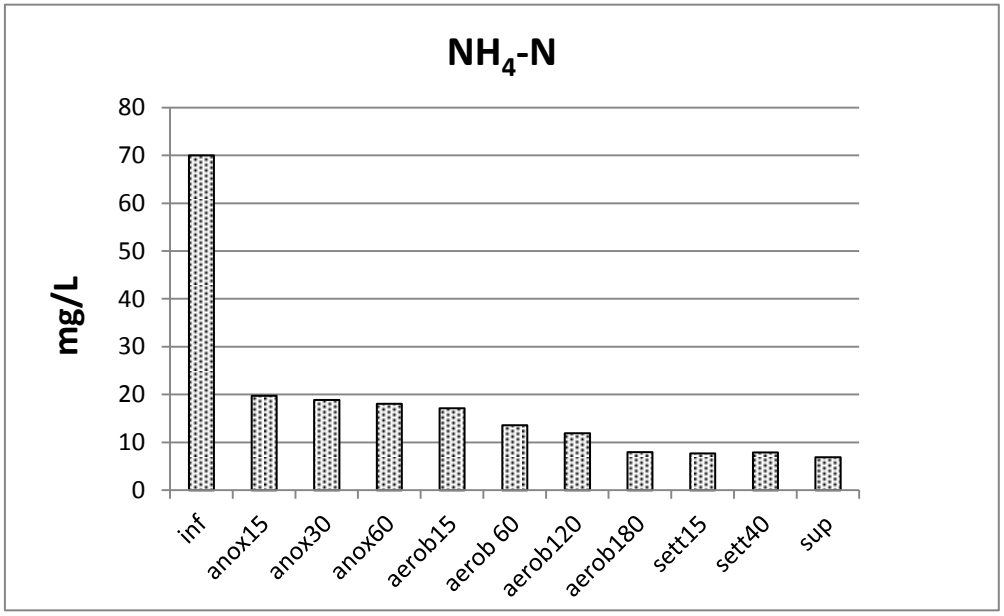

Figure 4.2.40 NH_4^+-N profile during the cycle

The NO_3^- -N profile is given in Figure 4.2.41. The NO_3^- -N, which was below 1 mg/L, increased to about 10 mg/L in the aerobic zone.

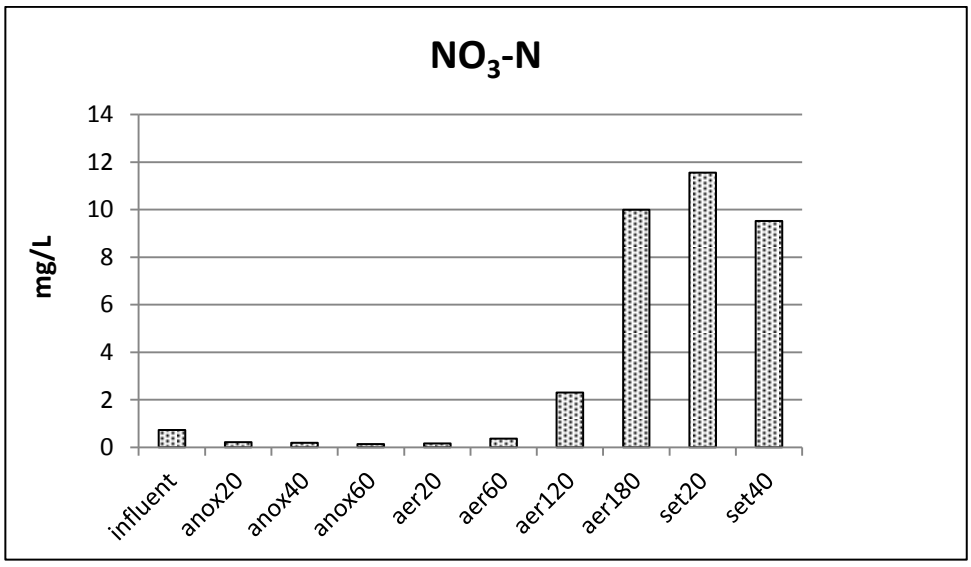

Figure 4.2.41 NO_3^- -N profile during the cycle

The PO_4^{-3-} -P profile in the SBR process is given in Figure 4.2.42. Influent PO_4^{2-} -P concentration was 8 mg/L but increased to over 70 mg/L after mixing

with the activated sludge in the anoxic zone. At the end of 'anoxic' zone, PO_4^{-3-}-P increased to 75 mg/L. Whereas in the aerobic zone PO_4^{-3-} -P decrease due to bacteria uptake. At the end of the cycle effluent PO_4^{-3-} -P concentration was less than 1 mg/L; as seen in Figure 4.2.42.

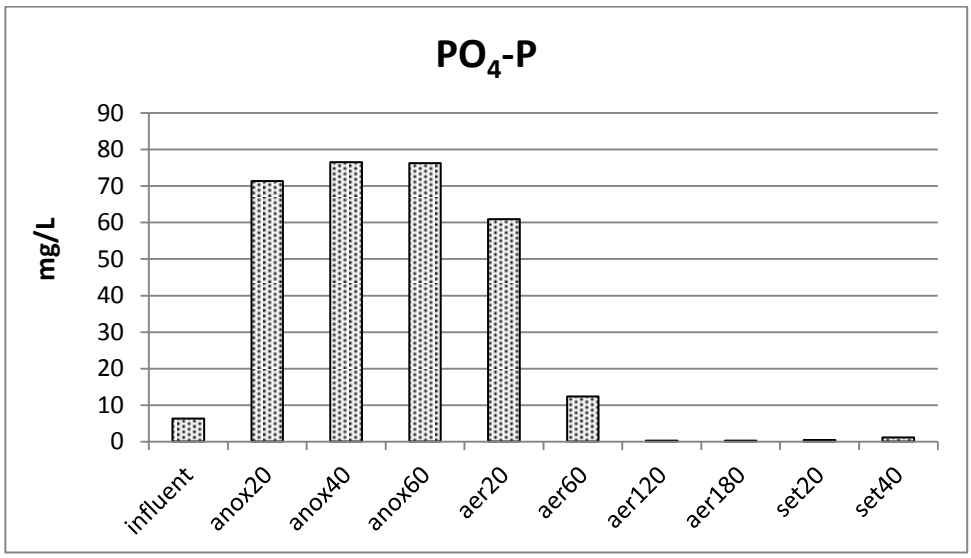

Figure 4.2.42 PO_4^{-3-} -P profile during one cycle

The pH and the oxygen profiles recorded during one cycle are given in Figure 4.2.43 and in 4.2.44 respectively.

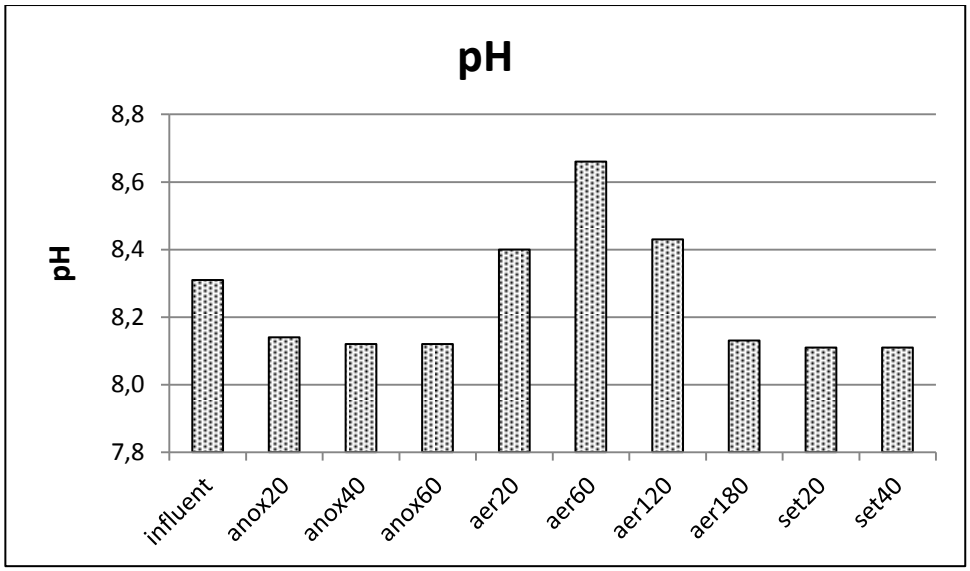

Figure 4.2.43 pH profile during the cycle

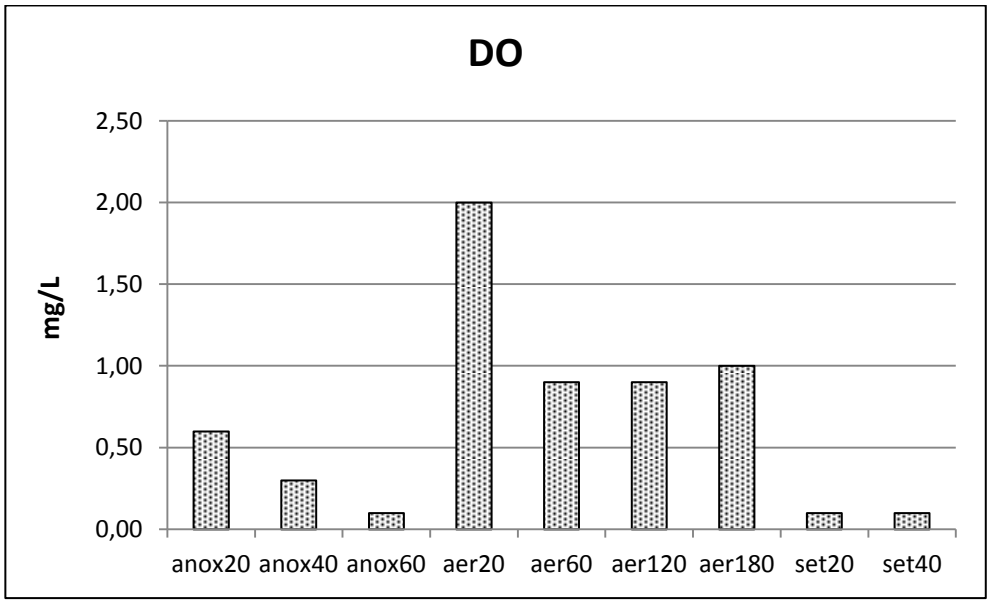

Figure 4.2.44 DO profile during the cycle

Apparently, what is termed here 'anoxic' was not anoxic after all and it behaved like anaerobic zone, as can be seen from Figure 4.2.41 and 4.2.44, with concomitant release of phosphorus by bacteria.

4.2.4.3 Removal of selected PPCPs by SRB combined with Membrane Separation with and without Powdered Activated Carbon (PAC) addition

Following the first period, where conventional pollution parameters were studied, selected PPCPs were spiked into the influent wastewater to determine removal of these compounds by the SBR+ membrane plant with and without PAC addition. In the second period of study which lasted for 76 days, PAC was not added to the system. PAC addition to the system was started after 108 days from the start-up. PAC addition to the SBR+ membrane separation is a patented process by Prof. Omil and his team under the brand name of SeMPAC. In order to characterize the system, liquid samples were taken from influent, supernatant of SBR, permeate of membrane; sludge samples were taken from SBR tank, membrane chamber, recycle line and sometimes from the surface of the membrane plates. Both results obtained with and without PAC addition were

plotted together on the same figures in the subsequent figures. Prior to experiments, background sludge concentration of the selected PPCPS was checked in the sludge initially transferred to the system.

The first compound studied was celestolide (CEL) which is a musk fragrance. Samples from influent, supernatant of SBR and permeate of membrane were analyzed and the results are given in Figure 4.2.45.

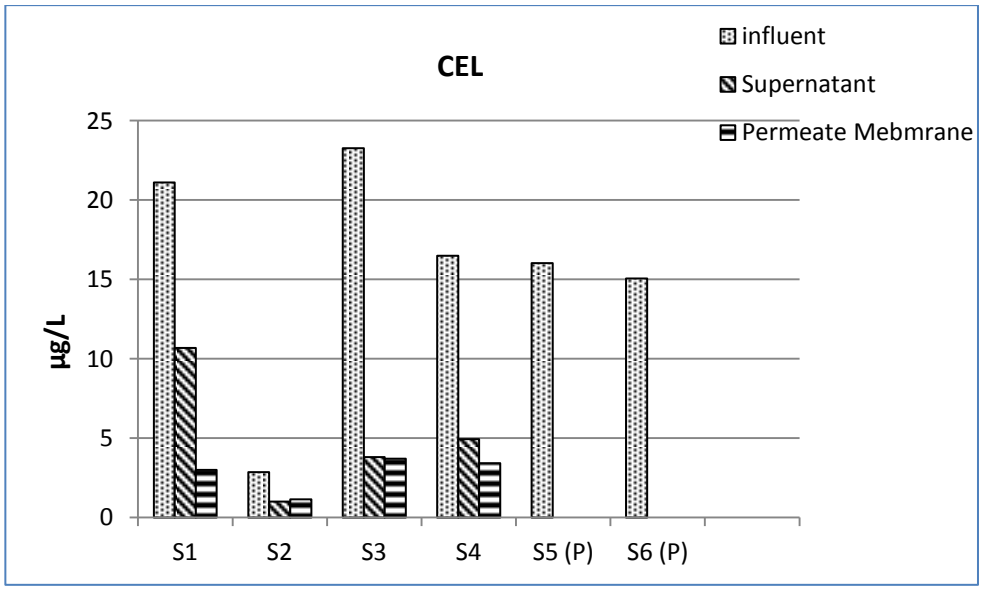

Figure 4.2.45 Concentration of CEL in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.45, influent CEL concentration was variable between 11 and 23 µg/L except for the second sample (S2). Evidently an error has occurred during the analysis of the second influent sample. The first four samples (S1-S4) were taken in the second period without PAC addition; and the last two (S5(P) and S6(P))were taken during the third period where PAC was added into the aeration tank. The removal in the SBR tank was about (from 50 to 80%). An extra (10-30%) removal was achieved by the membrane chamber. After addition of PAC all the CEL was adsorbed by PAC and it could not be detected in the membrane permeate or the SBR supernatant.

In order to determine the fate of the compounds during treatment, sludge samples were taken at the same time as with the liquid samples. Moreover, transferred sludge was also analyzed to learn the history of the sludge. At the beginning of the study, background CEL concentration in the transferred sludge was 0,597 µg/g. After spiking PPCPs, CEL concentration in the sludge increased as summarized in Figure 4.2.46.

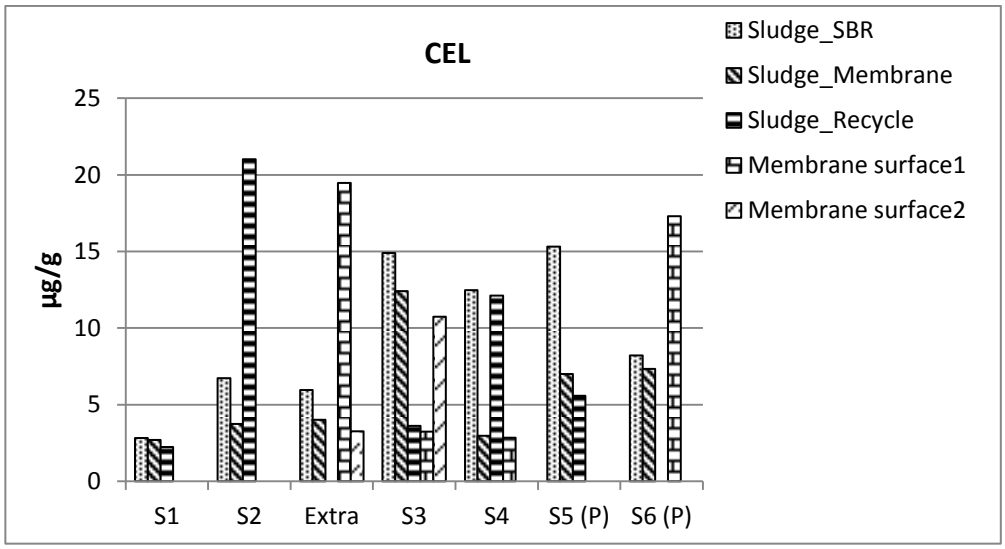

Figure 4.2.46 Concentration of CEL in the sludge samples.

As seen from this figure, CEL concentration started to accumulate in sludge after spiking the wastewater. Sometimes, it was possible to take samples from the bio-film on the membrane surface. Since there were two membrane plates, they were designated as *Membrane surface 1* and *Membrane surface 2*. Sampling from membrane surfaces were subject to availability at the time of sampling. Missing bars in this figure should not mean 'value below detection' but merely indicate that no sample has been taken at this sampling point. From Figure 4.2.46, it is seen that CEL concentration in sludge from the SBR chamber was mostly higher than sludge in the membrane chamber. In addition, concentration of CEL in the recycle sludge was higher than the membrane chamber. The higher concentration of CEL in the SBR chamber could be explained by the contribution by the recycled sludge. The CEL concentration was sometimes over 15 µg/g in the

membrane surface sludge. Sometimes concentration of CEL in one membrane surface was higher than the other. This shows that CEL could have deposited on the surface of the membranes and then sloughed and settled into the membrane chamber and recycled to the SBR chamber. It follows that high concentration of CEL in the recycled sludge could be explained the deposition of CEL-rich sludge at the bottom of the membrane chamber.

Columns shown at '*Extra*' position in relevant figures indicate sludge PPCP concentrations where simultaneous liquid samples could not have been taken. This 'extra' sample also supported the idea of deposition of compound on the membrane surface and settling to the bottom. Furthermore, in extra situation, it was not possible to get samples from the recycle line; hence, no value for the recycled sludge could be given. As can be seen from Figure 4.2.46., CEL concentration in the sludge started to increase after the addition of PAC since PAC adsorbed any remaining CEL into the sludge.

The other musk fragrance studied was galaxolide (GLX) which was analyzed by GC/MS. The liquid concentrations of this compound are given in Figure 4.2.47.

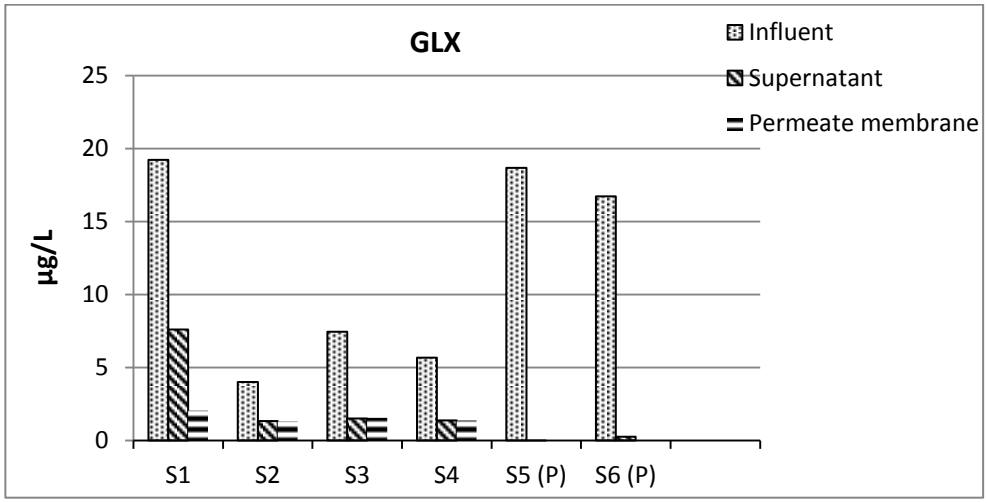

Figure 4.2.47 Concentration of GLX in influent, supernatant of SBR and permeate of membrane.

As clearly seen from Figure 4.2.47, influent GLX concentration was between 6 and 20 µg/L except for the second sample. In the SBR supernatant this decreased up to 1,5 µg/L. Membrane filtration did not affect any extra removal of GLX in most of the samples analyzed; and upon PAC addition, almost all the GLX was removed in the SBR chamber.

The GLX concentration in the sludge samples were also analyzed before and after spiking. The background concentration of this compound in sludge was 0,837 µg/g before spiking. Its concentration change in the sludge through process compartments is summarized in Figure 4.2.48.

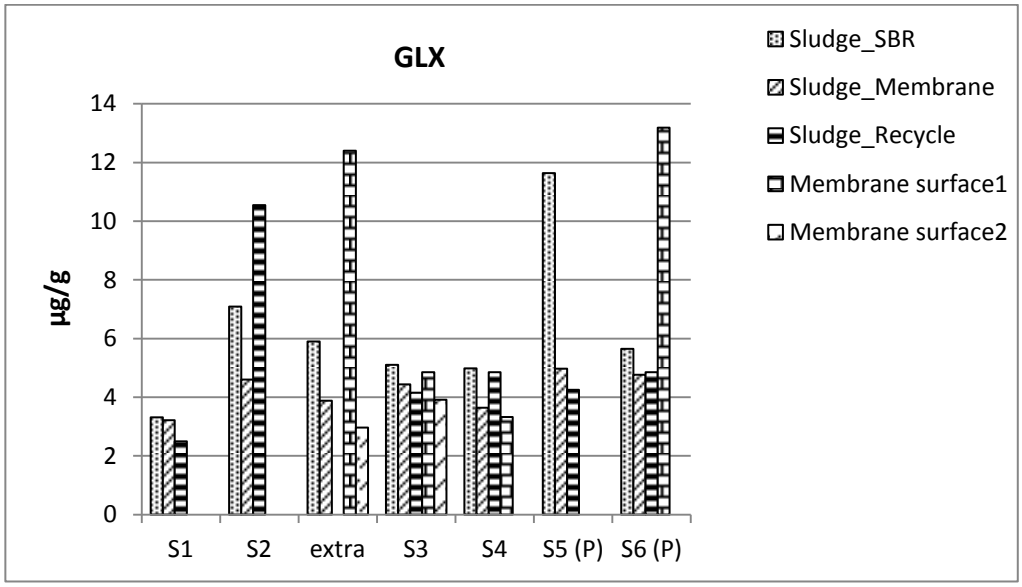

Figure 4.2.48 Concentration of GLX in the sludge samples.

As seen in this figure GLX rapidly accumulates in sludge and its concentration was higher in SBR tank than in the membrane chamber. Similar to CEL, the GLX tended to accumulated on the surfaces of the membranes.

The last musk fragrance investigated was tonalide (TON).

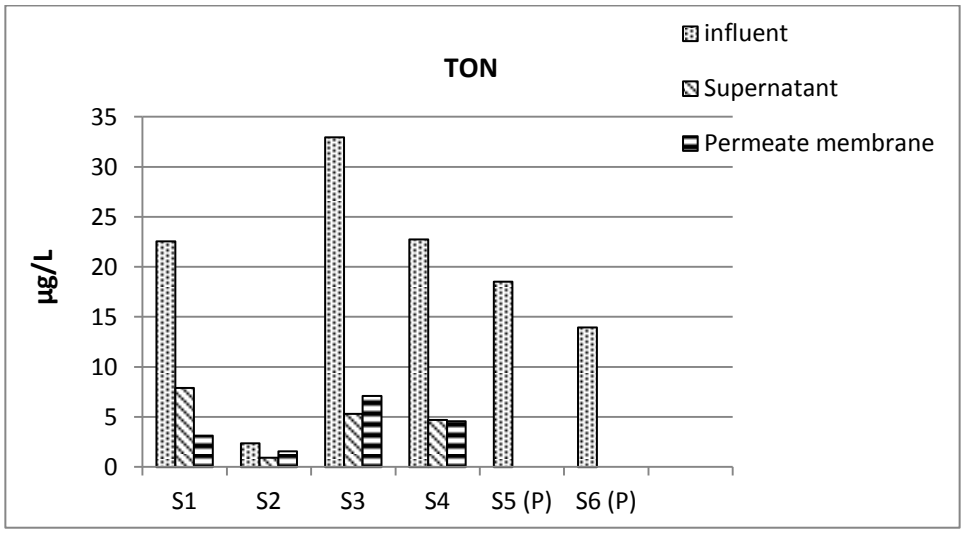

Figure 4.2.49 Concentration of TON in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.49. influent TON concentration was from 14 to 33 µg/L over 10 µg/L except for concentration in the second sampling point. After treatment concentration dropped to around 20 % in the SBR chamber was about 80%. Concentration of TON in SBR supernatant and membrane permeate were almost the same, indicating no additional removal by the membrane. After PAC addition TON was completely removed in the SBR supernatant.

Sludge was also sampled. Before spiking, background TON concentration was 0,96 µg/g in the sludge. Its concentration change in the sludge after spiking, and through the process compartments, is summarized in Figure 4.2.50.

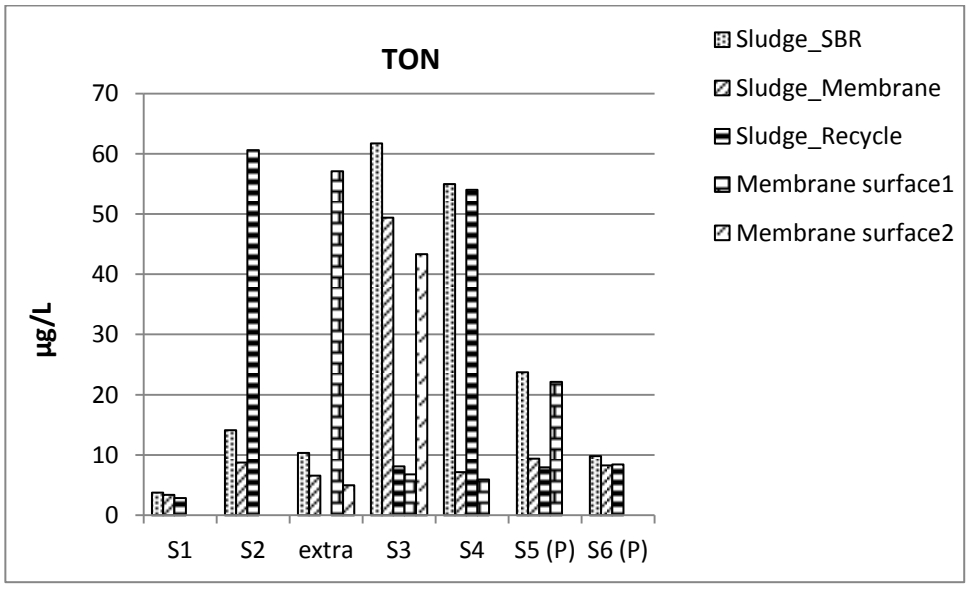

Figure 4.2.50 Concentration of TON in the sludge samples.

As seen in Figure 4.2.50, TON accumulated sharply during the study. Concentration of TON in the SBR tank was higher than that in the membrane chamber. The TON concentration in recycled sludge was sometimes higher than 50 µg/g. and was over 40 µg/g. on the surface of the membrane. Evidently, Like the other fragrances, bio-film where TON has concentrated settled into the membrane compartment and was recycled to the SBR tank. However, concentration of TON in the sludge did not increase by the addition of PAC.

Removal of carbamazepine (CBZ), which is used as anti-epileptic, was also investigated. The concentrations of CBZ in various effluents are given in Figure 4.2.51.

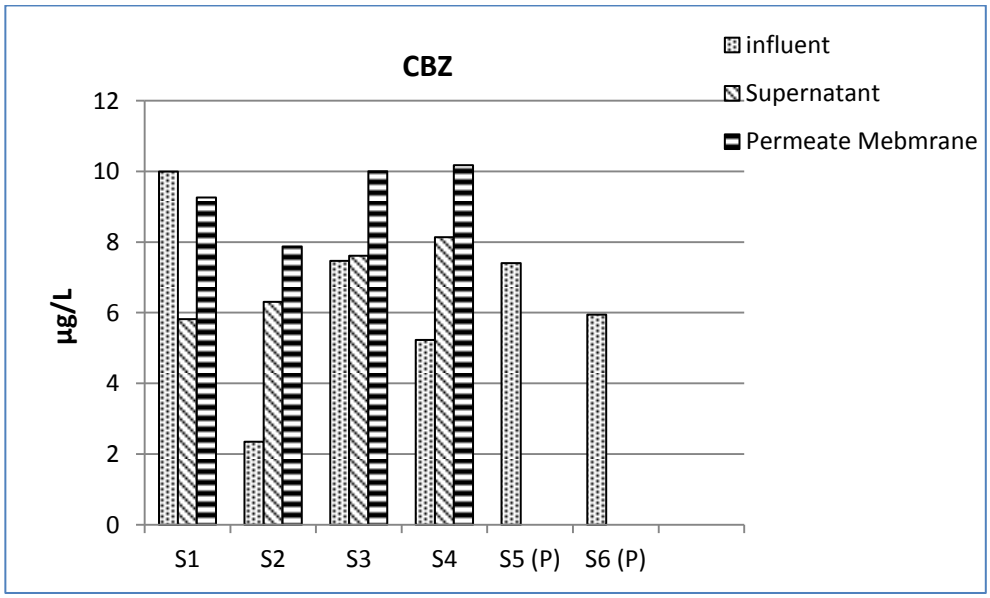

Figure 4.2.51 Concentration of CBZ in influent, supernatant of SBR and permeate of membrane.

As seen in this figure, sometimes influent CBZ concentration was higher than the supernatant and permeate. This may be attributed to an analytical error consistent with this compund throughout the thesis work. It is well known from literature and the other systems studied in this thesis work, that this compound is not an biodegraded. After addition of PAC to SBR tank CBZ was totally adsorbed by PAC and was under limit of detection in both the membrane permeate and the supernatant of the SBR.

Concentration of CBZ in all the sludge samples were under limit of detection (LOD). The CBZ in one surface sludge sample after PAC addition was determined as 3,17 µg/g, it was below detection in all other samples. Therefore, it was not plotted here.

Another compound investigated during the study was diezapam (DZP) which is a tranquillizer. Like the CBZ, DZP was not removed by this system and the concentration of DZP in influent, supernatant of SBR and permeate of membrane are given in Figure 4.2.52.

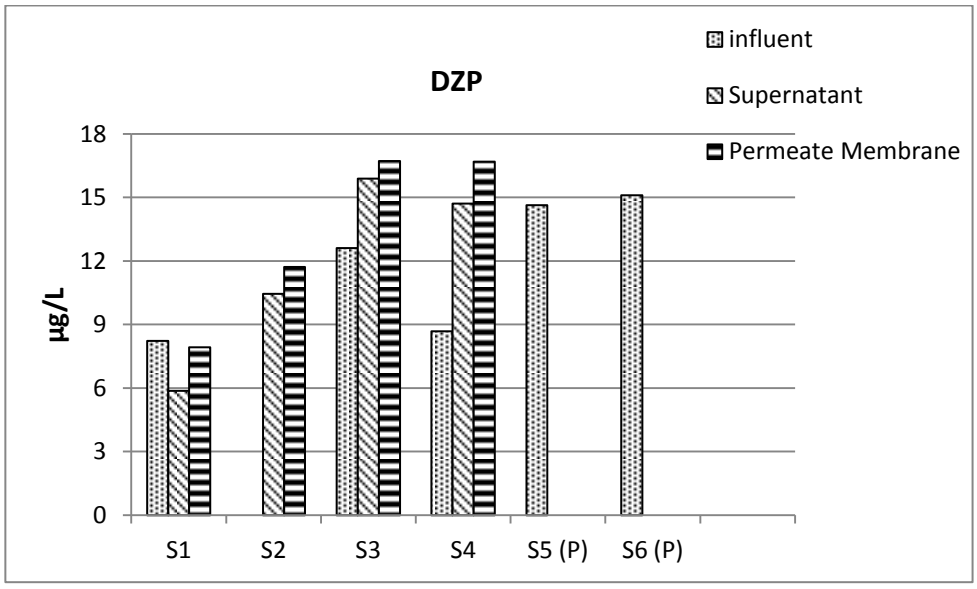

Figure 4.2.52 Concentration of DZP in influent, supernatant of SBR and permeate of membrane.

As seen in this figure, influent concentrations for the first four samples were less than the supernatant and permeate of membane. This may be attributed to an analytical error during the analyses. After PAC addition, all the DZP was adsorbed by powdered activated carbon and coud not measured in the effluent.

The DZP in sludge samples were always under limit of detection except for one surface sludge sample after PAC was added, which was 14,84 µg/g. Therefore, it was not plotted.

In this serious of studies removals of three different commonly used anti-inflammatories (ibuprofen, naproxen and diclofenac) were studied in SBR+membrane plant, with and without addition of PAC. The first anti-inflammatory compound studied was ibuprofen (IBP). The influent IBP concentration was mostly from 5,7 to 10,6 µg/L as seen in Figure 4.2.53.

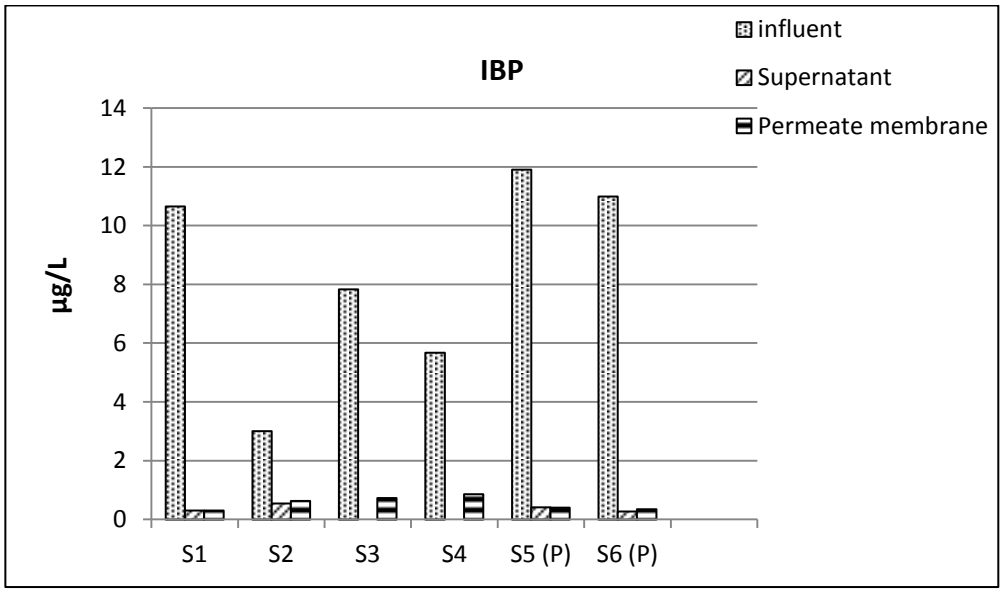

Figure 4.2.53 Concentration of IBP in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.53, removal of IBP by the SBR+ membrane plant was from 80 to 90%. There were no conspicuous differences between the SBR supernatant and membrane permeates. This indicates that there was no additional removal by the membrane on IBP removal. After PAC addition to the system the removal of IBP increased to over 99%. The sludge concentrations observed for IBP are summarized in Figure 4.2.54.

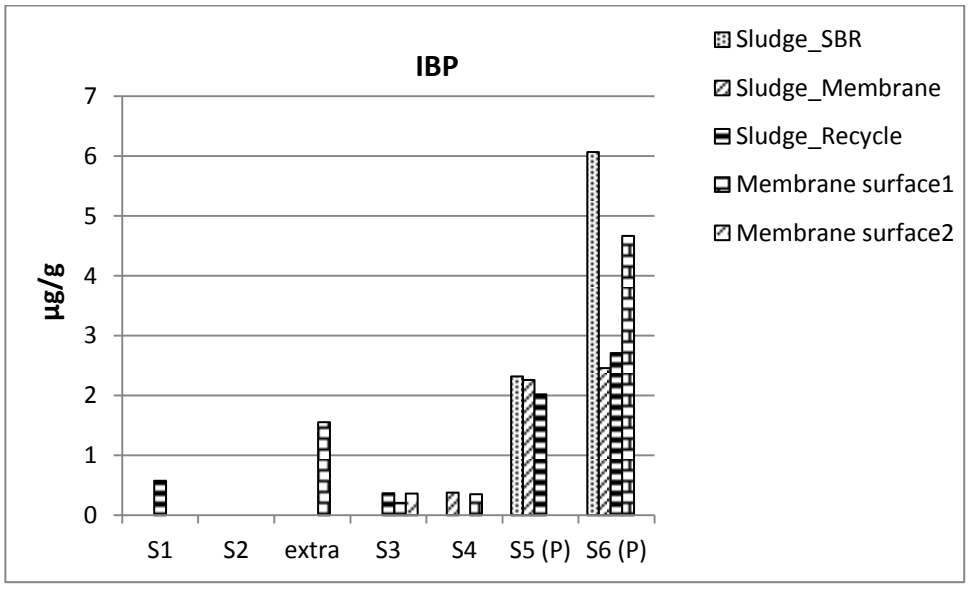

Figure 4.2.54 Concentration of IBP in the sludge samples.

Background concentration of IBP in sludge was determined as 1,18 µg/g, before spiking. After spiking IBP to the synthetic wastewater, it did not accumulate in sludge, as most samples were under limit of detection. This showed that IBP was removed by biodegradation. After addition of PAC to the system, it tended to accumulate in sludge of SBR tank, membrane chamber and in recycle line. IBP concentration in the membrane surface bio-film was also high and concentrated it in the recycle line. PAC Edition was counter productive in this case as it hampered biological degradation by making this compound unavailable to microorganisms.

Another anti-inflammatory compound studied was Naproxen (NPX). The results of affluent analysis are given in Figure 4.2.55.

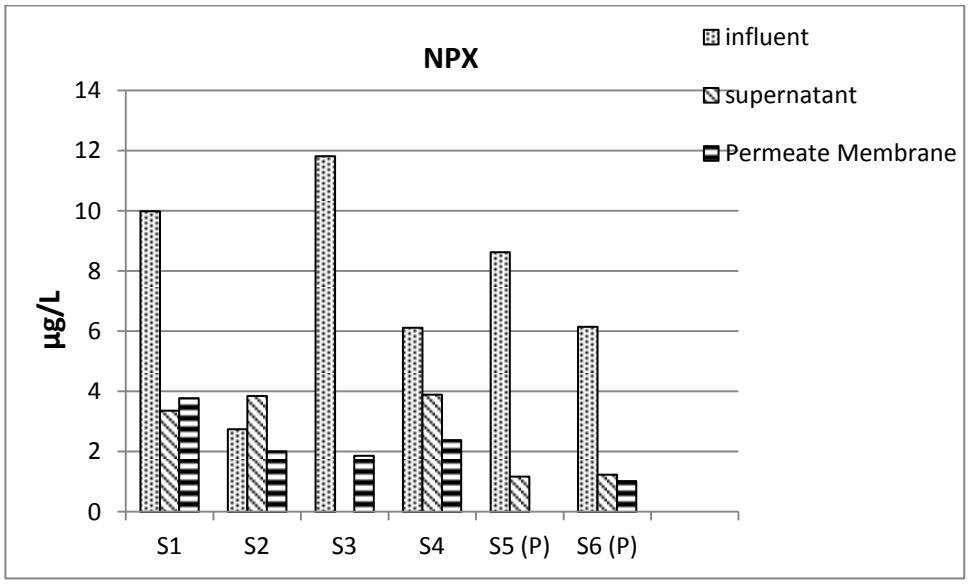

Figure 4.2.55 Concentration of NPX in influent, supernatant of SBR and permeate of membrane.

The influent concentration was 6-10 µg/L during the study. In the second sample there was the usual error associated with this analysis. There was an additional problem during the analysis of the supernatant of third sample. The removal of NPX was between 60 to 80% when the overall system considered. After PAC addition, removal increased to over 85%. Activated carbon did not adsorb all the NPX.

The NPX was also analyzed in sludge samples. The background concentration of NPX in sludge was 0, 824 µg/g. The concentration changes observed in the sludge samples are given in Figure 4.2.56.

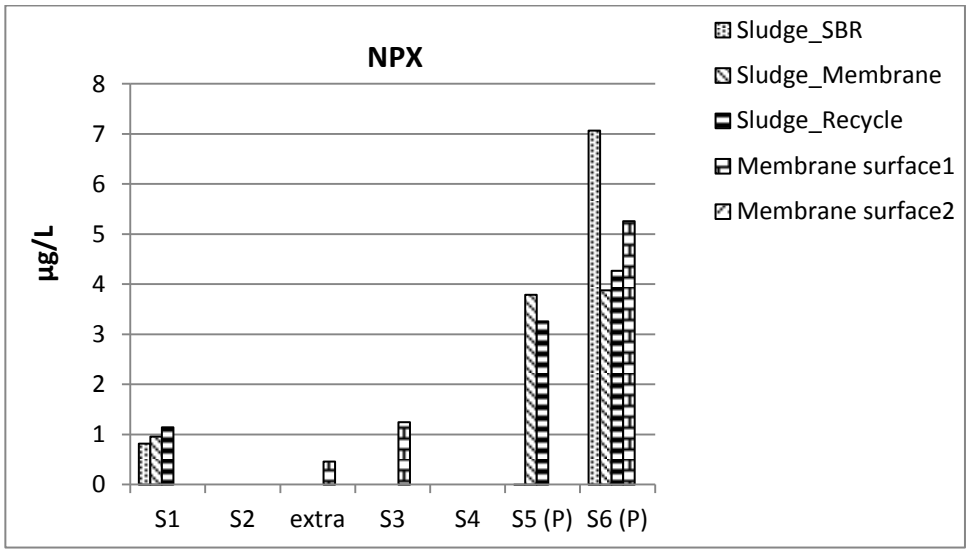

Figure 4.2.56 Concentration of NPX in the sludge samples.

The background concentration of NPX was detectable in the first sludge sample but later disappeared in samples. However, after addition of PAC, it could be detected in all the sludge samples.

The last anti-inflammatory compound investigated was Diclofenac (DCF). The DCF concentrations analyzed in liquid samples are given in Figure 4.2.57.

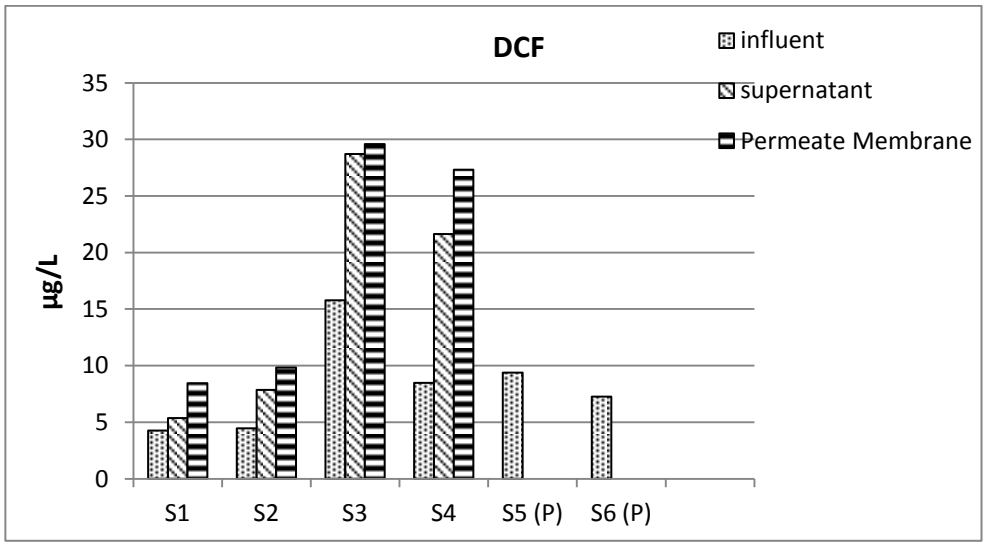

Figure 4.2.57 Concentration of DCF in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.57, influent concentrations of DCF was lower than that in SBR supernatant and permeate of membrane. An analytical error might have caused this outcome. Moreover, the DCF concentration observed in membrane permeate was higher than that observed in the supernatant of SBR. However, after PAC addition, all the DCF was absorbed by PAC and the concentration in the supernatant of SBR was under the limit of detection.

The observed concentration of DCF in sludge samples are given in Figure 4.2.58.

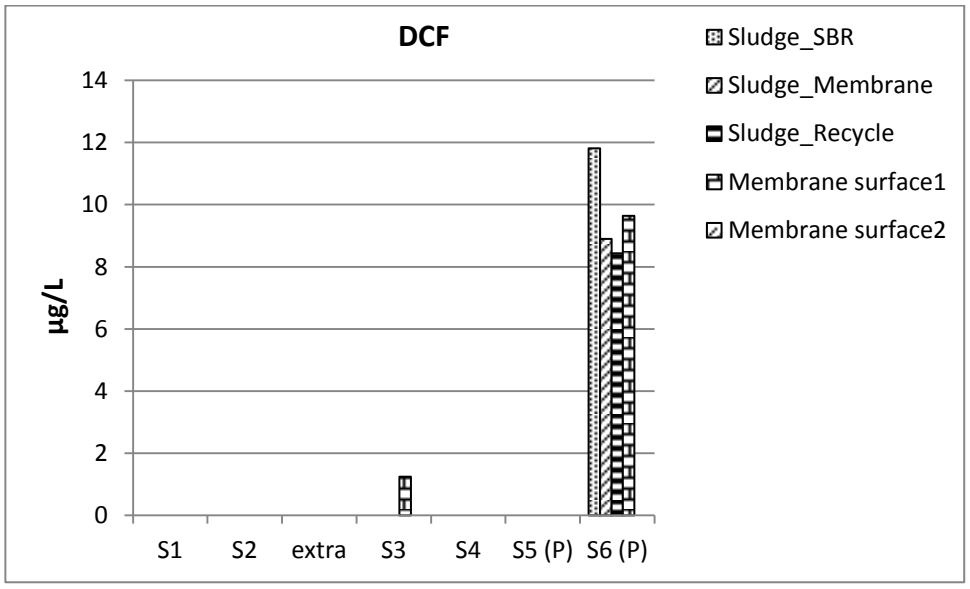

Figure 4.2.58 Concentration of DCF in the sludge samples.

Background DCF concentration of sludge was under limit of detection. After spiking, it did not accumulate in sludge and was under limit of detection, except for a membrane surface sludge in one sample. After addition of PAC, it could be detected at above 8 µg/g in all the samples.

During the course of this study, four different commonly prescribed antibiotics were investigated. The first of these was Erythromycin (ERY). The

concentration of ERY in the influent, supernatant of SBR and permeate of membrane are given in Figure 4.2.59.

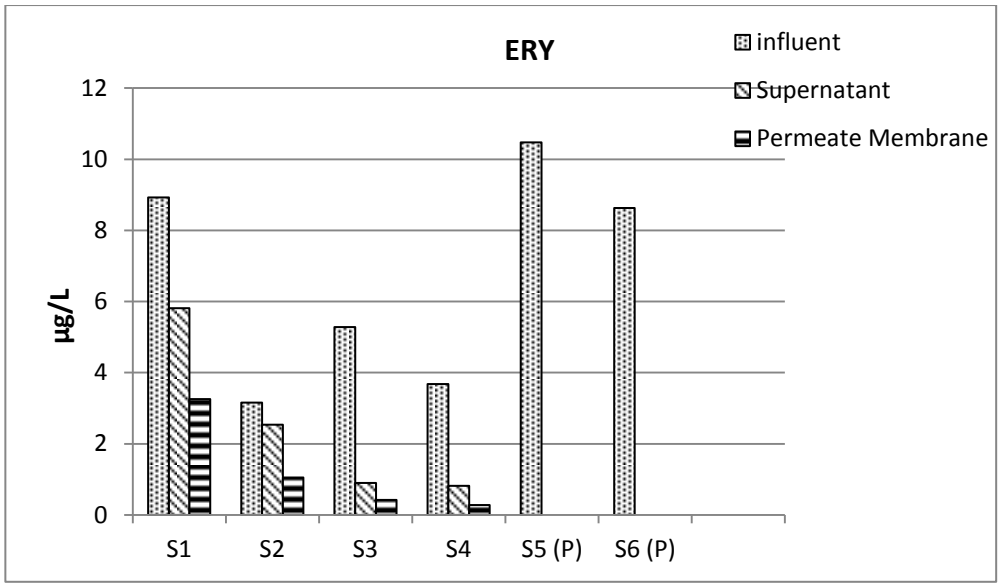

Figure 4.2.59 Concentration of ERY in influent, supernatant of SBR and permeate of membrane.

The removal of this compound in the SBR tank was between 20 to 80% before addition of PAC. After PAC addition all the ERY was adsorbed by PAC in the SBR chamber. A removal of 63 to 92% was observed without PAC. It is clear from Figure 4.2.59 that additional removal was observed with the membrane, since permeate value was lower than the SBR supernatant.

The ERY concentration in the sludge samples were also measured during the study as given in Figure 4.2.60.

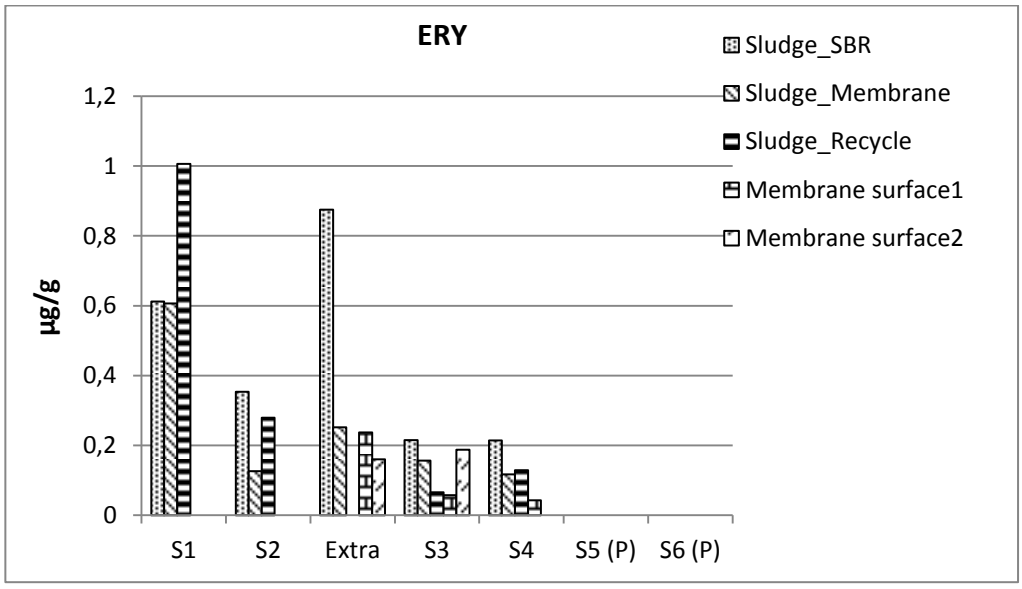

Figure 4.2.60 Concentration of ERY in the sludge samples.

The sludge concentrations of ERY is summarized in Figure 4.2.60. The background ERY concentration in the transferred sludge was 0,256 µg/g. After PAC addition ERY could not be detected in sludge samples. This indicated that ERY was biodegraded by activated sludge and PAC addition improved this.

Roxythromycin was another antibiotics investigated during the study. The concentration of ROX in the influent, supernatant of SBR and permeate of membrane are given in Figure 4.2.61.

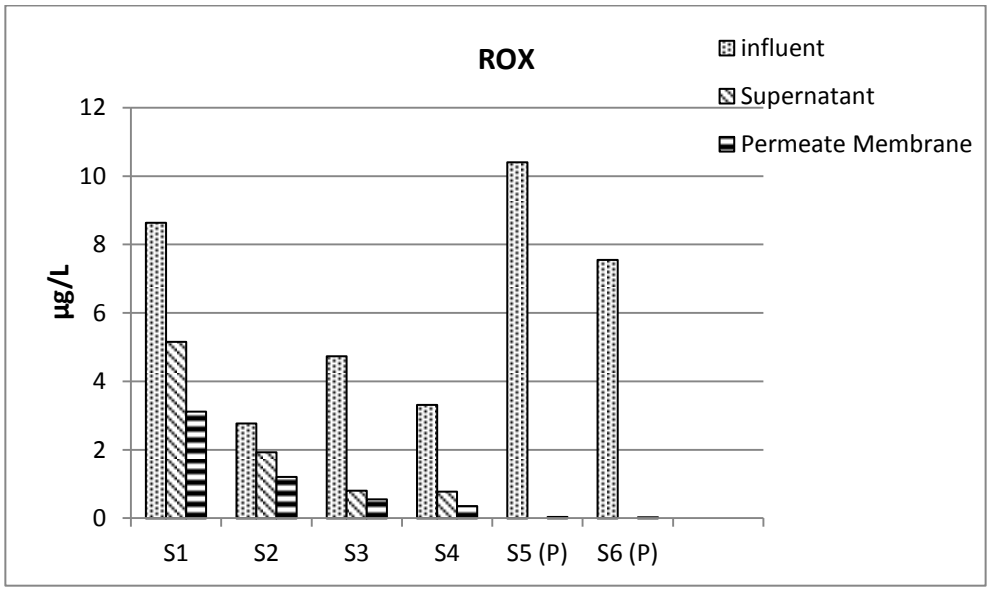

Figure 4.2.61 Concentration of ROX in influent, supernatant of SBR and permeate of membrane.

As seen in this figure, removal of ROX in the SBR tank was between 30 and 80%. In addition to this, a removal of 10 to 20% extra was achieved by the membrane chamber. The total ROX removal in SBR+ membrane plant was etween 56 to 90%. After addition of PAC, over 99% removal was achieved in the SBR tank. Therefore, in the membrane permeate ROX was almost zero.

The sludge concentration of ROX is presented in Figure 4.2.62.The background concentration of ROX in the transferred sludge was 0,213 µg/g.

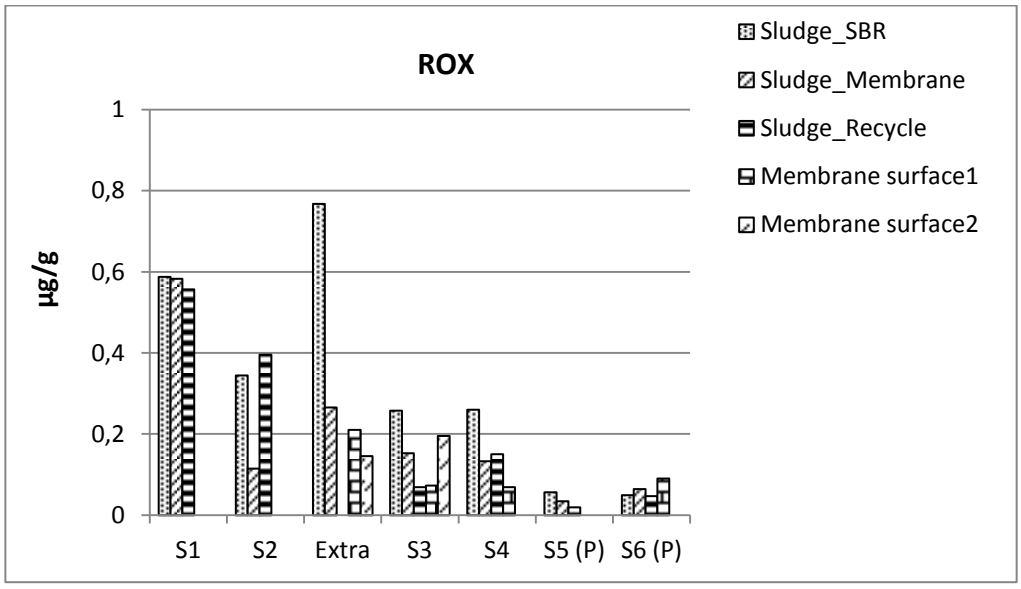

Figure 4.2.62 Concentration of ROX in the sludge samples.

As seen in Figure 4.2.62, after spiking ROX very little accumulation in the sludge could be observed. Moreover upon PAC addition, ROX concentration in sludge decreased. In the absence of any recorded accumulation of this compound in sludge it can be concluded that ROX was primarily removed by biodegradation and PAC addition improved this by adsorbing and concentrating this compound and making it more available to the microorganisms.

Removal of Sulfamethoxazol (SMX) by SBR+membrane process was also investigated. The influent, supernatant and permeate concentrations before and after PAC addition are given in Figure 4.2.63.

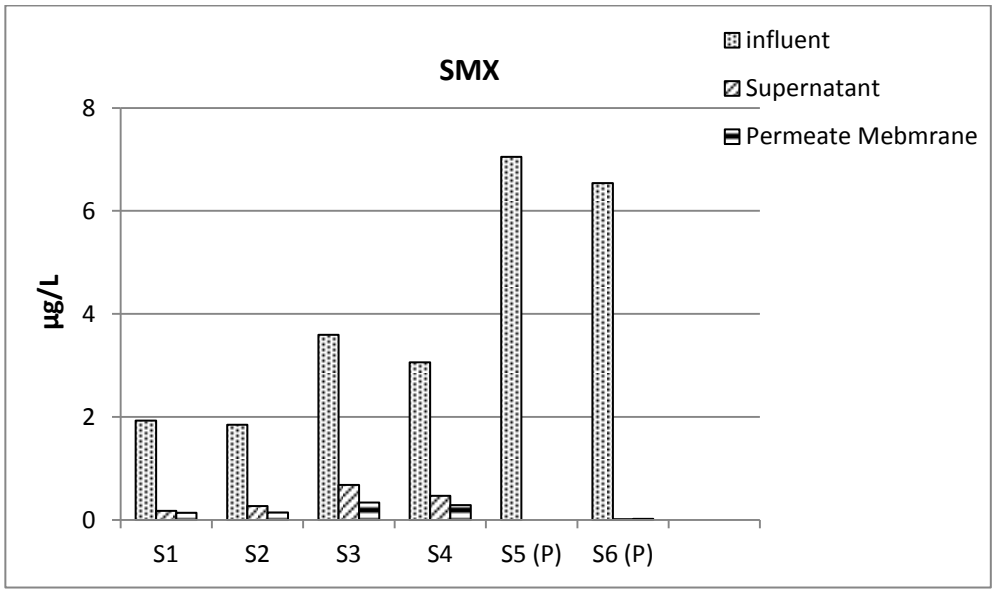

Figure 4.2.63 Concentration of SMX in influent, supernatant of SBR and permeate of membrane.

As seen from this figure, over 80% removal could be achieved just by the SBR tank. A slight contribution by the membrane chamber over the SBR removal is understood. Over 90% removal was achieved by the complete SBR+membrane system. After PAC addition, this has risen to over 99%.

Sludge samples were also analyzed for residual SMX, whose results are summarized in Figure 4.2.64.

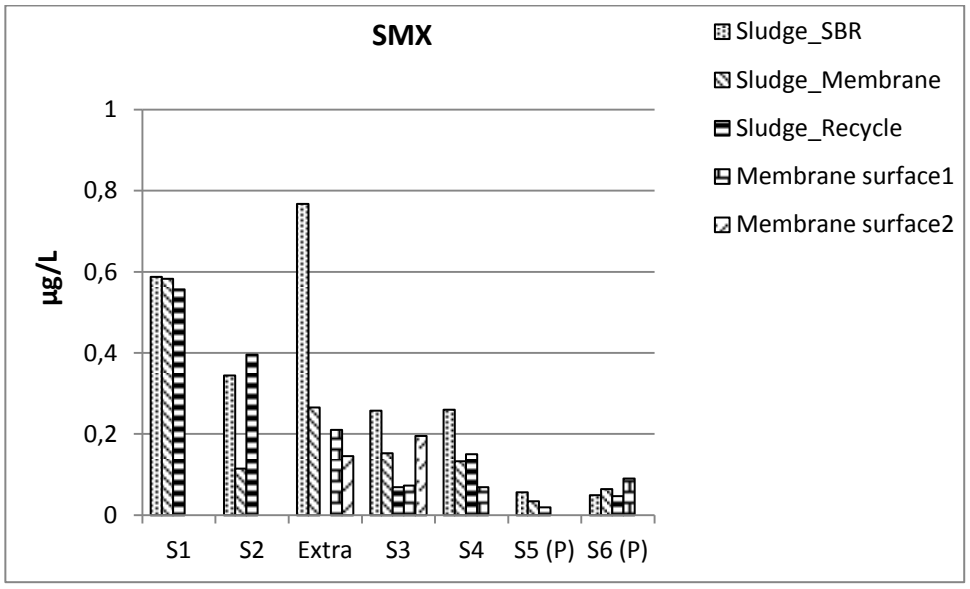

Figure 4.2.64 Concentration of SMX in the sludge samples.

Background SMX concentration in sludge was under limit of detection. The observed SMX in sludge was also very low in the spiked samples suggesting that SMX was mostly biodegraded by the microorganisms. The PAC addition only slightly improved biodegradation

The last antibiotic investigated during the study was trimethroprim (TMP). The concentration of TMP in the liquid samples before and after PAC addition into the SBR+membrane plant are given in Figure 4.2.65.

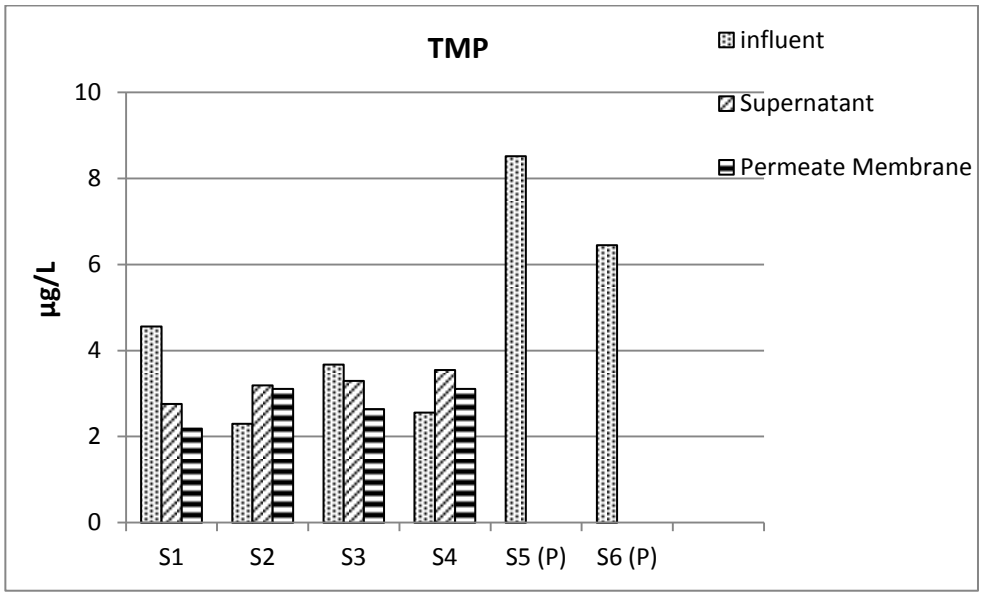

Figure 4.2.65 Concentration of TMP in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.65, of TMP was not removed in the SBR+membrane separation plant. In some cases influent concentration was lower than the effluent. The TMP concentration in the permeate was less than the supernatant of SBR. Unlike previous antibiotic compounds studied, TMP was not removed from the effluents without PAC addition. However after PAC addition all the TMP was adsorbed and in the supernatant of SBR TMP was under limit of detection.

The TMP concentrations in sludge samples are given in Figure 4.2.66.

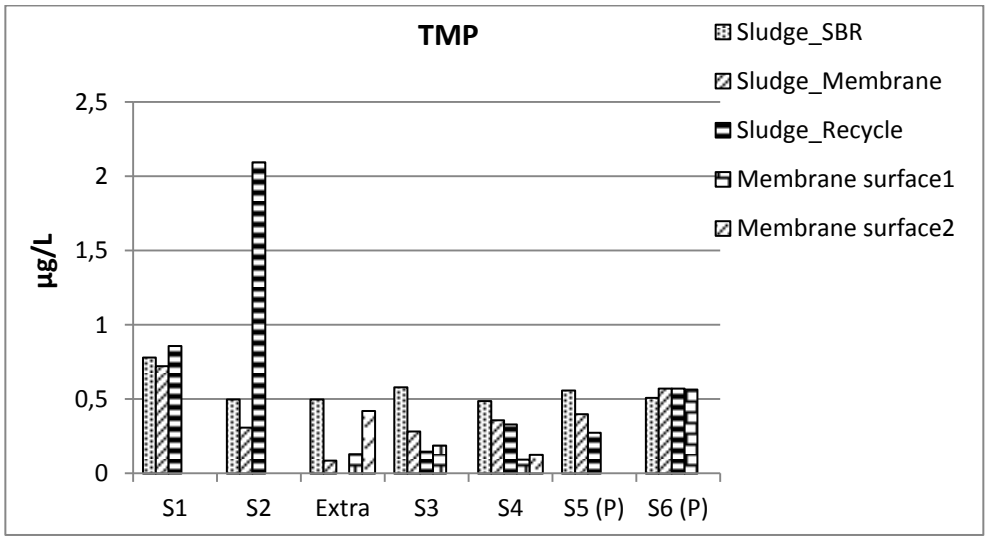

Figure 4.2.66 Concentration of TMP in the sludge samples.

The background TMP concentration in the transferred sludge was 0,551 µg/g. After spiking TMP into the wastewater, there was no increase in TMP concentration in the sludge samples. TMP did not accumulate in sludge samples.

The removal of Fluoxetine (FLX), which is used as antidepressant, in SBR+membrane system with and without PAC addition was investigated. The concentrations of FLX in influent, supernatant of SBR and permeate of membrane are given in Figure 4.2.67.

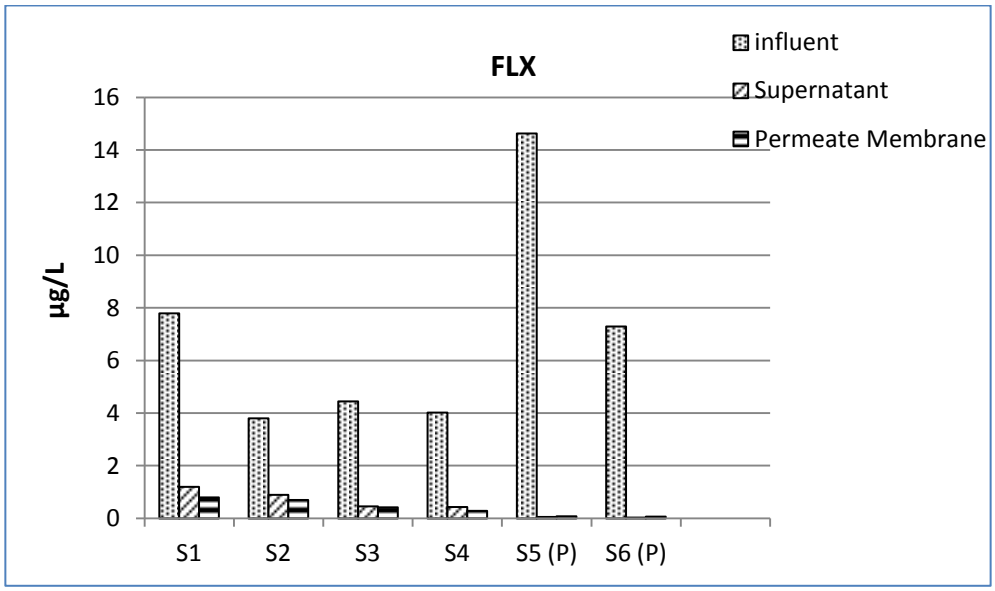

Figure 4.2.67 Concentration of FLX in influent, supernatant of SBR and permeate of membrane.

As seen in Figure 4.2.67, influent FLX concentration was vaiable between 4 µg/L and 14 µg/L., A removal from 75 to 90 % was achieved in the SBR tank before addition of PAC. A slight additional removal was achieved in membrane chamber. After addition of PAC, almost all the FLX was absorbed by PAC.

The FLX concentration in sludge samples was also investigated. Background FLX concentration was 0,073 µg/g in the activated sludge transferred from treatment plant of Calo e o Milladoiro. The sludge concentrations measured during the course of the study are given in Figure 4.2.68.

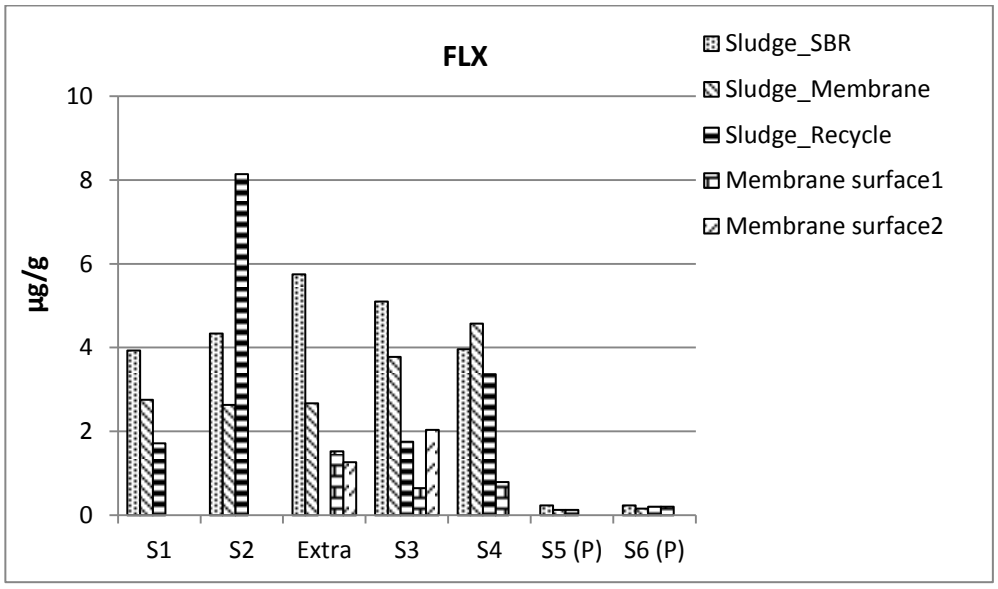

Figure 4.2.68 Concentration of FLX in the sludge samples.

As seen in this figure, spiked FLX accumulated in the sludge. FLX concentration in the SBR tank was higher than the membrane chamber. This may be due to FLX being initially sorbed by the SBR chamber and not transferring to the membrane chamber. Although removal of FLX after PAC addition was over 99%, still it could not be detected in the sludge samples after PAC addition. This is a good example of PAC assisted biodegradation, where PAC concentrates FLX on the carbon surfaces making it available to microorganisms at higher concentrations which in turn support higher microbial uptake rates and regeneration of the carbon. The sludge concentration of FLX in these samples decreased to below 1 µg/g after addition of PAC.

4.2.5 Effect of Different Zone for Removal of Selected PPCPs

In order to understand the domain of selected PPCPs' removal; supernatant samples were drawn at different zones in the SBR plant, at various periods of the operation cycle of SBR, and without the addition of PAC to the system. The first sample was taken from the influent. The 2nd sample was taken from the anoxic zone at the 5th minute of operation. At the end of the anoxic zone the third sample

was taken. The 4th sample was taken after one hour from the start of the aerobic zone and 5th after 3 hours from the start of the aerobic. The last sample was taken at the end of the cycle during the settling zone. The concentrations observed at different zones of the operating cycle are given in Figure 4.2.69.

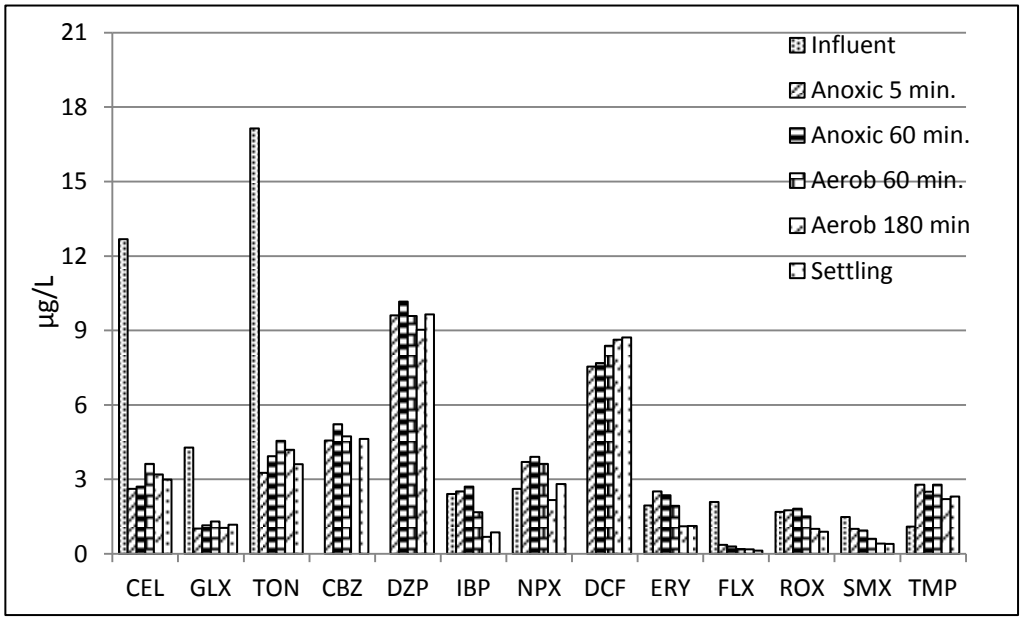

Figure 4.2.69 Concentration of selected PPCPs during each cycle.

As seen in this figure, all of the fragrances (CEL, GLX and TON) were fast sorbed by the activated sludge upon mixing and were removed from the supernatants in the anoxic zone. The CBZ and DZP concentrations were unchanged during the treatment cycle. There was no effect of the anoxic zone on the removal of IBP. Following the anoxic zone, there was about 65% removal of this compound in the aerobic zone. There was no NPX and DCF removal during the study. The ERY was not removed in the anoxic zone, but 40% of removal was achieved in the aerobic zone. The FLX was primarily removed in the anoxic zone. An additional 5% removal was achieved in the aerobic zone. The total removal during the cycle was over 93%. The ERY, ROX were not removed in the anoxic zone. A 40% removal was achieved in the aerobic zone. A 40% SMX was

removed in the anoxic zone. At the end of the aerobic zone 80% removal was achieved. The TMP was not removed during the SBR cycle.

During the study, four different MBR plants with configurations were used for understand the removal of selected EDCs. The summary for the removal was given in the below table.

Table 4.2.2 Summary for the removal of selected compounds by different MBR configurations

Compound	Biodegradation	Adsorption to sludge	Sludge age	Comments
VRM				
Diltiazem	+	-	25	A slightly removed by biodegradation
Prog.	+++	-	25	Completely removed by biodegradation
Estrone	+++	-	25	Completely removed by biodegradation
CBZ	-	-	25	No removal observed
Acetaminop.	+++	-	25	Completely removed by biodegradation
Clearbox				
Diltiazem	-	-	10,15,20,25	No removal observed
Prog.	+++	-	10,15,20,25	Completely removed by biodegradation in all SRTs
Estrone	+++	-	10,15,20,25	Completely removed by biodegradation when SRT was 10, 20 and 25 days. When SRT 15 days, 75% removal
CBZ	-	-	10,15,20,25	No removal observed in all SRTs
Acetaminop.	++	-	10,15,20,25	Higher SRT has high removal. When SRT was 25 days, Completely removed by biodegradation

Table 4.2.2cont. Summary for the removal of selected compounds by different MBR configurations (cont'd)

Compound	Biodegradation	Adsorption to sludge	Sludge age	Comments
Konacık				
Diltiazem	-	-	25	No removal observed
Prog.	-	-	25	Under LOD
Diltiazem	-	-	25	No removal observed
Estrone			25	No measured in the samples
CBZ	-	-	25	No removal observed
Acetaminop.	+++	-	25	Completely removed by biodegradation
SBR+MBR (+PAC) (SRT= Time of operation= 140 days)				
CEL	-	++	140 days	Removed by sorption to sludge. After PAC addition, all of them removed
GLX	-	++	140 days	Removed by sorption to sludge. After PAC addition, all of them removed
TON	-	++	140 days	Removed by sorption to sludge. After PAC addition, all of them removed

Table 4.2.2cont. Summary for the removal of selected compounds by different MBR configurations (cont'd)

Compound	Biodegradation	Adsorption to sludge	Sludge age	Comments
CBZ	-	-	140 days	No removal observed before PAC addition. After PAC addition, completely removed by sorption to PAC
DZP	-	-	140 days	No removal observed before PAC addition. After PAC addition, completely removed by sorption to PAC
IBP	++	-	140 days	Highly removed by biodegradation, completely removed by sorption to PAC
NPX	+	-	140 days	Half of them removed by biodegradation. After PAC addition, over 85% removal achieved.
DCF	-	-	140 days	No removal observed before PAC addition. After PAC addition, completely removed by sorption to PAC
ERY	++	-	140 days	Highly removed by biodegradation, completely removed by sorption to PAC, PAC was regenerated by microbial action
ROX	++	-	140 days	Highly removed by biodegradation, completely removed by sorption to PAC, PAC was regenerated by microbial action
SMX	++	-	140 days	Highly removed by biodegradation, completely removed by sorption to PAC, PAC was regenerated by microbial action

Table 4.2.2cont. Summary for the removal of selected compounds by different MBR configurations (cont'd)

Compound	Biodegradation	Adsorption to sludge	Sludge age	Comments
TMP	-	-	140 days	Removed by sorption to PAC,
FLX	-	++	140 days	Removed by sorption to sludge. After PAC addition, all of them removed

"-" = No removal observed

"+" = Less than 50% removal

"++" = Removal rate between 50-80%

"+++" = Over 80% removal

CHAPTER 5

CONCLUSION

In last few decades, an increasing concern has arisen on endocrine disrupting compounds in water cycle, whose source is the wastewaters. This study has therefore focused on analyses, occurrence and removals of selected EDCs in the wastewater. The following conclusions can be drawn from the present study:

- Optimization and determination of selected endocrine disrupter compounds, EDCs, which are present in environmental samples at ultra trace levels, is problematic due to the complicated nature of detection instrumentation and complex background matrix. The selected EDCs, namely, diltiazem, progesterone, estrone, carbamazepine, benzyl butyl phthalate and acetaminophen, in liquid and sludge samples, were analyzed using HPLC coupled with ESI-MS/MS. Following optimization of the analyses conditions on the instrument, appropriate analytical methods were developed for the extraction and concentration and simultaneous determination of the selected EDCs in influent and effluent wastewater samples. Whit this approach the ppt levels could be reached for the analytes of interest. As for the sludge samples recoveries exceeding 93.0 and 97.5% could be achieved.

- Following method optimization and development, four different membrane bioreactor (MBR) treatment plants were investigated for EDCs' fate and removal. Three of these were located in Turkey and one was in Spain. The two of the Turkish MBRs were full scale, and one was a pilot scale MBR, namely Clear-Box, located at METU Campus. One of the full

scales was also operating at METU, named VRM, for its rotating membrane holder drum. The other full scale MBR was a static MBR located in Konacık, Turkey.

- The CBZ and DZP were not removed in the VRM plant during the study. Progesterone and estrone were completely removed by biodegradation and were under limit of detection in sludge samples. Over 90% acetaminophen removal could be achieved in VRM. Only acetaminophen could be detected in the sludge samples.

- In Clear-Box MBR system 4 different SRTs, 10, 15, 20 and 25 days, were tried with different flux rates to understand the effect of SRT and flux rate on the removal of selected EDCs. At 10 days SRT, selected compounds were spiked into the influent. Measured permeate diltiazem concentrations were found proportional with the flux-rates. For example at lower flux rates permeate concentrations were also low; or vice verse. This was observed at all SRTs. Progesterone was almost entirely removed at all the SRTs and fluxes tested, except for 25 days SRT, where flux increase caused decrease in CBZ removal. Moreover, CBZ concentration in the permeate taken in the first minute of suction was higher than that was taken at the last minute of suction cycle. This clearly indicates effect of concentration polarization over the membrane surface. Estrone was removed completely during the study. However, lower flux rates had negative effect on the removal of estrone when SRT was 15 days. The last compound studied was acetaminophen. At long SRTs all the acetaminophen was removed; higher flux rates supported higher acetaminophen removals.

- In Konacık MBR Plant, diltiazem, CBZ and acetaminophen were detected in all the influent samples. Although acetaminophen was removed completely in this MBR process, CBZ and diltiazem were not removed at

all in this system. Only acetaminophen was detected in the sludge samples; and progesterone, estrone and BBP were under limit of detection in influent, effluent and sludge samples.

- Thirteen selected compounds falling into the category of personal care products and medication, namely, Fluoxetine (FLX), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), Carbamazepine (CBZ), Trimethoprim (TMP), Roxithromycin (ROX), Erythromycin (ERY), Sulfamethoxazole (SMX), Diazepam (DZP), Galaxolide (GLX), Tonalide (TON), Celestolide (CEL), were investigated in a sequencing batch reactor (SBR) combined with a membrane separation unit, with and without powdered activated carbon addition. Treatment studies on this lab scale unit was divided into three periods. In the first period only general parameters were investigated. Over 95% COD removal, 70% total nitrogen and over 99% PO_4-P removal by this plant was demonstrated. In the second period, removal of selected compounds were investigated in this lab unit. For the musk fragrances CEL, GLX and TON, around 50-60% removal was observed in the SBR tank and sometimes additional removal was achieved in the membrane chamber. Much of the musk fragrances were found to have accumulated in the sludge.

- Antibiotics, ROX, ERY and SMX except for TMP, were removed by biodegradation. Extra 30% removals of these compounds have been achieved in the aeration tank. TMP was not removed at all during the study. These compounds did not sorb onto the activated sludge. However, upon PAC addition, all the non-degraded compounds were removed to completion and accumulated in the sludge. Concentration of some of the PAC-sorbed compounds did not increase in the sludge, though there was no wastage of sludge from the system. These suggest that compounds concentrated in the sludge were amenable to further degradation by microorganisms at that state.

- The CBZ, DCF and DZP were not removed at all in the reactor system before the addition of PAC. Sometimes, influent concentrations were lower than effluent concentrations owing to analytical error. After addition of PAC, permeate concentrations were under limit of detection.

- The IBP and NPX were removed by biodegradation during the study. After addition of PAC, these compounds were still removed but were concentrated in sludge.

- The last compound studied was FLX and was removed by adsorption to the sludge. PAC addition also had the same effect.

CHAPTER 6

FUTURE WORKS

Analyses, effects, and treatments of endocrine disrupter compounds is one of the hot topics in Environmental Engineering. Therefore, there are many studies could be developed and continuation of research on EDCs with national and international projects.

In this study, different types of EDCs were optimized and analyses in sludge and wastewater samples. The removal efficiency of them was determined. In the future works, optimization of different EDCs can be done and removal efficiency of them can be investigated in different systems.

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APPENDIX A

CALIBRATION CURVES FOR EDCs IN SPAİN

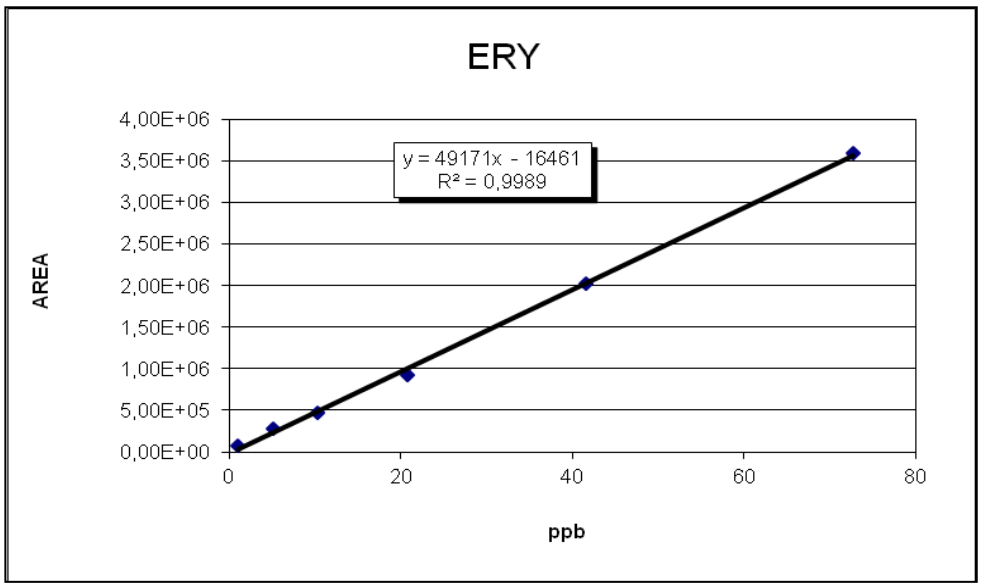

Figure A 1 Calibration curve for ERY

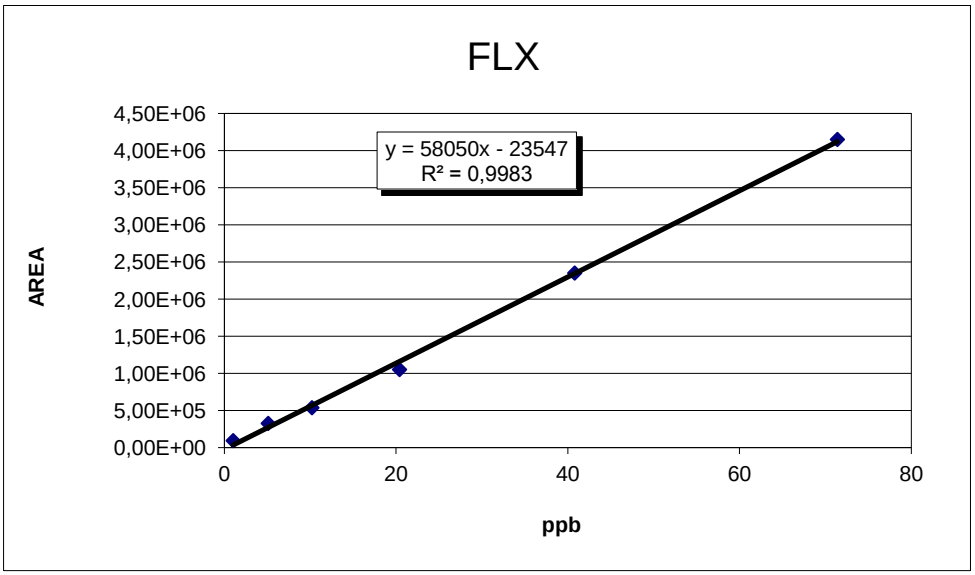

Figure A 2. Calibration curve for FLX

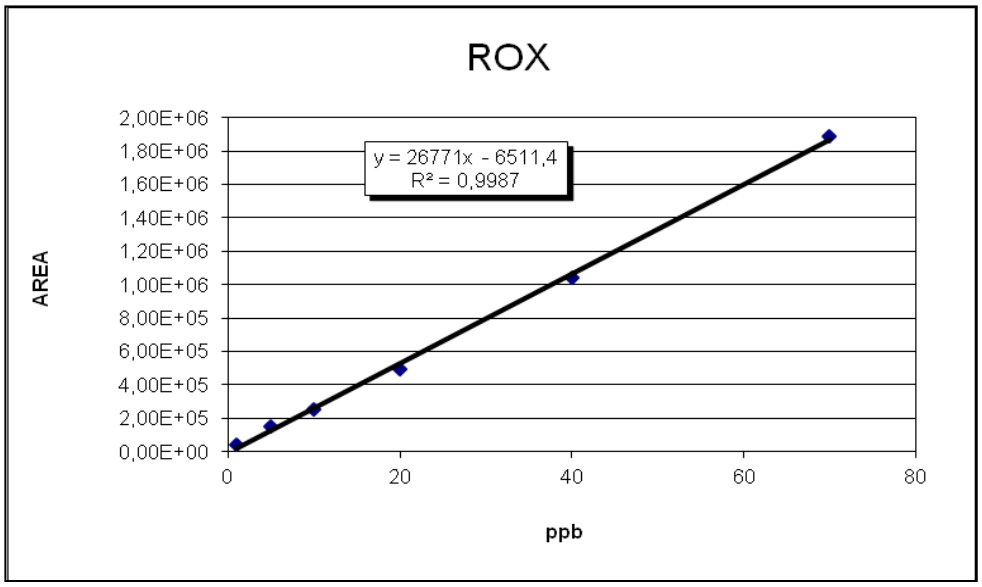

Figure A 3.Calibration curve for ROX

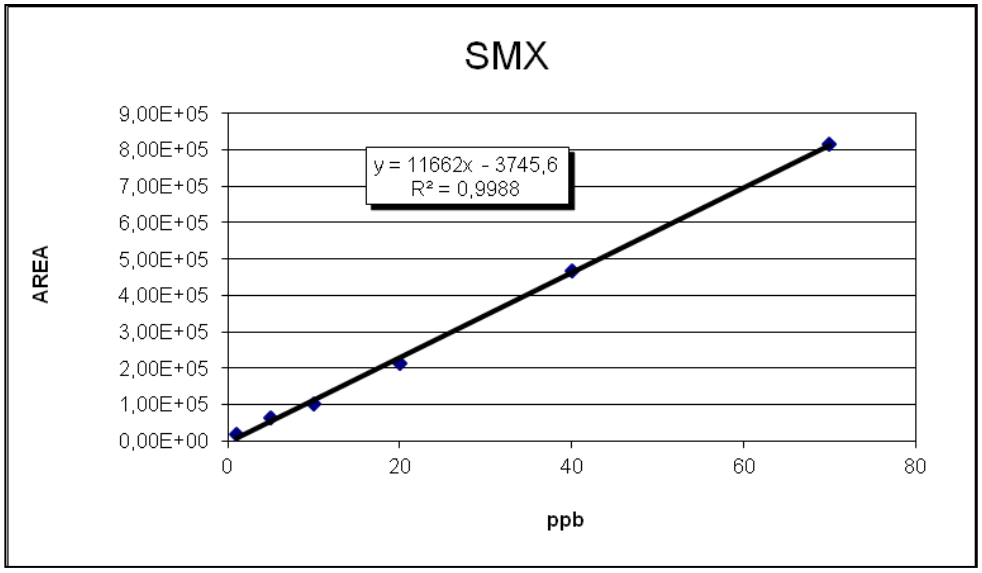

Figure A 4.Calibration curve for SMX

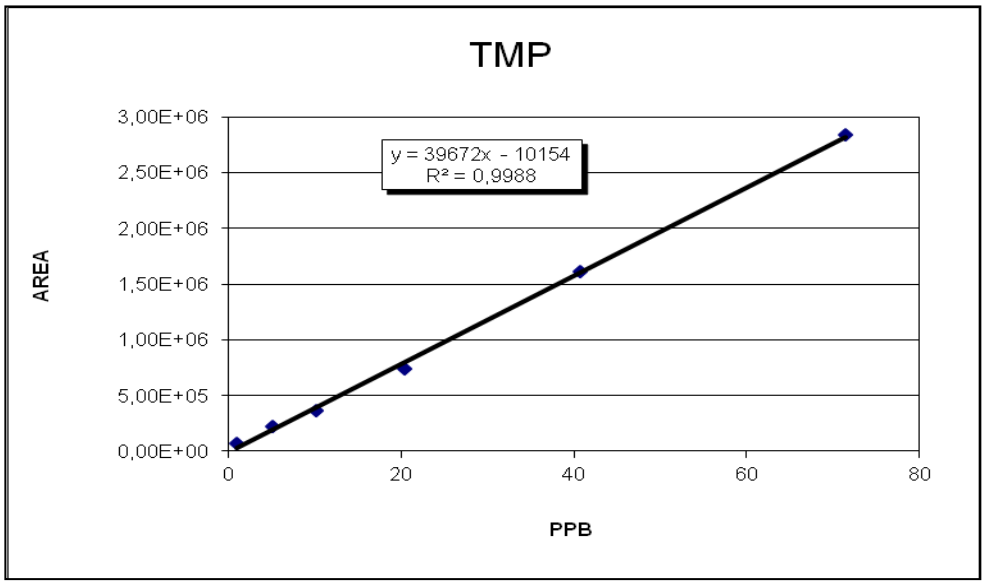

Figure A 5.Calibration curve for TMP

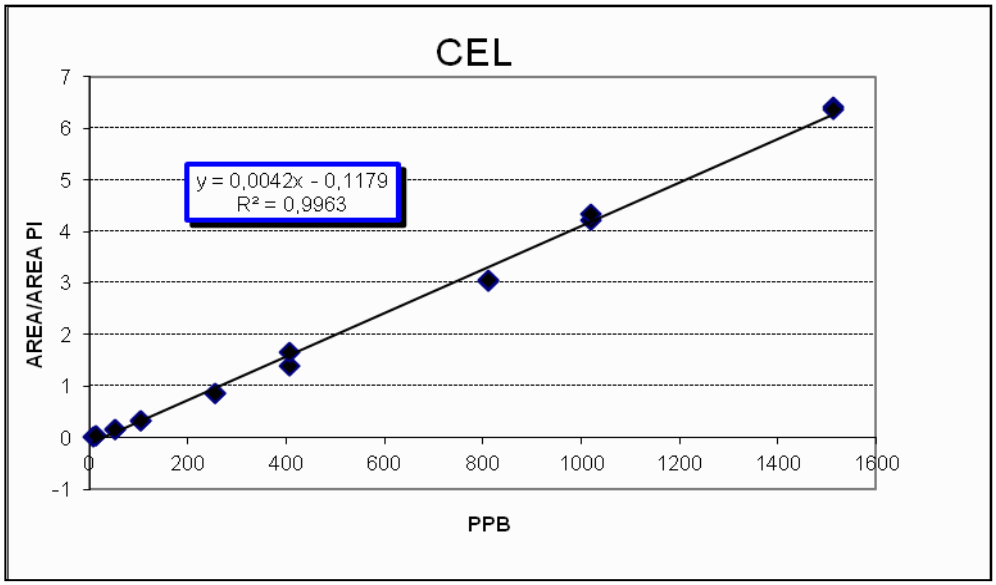

Figure A 6.Calibration curve for CEL

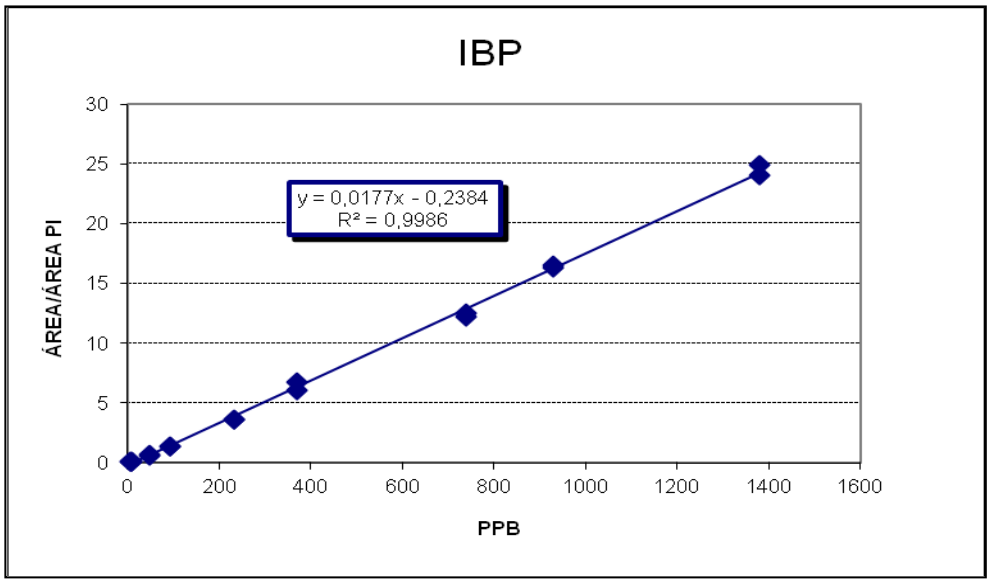

Figure A 7.Calibration curve for IBP

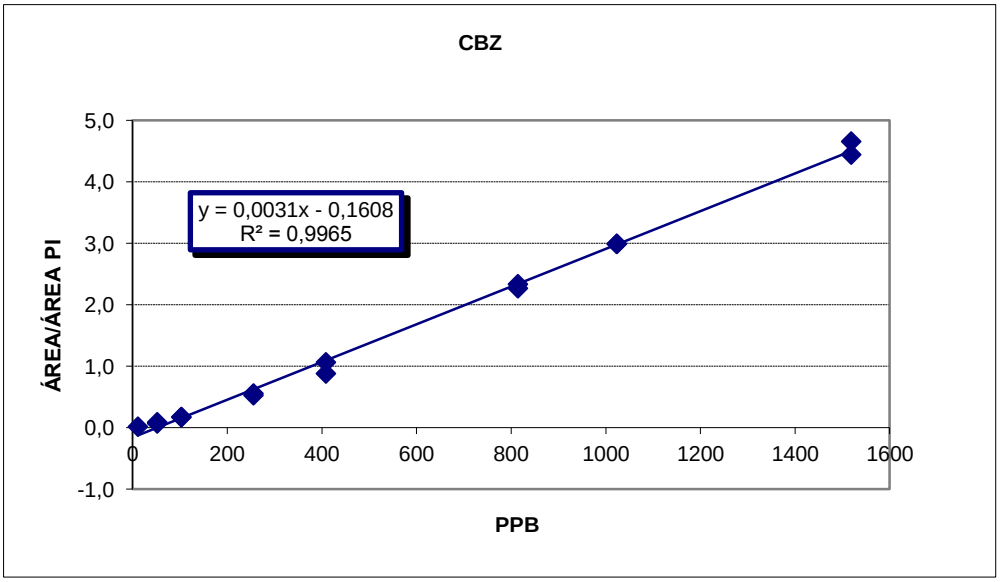

Figure A 8.Calibration curve for CBZ

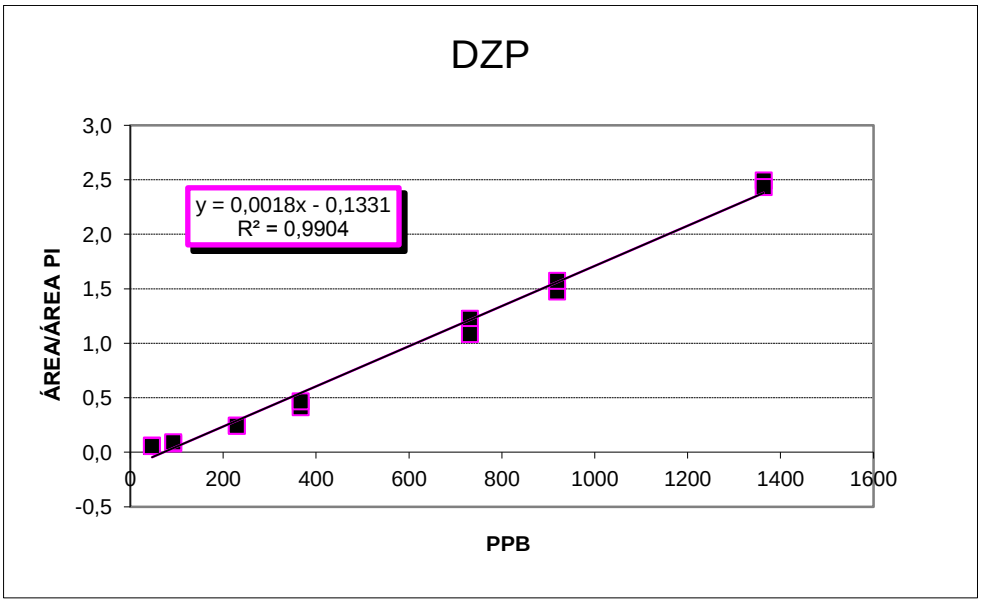

Figure A 9.Calibration curve for DZP

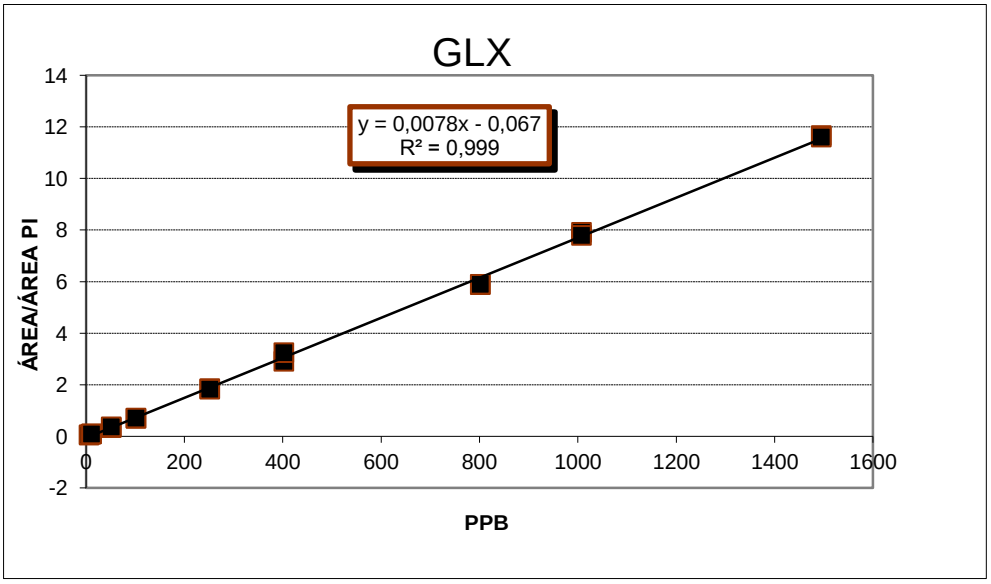

Figure A 10.Calibration curve for GLX

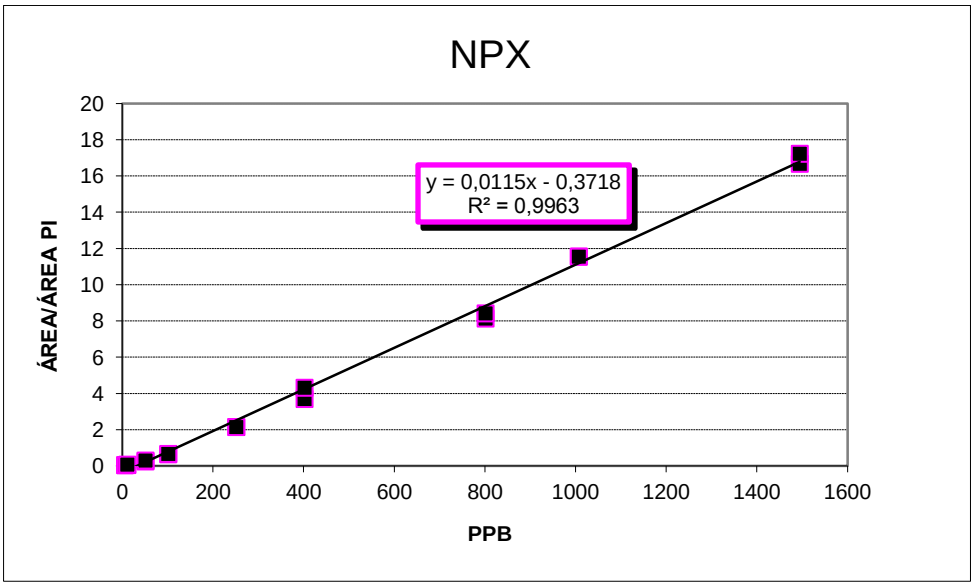

Figure A 11.Calibration curve for NPX

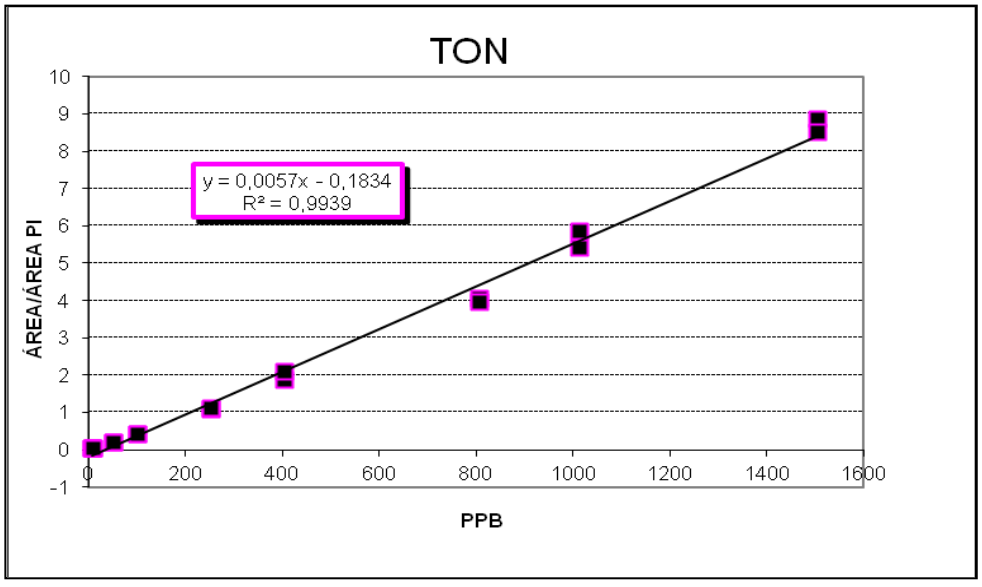

Figure A 12.Calibration curve for TON

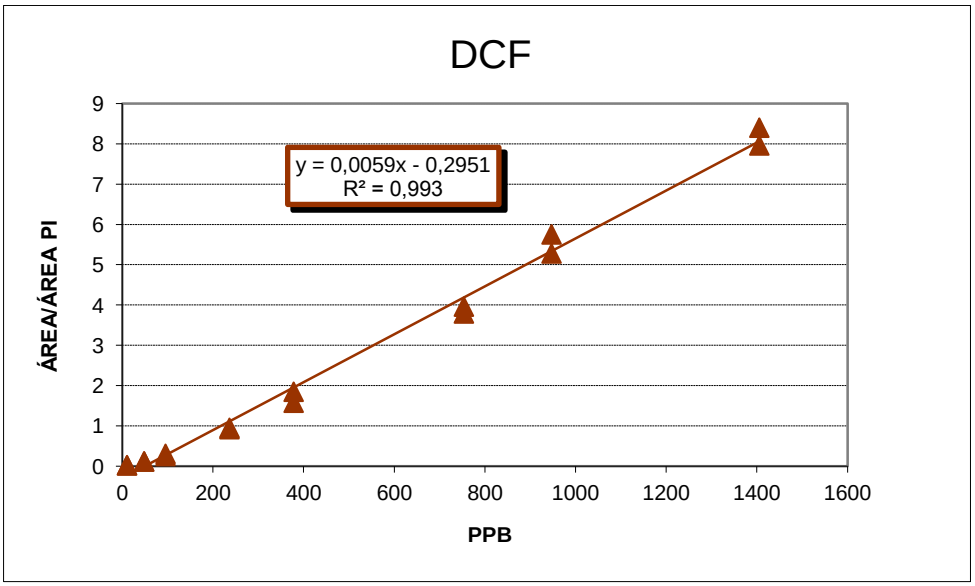

Figure A 13.Calibration curve for DCF

APPENDIX B

OPTIMIZATION OF SELECTED EDCs IN TURKEY

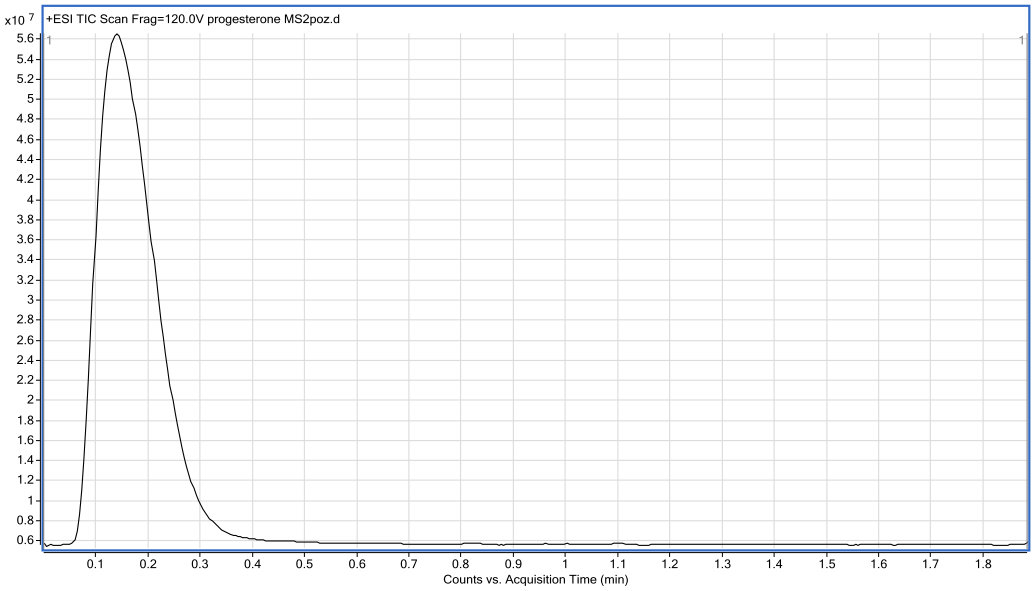

Figure B 1. Positive ion scan for Progesterone

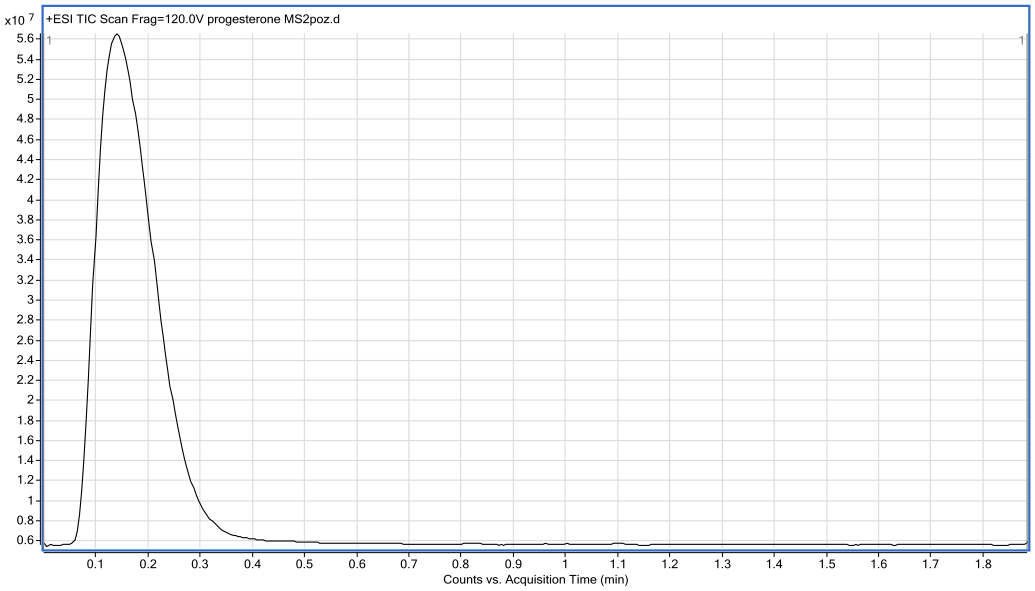

Figure B 2. Negative ion scan for Progesterone

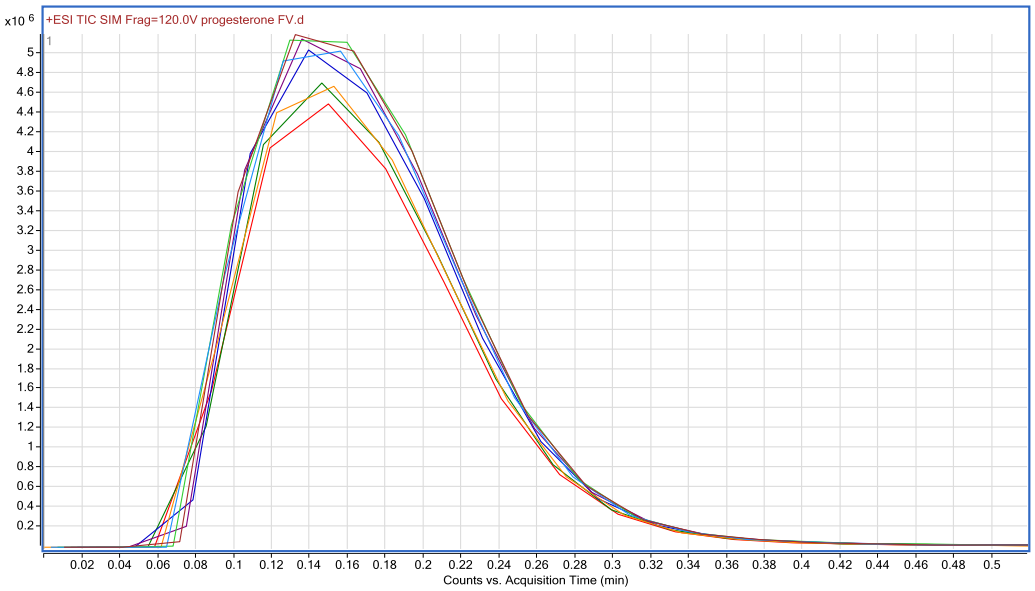

Figure B 3. Optimization of Fragmentor Voltage for Progesterone.

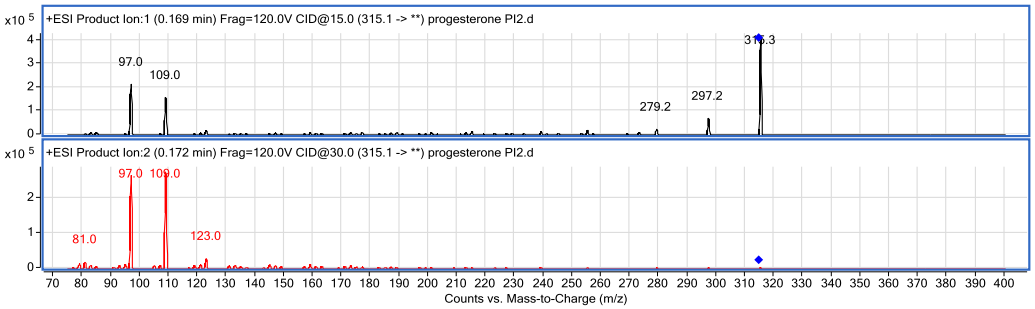

Figure B 4. Product ions for progesterone.

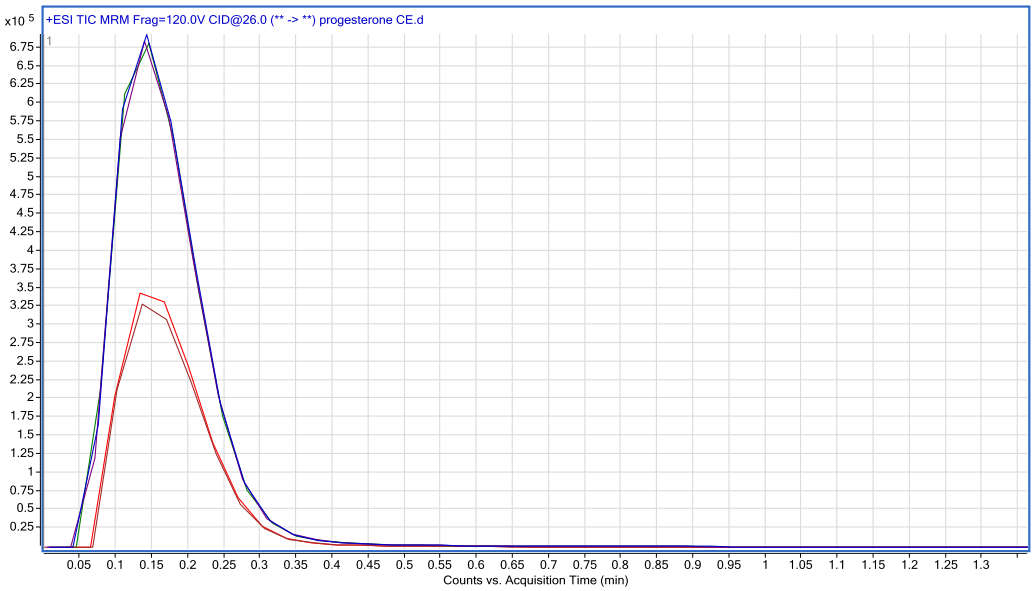

Figure B 5. Optimization of Collosion Energy for Progesterone.

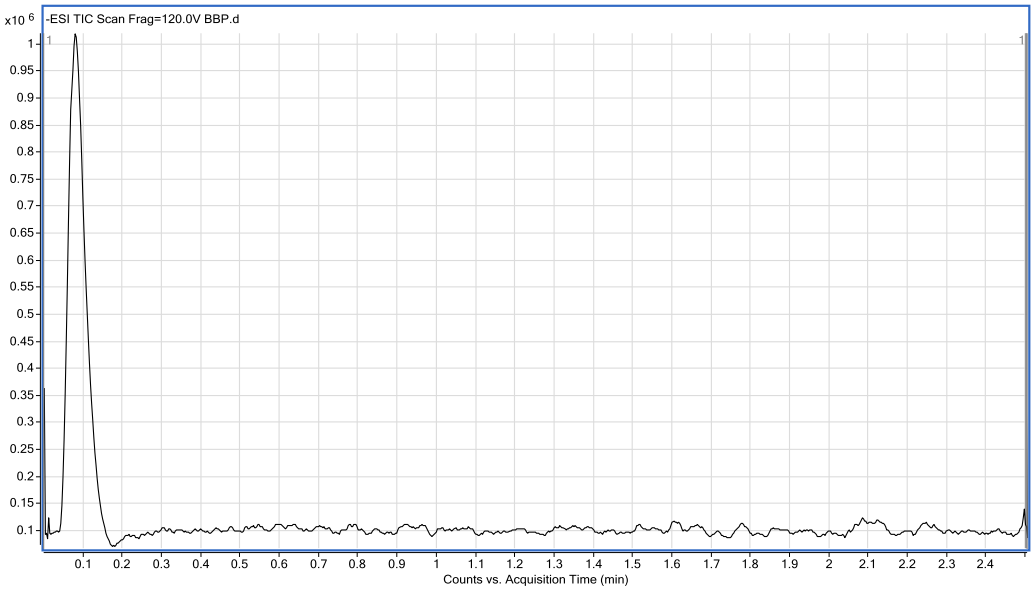

Figure B 6. Negative ion scan for BBP

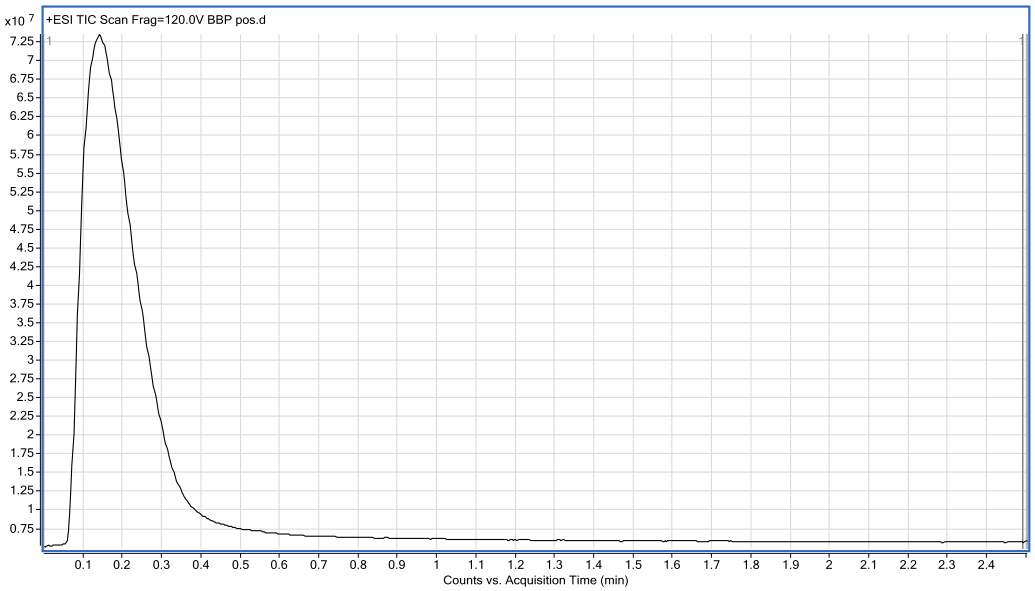

Figure B 7. Positive ion scan for BBP

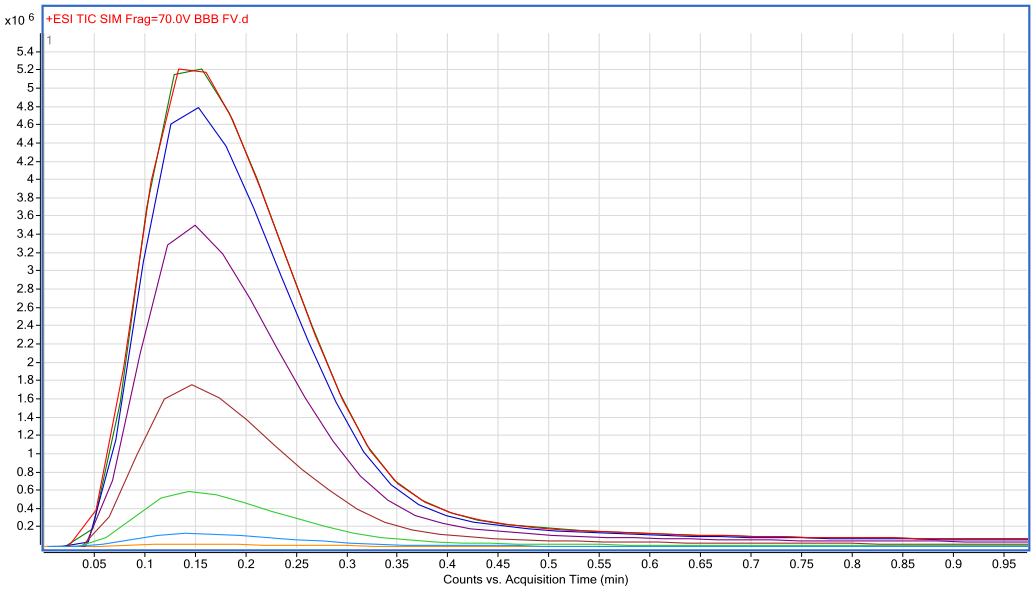

Figure B 8. Optimization of Fragmentor Voltage for BBP

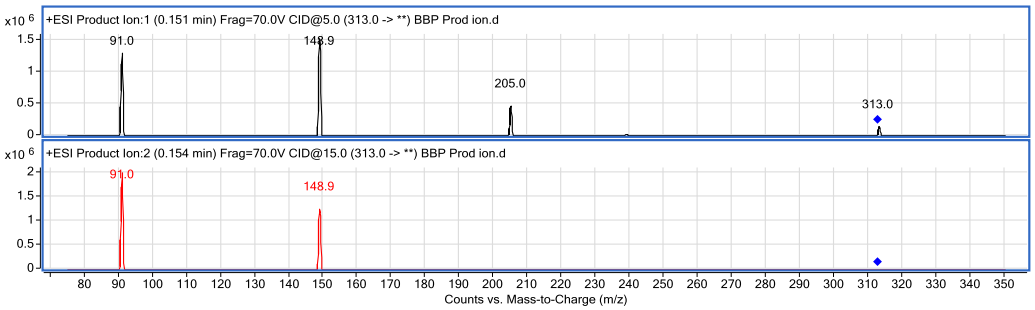

Figure B 9. Product ions for BBP.

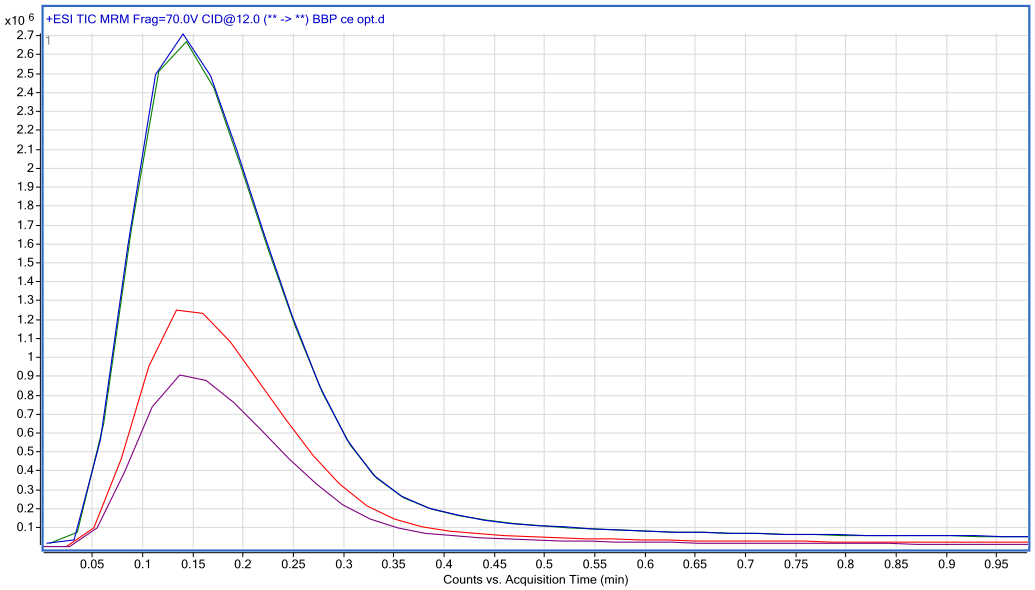

Figure B 10. Optimization of Collosion Energy for BBP.

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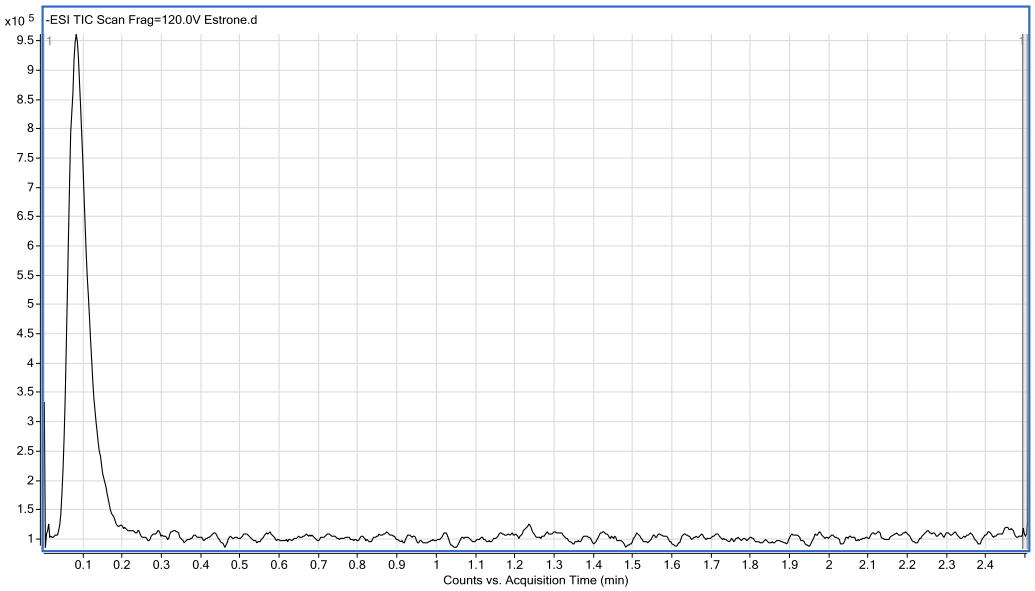

Figure B 11. Negative ion scan for Estrone

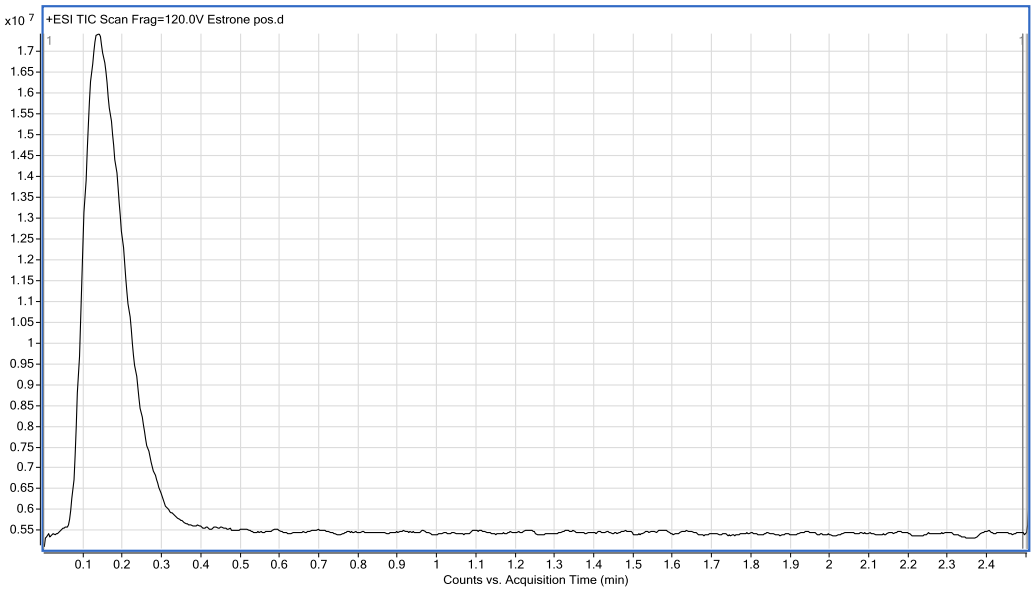

Figure B 12. Positive ion scan for Estrone

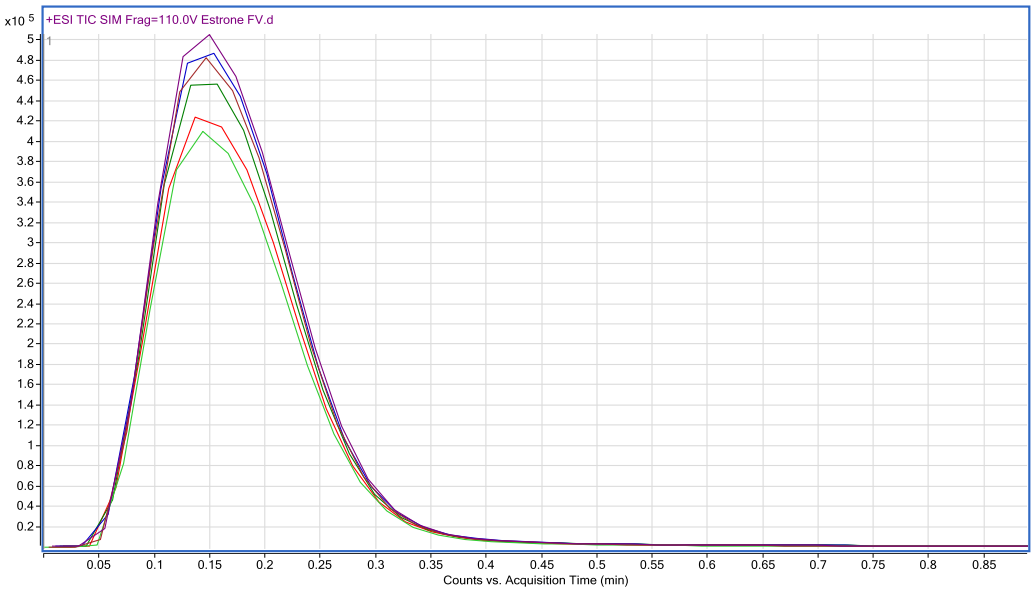

Figure B 13. Optimization of Fragmentor Voltage for Estrone.

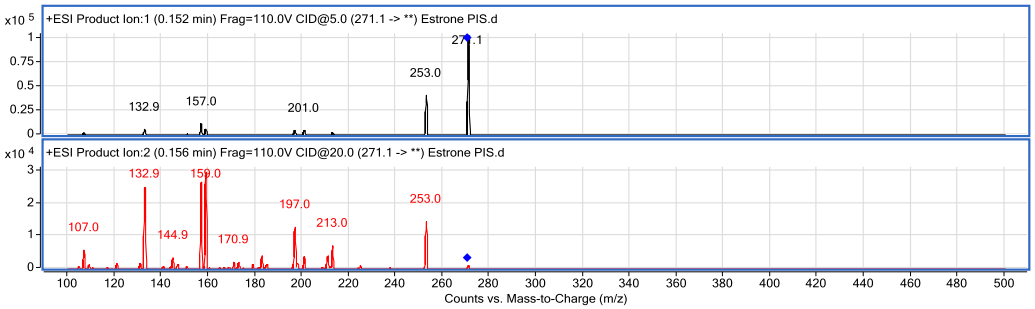

Figure B 14. Product ions for Estrone.

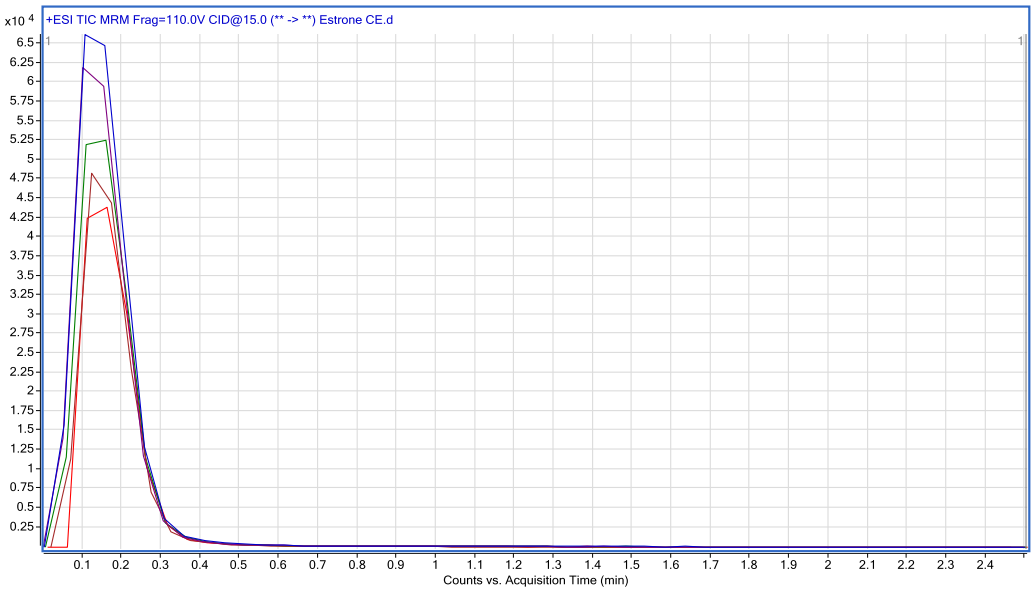

Figure B 15. Optimization of Collosion Energy for Estrone.

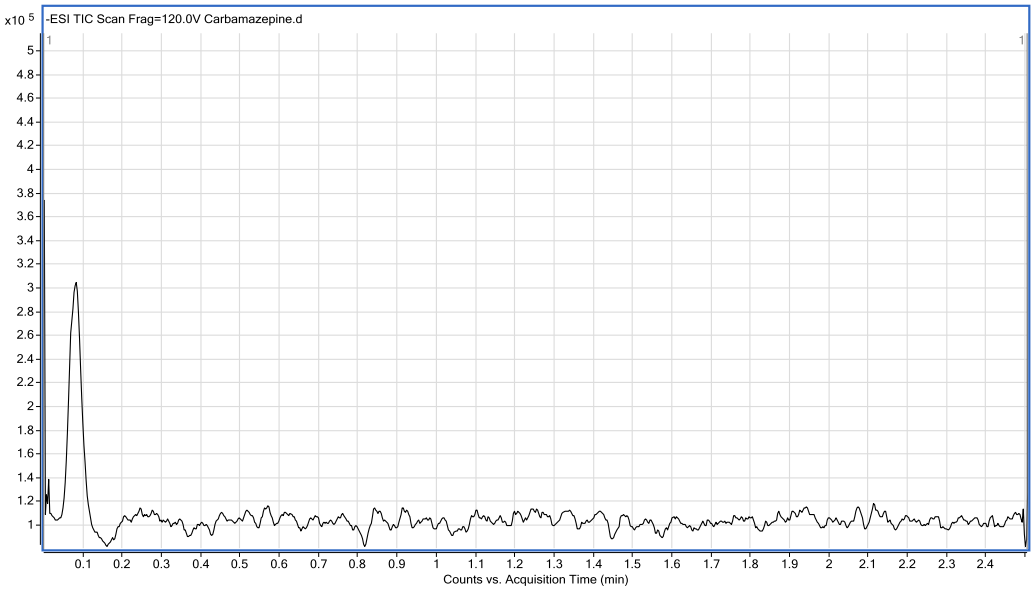

Figure B 16. Negative ion scan for CBZ

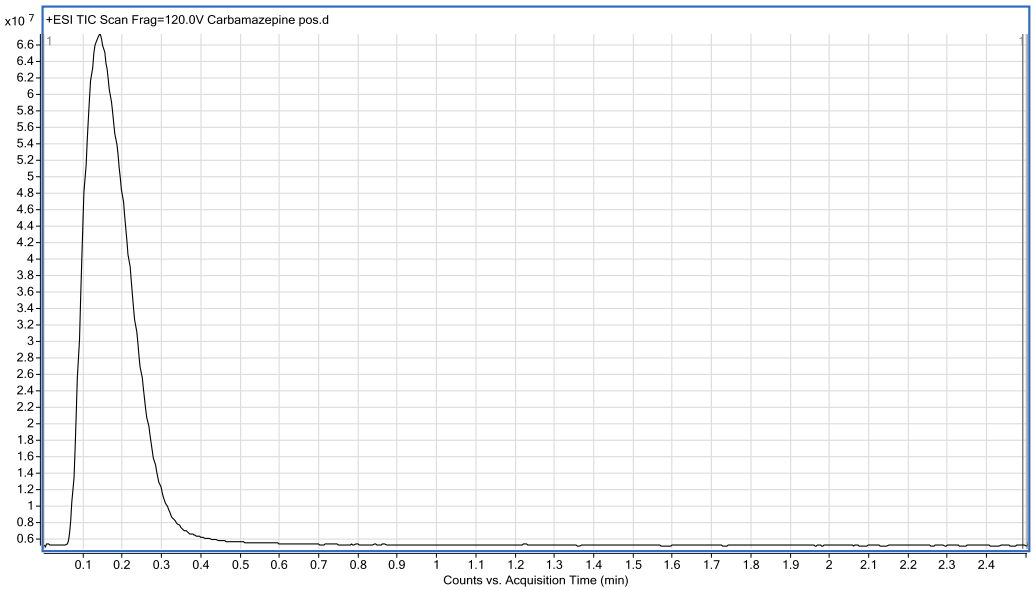

Figure B 17. Positive ion scan for Estrone

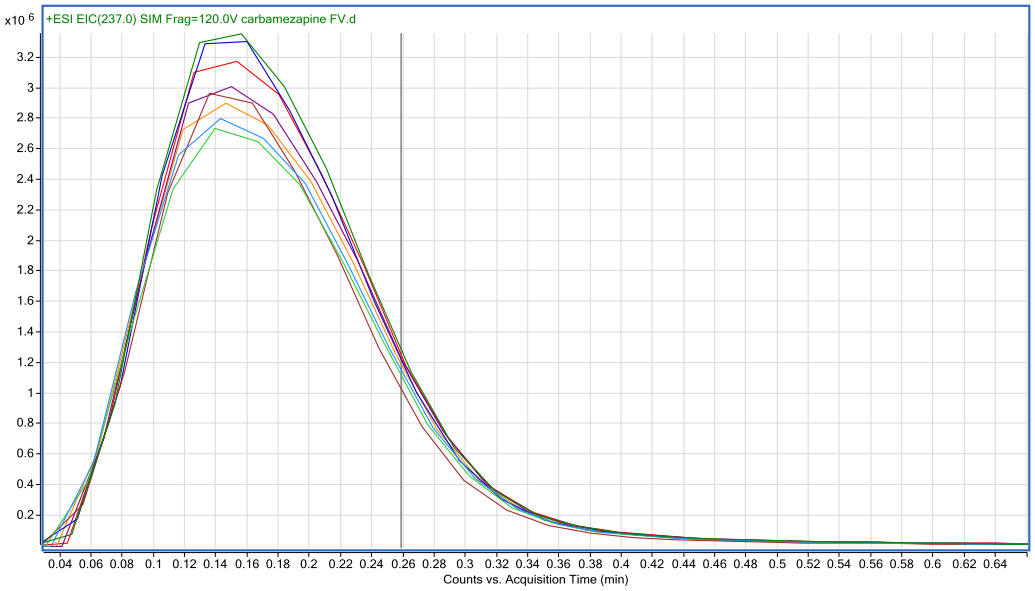

Figure B 18. Optimization of Fragmentor Voltage for Carbamazepine

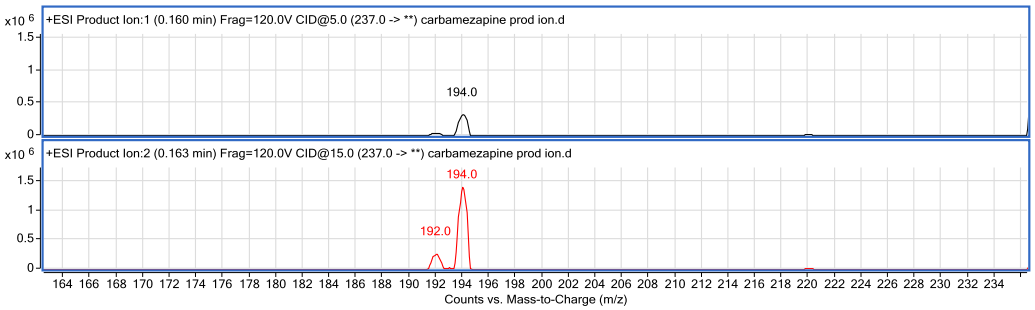

Figure B 19. Product ions for Carbamazepine.

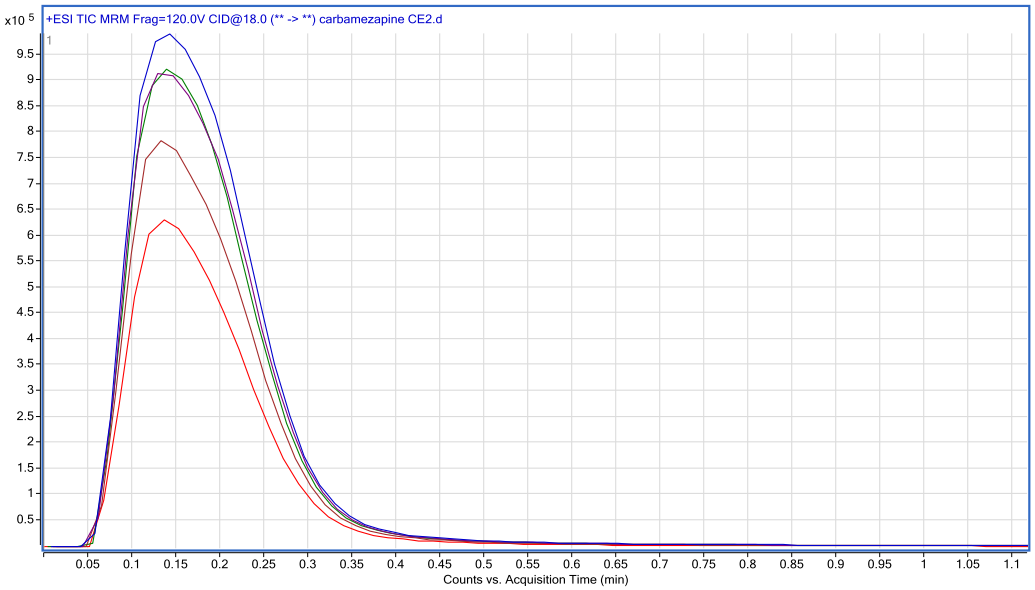

Figure B 20. Optimization of Collosion Energy for Carbamazepine

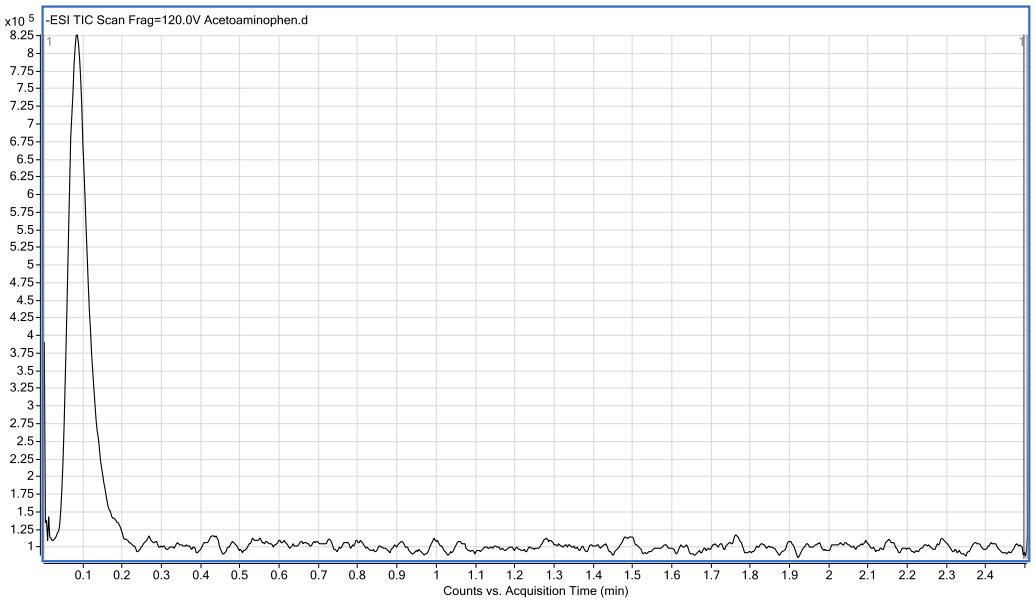

Figure B 21. Negative ion scan for Acetaminophen

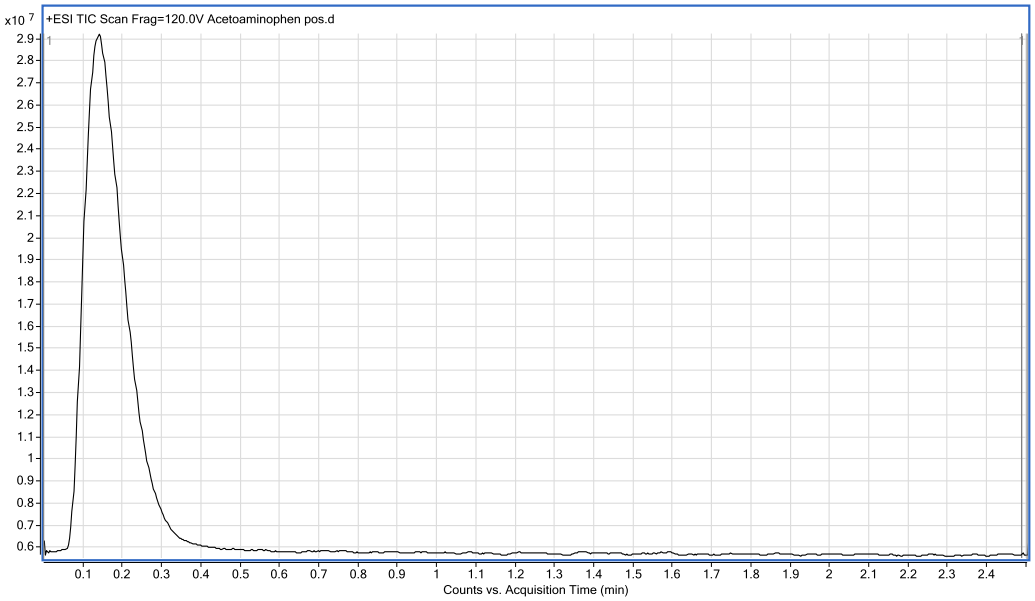

Figure B 22. Positive ion scan for Acetaminophen

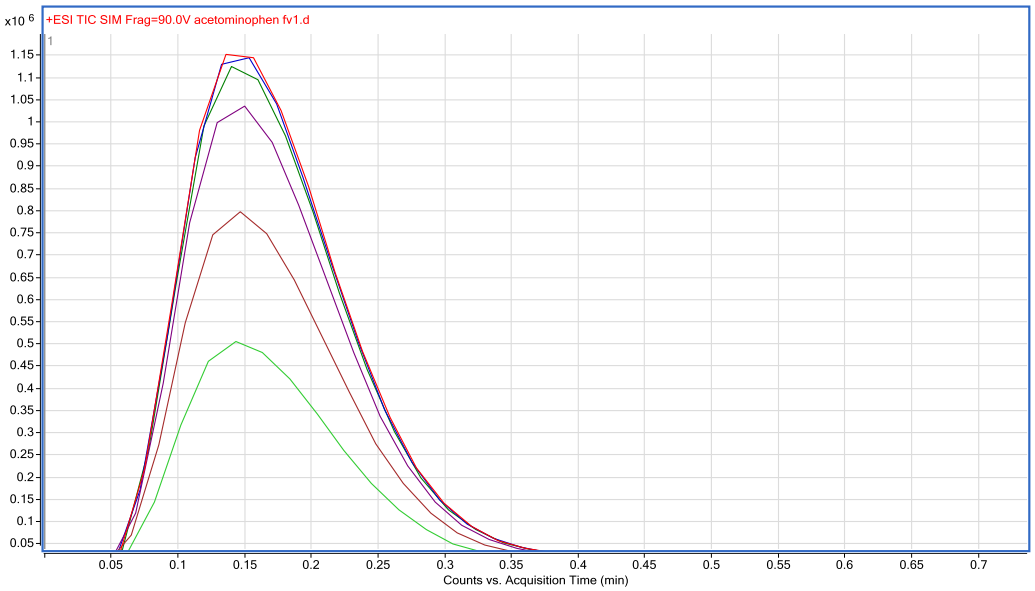

Figure B 23. Optimization of Fragmentor Voltage for Acetaminophen

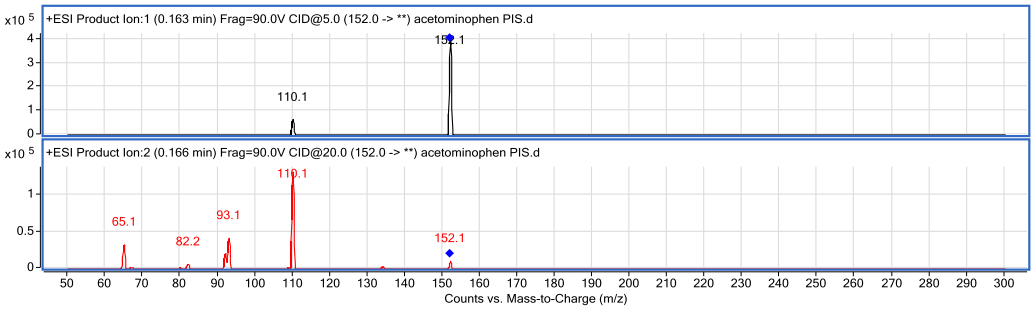

Figure B 24. Product ions for Acetaminophen.

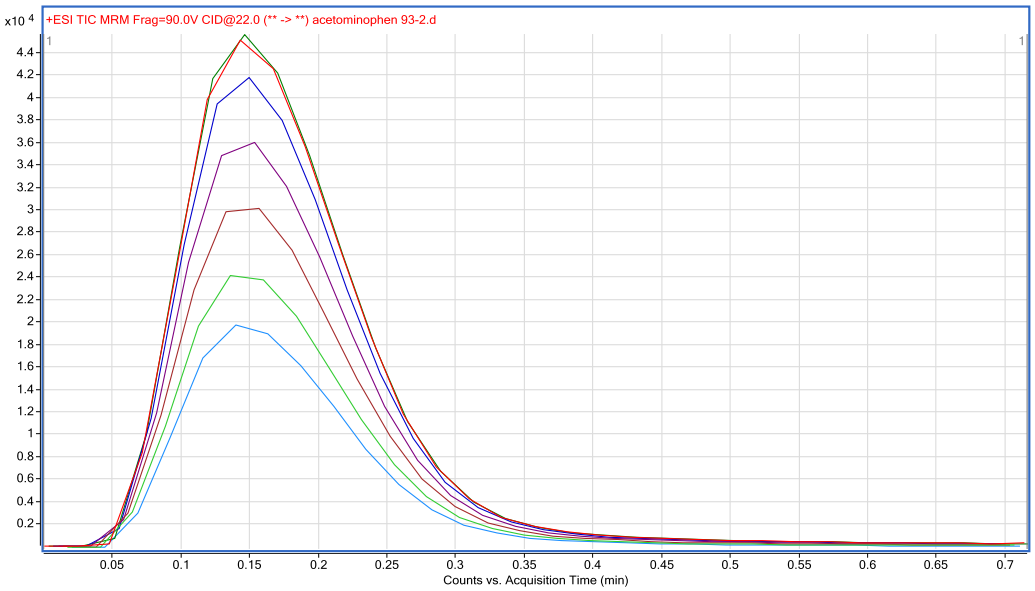

Figure B 25. Optimization of Collosion Energy for Acetaminophen

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Komesli, Okan Tarık
Nationality: Turkish (TC)
Date and Place of Birth: 09 February 1979, Erzurum
Marital Status: Married
Phone: +90 312 210 58 65
Fax: +90 312 210 26 46
email: okan.komesli@gmail.com

EDUCATION

Degree	Institution	Year of Graduation
MS	METU .Environmental Engineering	2003
BS	Atatürk U. Environmental Engineering	1999

WORK EXPERIENCE

Year	Place	Enrollment
2002- Present	METU Dep. of Env. E.	Teaching Assistant

FOREIGN LANGUAGES

Advanced English, Beginning Spanish

PUBLICATIONS

1. **Komesli O.T.,** Teschner K., Hegemann W.,Gokcay C. F., *"Vacuum membrane applications in domestic wastewater reuse*", ***Desalination,*** 215 (2007) 22–28.

2. Muz M., Sönmez M.S., **Komesli* O.T.,** Bakırdere S., Gökçay C.F. *"Determination of Selected Natural Hormones and Endocrine Disrupting Compounds in Domestic Wastewater Treatment Plants by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry after Solid Phase Extraction"* ***Analyst***, 2012, **137** (4) 884-889.

3. Bakırdere S., Bora S, Bakırdere E.G., Aydın F, Arslan Y., **Komesli O.T.,** Aydın I., Yıldırım E. *"Selective and Sensitive Determination of Aflatoxin Species in Foodstuffs"* ***Central European Journal of Chemistry*** 10 (3) (2012) 675-685.

4. **Komesli O.T.,** Bakırdere S., Bayören C., Gökçay C.F., *"Simultaneous Determination of Selected Endocrine Disrupter Compounds in Wastewater Samples using HPLC-ES-MS/MS"* ***Environmental Monitoring & Assessment,*** DOI 10.1007/s10661-011-2334-x, 184(8), 2012, 5215-24.

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1. Muz M, Sönmez M. S., **Komesli O.T.,** Gökçay C.F., "*Kesikli ozonlama yöntemi ile atık çamur azaltımı*" Su Kirlenmesi Kontrolü, (2010) No: 20 (1)

2. Gökçay C.F., **Komesli O. T.,** "*ODTÜ Yeni Biyomembran Arıtma Tesisi*, *Su ve Çevre Teknolojileri*, Ocak Şubat 2007 No: 12

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1. Bayören C. **Komesli O.T.,** Gökçay C.F. *"Determination of phage removal efficiencies of vacuum rotating membrane and UV disinfection unit*" IWA MTWR, Regional Conference and Exhibition on Membrane Technology and Reuse, 18-22 October 2010, İstanbul, Turkey.

2. **Komesli O. T**., Gökçay C. F., "*Operational experience on VRM plant at METU*", 26-28 September 2007, Thessaloniki, Greece.

3. Teschner K., Hegemann W., Gökcay C.F. and **Komesli O. T.,** "*Submerged membranes in wastewater lagoons: a newapproach to wastewater reuse in agriculture, 2nd IWA National Young Water Professionals Conference, Germany"* Membrane Technologies for Wastewater Treatment and Reuse"4-5 June 2007, Berlin, Germany

4. **Komesli O. T.,** Gürkan Z., Gökçay C. F., *"Sludge Characteristics of a Vacuum Rotation Membrane Bioreactor (MBR)"*, 28-30 March 2007 Antalya, Turkey

5. **Komesli O. T.,** Gökçay C. F.*, "Treatment and Resue of Domestic Wastewater by MBR (Membrane Bioreactor): The Energy Consumption of MBR"*, 19-22 October 2006, Phocaia, Turkey

6. Hagemann, Werner; Teschner, Katharina; Gökcay, Celal; **Komesli, Okan;** Bischof, Franz, "*Verbesserung der Ablaufqualität von Abwasserteichanlagen durch den Einsatz von Membranverfahren"*, Aachener Kongress Dezentrale Infrastruktur, .17 und 18. Oktober 2006 im Eurogress, Aachen, Deutschland.

7. **Komesli O. T.,** Gökçay C. F., *"Performance of Full Scale Membrane Bioreactor to Treatment and Reuse of Wastewater",* 21-23 September 2006, Rende, Italy.

8. Teschner K., **Komesli O. T.,** *"Upgrading of Wastewater Lagoons by Membrane Filtration; Productionof High Quality Effluent for Unrestricted Irrigation Losses*", 21 -23 June 2006, Bucharest, Romania.

9. **Komesli O. T.,** Teschner K., Hegeman W., Gökçay C.F., *"Vacuum Mambrane Applications in Domestic Wastewater Reuse"*, 8-10 June 2006, Marrakech, Morocco.

10. **Komesli O. T.,** Gökçay C.F., *"Akdeniz Ülkeleri İçin Sürdürülebilir Evsel Atıksu Arıtımı ve Atıksuların Tarımda Geri Kullanılmı ile İlgili Enstrümanların ve Yöntemlarin Geliştirilmesi",* Workshop, 9-10 June 2005, Ankara, Turkey.

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1 *Muz M., Sönmez M.S., Komesli O.T., Bakırdere S., Gökçay C.F., "*Determination of Selected Natural Hormones and Endocrine Disrupting Compounds in Domestic Wastewater Treatment Plant by LC-ESI-MS/MS after Solid Phase Extraction" *16th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region September 24 to 27, 2011, Ioannina – Greece.*

2 Muz M., Sönmez M.S., **Komesli O.T.,** Bakırdere S., Yıldırım E., Gökçay C.F., "*Treatment of Endocrine Disrupting Compounds in Domestic Wastewater Treatment Processes in Turkey"* USAYS, International Sustainable Water Wastewater Management Symposium, 26-28 October 2010, Konya, Turkey.

3 **Komesli O.T.,** Berberoglu M.,Arıkan M., Muz M., Sönmez M.S., Gökçay C.F., "*Treatement and Reuse of Domestic Wastewater by Membrane Bioreactor: METU Application"* USAYS, International Sustainable Water Wastewater Management Symposium, 26-28 October 2010, Konya, Turkey.

4 Sönmez M.S., Muz M., **Komesli O.T.,** Gökçay C.F., *"A New Patented Sludge Treatment Process: Simultaneous Treatment of Endocrine Disrupting Chemicals During Aerobic Ozone-Pulsed Sludge Stabilization"* USAYS, International Sustainable Water Wastewater Management Symposium, 26-28 October 2010, Konya, Turkey.

5 Sönmez M.S., Muz M., **Komesli O.T.,** Celal Ferdi Gökçay C.F. *"Minimizing MBR Sludges by Pulse Ozonation"* IWA MTWR, Regional Conference and Exhibition on Membrane Technology and Reuse, 18-22 October 2010, İstanbul, Turkey.

6 Yılmaz B., Sandal S., Bulmuş Ö., **Komesli O.T.,** Gökçay C.F. *"Analysis of adsorbable Organic Halogen and Total Organic Carbon Levels in Environmental and Drinking Water Samples in Turkey"*, The 3rd Euro-Asian Conference on Hazardous Waste& Human Health, 27-30 March 2008, İstanbul, Turkey.

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1. Sönmez M.S., Muz M., **Komesli O.T.,** Celal Ferdi Gökçay C.F., *"Kesikli Ozonlama Yöntemiyle Atık Çamur Miktarının Azaltılması",* ACS2009, 04-06 Kasım 2009, İzmir, Türkiye.

2. Codal A., Özkan U.G.Y., **Komesli O.T.,** Gökçay C.F., *"Membran Biyolojik Arıtma Sistemlerinin Modellenmesi",* Memtek 2009, 01-02 Kasım 2009, İstanbul, Türkiye.

National Poster Papers

1. Bayören C., **Komesli O.T.,** Gökçay C.F. "*HPLC-ESI-MS Cihazı ile Atıksularda Endokrin Bozucu Maddelerin Tayini*" 1. Eser Analiz Çalıştayı, 22-25 Nisan 2010, Denizli, Türkiye.

2. Komesli O.T., **Gökçay C.F.,** ODTÜ-VRM *"Membran Biyoreaktör Sisteminin Enerji Kullanım Analizi"*, Memtek 2009, 01-02 Kasım 2009, İstanbul, Türkiye.

3. Bayören C., **Komesli O.T.,** Gökçay C. F., *"Döner Vakumlu Membran Tesisi ve UV Dezenfeksiyonu Üzerinde Faj Uzaklaştırma Verimliliklerinin Belirlenmesi*", Memtek 2009, 01-02 Kasım 2009, İstanbul, Türkiye.

Research Project

1. Researcher, *Upgrading of Wastewater Treatment Plants by the Installation of Membranes for the Separation of Bacteria from the Wastewater*, Federal Ministry of Education and Research (BMBF), Germany. 2004-2007.

2. Researcher, *Use of Membrane Bioreactors in Domestic Wastewater Treatment*, the Turkish Scientific and Technical Research Council (TUBITAK), Project Manager: Prof. Dr. Celal Ferdi GÖKÇAY, Project No: CAYDAG 105Y100, 2005-2007.

3. Researcher, *Vacuum Membrane Application in Turkey*, Federal Ministry of Education and Research (BMBF), 2004-2006.

4. Researcher, *Treatment of Endocrine Disrupting Chemicals (EDSs) in Classical Wastewater Treatment Plants and in Membrane Bioreactor Systems and Development of a Generic Wholesale Bioassay Parameter for their Analysis,* TUBITAK, Project Manager: Prof. Dr. Celal Ferdi GÖKÇAY, Project No: CAYDAG 108Y272, 2009-2011.

Certificate

1- 3rd Summerschool "Innovative technologies for urban wastewater treatment plants" 5-9 July 2010, Santiago de Compostela, Spain.

2- Education Seminar for FISH Applications in Biotechnology, 20-22 June 2007, İstanbul Technical University, Bogazici University, Istanbul, Turkey.