Comparison of EDCs Removal in Full and Pilot Scale Membrane Bioreactor Plants: Effect of flux rate on EDCs removal in short SRT

O.T. Komesli[1*], M. Muz[2], M. S. Ak[3], S. Bakırdere[4], C.F. Gokcay[3]

[1]Ataturk University, Department of Environmental Engineering, 25250 Erzurum, TURKEY

[2]Department Effect-Directed Analysis Helmholtz-Zentrum für Umweltforschung GmbH – UFZ Helmholtz Centre for Environmental Research – UFZ Permoserstraße 15/04318 Leipzig, Germany

[3]Department of Environmental Engineering, Middle East Technical University, 06100, Ankara, TURKEY

[4]Yıldız Technical University, Department of Chemistry, 34349 İstanbul, TURKEY

**Corresponding author email: okan.komesli@gmail.com phone:+ 90 442 231 4822*

ABSTRACT

Membrane bioreactor (MBR) is used for tertiary treatment of wastewater. Sludge Retention Time (SRT) and flux are two important parameters for MBR processes. In this study, the removal of selected endocrine disrupter compounds (EDCs),diltiazem, progesterone, estroneand carbamazepine (Cbz) by one full scale and one pilot scale MBR plantswere investigated.In this study, sludge age was arranged for 10 days and the sludge concentration was not changed about 5 g/L. The transmembrane pressure (TMP) increases either increasing of flux increase or increasing of sludge concentration in the membrane chamber. Therefore, TMP increased by the increasing of flux from 13 to 30 L/m^2-h in both plants. . TMP increases from -25 to -300 mbar in pilot scale and from -160 to over -350 in full scale MBRs. It was understood that flux had very little effect on the removal of EDCs in very low concentrations. Moreover, diltiazem was completely removed in full scale where as no removal was achieved in pilot scale. Estrone and progesterone were completely removed by biodegradation in both plants. Acetaminophen removed completely in full scale plant whereas over95% removal was achieved in Pilot scale MBR. Moreover, when the flux increased removal efficiency decreased.

Keywords: membrane bioreactor, transmembrane pressure, flux, endocrine disrupter compounds, suction time

1. Introduction

Following urbanization and industrialization, there has been over 100000 chemicals produced during 1930s [1]. 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 [1]. After their appearance in the environment during 1950s, observations on the wildlife indicated that population of fish, birds, reptiles and mammals were decreasing [2-4]. 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 (5-6). However, concern over these compounds in waters and wastewaters had not grown until 1990s until realizing their effects on living organisms [5, 7, 8].In 1994 some sexual abnormalities in fish living near wastewater treatment plant outfalls were noticed [9]. 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. Owing to their disrupting effect on the endocrine system, they are called Endocrine Disrupter Compounds (EDCs). 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 [7, 10]. As such, the most pressing issue is to identify the effective treatment methods which can remove these compounds from wastewaters. 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. 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. In addition to these treatment systems, membrane bioreactor systems also investigated to removal of EDCs. 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. 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. The studies on removal of EDCs in WWTPs are summarized in Table 1.

Table 1. Studies on removal of EDCs in WWTPs

Compound	**Location of Study**	**Removal %**	**Type of treatment**	**Reference**
Diltiazem	TanCheon, Korea (FS)	-	CAS	[11]
	JungRang, Korea (FS)	-	CAS	[11]
	NanJi, Korea (FS)	-	CAS	[11]
	SeoNam, Korea (FS)	-	CAS	[11]
Prog.	USA (PS)	99	CAS	[12]
E_1	Kristianstad, Sweden (FS)	78	CAS+ CT	[13]
	U.K.	88	CAS	[14]
	USA	64	CAS	[1]
	WWTPs, Korea	87.1	CAS+BNR+UV	[15]
	USA (PS)	99.9	CAS	[12]
CBZ	Netherlands	9	CAS	[16]
	Austria	-	CAS	[17]
	Terrassa, Spain (FS)	-	CAS	[18]
	Terrassa, Spain (PS)	-	FSh-MBR	[18]
	Terrassa, Spain (PS)	-	HF-MBR	[18]
	Alcala´de Henares, Spain (FS)	9.5	CAS	[19]
	Korea	23.1	CAS+BNR+UV	[15]
	TanCheon, Korea (FS)	50	CAS	[11]
	JungRang, Korea (FS)	50	CAS	[11]
	NanJi, Korea (FS)	-	CAS	[11]
	SeoNam, Korea (FS)	50	CAS	[11]
	Galicia, Spain (PS)	99	SBR+AC+MBR	[20]
	Belgium (LS)	99.9	MBR	[21]

Acet	Spain (FS)	99	CAS	[22]
	Terrassa, Spain (FS)	99	CAS	[18]
	Terrassa, Spain (PS)	99.9	FSh-MBR	[18]
	Terrassa, Spain (PS)	99.9	HF-MBR	[18]
	Alcala´de Henares, Spain (FS)	100	CAS	[19]
	WWTPs, Korea (FS)	99.9	CAS+UV	[15]
	TanCheon, Korea (FS)	99	CAS	[11]
	JungRang, Korea (FS)	99	CAS	[11]
	NanJi, Korea (FS)	99	CAS	[11]
	SeoNam, Korea (FS)	99	CAS	[11]

FS= Full Scale, PS= Pilot Scale, HF= Hollow fiber, FSh= Ftale Sheet, CAS=conventional activated sludge, MBR= membrane bioreactor, UV=ultraviolet, AC=Activated carbon, BNR= biological nutrient removal, CT= chemical treatment, LS=laboratory scale

As it was seen in above table, there were many studies about removal of EDCs in different type of MBRs. However in all these studies, researchers only focused on the removal efficiencies without the effect of flux on the removal of these compounds. In this study, removal of five different EDCs including natural hormones and pharmaceuticals were investigated by one full scale and one pilot scale MBR plant considering the effect of flux in short SRT.

2.Material and Methods

2.1 Chemicals

Analytical reagent grade chemicals used during the study. The selected Endocrine Disrupting Compounds, EDCs, diltiazem (>99%), progesterone (>99%), estrone (>99%) were obtained from Sigma, carbamazepine (>99%) and acetaminophen (>99%) were purchased from Sigma-Aldrich.

HPLC grade methanol, acetonitrile, toluene and acetone were purchased from Merck (Darmstadt, Germany). 0.7 µm pore size, 47 mm diameter glass-fiber pre-filters used for get rid of impurities were obtained from PAL Life Sciences (Mexico).Milli-Q water purification system (Millipore, USA) was used for getting ultrapure de-ionized water used in all dilutions and sample preparations. Ammonia (Merck) and formic acid (Merck) were used for the preparation of mobile phase. The main stock solutions of analytes were prepared in methanol as 1000 mg/L in concentration and stored at 4 °C in refrigerator. In the preparation of working standard solutions, Milli-Q water was used to dilute the stock solutions into working standards just prior to experimentation.LC-ESI-MS/MS used for determined of EDCs were optimized and optimization studies and extraction recoveries were given in the other studies [23-25].

2.2. 2.2. Laboratory analysis

All parameters were analyzed in parallel. Analytic methods were performed according to Standards Methods [26]. The COD, ammonium nitrogen, and nitrate were measured using HachDr 2000 Model Spectrophotometer. The Hach COD reagent (Cat No.21259-51) for COD, Ammonia Salicylate (Cat No.23953-66) reagent powders and Ammonia Cyanurate (Cat No.23955-66) for ammonium nitrogen and The HachNitriver reagent (Cat No.14065-99) and Hachnitraver reagent (Cat No.14119-99) for nitrate measurements were used. Dissolved oxygen was determined by JumodTrans O_2-01 model DO-meter.

2.3. Description of MBR Systems

During the study, one full scale MBR plant described as vacuum rotating membrane, VRM, capacity was from 100 to 200 m^3/d,and one pilot scale MBR plant, capacity was from 600 to 1500 L/dwas used to understand the effect of flux in short SRT on the removal of EDCs.The full scale VRM plant unit was composed to two tanks; first one in sequence of operation being the aeration tank used for biological treatment and the second one is the so called filter chamber, where the rotating membrane filter is housed (Figure 1).After filtration by membranes, treated wastewater passed through from UV.

Figure 1 The Full-Scale MBR Unit at METU Campus, Turkey.

The pilot MBR plant was near to the full scale plant and the same real wastewater after 3 mm fine screen used during the study. After fine screen, there was a storage tank about 350 L and wastewater was transferred from storage tank to MBR plant by submerged pump (Figure 2).

Figure 2 Clear-Box MBR Plant

Properties of two treatment facilities were given in Table 2.

Table 2. Properties of the MBR Plants

	Clear-Box Pilot Plant	VRM Plant
Storage tank volume (m^3)	0,35	10
Aeration Tank Volume (m^3)	0,75	85
MBR tank volume (m^3)	0,75	23
Membrane Type	plate and frame	plate and frame
Total Membrane Area (m^2)	3	540
Membrane Material	polyethersulfones (PES)	PES
Nominal Pore Size (µm)	0.038	0.038
Sludge Retention Time (days)	10	10
Flux (L/h-m^2)	13.3-26	13.3-30

Results and Discussion

Steady-State Conditions of the Plants

The removal of selected EDCs in short SRT was investigated during the study. At the beginning of the study, the SRT was arranged as 10 days which was very short compared to the classic arranged SRTs for MBR plant operations[20, 27]. The aim of arrangement of short SRT was to refreshed activated sludge and overcome the sludge aging. At the beginning of the study, activated sludge in the Clear-Box MBR plant was inoculated from activated sludge in VRM unit. About 30 days was waited for the steady-state conditions of both plants.After 30 days of the operations, the MLSS concentration in both plants was about 5 g/L and it was under steady-state. The oxygen concentration in both plants was between 1 and 2 mg/L during the study. The COD removal in both plants was over 95% during the steady state period.

After steady-state conditions were reached, fluxes were arranged from 13,3 to 30 L/m^2-h in both plants. The TMP was increased from -25 to -300 mbar in Clear-box plant and from -165 to -350 (and over -350 when flux 30 L/m^2-h)in VRM plant. When TMP exceeded -350 mbar, extra-flushing period started and suction was stopped. As a result, flux was arranged from 13,3 to 26 L/m^2-h in VRM plant. The TMP changed with flux were given in Figure 3.

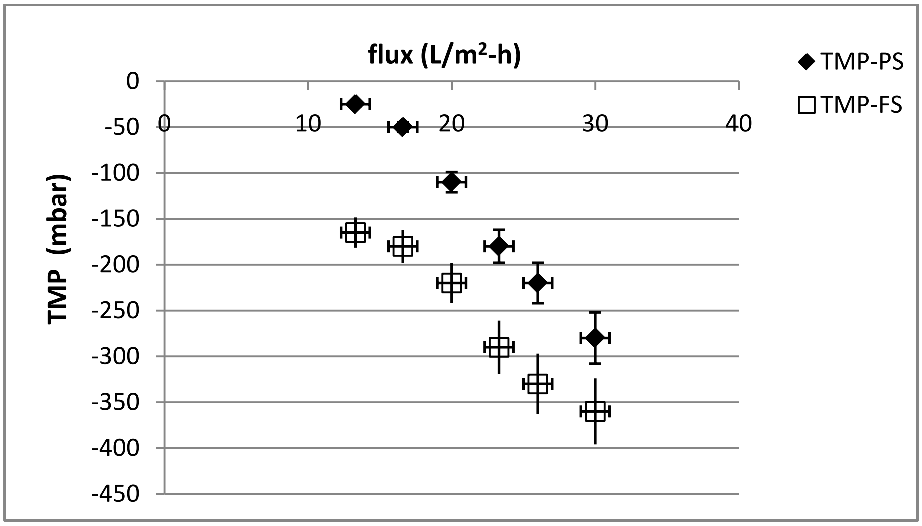

Figure 3 COD concentration in the influent, supernatant and permeate of both plants.

As it was seen in above figure, TMP was increased from -25 to -300 mbar in pilot scale MBR and from -165 to over -350 mbar in full scale MBR with increased fluxes. Composite samples, at 4 ^{o}C, were collected from influent, VRM effluent (before and after UV disinfection point) and Clear-Box effluent. In order to see the effect of membrane filtration with different fluxes, grab sample from the aeration tank were also taken.Grab sample indicated the conventional activated sludge systems.In addition to these samples, sludge samples were taken to understand the removal mechanisms of the plants. Samples were immediately transferred to the laboratory for analysis. In order to see the effect on COD removal, COD efficiency in all fluxes was investigated and given in Figure 4.

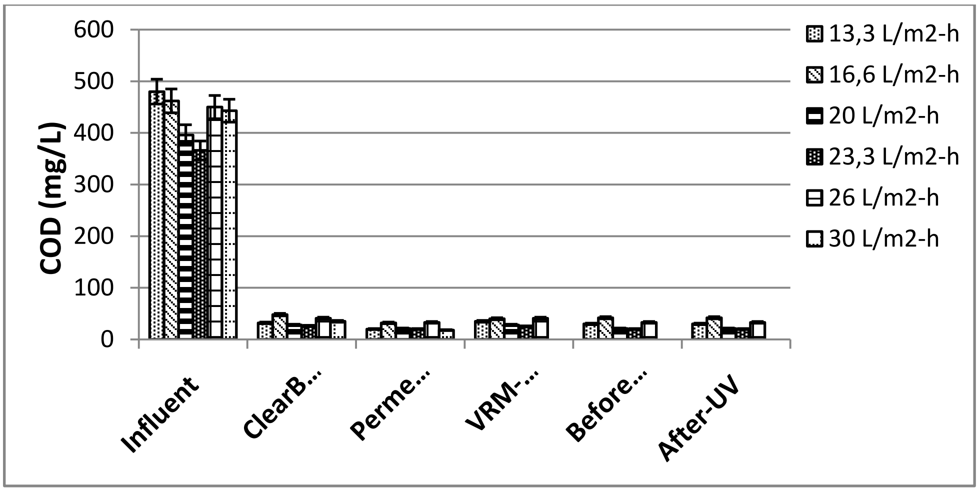

Figure 4 COD concentration in the influent, supernatant and permeate of both plants.

As it was clearly understood from the above figure, flux had no effect on the removal of COD. Not only in supernatant of both systems but also permeate of the membrane had over 95% removal of COD.

In order to see the effect of flux and comparison of full and pilot scale MBR plants on the removal of selected EDCs composite samples were directly taken from the wastewaters and analyzed by the developed methods. During the study, the first compound was diltiazem used for the treatment of hypertension and preventive medication for migraine. The diltiazem concentration in all samples was given in Figure 5.

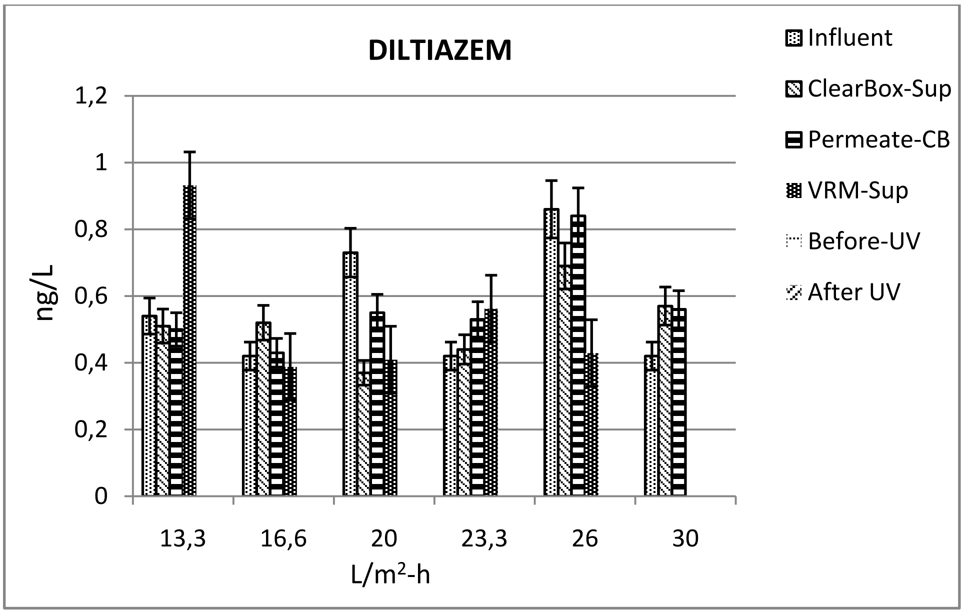

Figure 5. Diltiazem concentrations in all samples with different fluxesin both plants

The influent concentration of the diltiazem was between 0.4 and 0.85 ng/L.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. At fluxes between 13 and 16 L/m^2-h the influent, supernatant and membrane permeate concentrations of pilot scale MBR were almost the same. However, when the flux was 23 and 26 L/m^2-h, the diltiazem concentration in permeate of pilot scale MBR 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. Moreover, diltiazem concentration in sludge samples was under limit of detection.

Progesterone was the second compound investigated during the study and the results were given in Figure 6.

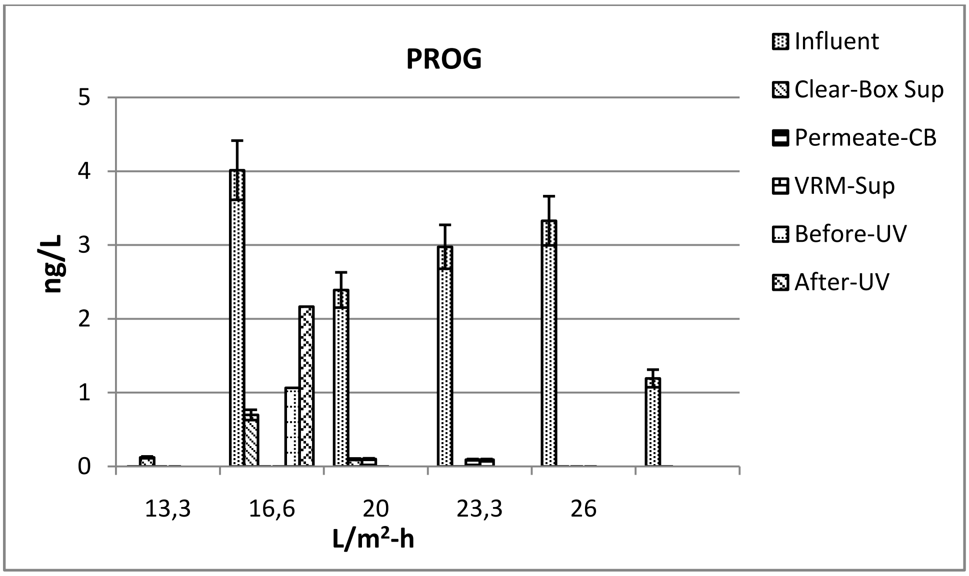

Figure 6. Progesterone concentrations in all samples with different fluxes in both plants

Since this is a natural hormone excreted by the pregnant women, the concentration was very low between 0.29 and 4.01 ng/L as seen in Figure6. Concentration of progesterone was under the limit of detection in supernatants and permeates of both plants. Observations at flux 16,6 L/m^2-h is obviously erroneous due to trace level analysis. Absence of any detectable amount in sludge suggested complete biodegradation. This results clearly show that progesterone was removed biologically and no noticeable effect of permeate flux exists.

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. The estrone removal in full and pilot scale MBR plants at differing membrane fluxes is given in Figure 7.

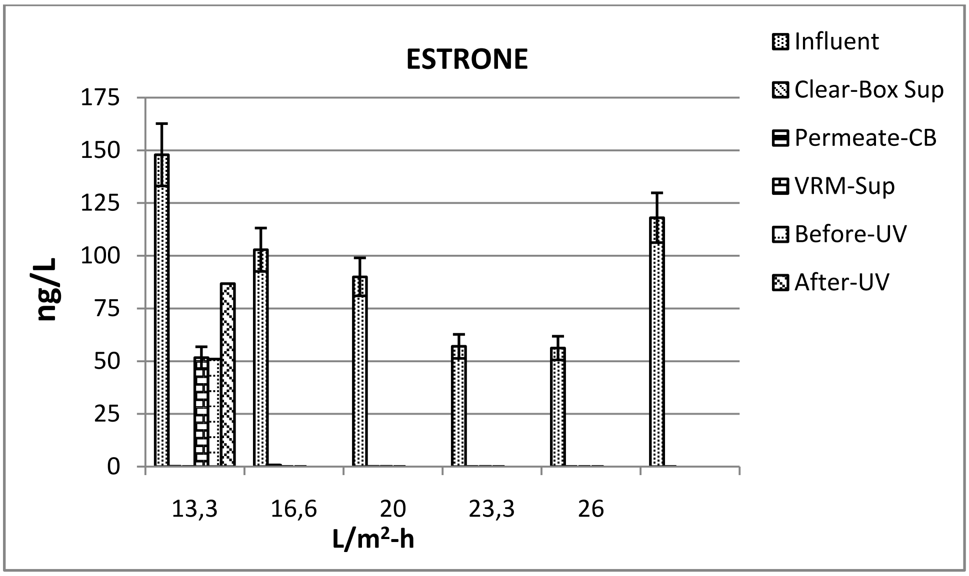

Figure 7. Estrone concentrations in all samples with different fluxes in both plants

The influent estrone concentration was between 52 and 150 ng/L. Estrone concentration was under limit of detection in supernatants and permeates of both systems in all fluxes except for when flux was 13,3 L/m^2-h due to the erroneous measurements. Since the log k_d value for estrone is low, e.i. 2.4-2.9 [28] it was not deposited in the sludge. Moreover, k_{biol} of estrone is between 200 and 300 (L g^{-1} SS day^{-1}) [28] all the estrone was biodegraded in the system.Estrone could not be detected in sludge samples.

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 all samples was given in Figure 8.

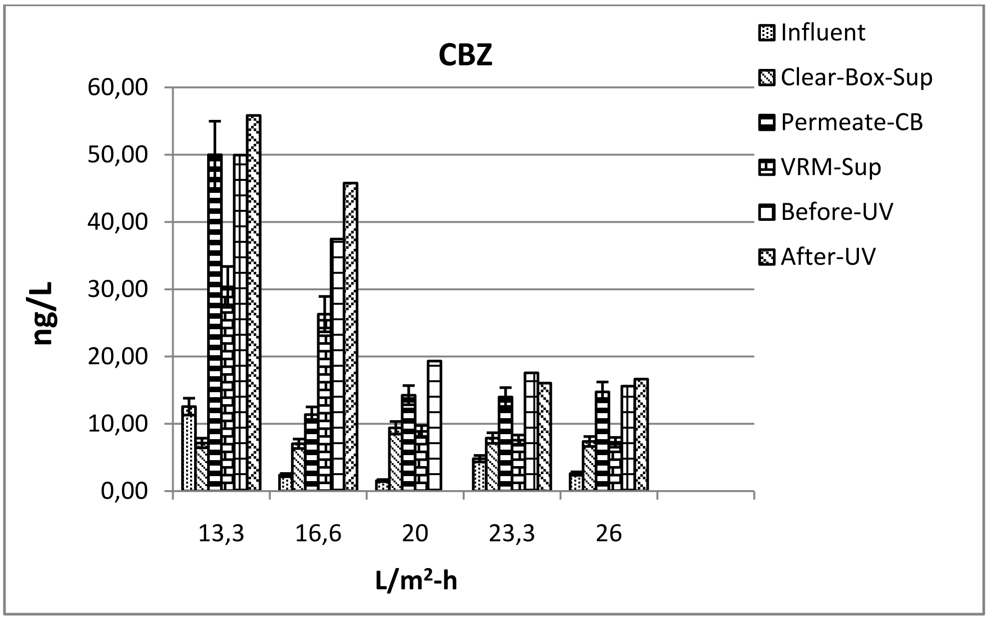

Figure 7.CBZ concentrations in all samples with different fluxes in both plants

As it was seen in Figure 8, the influent concentration of CBZ was between 2 and 12 ng/L. It is evident from Figure 8 that this compound was concentrated on the membrane and permeate effluent concentrations were higher than the supernatants. This finding was consistent with the with VRM results. 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. Since the log k_d and k_{biol} for CBZ are both very low [28], meaning that this compound is neither biodegradable nor removed by sorption onto the sludge. For that reason, it could not be detected in sludge. 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 was given in Figure 8.

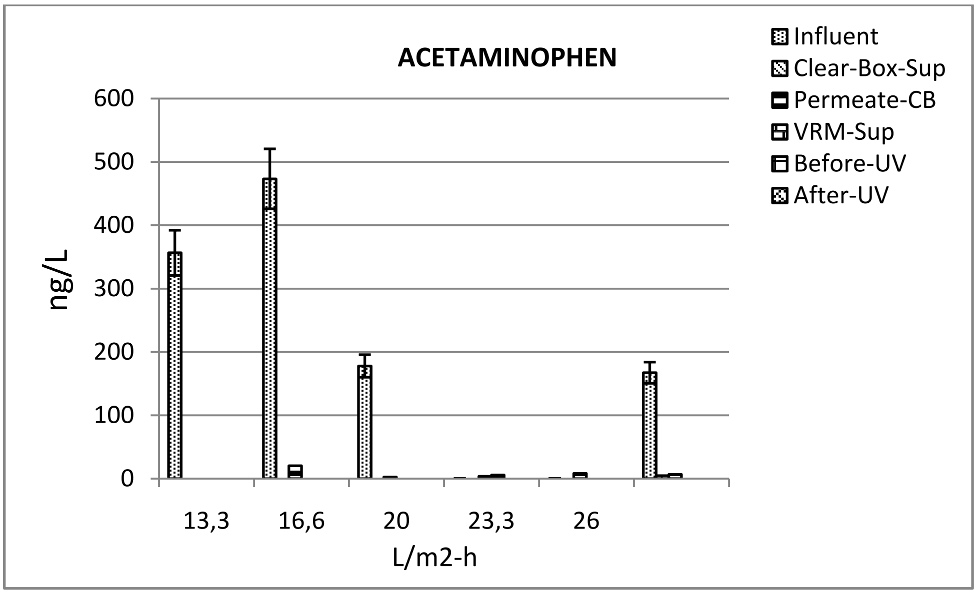

Figure 8 Acetaminophen concentrations in all samples with different fluxes in both plants

As seen in Figure 8, Acetaminophen concentration in the wastewater influent was between 167–480 ng/L. Although, biodegradation and sludge-water partition coefficients are quite low for this compound, 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 of pilot scale MBR. 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. In full scale application, Acetaminophen was completely removed.

Conclusion

During the study, removal of five different EDCs in full and pilot scale MBR plants with different fluxes was investigated. The real wastewater was used and there was not any spiked of these compounds. It was concluded from these study that it was not any important effects of flux or TMP on the removal of EDCs in short SRTs. It was also understood from the study removal of EDCs in full scale MBR applications was higher than removal of these compounds in pilot scale applications.Estrone, progesterone and acetaminophen were mostly removed by biodegradation since concentrations of these compounds were mostly under limit of detection in supernatants of the activated sludge inside the reactors. Diltiazem was completely removed in full scale but there was not any removal in pilot scale applications. In addition, although acetaminophen was completely removed in full scale MBR, over 85% removal was achieved in pilot scale. This was explained by the occurring of vibration on the membrane surface in full scale applications. CBZ was not removed in both plants.

Acknowledgement

This research was funded by TUBITAK project no 1008Y272. We thankMETU-Technopolis for their contributions.

References

References

[1] Snyder S.A., Westerhoff P., Yoon Y., SedlacD.L.,Pharmaceuticals, Personal Care Products, and Endocrine Disruptors in Water: Implications for the Water Industry,*Environmental Engineering Science*, 20-5 (2003)

[2] Bowden J., Novel analytical strategies for the characterization of streods and endocrine disrupting compounds (EDCs) in biological and environmental samples, PhD Thesis, University of Florida, USA, 2009.

[3] Colborn T., Dumanoski D., Myers J.P., Our Stolen Future, Dutton: New York, NY, 1996, p306.

[4] Fossi C., Marsili L., Effects of the endocrine disrupters in aquatic mammals, *Pure Applied Chemistry,* 75 (11-12), 2235-2247 (2003)

[5] Snyder S.A. and Benotti M.J., 2010, Endocrine disruptors and pharmaceuticals: implications for water sustainability, *Water Science and Technology*, 61(1), 145-154 (2010)

[6] Stumm-Zollinger, E. and Fair, G. M., Biodegradation of steroid hormones. *J. Water Pollut. Control Fed. 37*, 1506–1510 (1965)

[7] Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and, Waldock, M., Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. *Environ. Sci. Technol.* 32(11), 1549–1558 (1998)

[8] Routledge, E. J., Sheahan, D., Desbrow, C., Brighty, G. C., Waldock, M. &Sumpter, J. P., Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. *Environ. Sci. Technol.* 32(11), 1559–1565 (1998)

[9] Purdom C.E., Hardiman P.A., Eno N.C., Tyler C.R., Sumpter J.P., Estrogen effects of effluents from sewage treatment works, *Chemical Ecology*, Vol. 8, 75 (1994)

[10] Song H.L., Nakano K., Taniguchi T., Nomura M., Nishimura O., Estrogen removal from treated municipal effluent in small-scale constructed wetland with different depth, Bioresource Technology 100, 2945–2951 (2009)

[11] Choi K., Kim Y., Park J., Park C.K., Kim M.Y, Kim H.S., Kim P., Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea, Science of the Total Environment, 405, 120-128 (2008)

[12] Esperanza M., Suidan M.T., Marfil-Vega R., Gonzalez C., Sorial G.A., McCauley P., Brenner R., Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment Chemosphere, 66, 1535-1544 (2007)

[13] Zorita S., Mårtensson L., Mathiasson L., Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden, Science of the Total Environment, 407, 2760-2770 (2009)

[14] Ternes T.A., Stumpf M., Mueller J., Haberer K., Wiken R. D., Servos M., Behavior and occurrence of estrogens in municipal sewage treatment plants- Investigations in Germany, Canada and Brazil, *Science of the Total Environment*, 225 (1-2), 81-90 (1999)

[15] Behera S. K., Kim H.W, Oh J. E., Park H.S., Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea.Sci Total Environ., 409 (20) 4351-4360 ()

[16] Heberer T., Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data, Toxicology Letters, 131 (1-2), 5-17 (2002)

[17] Clara M., Strenn B. , Gans O., Martinez E., Kreuzinger N., Kroiss H., Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants, Water Research, 39, 4797–4807 (2005)

[18] Radjenovic, J., Petrovic, M., Barcelo, D., Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Research 43 (3), 831-841 (2009)

[19] Rosal R., Rodrı´guez A., Perdigo´n-Melo´n J. A., Petre A., Garcı´a-Calvo E., Go´mez M., Agu¨ era A., Ferna´ndez-Alba A. R., Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation, Water Research, 44 (2), 578-588 (2010)

[20] Serrano D., Suarez S., Lema J.M., Omil F., Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in a sequential membrane bioreactor, Water Research, 45, 5323-5333 (2011)

[21] Gusseme B. D., Vanhaecke L., Verstraete W., Boon N., Degradation of acetaminophen by Delftiatsuruhatensis and Pseudomonas aeruginosa in a membrane bioreactor, *Water Research*, 45. 1829-183 (2011)

[22] Gomez M.J., Martinez Bueno M.J., Lacorte S., Fernandez-Alba A.R., Agüera A., Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast, Chemosphere 66, 993–1002 (2007)

[23] 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, 184(8), 5215-24 (2012)

[24] 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 ExtractionAnalyst, 137 (4) 884-889 (2012)

[25] M.S. Sönmez, M. Muz, O.T. Komesli, S. Bakırdere, C.F. Gökçay, Ultra Trace Determination of Selected Endocrine Disrupter Compounds in Sludge Samples using HPLC-ESI-MS/MS after Ultrasound-Aided Sequential Extraction, Clean, (9) 980–985 (2012)

[26] Standard Methods for the Examination of Water and Wastewater, twentieth ed., APHA American Public Health Association, Washington, DC, 2001.

[27] Komesli O.T., Tescher K., Hegemann W., Gokcay C.F., 2007. Vacuum membrane applications in domestic wastewater reuse, *Desalination*, 215 22–28 (2007)

[28] Suarez S., Carballa M. Omil F., Lema J. M., How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters?, Rev Environ SciBiotechnol, 7, 125–138 (2008)