INVESTIGATION OF ENDOCRINE DISRUPTING COMPOUNDS IN MEMBRANE BIOREACTOR AND UV PROCESSES

By

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ABSTRACT

Endocrine disrupting compounds (EDCs) in the environment have recently emerged as a major issue in Canada and around the globe. The primary objective of this thesis was to investigate the fate of EDCs in two wastewater treatment processes, membrane bioreactors (MBRs) and ultraviolet (UV) disinfection. Two submerged MBR systems using hollow fiber membranes from two membrane manufacturers were tested. The results from a bench-scale and a pilot scale MBR for the treatment of swine wastewater with high concentration of EDCs showed that over 94% of the estrogenic activity (EA) in the influent was reduced through the MBR process. Biological degradation was the dominant removal mechanism for the removal of EDCs in MBRs. Over 85% of the influent EA was reduced by biodegradation through the MBR process. The other MBR system was built to study the removal mechanisms of two estrogens in a hybrid MBR with the addition of powdered activated carbon (PAC). The effects of PAC dosing on MBR overall performance was studied as well. It was found that PAC dosing could increase the removal rates of 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) by 3.4% and 15.8%, respectively and result in a slower rate of trans-membrane pressure (TMP) increase during MBR operation, which could significantly reduce the operating cost for membrane cleaning and/or replacement. The operating cost for PAC dosing could be offset by the benefit achieved from reducing the cost for membrane maintenance. The slower rate of TMP increase in the PAC-MBR was associated with the lower concentrations of soluble extracellular polymeric substances and colloidal organic compounds in the PAC-MBR sludge.

The degradation kinetics of three estrogens, estrone (E1), E2, and EE2 in de-ionized water by UV irradiation was studied. The experimental results showed both the apparent concentrations and overall EA of all three investigated estrogens in water decreased with direct UV irradiation. To further study the impact of UV on the overall EA of wastewater, the EA of pre-UV and post-UV samples from five wastewater treatment plants were measured in both liquid and solid phase by Yeast Estrogen Screen assay. It was found that the EA of wastewater decreased after UV disinfection in three of the investigated plants whereas it increased in the other two plants. This observation needs to be further studied because it might have significant impacts on the application of UV systems for wastewater disinfection.

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

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ABBREVIATIONS AND NOMENCLATURE

AC   Activated carbon

AOB   ammonia oxidizing bacteria

AOP   advanced oxidation process

APEs   alkylphenol polyethoxyates

AS   activated sludge

ASP   activated sludge process

BOD   biological oxygen demand

BPA   bisphenol A

COD   chemical oxygen demand

CPRG   chlorophenol red β-D-galactopyranoside

DO   dissolved oxygen

E1   estrone

E2   17β-estradiol

E3   estriol

E2-Eq   17β-estradiol equivalence

EA   estrogenic activity

EDCs   endocrine disrupting compounds or chemicals

EE2   17α-ethinylestradiol

EPS   extracellular polymeric substances

GAC   granular activated carbon

GC	gas chromatography
Hc	Henry's law constant
hER	human estrogen receptor
HPLC	high-performance liquid chromatography
HRT	hydraulic retention time
k	UV photodegradation rate constant
k_{bio}	biodegradation constant
k_D	adsorption coefficient
LOQ	limits of quantification
MBR	membrane bioreactor
MLD	million liter per day
MF	microfiltration
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
MS	mass spectrometry
MWCO	molecular weight cut-off
NF	nanofiltration
NP	nonylphenol
NH_3-N	ammomia nitrogen
ONPG	o-nitrophenyl-β-galactosidase
OP	octylphenol
ORP	oxidation and reduction position

PAC	powdered activated carbon
RO	reverse osmosis
SBR	sequencing batch reactor
SPE	solid phase extraction
SRT	solids retention time
TCU	true color unit
TMP	trans-membrane pressure
TOC	total organic carbon
TSS	total suspended solids
UASB	anaerobic sludge blanket
UF	ultrafiltration
UV	ultraviolet
VSS	volatile suspended solids
WAS	waste activated sludge
WWTPs	wastewater treatment plants
YES	yeast estrogen screen

CHAPTER 1: INTRODUCTION

1.1 Background and Research Needs

1.1.1 Concerns of endocrine disrupting compounds in the environment

Endocrine disrupting compounds or chemicals (EDCs) generally refer to chemical substances with the capacity to disrupt the endocrine system of animals. A variety of such chemicals present in the environment could have serious long-term impacts on the health of wildlife and humans. Despite controversial opinions on the impact of EDCs on human beings and overall ecology, the adverse effects of EDCs on aquatic species such as fish have been well documented. The negative impacts include the feminization due to hormonal imbalance and reduced reproductive success in male fish and avian (Jobling et al., 1998; Kidd et al., 2007; Rodgers-Gray et al., 2000). Some researchers have attributed the decreases in human sperm quality and quantity over the past five decades to EDCs in the environment (Carlsen et al., 1995). Likewise, it has been suggested that sharp increases in breast, testicular, and prostate cancers reported over the past 40 years were related to EDCs in the environment (USEPA, 1997). However, other scientists believe that it is unlikely that these effects would be caused by EDCs in water due to their extremely low concentrations and small doses compared to estrogenic compounds present in food sources (Snyder et al., 2003).

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Since the mid-1990s, the occurrence and behavior of EDCs in the environment, especially in wastewater treatment plants (WWTPs), have gained considerable attention worldwide (Baronti et al., 2000; Esperanza et al., 2007; Fernandez et al., 2007; Holbrook et al., 2002; Khanal et al., 2006; Liu et al., 2009; Servvos et al., 2005). It has been widely accepted that the most likely source of EDCs in aquatic environments is from the discharge of municipal and industrial effluents along with runoff from agricultural production (de Alda and Barcelo, 2001; Hewitt and Servos, 2001; Layton et al., 2000). Some scientists concluded that discharge from WWTPs is one of the largest sources of EDCs in the aquatic environment (Chambers et al., 1997; Nakada et al., 2006; Servos et al., 2005; Tan et al., 2007). Whereas, other researchers considered that estrogens in the aquatic environment come primarily from untreated (or not biologically treated) wastewater rather than from WWTPs effluents (Baronti et al., 2000). Most of the previous studies focused on investigating the occurrence or removal efficiencies of EDCs in WWPTs. However, definitive understanding of the fate and removal mechanisms of EDCs in wastewater treatment unit operations has not been established. Efforts are now ongoing to comprehensively understand the fate of EDCs in wastewater treatment processes and to develop effective ways to remove them to environmentally acceptable levels (Lee et al., 2003b).

1.1.2 Research need on EDCs in membrane bioreactor

The membrane bioreactor (MBR) is an emerging technology, which couples biological degradation with membrane filtration replacing the secondary clarifier in the conventional activated sludge process. Since research on MBR technology began over

30 years ago, several generations of MBR systems have evolved (Crawford et. al., 2002). Up to this date, MBR systems have mostly been used to treat industrial wastewater, domestic wastewater and specific municipal wastewater, where a small footprint, water reuse, or stringent discharge standards were required. It is expected, however, that MBR systems will increase in capacity and broaden in application area in the future due to more stringent regulations and water reuse initiatives (Cicek, 2003; Visvanathan et al., 2000; Yang et al., 2006). As shown in Figure 1.1, an increasing number of studies on MBR are being conducted all over the world.
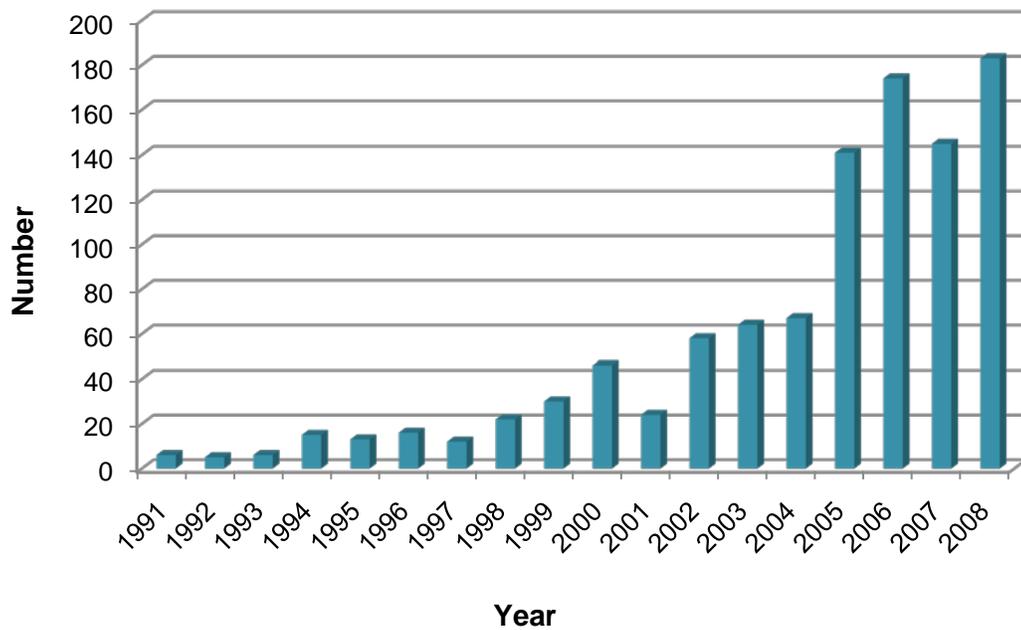
Figure 1.1 Chronological distributions of peer-reviewed articles involving studies on MBR

MBR systems are characterized by two configurations: submerged (immersed or integrated) MBRs and external (recirculated or side-stream) MBRs. Yamamoto et al. (1989) were the first to introduce submerged membranes in an aeration tank for solid/liquid separation. Prior to this, researchers concentrated on membrane separation

in external circuits. Due to the absence of a high-flow recirculation pump, submerged MBRs consume much lower power than external MBRs. This was the primary driver for propelling submerged MBRs into the purview of large-scale wastewater treatment plants in a few dozen countries around the world. External MBRs were considered to be more suitable for wastewater streams characterized by high temperature, high organic strength, extreme pH, high toxicity, and low filterability. Most Studies on the treatment of municipal wastewater with MBRs utilized the submerged configuration.

Full-scale commercial applications of MBR technology in North America for treatment of industrial wastewaters date back to 1991 (Sutton et al., 2002; Sutton, 2003). In the early 1990s, MBR installations were mostly constructed in external configuration, in which case the membrane modules were outside the bioreactor and biomass was re-circulated through a filtration loop. This limited wider application of MBRs in the treatment of municipal wastewater in North America because of high power consumption. After the mid-1990s, with the development of a submerged MBR system, MBR applications in municipal wastewater extended widely.

During the past two decades, MBRs have been proven quite effective in removing both organic and inorganic pollutants as well as biological entities from water or wastewater streams (Cicek et al., 1998; Cicek, 2003; Howell, 2004; Yang et al., 2006). Due to their advantages such as high sludge age, high biomass concentration and complete particle retention, MBRs are expected to achieve enhanced performance in removing EDCs. With the expansion of MBR installations for wastewater treatment and water reuse,

there needs to be an assessment as to whether MBR-based processes are able to improve

EDCs removal from wastewater.

1.1.3 Research need on EDCs in UV process

UV light is electromagnetic radiation with a wavelength shorter than that of visible light,

but longer than soft X-rays. When considering the effect of UV radiation on human

health and the environment, the range of UV wavelengths is often subdivided into UVA

(400–320 nm), also called long wave or "black light"; UVB (320–280 nm), also called

medium wave; and UVC (< 280 nm), also called short wave (Metcalf & Eddy, 2003).

The germicidal portion of the UV irradiation wavelength is between 220 and 320 nm.

The 254 nm wavelength produces the strongest effect in controlling microorganisms

and is called the germicidal wavelength.

UV radiation as a disinfecting technique has been proven in multiple industrial

applications, including drinking water disinfection and wastewater treatment. The

effectiveness of this technology is directly related to the amount of UV radiation

received by the target organisms. In general, UV light in the germicidal range

inactivates microorganisms by causing photochemical damage to their genetic material

(i.e., DNA or RNA). When the genetic material of a cell is damaged, it is unable to

reproduce and is then inactivated.

UV technology has been available since the early 1920s but has only been applied

commercially for full-scale wastewater applications since 1980. During the past decades,

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UV irradiation has been proven to be a viable and economical option for disinfection of secondary wastewater effluents (Zhou and Smith, 2001). One of the advantages of UV disinfection is that it does not need any chemical addition, therefore not contributing to the formation of toxic by-products (USEPA, 1999). As shown in Figure 1.2, the application of UV disinfection for wastewater treatment has been expanding dramatically in the USA in the past decades.
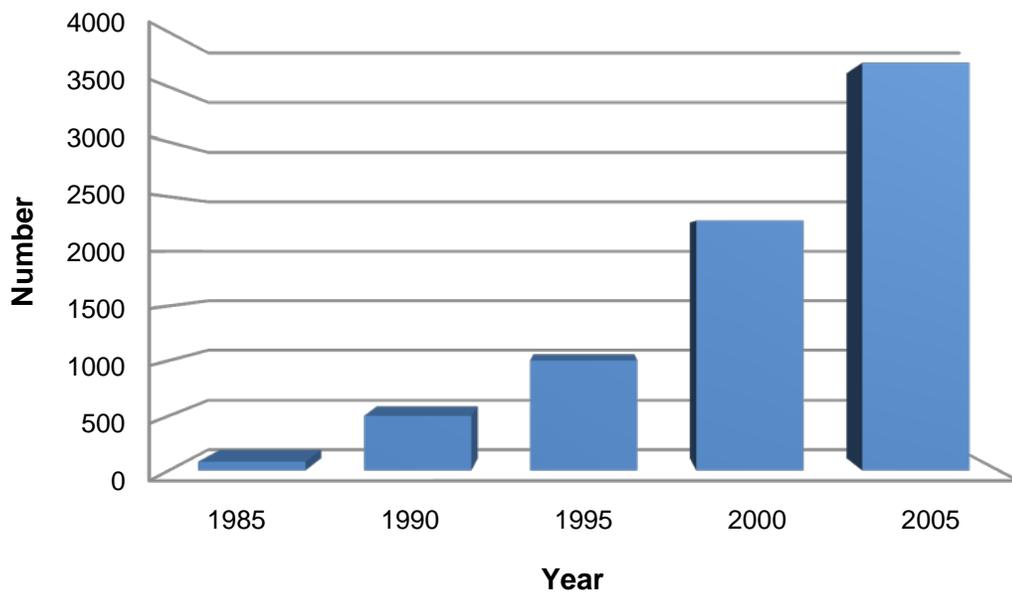
Figure 1.2 Number of existing WWTPs with UV disinfection in USA (WEF/ USEPA)

In recent years, a number of researchers have investigated the degradation of estrogens by direct UV irradiation or UV-based advanced oxidation processes (Benotti et al., 2009; Chen et al., 2006; Feng et al., 2005; Liu and Liu, 2004; Nomiyama et al., 2007; Ohko et al., 2002; Rosenfeldt and Linden, 2004). However, the fate of specific estrogens and overall estrogenic activity in the UV irradiation process has not been well documented.

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Since UV has been more widely used as an option for the disinfection of drinking water and wastewater, further research on the fate of EDCs in UV-based processes is required.

1.2 Objectives of the Thesis

A recent study by Cicek et al. (2007) showed that the effluents from a Canadian full-scale municipal WWTP contained similar amounts of steroid estrogens in soluble and particulate phases. Because membrane filtration in MBRs can retain all particulate substances, it was hypothesized that MBRs could enhance the removal of estrogens in wastewater by retaining estrogens associated with particulate phase. In the study of Cicek et al. (2007), it was also found that the UV process appeared to result in a slight increase of soluble phase estrogens. The authors concluded that uncertainties remain with regards to the exact impact of UV treatment on estrogens in WWTPs. As UV irradiation has been reported to be able to reduce the concentrations of estrogens in water (Chen et al., 2006; Liu and Liu, 2004), it was hypothesized that the impacts of UV irradiation on EDCs in water and in wastewater might be different. The research in this Ph.D. thesis was built on these hypotheses and focused on studying the fate of EDCs in MBR and UV processes.

At the beginning of the project, UV was proposed to be a post-treatment process following the MBR process. With the research proceeding, it was found that the impact of UV irradiation on the EDCs in wastewater was affected by specific field conditions. The feasibility of UV as a post-treatment to further remove EDCs from wastewater was

in doubt. Therefore, the proposal was modified to investigate the fate of EDCs in MBR and UV processes separately. The specific objectives of this Ph.D. thesis were:

Objective (1): To evaluate technical feasibility of the treatment of swine wastewater by MBRs with consideration of EDCs removal;

Objective (2): To assess the feasibility of powdered activated carbon (PAC) dosing in MBRs to improve MBR performance and EDCs removal, and to evaluate the cost effectiveness of PAC dosing in MBRs;

Objective (3): To evaluate feasibility of UV irradiation for EDCs removal in water by kinetics study;

Objective (4): To assess the impacts of UV disinfection on the fate of EDCs in wastewater treatment plants.

1.3 Literature Review

1.3.1 EDCs in the environment

A large variety of natural and synthetic compounds have been identified the potential to be endocrine disrupting. The United States Environmental Protection Agency (USEPA) has proposed a definition of an EDC 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." (USEPA, 1997). Many EDCs, or potential EDCs, were

classified as organic micropollutants such as steroid hormones, surfactants, pesticides, herbicides, pharmaceuticals, polyaromatic compounds etc. However, some inorganic substances, such as metals and nitrate, have also been reported to have potential to exhibit endocrine disrupting activity (Birkett and Lester, 2003; Guillette and Edwards, 2005; Hansen et al., 2009).

The EDCs of particular concern for wastewater treatment can be primarily categorized into one of two groups: steroid estrogens and alkylphenol polyethoxyates (APEs) and their associated metabolites (Holbrook et al., 2002; Lee et al., 2008). Steroid estrogens include natural estrogens (estrone E1, 17β-estradiol E2 and estriol E3) and the artificial estrogen 17α-ethinylestradiol (EE2) which is one of the main components of birth control pills. The primary chemicals in the APE group include nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA) (Holbrook et al., 2002; Ying and Kookana, 2003). The properties and structure of the six selected EDCs, i.e. E1, E2, EE2, NP, OP and BPA, are presented in Table 1.1 and Figure 1.3 (Birkett and Lester, 2003; Hanselman et al., 2003; Murk et al., 2002; Ying and Kookana, 2003). Although estrogens are present in very low concentrations in wastewater streams (at ng/L range), they are responsible for a significant part of the endocrine-disrupting effects observed in the aquatic environment due to their high endocrine-disrupting potency.

Table 1.1 Properties of the selected EDCs

Chemical name	Formula	MW [a]	Sw [b] mg/L)	Log Kow[c]	EEF[d]
estrone (E1)	$C_{18}H_{22}O_2$	270.4	0.8-12.4	3.1-3.4	0.01
17β-estradiol (E2)	$C_{18}H_{24}O_2$	272.4	3.9-13.3	3.1-4.0	1
17α-ethinylestradiol (EE2)	$C_{20}H_{24}O_2$	296.4	4.8	4.15	1.2
nonylphenol (NP)	$C_{15}H_{24}O$	220.4	1.57	5.76	5.7×10^{-4}
octylphenol (OP)	$C_{14}H_{22}O$	206.3	12.6	4.12	$<1.0 \times 10^{-5}$
bisphenol A (BPA)	$C_{15}H_{16}O_2$	228.3	120	3.32	1.0×10^{-5}

[a]MW - Molecular weight; [b]Sw - Water solubility at 20 ℃; [c]Kow - Octanol-water partition coefficient
[d]EEF – Estradiol equivalency factors (estrogenic potency relative to estradiol)
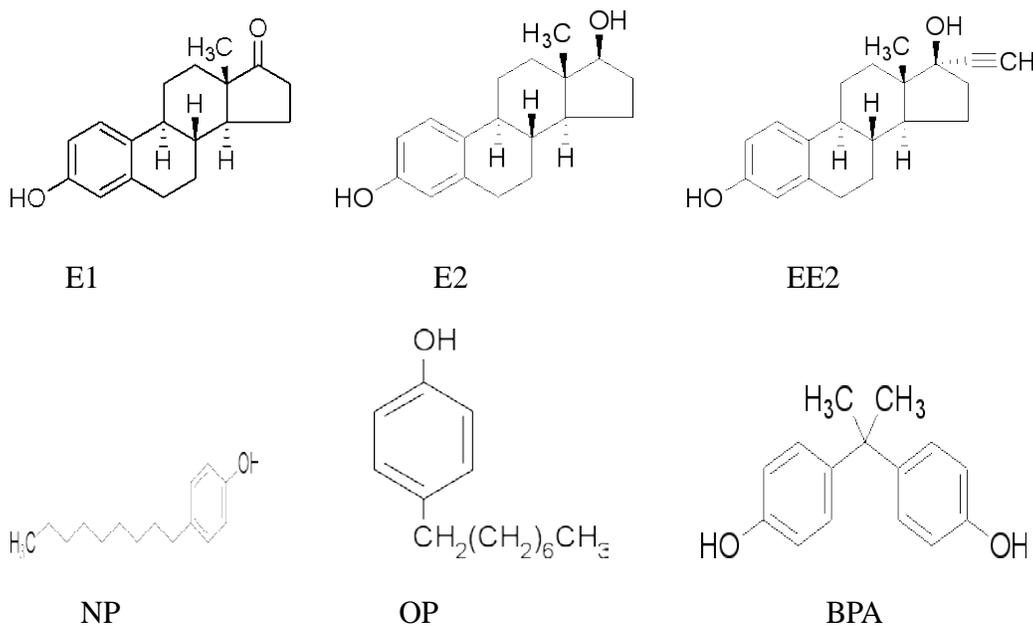
Figure 1.3 Structure of the selected EDCs (Birkett and Lester, 2003)

1.3.2 Removal of EDCs in wastewater treatment processes

Since the discharge from WWTPs was reported to be one of the major sources of EDCs in the aquatic environment, many researchers have studied the occurrence and behavior of EDCs in WWTPs in the past decades. Conventional biological treatment processes, such as the activated sludge process (ASP), have been reported effective in reducing levels of some EDCs in wastewater and sewage. Most studies on EDCs have been done in WWTPs that utilize the activated sludge processes.

Previous studies on the occurrence and fate of EDCs in WWTPs have showed a wide range of removal efficiencies. Most of the EDCs were not completely removed by exiting WWTPs and they remained with fluctuating concentrations in effluents (Liu et al., 2009). Fernandez et al. (2007) measured the concentrations of 30 estrogenic contaminants in influents and effluents from 4 WWTPs and found the greatest levels of steroidal estrogens in municipal effluents were E1 > E2 > E3 which were all < 20 ng/L. Field data suggested that conventional ASP can remove over 85% of E2, E3 and EE2 (Johnson and Sumpter, 2001) from wastewater influents, although a wide range of data were reported in previous studies (Anderson et al., 2003; Chimchirian et al., 2007; D'Ascenzo et al., 2003; Servos et al., 2005). On occasions, 99% removal of steroid estrogens has been observed within existing WWTPs. The synthetic estrogen, EE2, appeared to be removed less than natural estrogens. Andersen et al. (2003) investigated the fate of E1, E2 and EE2 at a German sewage treatment plant and found that the elimination efficiency of EE2 (90%) was slightly lower than E1 and E2 (>98%). However, a couple of studies showed that the removal efficiency of E1 was remarkably

lower than those of the other estrogens; with the average value at 61% (Baronti et al., 2000; D'Ascenzo et al., 2003). In some cases, E1 concentrations in WWTP effluents were observed even higher than in influents, which was explained due to the biotransformation of E2 into E1 in biological treatment processes (Baronti et al., 2001; Johnson et al., 2007; Joss et al., 2004).

It should be pointed out that the removal efficiencies of estrogens in previous studies were normally calculated from the concentrations of estrogens in influents and effluents, which did not represent the exact removal rates since the transformation between E1 and E2 might take place during biological processes. The conversion of E2 to E1 in activated sludge process has been reported in recent studies (Chang et al., 2006; Dytczak et al., 2008). It has been found that E2 was converted to E1 faster under aerobic than anoxic conditions. This conversion could cause a decrease in the estrogenicity of the final effluent as E1 is less estrogenic than E2, but this removal could be short-lived as E1 could easily be transformed back to E2 under other conditions (Routledge et al. 1998).

The high variation in the observed data suggests that specific treatment sequences and operational conditions significantly affect the extent of EDCs removal by each treatment process. The removal efficiencies of EDCs could be influenced by the fluctuation of EDCs levels in the influent, the process of the WWTPs and operational conditions as well (Liu et al., 2009). The following operational parameters have been reported in previous studies as major factors affecting the removal of EDCs in

wastewater treatment processes.

- Solids retention time (SRT)

The solids retention time (SRT) is the average amount of time that sludge remains in a wastewater treatment process. SRT is a critical parameter for WWTPs design because of its ability to affect plant size and design, as well as performance (Metcalf & Eddy, 2003). In activated sludge systems, high SRT is essential for the removal and degradation of EDCs from wastewater (Koh et al., 2008). High SRT could allow for the enrichment of slowly growing bacteria and also the establishment of more diverse microorganisms which are able to degrade a large number of EDCs (Cirja et al., 2008). Several researchers have reported that longer SRTs correlated higher removal rates of EDCs (Johnson et al. 2007; Servos et al. 2005).

Clara et al. (2005a) investigated the relationship between SRT and removal rates of EDCs based on the data from four lab-scale and five full-scale wastewater treatment facilities. High removal rates of micro-pollutants and low effluent concentrations were achieved at SRTs higher than 10 d. This observation was in agreement with previously reported results suggesting activated sludge processes that favor nitrifying bacteria with long sludge retention times and warm summer temperatures would successfully eliminate EE2 (Vader et al., 2000). A similar conclusion was drawn in another study that WWTPs with high SRT were more effective in eliminating estrogens, although the apparent removal rates were not statistically correlated with SRTs (Servos et al., 2005). Johnson et al. (2005) studied the effluents from 17 different WWTPs across Europe,

with SRT values ranged from 3 to 30 d. They found a weak but statistically significant correlation between removal rates and SRT ($p < 0.5\%$, $r^2=0.28$), with satisfying removal rates at a high SRTs (30 d).

- Hydraulic retention time (HRT)

Like the SRT, HRT can also have impacts on the ability of WWTPs to reduce EDCs. Svenson et al. (2003) compared 20 different WWTPs across Sweden, representing different treatment processes. It was found that >97% removal efficiencies occurred when the HRT was greater than 12 h in two of the WWTPs. In their comparison of WWTPs across Europe, Johnson et al. (2005) found that EDC removal efficiencies for E1 had significant correlation with HRT ($p < 5\%$, $r^2=0.16$) in the biological process of the plant. Removal efficiencies were 59%, 80%, and 82% for HRTs of 12, 8, and 19 h respectively. Servos et al (2005) compared 18 different WWTPs across Canada and found that longer HRTs (greater than 10 hours) were associated with high estrogen reduction (greater than 50%). However, a statistically significant relationship between removal efficiencies and HRT was not established.

- Conditions of the biological process

Environmental conditions such as dissolved oxygen (DO) are also important factors affecting EDCs removal. Generally speaking, the removal efficiency of EDCs in aerobic conditions was higher than in anaerobic conditions (Ermawati et al., 2007; Liu et al., 2009). Furuichi et al. (2006) investigated the removal of estrogenic compounds from swine wastewater in a farm wastewater treatment plant consisted of a series of an up-

flow anaerobic sludge blanket (UASB) and a trickling filter. It was found that the removal rate of estrogenic compounds between the effluent and the influent was greater than 97% and the trickling filter removed the majority of estrogenic compounds.

During biological treatment processes in WWTPs, nitrification can occur by oxidizing ammonia to nitrite and further to nitrate. Nitrification may have benefits to the removal of trace organic contaminants, including EDCs (Birkett and Lester, 2003). Vader et al. (2000) utilized nitrifying activated sludge in a laboratory reactor with ammonium and hydrazine as energy sources and found good EE2 removal with nitrifying sludge. The sludge that failed to nitrify also failed to degrade EE2. The sludge lost the nitrification capacity due to the drop of temperature. Therefore, the failure of EE2 degradation of EE2 might be due to the low temperature, rather than the failure of nitrification. Andersen et al. (2003) investigated the fate of E1, E2, and EE2 at a municipal WWTP in Germany. The natural estrogens E1 and E2 were largely biodegraded in the denitrifying and nitrifying tanks of the activated sludge system, whereas EE2 was only degraded in the nitrifying tank. This was proven by Yi and Harper (2007), in which the biotransformation rate of EE2 had a linear relationship with nitrification rates in a series of batch experiments. In another study, a significant degradation of EE2 was observed in nitrifying activated sludge with the presence of ammonia-oxidizing bacterium (Shi et al., 2004). Whether ammonia oxidizing bacteria (AOB) are responsible for the biodegradation of EE2 was studied by the researchers in University of Washington. It was suggested that EE2 removal at low concentrations in municipal treatment activated sludge systems was not due to cometabolic degradation by AOB, or to abiotic nitration,

but most likely due to heterotrophic bacteria (Gaulke et al., 2008). Another study also suggested that the removal of natural estrogens E1 and E2 by activated sludge was independent of nitrification (Suzuki and Maruyama, 2006). In a more recent study, it was concluded that the AOB were most likely responsible for the first degradation step of EE2, whereas the heterotrophic bacteria might play a role in the subsequent removal of the metabolites (De Gusseme et al., 2009)

- Temperature

Temperature is an important parameter for WWTPs operation and can affect the overall efficiency of a biological treatment process (Metcalf & Eddy, 2003). It has been suggested that temperature would impact the ability of specific slow-growing bacteria to degrade EDCs (Koh et al., 2008). A number of studies have suggested that warmer temperatures were more advantageous and correlating with higher removal rates compared to colder conditions (Andersen et al., 2003; Soares et al., 2006; Ternes et al., 1999; Vader et al., 2000)

However, other studies suggested that temperature might not have a great impact on EDCs removal. Cargouet et al. (2004) examined E1, E2, E3, and EE2 concentrations in 4 WWTPs in France and found no correlation between temperature and the removal efficiencies of estrogens. Johnson et al. (2000) investigated the removal of estrogens in municipal WWTPs in Rome and the Netherlands. Regression analysis for E2 showed no significant correlation between the estrogen removal rates and temperature (r^2=0.031). In another study, a comparison of the presence of E1, E2, EE2 and NP in 17

WWTPs in Europe showed that temperature had no significant influence on the removal of E1 (Johnson et al., 2005).

- pH

The sorption of estrogens to the organic matrix was reported to be strongly dependent on the pH value (Cirja et al., 2008). Higher pH values (approximately 9-10) were disadvantageous because lower pH could likely increase the tendency of adsorption of EDCs to sludge particles (Urase et al., 2005). Clara et al. (2004b) investigated the adsorption of BPA, E2 and EE2 to activated and inactivated sludge; they found that increasing solubility as the pH increased from 7 to 12 with 30-50% desorption of initially adsorbed compounds at a pH of 10. In a more recent study, the effect of pH on the sorption of steroid hormones was investigated (Neale et al., 2009). Strong sorption was observed at acidic and neutral pH, whereas the sorption decreased considerably at alkaline conditions.

1.3.3 Removal mechanisms of estrogens in biological treatment processes

The fate of EDCs in wastewater treatment processes depends on physic-chemical properties of the compound, wastewater characteristics and operational conditions. In the literature, adsorption and biodegradation have been reported to be two principal removal mechanisms of steroid estrogens in the activated sludge process (Clara et al., 2004a; Johnson and Sumpter, 2001; Liu et al., 2009; Svenson et al., 2003).

In the case of removal by biodegradation, Ternes et al. (1999) investigated the persistence of natural estrogens and contraceptives under aerobic conditions using an experimental activated sludge system. The results showed that while in contact with activated sludge, E2 was oxidized to E1 but further degradation products of E1 were not observed. On the other hand, EE2 was principally persistent under the selected aerobic conditions with little or no transformation. Suzuki and Maruyama (2006) studied the fate of E1 and EE2 in batch mixing experiments using municipal sewage and activated sludge. It was found that E1 and E2 were initially adsorbed onto the activated sludge and then biodegraded within a few hours, when the sewage was satisfactorily mixed with the activated sludge.

EDCs preferentially adsorb onto suspended microorganisms in secondary sludge due to their hydrophobic properties (Langford and Lester, 2003). Natural estrogens E1 and E2 are considered to be weakly hydrophobic. So, binding to sludge would be unlikely to dominate their fates. However, the synthetic estrogen EE2 is somewhat more hydrophobic, approximately 10 times greater than E2. Therefore, removal of EE2 by sorption to sludge would be likely to play a more important role in wastewater treatment processes (Lai et al., 2000).

Volatilization is another pathway for the removal of estrogens in wastewater treatment processes (Cirja et al., 2008; Khanal et al., 2006). The loss of estrogens through volatilization could be predicted by their Henry's law constants (Hc). Generally, compounds with an Hc greater than 10^{-3} atm m^3 mol^{-1} can be removed by volatilization.

Estrogens E1, E2 and EE2 have very small Hc at the magnitude of 10^{-7} (Birkett and Lester, 2003). Thus, estrogens are not easily volatilized under normal temperature and pressure conditions. The removal of estrogens from water or wastewater by volatilization is likely to be limited.

1.3.4 Removal of EDCs in MBRs

Through their specific design and operational features, MBRs may improve the removal mechanisms in activated sludge processes in terms of adsorption, biodegradation and membrane filtration. The improved physical sludge characteristics of MBRs, including higher biomass concentration, finer granulometry with more effective contact surface and more hydrophobic sludge, could enhance the adsorption of EDCs onto the sludge (Lee et al., 2003a). In MBRs, high sludge age favors slow growing microorganisms and low mass organic load favors the biological synthesis of a broader substrate spectrum. In addition, complete retention of particles through membrane filtration provides full removal of all contaminants bound to colloidal and particulate matters. Most of EDCs range from 150 to 500 Daltons in molecular size. As a result, only those compounds associated with particles or colloidal organic matter will be removed by microfiltration (MF) and ultrafiltration (UF) membranes, which are the two most widely used types of membranes in MBRs (Clara et al., 2004a).

Since MBR technology has been considered as one of the promising technologies for wastewater treatment (Cirja et al., 2008; Yang et al., 2006), many studies on the removal of EDCs in MBRs have been conducted in recent years. Table 1.2 shows a summary of

some studies on the removal of selected EDCs in MBRs during the past 5 years. The majority of the studies were conducted by measuring the concentrations of EDCs in influents and effluents in pilot-scale MBRs. Further research is needed to reveal specific removal mechanisms of EDCs in MBRs and to investigate the performance of full-scale MBRs in removing EDCs. As shown in Table 1.2, it is clear that most of the investigated EDCs were not completely removed by MBRs and they remained with fluctuating concentrations in MBR effluents. The performance of MBRs in removing EDCs depends not only on the concentrations of EDCs in influents, but also on operating parameters such as SRT, HRT, temperature, DO, pH and other influencing conditions.

Table 1.2 Summary of the studies on the removal of selected EDCs in MBRs

Compound	Scale	Influent (ng/L)	Effluent (ng/L)	Removal (%)	Reference
E1	Pilot	16.5-44.05	1.71-12.12	80.2-82.3	Hu et al., 2007
	Lab	16.63-49.95	N.D.*-3.78	84.0-91.4	Hu et al., 2007
	Pilot	N.A.**	N.D.-1.2	N.A.	Spring et al., 2007
	Pilot	108-231	4.4[a]	93-99	Zuehlke et al., 2006
	Pilot	N.D.-55.6	N.D.-27.12	64±16	Lee et al., 2008
	Pilot	29-81	N.D.-21	--	Clara et al., 2005a
	Pilot	25±5	2.4±0.5	96±1	Joss et al., 2004
E2	Pilot	N.D.-5.74	N.D.-2.83	49.3-63.1	Hu et al., 2007
	Lab	N.D.-6.21	N.D.	60-66.5	Hu et al., 2007
	Pilot	N.A.	N.D.-1.1	N.A.	Spring et al., 2007
	Pilot	11-43	0.8[a]	94-99	Zuehlke et al., 2006
	Pilot	N.D.	N.D.	--	Lee et al., 2008
	Pilot	67-125	N.D.-6	--	Clara et al., 2005a
	Pilot	6.3±1.3	≤ 0.5	≥ 98	Joss et al., 2004
EE2	Lab	750, 000	--	58.4-97.7[b]	De Gusseme et al., 2009
	Pilot	N.A.	0.9-1.6	N.A.	Spring et al., 2007
	Lab	100, 000		~80.0	Cirja et al., 2007
	Pilot	5-23	1.3[a]	82-94	Zuehlke et al., 2006
	Pilot	N.D.-38.6	N.D.	>71	Lee et al., 2008
	Pilot	3-20	N.D.-4	--	Clara et al., 2005a
	Pilot	1.6±0.3	≤ 0.5	≥ 75	Joss et al., 2004
BPA	Lab	750,000	--	90	Nghiem et al., 2009
	Pilot	0.01-2.05	N.D.-0.60	68.9-77.9	Hu et al., 2007
	Lab	N.D.-1.20	N.D.-0.25	82.2-90.1	Hu et al., 2007
	Pilot	N.A.	2.5-12.6	N.A.	Spring et al., 2007
	Pilot	23.96-159.52	2.51-10.12	93±6	Lee et al., 2008
	Pilot	2025-2376	16-158	--	Clara et al., 2005b
NP	Pilot	N.D.-1.21	0.002-6.70	-439.5~-161.1	Hu et al., 2007
	Lab	N.D.-9.07	N.D.-2.12	-313.4~-269.3	Hu et al., 2007
	Pilot	0.41-4.6	0.22-1.68	55±25	Lee et al., 2008
	Pilot	2673-4031	297-482	--	Clara et al., 2005b
OP	Pilot	N.D.	N.D.	--	Lee et al., 2008
	Pilot	118-436	N.D.-74	--	Clara et al., 2005b

* N.D. - not detected; ** N.A. – not available; a – average; b – HRT=4 days

A number of studies have shown higher removal rates of EDCs in MBRs than in conventional treatment systems. The improved removal of EDCs in MBRs might be due to their higher SRT compared to conventional ASP. Joss et al. (2004) ran a pilot-scale MBR system (SRT=30d) fed with primary effluent in parallel with an ASP treatment plant (SRT=11d). For natural estrogens E1 and E2, degradation activity was observed to be higher in the MBR sludge than in the AS sludge. The researchers considered the sludge age might contribute to this difference in degradation activity. Similar results were reported in another study, where MBR showed better E2 reduction than the conventional treatment system that received the same influent wastewater (Holbrook et al., 2002). Higher removal rates for all three investigated estrogens E1, E2 and EE2 were obtained in two pilot-scale MBR plants than a full-scale WWTP fed with the same municipal raw wastewater (Zuehlke et al., 2006). Low removal rates were found during the winter, indicating that operating temperature might impact the removal efficiency of estrogens in biological treatment processes. However, contradicting results were reported in another study. Clara et al. (2005b) investigated the removal of 9 EDCs including EE2 in a pilot-scale MBR and conventional WWPTs with comparables SRTs. In this case, no significant difference was observed in the removal rates of EE2 achieved in the two systems.

A study on the fate of E1 and E2 in a MBR revealed that the bio-cake, which accumulated on membrane surface due to membrane fouling, played an important role in reducing estrogens. The membrane with bio-cake provided a better removal of

estrogens than the virgin membrane (Chang et al., 2006). In a more recent study, the removal efficiencies of EDCs from effluents using pilot scale sewage treatment processes, including MBR, nanofiltration (NF) and reverse osmosis (RO) for the purpose of water reuse, were estimated and compared. It was found that the removal efficiency for E1 using the MBR process was relatively lower than the other investigated compounds (Lee et al., 2008).

1.3.5 Rejection of EDCs by membranes

The membrane process is being widely used for the removal of contaminants in advanced water and wastewater treatment (Liu et al., 2009). In recent years, research on the removal of EDCs by membrane filtration has greatly increased. Studies have shown that the rejection efficiency of EDCs by membranes strongly depends on the membrane characteristics and EDCs' physic-chemical properties, such as molecule weight, Kow, water solubility, electrostatic property and so on. In comparing membrane types, EDCs rejection rate by RO was the highest, followed by NF membrane, then UF membranes, with the rejection of MF membranes as the lowest (Chang et al., 2003; Snyder et al., 2007; Yoon et al., 2007). Snyder et al. (2007) demonstrated that RO could remove above 99% of natural and synthetic estrogens.

The rejection of EDCs by membrane processes is mainly due to charge repulsion, adsorption and size exclusion (Liu et al., 2009). Chang et al. (2003) studied the rejection of E1 by a MF membrane and attained high rejection results. However, the rejection of E1 by membranes decreased with the increase of filtration time. Because the pore size

of the MF membrane tested was several orders of magnitude larger than the E1 molecules and the rejection was found to be reversible, the rejection of E1 by MF membranes was regarded as a result of adsorption rather than size exclusion. In the case of NF and RO membranes, molecular weight cut-off (MWCO) was suggested to be useful for the performance prediction of EDCs rejection. Jung et al. (2007) examined the rejection properties for 10 alkylphenols with four kinds of flat-sheet NF membranes and found a linear relationship between the rejection and MWCO.

The performance of the rejection of EDCs by membranes may be affected by membrane fouling. Both positive and negative impacts of membrane fouling on membrane rejection of EDCs, depending on the type of membranes used, were observed. Agenson and Urase (2007) studied the performance of NF and RO membranes under conditions of organic fouling. For NF membranes, rejection of BPA increased with fouled membranes compared to virgin membranes, from 21% (virgin) to 60.8% (fouled by activated sludge); while for RO membranes, rejection of BPA decreased from 96.6% (virgin) to 82% (fouled by activated sludge).

1.3.6 Adsorption of EDCs by activated carbon

Activated carbon (AC) is a material with exceptionally high surface area. A gram of activated carbon may have a surface area in excess of 400 m², with 1500 m² being readily achievable (Metcalf & Eddy, 2003). The two kinds of AC in market are PAC and granular activated carbon (GAC). Both PAC and GAC have been studied for the removal of EDCs from water and wastewater.

Previous studies on the removal of EDCs by AC mainly focused on the removal efficiency and adsorption isotherms of selected EDCs in lab waters (de-ionized or distilled) or drinking water (Yoon et al., 2003; Zhang and Zhou, 2005; Zhou et al., 2007). All of those studies concluded that AC could effectively remove selected EDCs from water streams. It was found that PAC treatment was feasible for removing more than 99% of three estrogenic compounds (BPA, E2 and EE2) from raw drinking waters containing such compounds (Yoon et al., 2003). In another study, GAC was reported to have very high adsorption capacities for the two investigated estrogens, with a maximum adsorption constant of 9290 ml/g for E1 and 12200 ml/g for E2 (Zhang and Zhou 2005).

The types of AC produced from different materials might affect the removal of EDCs by AC adsorption. Choi et al. (2005) studied the removal performances for three EDCs including NP and BPA by using a GAC fixed bed at lab scale. Among the three AC types, the coal-based carbon was found more effective than the other two due to its larger pore volume. Other environmental parameters could also influence the removal performance of EDCs by AC adsorption. It was also found that the adsorption capacities of the investigated carbons were reduced with an increase of adsorbent concentration and by the presence of surfactant and humic acid.

In the past few years, a number of researchers have demonstrated that AC has a strong capacity of removing various EDCs from simulated and real wastewater (Choi et al.,

2005; Fukuhara et al., 2006; Ifelebuegu et al., 2006; Snyder et al., 2007). Ifelebuegu et al. (2006) investigated the removal of EE2 from wastewater final effluent by three types of GAC (wood, coconut and coal based) in batch adsorption experiments. More than 96% removal of EE2 from wastewater final effluent was achieved by all three types of GAC. The effects of other competing adsorbates in the wastewater effluent reduced the adsorption capacities of the carbons.

Many researchers observed a substantial difference in EDCs removal by AC adsorption between their presence in water and wastewater. Fukuhara et al. (2006) observed that the amounts of E2 adsorbed by AC in river water and WWTP secondary effluent were about one-thousandth of that in pure water solution. This was due to the large amount of co-present substances in river water and WWTP secondary effluent that compete with E2 for adsorption. Severe deterioration of AC adsorption capacity was also found in the effluent of WWTPs by Snyder et al. (2007), who compared the removal efficiencies of EDCs in WWTP effluents by AC with those in surface water.

1.3.7 Degradation of EDCs in UV-based processes

Ultraviolet (UV) is gaining wide application for microbial disinfection both in drinking water and wastewater treatment. In addition to its disinfection effectiveness by inactivating microorganisms, UV can also degrade organic compounds, including EDCs, by direct photolysis as a consequence of UV light absorption. For a compound to be photolabile, it needs to have the capacity to absorb photons of the incident light emitted by a UV lamp. Liu and Liu (2004) studied the direct photolysis of natural

estrogens E1 and E2 in aqueous solutions irradiated by UV-light and UV-Vis-light. The results showed that both E1 and E2 concentrations decreased after irradiation with a UV lamp. However, the UV doses used in this study were from 900 to 5400 mJ/cm^2, which were much higher than those commonly used for WWTP effluent disinfection (i.e., 20 to 120 mJ/cm^2). Similar result was reported in another study that approximate 3000 mJ/cm^2 was required in laboratory experiments to achieve up to 80% removal of EE2 in an aqueous solution (Liu et al., 2003).

The difference in degradation efficiency of EDCs was observed between direct UV irradiation by low-pressure and medium-pressure lamps. Low-pressure UV lamps generate essentially monochromatic radiation at a wavelength of 254 nm; while medium-pressure high-intensity lamps generate polychromatic radiation (Metcalf & Eddy, 2003). Rosenfeldt and Linden (2004) reported that 14.5%, 17.7% and 21.6% degradation occurred for BPA, E2 and EE2, respectively, at a UV fluence of 1000 mJ/cm^2 from the medium-pressure lamp. However, no more than 5% degradation for all three investigated EDCs was observed under the same fluence from low-pressure lamps. The reason for the low degradation by low-pressure lamps was that radiation emitted from low-pressure lamps (254 nm) had a very low probability of being absorbed by the EDCs in the water. In a more recent study, results demonstrated that medium-pressure UV direct photolysis more effectively removed BPA compared to low-pressure UV lamps (Chen et al., 2007a).

Other factors, such as UV spectra and presence of natural organic matter, may also

affect the degradation of EDCs by UV irradiation. Leech et al. (2009) conducted photolytic experiments with E2 in laboratory grade water under simulated sunlight between 290 and 720 nm. Results showed that photodegradation of E2 was greatest in full sunlight containing UV-B (290-320 nm) although degradation was also detected with UV-A (320-400 nm) and visible light (400-720 nm) alone. It was also found that photodegradation was accelerated in the presence of natural organic matter, primarily due to the production of radicals. In another study, it was found UV at wavelength of 254 nm degraded E1 in water much more effectively than long-wave UV at wavelength of 365 nm (Wen et al., 2009).

A number of studies have proven that UV as part of an advanced oxidation process (AOP) degraded EDCs more effectively than direct UV photolysis treatment (Chen et al., 2006; Coleman et al., 2004; Feng et al., 2005; Nomiyama et al., 2007; Rosenfeldt and Linden, 2004). Table 1.3 presents a summary of studies on the fate of selected EDCs in UV-based AOPs. Research on the removal of EDCs by UV-based AOP can be sorted into two categories: (1) removal effectiveness and optimization of operational conditions for AOP and (2) degradation pathways of EDCs by AOP. The investigated AOP systems include UV/H_2O_2, UV/Fenton, UV/TiO_2 and UV/O_3. The ultrasound/UV treatment of EDCs in aqueous medium could result in hydrogen peroxide formation and organic or inorganic structure oxidation. In those reactions, hydroxyl radical was reported as oxidative reagent (Torres et al., 2007). Therefore, ultrasound/UV was regarded as a UV-based AOP in this literature review as well.

Table 1.3 Summary of studies on the fate of selected EDCs in UV-based AOPs

Target EDCs	AOP	Medium	UV lamp	Reference
E2	UV/TiO$_2$	Lab water	Hg-Xe lamp(365nm)	Ohko et al., 2002
BPA	UV/TiO$_2$	Lab water	Black light (359nm)	Nomiyama et al., 2007
E1, E2 , EE2	UV/TiO$_2$	Lab water	High pressure mercury	Coleman et al., 2004
E1, E2	UV/TiO$_2$	Lab water	UV lamp (253nm, 238-579nm)	Zhang et al., 2007
E1, E2		Wastewater	UV lamp (253nm)	Zhang and Zhou, 2008
BPA, E1, E2, EE2, OP	UV/TiO$_2$ and UV/H$_2$O$_2$	River water	UV lamps (254 and 185nm)	Benotti et al., 2009
BPA, E2, EE2	UV/H$_2$O$_2$	Lab water	Low- and medium-pressure lamps	Rosenfeldt and Linden, 2004
BPA	UV/H$_2$O$_2$	Lab water	Low-pressure Hg lamp	Chen et al., 2006
BPA	UV/H$_2$O$_2$	Lab water	Low- and medium-pressure Hg lamp	Chen et al., 2007a
E2, EE2, BPA, NP	UV/H$_2$O$_2$	Lab and natural river waters	Low-pressure Hg lamps	Chen et al., 2007b
E2, EE2	UV/H$_2$O$_2$	Lab and natural waters	Low- and medium-pressure lamps	Rosenfeldt et al., 2007
E1	UV-VIS /Fenton	Lab water	Metal halide lamp ($\geq$313nm)	Feng et at., 2005
BPA	UV/Fenton	Lab water	Xe lamp(>300nm)	Katsumata et al., 2004
E2	UV/Fenton	Lab water	Black light lamps (365nm)	Zhao et al., 2008
E2, BPA	UV/O$_3$	Lab water	Low-pressure mercury UV lamp	Irmak et al., 2005
BPA	Ultrasound/UV /Fe^{2+}	Lab water	Low-pressure mercury lamp	Torres et al., 2007

Most of the previous studies on the fate of EDCs in UV or UV-based processes were done in the lab by using prepared EDC solutions in lab waters, with a few of them using natural river waters or wastewater samples. The fate of EDCs in wastewater during UV-based processes has not been well studied. Spring et al. (2007) treated the effluent from a pilot-scale MBR plant with 4 different disinfection methods including UV treatment.

It was found UV radiation significantly decreased concentrations of EE2 and on average, decreased concentrations of E2 from MBR effluent. However, the authors suggested that the removal of estrogens during the UV treatment was not because of the UV degradation itself but instead because of the binding of estrogens to the tubing in the flow-through system. In another study, the removal of estrogens in a full-scale WWTP was investigated by sampling at each of treatment units. It was found that the UV treatment process did not reduce the amount of estrogens (Cicek et al., 2007). Further research is strongly required to determine the fate of EDCs during UV disinfection process in WWTPs. In a more recent study (Zhang and Zhou, 2008), photodegradation aided by a catalyst TiO_2 was studied for its removal efficiency of EDCs from wastewater. In comparison with sunlight treatment, more efficient degradation was observed with UV irradiation. By applying the photocatalysis treatment to real WWTP effluent, no target compounds were detected after the treatment.

1.3.8 Measurement of EDCs in environmental samples

- Sample preparation

Measurement of EDCs in wastewater is quite a difficult task because of their very low concentrations (from ng/L to µg/L range) and the complexity of the wastewater components. Therefore, very low detection limits are required and a number of pre-treatment steps are needed before final analysis. In general, complicated extraction and purification are performed before final quantification either by chemical methods or by biological assay (Pacakova et al., 2009). The process of sample preparation for liquid

samples often involves the application of filtration and solid phase extraction (SPE). Wang et al. (2008) developed an on-line SPE for the determination of estrogens in aqueous samples. This method could continuously perform extraction of E1, E2, E3 and EE3 from aqueous samples without any other pretreatments. Besides SPE, liquid-liquid extraction was also used for dealing with liquid samples in some studies (Fang et al., 2006; Raman et al., 2004).

The analysis of EDCs in solid matrices requires complicated time- and labor-consuming procedures and high-level analytical skills due to both the complexity of the solid matrices and the requirement of very low detection limits (Kuster et al., 2004). A limited number of studies for the determination of EDCs in solid matrices (soil, sludge and precipitate) have been reported. Ternes et al. (2002) developed a method for the determination of estrogens in digested and activated sludge from WWTPs, including solvent extraction, a gel permeation chromatography cleanup, a silica gel cleanup, derivatization with N-methyl-N-trimethylsilyltrifluoroacetamide, and a final detection by GC-ion trap-MS-MS. The limits of quantification (LOQ) were 2 ng/g for E1, E2 and EE2. In other studies, a variety of organic solvents such as dichloromethane, ethyl acetate, ethyl ether, cyclohexane and hexane have been used in liquid extraction (Braga et al., 2005; Fang et al., 2006; Finlay-Moore et al., 2000; Gobel et al., 2005; Gomes et al., 2004; Lorenzen et al., 2004; Oh et al., 2000; Ramam et al., 2004; Ying and Kookana, 2003). The shaking time varied from 2 hours to overnight. In some studies, ultrasonication was implemented to improve the extraction efficiency (Ternes et al., 2002; Williams et al., 2003). Table 1.4 presents a summary of studies using liquid

extraction for the determination of EDCs in solid samples.

Table 1.4 Summary of studies using liquid extraction for the determination of EDCs in solid samples

Solvent	Method	Solid matrix	Reference
Dichloromethane (DCM)	Shaking 4 h	River sediments	Oh et al., 2000
Ethyl acetate	Shaking overnight	Soil	Finlay-Moore et al., 2000
Acetone/hexane (50:50)	Liquid phase extraction	Ocean sediments	Braga et al., 2005
Ethyl ether	Shake for 2h	Swine waste	Raman et al., 2004
Methanol and acetone	4-step serial ultrasonication	Sludge and sediments	Gobel et al., 2005; Ternes et al., 2002
cyclohexane	--	Sludge	Fang et al., 2006
Hexane/acetone (1:1) Diethyl ether/hexane (10:1)	Shaking	Sediments and sludge	Gomes et al., 2004
Ethyl acetate	Shaking	Biosolid and animal manure	Lorenzen et al., 2004
Ethyl acetate	2-step shaking	Marine sediments	Ying and Kookana, 2003
Groundwater/DCM (1:1)	Sonication and shaking	River sediments	Williams et al., 2003

- Chromatographic analysis

Chemical quantification is generally performed by chromatographic analysis by high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS). GC-MS has been the most widely used for measuring estrogens in wastewater (de Alda and Barcelo, 2001; Desbrow et al., 1998; Stuart, 2007). The advantages of GC technology include high separation efficiencies, a high speed of analysis, the availability of highly sensitive detectors, and environmental friendliness owing to the utilization of inert gases as mobile phases (Pacakova et al., 2009). At present, the most widely used detection technique is based on universal MS. To enhance

spectral resolution and thus improve the detection selectivity, tandem GC-MS/MS could be used for the determination of estrogens in complex matrices such as sludge from WWTPs (Ternes et al., 2002). One disadvantage of this method is that the derivatization step usually required for subsequent GC-MS or GC-MS-MS is time-consuming and a likely source of inaccuracy. The detection limits achieved with GC-MS or GC-MS-MS varied in previous studies, depending on the performance of instruments and the quality of the samples, but generally 1.0 ng/L could be achieved (Desbrow et al., 1998; Huang and Sedlak, 2001; Williams et al., 2003)

The other chromatographic analysis is based on HPLC technology. HPLC has some important advantages over GC techniques. For instance, samples containing EDCs can be analyzed directly, without derivatization; and aqueous samples do not require drying which is necessary in GC analyses. On the other hand, a drawback of HPLC lies in a high consumption of solvents (Pacakova et al., 2009). A number of sensitive detectors are available in HPLC analysis. MS is best suited to determinate low concentrations of EDCs in environmental samples. The detection limit of the on-line solid-phase extraction HPLC achieved 10 ng/L, whereas the detection limit of the HPLC method with normal auto sampler injection was 10 μg/L for E2 and EE2 (Ying and Kookana, 2003). Baronti et al. (2000) monitored E1, E2, E3 and EE2 in influent and effluent samples from six WWPTs by using LC-tandem MS with electro-spray ionization. The LOQs for 4 investigated estrogens in WWTP influents, effluents and river water were from 0.008 to 0.9 ng/L.

- Biological assays

Biological assays for estrogen analysis include *in vitro* and *in vivo* tests. *In vitro* assays are based on the mode of action of EDCs, which are binding to the estrogen receptor (ER). The implication of *in vitro* assays is that the binding of EDCs to the ER may result in subsequent effects on biological activity (Birkett and Lester, 2003). The most widely used *in vitro* assay for the measurement of EDCs in environmental samples is the yeast estrogen-screening (YES) assay, which uses recombinant yeast strains containing the human estrogen receptor (hER). *In vitro* assays have the potential for high-throughout screening of a large number of compounds in environmental samples. However, those assays have limitations that might result in unreliable predictions. A combination of test methods, including *in vivo* assays, has been suggested as most appropriate to determine the overall endocrine disrupting effects of EDCs on individual organisms and ecosystems (Murphy et al., 2009).

In vivo assays are able to evaluate the impacts of EDCs on the endocrine systems as a whole, which often use living organisms such as fish in the tests (Huggett et al. 2003). In this study, the vitellogenin (VTG) tests by measuring blood plasma VTG concentrations in male fish were used to confirm and interpret the YES assay results. VTG is normally only present in the plasma of female fish, but male fish carry the gene for VTG. Estrogenic compounds can trigger the expression of the VTG gene in male fish and lead to increased levels of VTG in the blood. Attention should be drawn that the sole use of *in vitro* assays to screen for estrogenicity might underestimate or

overestimate estrogenic potential of wastewater. Huggett et al. (2003) observed that *in vivo* estrogenic activity was nearly 10-fold greater than the YES activity. However, Aerni et al. (2004) concluded that although all five investigated WWTP effluents contained measurable concentrations of estrogens and gave positive YES responses, the male fish significantly increased blood plasma VTG concentrations at only two out of five sites.

The YES assay has been most commonly used as an *in vitro* bioassay to investigate EDCs present in raw sewage, treated effluents and receiving waters (Aerni et al., 2004; Gaido et al., 1997; Holbrook et al., 2005; Murphy et al., 2009; Nelson et al., 2007; Pawlowski et al., 2004; Routledge and Sumpter, 1996; Yu and Chu, 2009). The YES assay has been developed and modified by many investigators. The schematic of the YES assay mechanism is illustrated in Figure 1.4. In brief, a recombinant yeast strain containing hER gene and an expression plasmid carrying estrogen response elements is grown in a selective growth medium. When the cells are incubated in the presence of estrogenic compounds, the lac-Z product β-galactosidase is secreted into the medium and causes the chromogenic substrate to change the color. This color change can be quantified by measuring absorbance by a spectrophotometer or micro-plate reader. There are two recombinant yeast strains available, one developed by Routledge and Sumper (1996) and the other developed by Gaido et al. (1997). These two strains use different chromogenic substrate, the former using chlorophenol red β-D-galactopyranoside (CPRG) while the latter using o-nitrophenyl-β-galactosidase (ONPG). In the YES assay, the overall estrogenic activity (EA) of the EDCs in environmental

samples is typically expressed as 17β-estradiol equivalence (E2-Eq) concentrations.
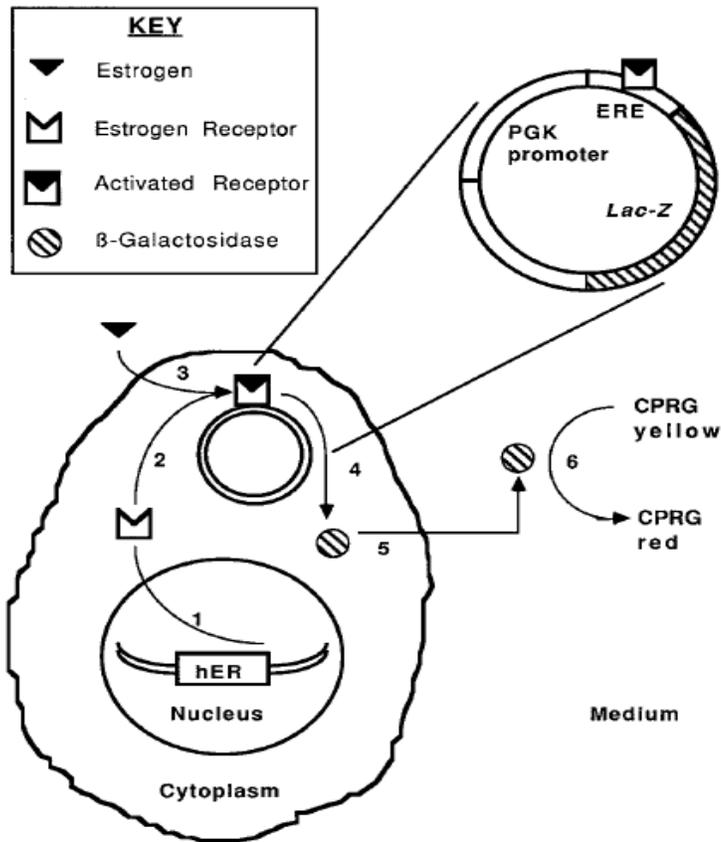
Figure 1.4 Schematic of the estrogen-inducible expression system in yeast*
*(Taken from Routledge and Supter 1996 with permission, "The human estrogen receptor gene is integrated into the main genome and is expressed (1) in a form capable of binding to estrogen response elements (ERE) within a hybrid promoter on the expression plasmid (2). Activation of the receptor (3), by binding of ligand, causes expression of the reporter gene *Lac*-Z (4) which produces the enzyme β-galactosidase. This enzyme is secreted into the medium (5) and metabolizes the chromogenic substrate CPRG (normally yellow) into a red product (6), which can be measured by absorbance.")

The YES assay possesses a couple of advantages over chemical analysis and other biological assays. Firstly, the YES assay can detect estrogenic activity in the ng/L range. Kirk et al. (2002) reported a detection limit for E2 of 3 ng / L. The low detection limit makes it well-suited to test estrogenic chemicals in the environment. A second advantage of the YES assay is its capacity to detect the overall EA of the samples

including both the parent compounds and any intermediate degradation products that can activate estrogen receptors, regardless of their identity. Thus, this approach provides a real indication of the ability of a process to reduce EA of the investigated wastewater; such information cannot be obtained by chemical analysis alone, because some of the intermediate degradation products that might possess a certain extent of EA cannot be measured by chemical analysis of the parent compound. Lastly, the YES assay is relatively easy to perform because yeast cells are easily grown under laboratory conditions. Disadvantages of the YES assay include high false-positive rate due to contamination from numerous sources, being unable to identify the specific chemicals in samples and lack of consistency throughout the research community as there is no strict protocol available for YES assay so far. In addition, transformations between different EDCs in treatment processes cannot be detected by the YES assay.

Apart from the research on the occurrence and fate of EDCs in WWTPs by the YES assay (Servos et al., 2005; Yu and Chu, 2009), the links between chemical analysis and the YES assay were also studied by many researchers. Nakada et al. (2004) studied secondary effluent samples from WWTPs in Japan by GC-MS and the YES assay. The results showed that E1 and E2 represented more than 98% of the total EA of WWTP effluents detected by the YES assay. Although it was concluded that E1 and E2 were the dominant environmental estrogens in WWTP effluents, the authors suggested a significant contribution to the EA of WWTP effluents from unidentified components in the effluents. Similar results from Nelson et al. (2007) suggested that about 95% of the total EA were derived from E1, E2 and E3 in WWTP effluents by using the YES assay.

In this study, the E-Screen bioassay was also conducted with the MCF-7 human breast cancer cell line while the YES bioassay employed two different types of recombinant yeast. It was observed that the results of the E-Screen and the YES bioassay varied by up to 4-fold on the same split sample of a nominal E2 concentration.

The measurement of EDCs in environmental samples by the YES assay may be affected by the presence of dissolved or colloidal matters in samples. Tanghe et al. (1999) firstly found that the presence of humic acid in the growth medium could reduce the sensitivity of the YES assay to E2. The authors suggested that humic acid was binding with E2, which reduced the binding of E2 directly to the hER in the YES assay. Holbrook et al. (2005) evaluated the impact of colloidal organic carbon on the sensitivity of the YES assay and concluded that the results generated by the YES assay had significantly variability in the presence of wastewater-derived colloidal organic carbon.

CHAPTER 2: METHODOLOGY

2.1 Overview of the Methodology for Objectives (1) & (2)

Two bench-scale MBR systems and a pilot-scale MBR system with hollow fiber membrane modules submerged into aerobic bioreactors were tested for the investigation of EDCs in the MBR process. Details of the MBR systems will be described in the following chapters. Two types of membrane modules from two manufacturers, one from GE/Zenon (Canada) and the other from Korea Membrane Separation Co., Ltd., were used in the MBR systems. Oxygen required by aerobic bacteria and membrane surface scouring was provided by compressed air via air diffusers. Membrane permeate was sucked out by vacuum pumps. Process control parameters including pH, oxidation and reduction potential (ORP), trans-membrane pressure (TMP) and temperature were monitored regularly.

A model laboratory scale MBR for the treatment of swine wastewater was used to study the removal of EDCs in terms of overall estrogenic activity (EA) at high concentrations. The results were confirmed in a pilot-scale MBR at similar conditions. To reveal removal mechanisms of specific EDCs at lower concentrations in MBRs, synthetic wastewater spiked with the selected EDCs was fed to a submerged MBR system using membrane modules with a thin-spread shape. A natural estrogen E2 and a synthetic estrogen EE2, which are widely considered as the major contributions to the estrogenicity in WWTPs effluents (Desbrow et al., 1998), were spiked into the

wastewater as target estrogens. In parallel, a PAC-MBR (MBR with PAC dosing) was tested with the same feed at similar conditions. The effects of PAC dosing on MBR performance and estrogen removal was investigated.

By monitoring EDC concentrations in terms of EA at designed sampling points, removal rates of EDCs by MBR systems were determined. The sampling points included the influent, mixed liquor in the bioreactor, and the membrane permeate. The samples of mixed liquor were separated by GF/C filters. The EA concentrations in the filtrate and the sludge were measured respectively. With the data of the concentrations and corresponding flow rates, the mass balance (Eq-1) of each estrogen was established on the basis of loading rates. For liquid phase samples, the load was simply concentration multiplied by flow rate. For solid phase samples, the load was determined using the concentrations based on a dry mass, flow rates of the stream and solid content of the stream. The load of sludge was the sum of liquid phase load and solid phase load.

$$L_{\text{inf}} = L_{eff} + L_{ws} + R_b \qquad \text{(Eq-1)}$$

where L_{inf} is the influent load (gd^{-1}), L_{eff} is the effluent load (gd^{-1}), L_{ws} is the load removed with the waste sludge(gd^{-1}), and R_b is the load biodegraded (gd^{-1}).

Removal mechanisms were investigated to differentiate between biological degradation, sludge adsorption and physical retention by membrane filtration. The reduction of the EA with the solid phase of waste sludge was contributed to the mechanism of sludge adsorption. From Eq-1, the R_b can be calculated by $R_b = L_{\text{inf}} - L_{\text{ws}} - L_{\text{eff}}$. In conventional

activated sludge process (ASP), the effluent is separated by secondary clarifiers. The difference of the EA amount in the filtrate of mixed liquor and membrane permeate is considered due to the mechanism of membrane filtration. Based on the removal rates by the mechanisms of biodegradation, sorption and membrane filtration, the dominant removal mechanism in MBRs was identified.

2.2 Overview of the Methodology for Objectives (3) & (4)

The study of EDCs in UV process was initiated by investigating the degradation kinetics of estrogens in water solution by UV irradiation. This study was conducted in a UV reactor (Trojan UVMax) provided by Trojan Technologies (Canada). Two natural estrogens E1 and E2 and a synthetic estrogen EE2, which are widely considered as the major contributions to the estrogenicity in WWTP effluents, were selected as target EDCs. Samples were taken at certain intervals of UV irradiation and analyzed by HPLC. Plotting the concentrations of estrogens against irradiation time, the parameters of UV degradation kinetics were obtained. With irradiation experiments of the mixture of the three investigated estrogens in water solution, the inhibition effects of co-present substances on the degradation of estrogens were investigated.

In order to understand the impacts of UV irradiation on the fate of EDCs in wastewater, a survey of EA change through UV disinfection in five full-scale WWPTs was conducted. Pre-UV and post-UV samples were taken from four municipal WWTPs and one industrial WWTPs and their EA was measured by the YES assay. The selection of

the WWTPs was based on the hypothesis that field conditions in WWTPs such as the source of the wastewater, biological treatment processes and UV system itself could affect the fate of EDCs in UV disinfection process.

2.3 Analytical Method of EDCs

The yeast estrogen screen (YES) assay was used as the major method for the measurement of EDCs throughout the studies. Details of the yeast estrogen assay and preparation of the medium components have been described previously by Routledge and Sumpter (1996). A step-by-step procedure of the YES assay is presented in Appendix A at the end of the thesis. A general description of the method is also given in chapter 1.3.8. In this research, the above procedure was modified to allow for high-throughput testing using a 96-well micro-titration plate reader (PowerWave™, BioTec). Samples or standard solutions (10 μl) were allocated to the wells of a 96-well micro-titration plate. Aliquots (190μl) of the seeded assay medium (containing recombinant yeast and the chromogenic substrate CPRG) were then dispensed into each well using a multichannel pipettor. Each plate contained one row of blanks, triplicate rows of standard E2 solutions and duplicate rows of samples. The plate was sealed with Parafilm wrapping film and shaken vigorously for 2 min prior to incubation at 30 °C for 72 h. After incubation, the plate was shaken and allowed to settle for 1h, after which the absorbance was checked at 540 nm (A_{540}) and at 620 nm (A_{620}) using a micro-plate reader (PowerWave™, BioTec). In order to correct the estrogenic response of each tested sample for turbidity, the following equation was applied to calculate the corrected

absorbance (A_{corr}) in each well.

$$A_{corr}Sample = A_{540}Sample - (A_{620}Sample - A_{620}Blank)$$

The EA was evaluated in terms of E2-equivalent concentration. The standard curve used to determine EA in ng/L unit was determined from the mean A_{corr} of the 3 wells containing the same E2 concentration. The concentrations of standard E2 solutions ranged from 2.5 to 50,000 ng / L. The linear portion of the E2 standard curve was used to calculate the EA of the samples in terms of 17β-estradiol equivalence (E2-Eq) concentrations.

CHAPTER 3: TREATMENT OF SWINE WASTEWATER BY SUBMERGED MEMBRANE BIOREACTORS WITH CONSIDERATION OF ESTROGENIC ACTIVITY REMOVAL

3.1 Abstract

A model laboratory scale membrane bioreactor (MBR) for the treatment of swine wastewater has been studied to illustrate the removal mechanisms of selected endocrine disrupting compounds (EDCs). A pilot scale MBR was also operated to confirm the results from the laboratory scale study. Liquid extraction with cyclohexane followed by yeast estrogen screen (YES) assay was used to determine the estrogenic activity (EA) of wastewater and sludge samples. Both laboratory and pilot scale MBRs were demonstrated to be effective systems for EA removal from swine wastewater. The average removal rate was 93.5% in terms of EA in the soluble phase of swine wastewater, and 94.5% in terms of total EA. During the pseudo-stable and stable operation periods, total COD removal efficiencies ranged from 68.5% to 82.7% and up to 99.9% removal of NH_3-N was achieved with proper pH control. A model mass balance revealed that over 85% of the influent EA was reduced through MBR process by degradation or volatilization. An average of 9.4% of the influent EA was removed from the MBR system in the wasted sludge, whereas 5.4% of the EA in the influent was found in the treated effluent.

3.2 Introduction

Swine wastewater has been considered as one of the major sources of water pollution. Besides high concentration of organic matter and nutrients (nitrogen and phosphorus), livestock waste contains appreciable amounts of estrogenic compounds, which are classified under the category of endocrine disrupting compounds (EDCs). The potential contamination of surface water and groundwater resources with EDCs can lead to significant ecological problems (Rodgers-Gray et al., 2000). Presence of EDCs in surface water has caused great concerns because even at very low concentrations (ng/L), these chemicals could have serious long-term impacts on the health of wildlife and humans. It has been widely accepted that the most likely source of EDCs in aquatic environments is from discharge of municipal and industrial effluents along with runoff from agricultural production (Hewitt and Servos, 2001). Farm animals may be a major emitter of EDCs. More EDCs might be present in the liquid phase than in the solid phase of swine waste as swine excrete estrogens mostly (96%) in urine (Hanselman et al., 2003). In Canada and the USA, land application is the most widely used swine waste management technique. Because of the tremendous growth of swine production and limited cropland where swine manure can be utilized with acceptable transportation fees, there are growing environmental concerns about current swine waste management practices. Therefore there is a need to establish feasible treatment processes that are able to remove EDCs to satisfactory levels, while also meeting discharge standards in terms of organic matter and nutrients.

Conventional activated sludge processes are problematic for the treatment of swine

wastewater due to the presence of a high fraction of non-degradable organic matter in the wastewater and the need for post-treatment for reuse/disposal of treated effluent (Shin et al., 2005). Membrane bioreactor (MBR) is a hybrid process in which biological degradation and membrane filtration are integrated for wastewater treatment. Some of the advantages of MBRs over conventional processes include the flexibility in operation, compact plant size, and high treated effluent quality suitable for re-use. Although MBRs have been recognized and used as suitable biological processes for treating high COD or BOD wastewater (Yang et al., 2006), swine wastewater treatment by MBRs has not been well studied or documented. A few such studies, which were carried out in South Korea (Kim et at., 2005; Shin et al., 2005), have shown that MBRs could achieve high COD and nitrogen removal.

The main objective of this research was to establish a model MBR system illustrating the removal mechanisms of EDCs from swine wastewater. Establishing a reliable and time-saving analytical procedure for estrogenic activity determination in wastewater and sludge samples was another goal of this research. Based on the results of the laboratory-scale MBR operation, a pilot scale MBR was also operated to demonstrate the larger scale applicability of MBRs for EDCs removal as well as organic matter and ammonia reduction from swine wastewater.

3.3 Materials and Methods

3.3.1 Experimental membrane bioreactors

As shown in Figure 3.1, a laboratory scale MBR system was set up and operated in continuous mode with dilute swine wastewater (resulting from the flushing of animal pens) as feed. A hollow fiber membrane module with membrane surface area of 0.047 m^2 was submerged in a process tank with a working volume of 7.2 L. An air compressor was used to provide air for membrane scouring and aeration. Automatic backwashing was employed for 30 seconds out of every 10 minutes to minimize membrane fouling. The laboratory MBR was operated at a hydraulic retention time (HRT) of 6 d and solids retention time (SRT) at 60 d. Temperature, pH, oxidation/reduction potential (ORP), trans-membrane pressure (TMP), membrane permeate flow rates, and effluent flow rates were continuously monitored. Samples of influent, effluent and mixed liquor were taken at least once a week. At the last phase of testing, a pH controller and a sodium carbonate solution were utilized to control pH at a range of 7.3-7.6.

A pilot-scale MBR (ZeeWeed-10, Zenon Environmental Inc.) was also operated under similar conditions. Raw swine wastewater was settled in two storage tanks and the supernatant was used as feed. The membrane surface area for the pilot-scale system was 0.93 m^2 and the working volume of the process tank was 227 L. Automatic backwashing was similarly employed by a reversible gear pump to minimize membrane fouling.
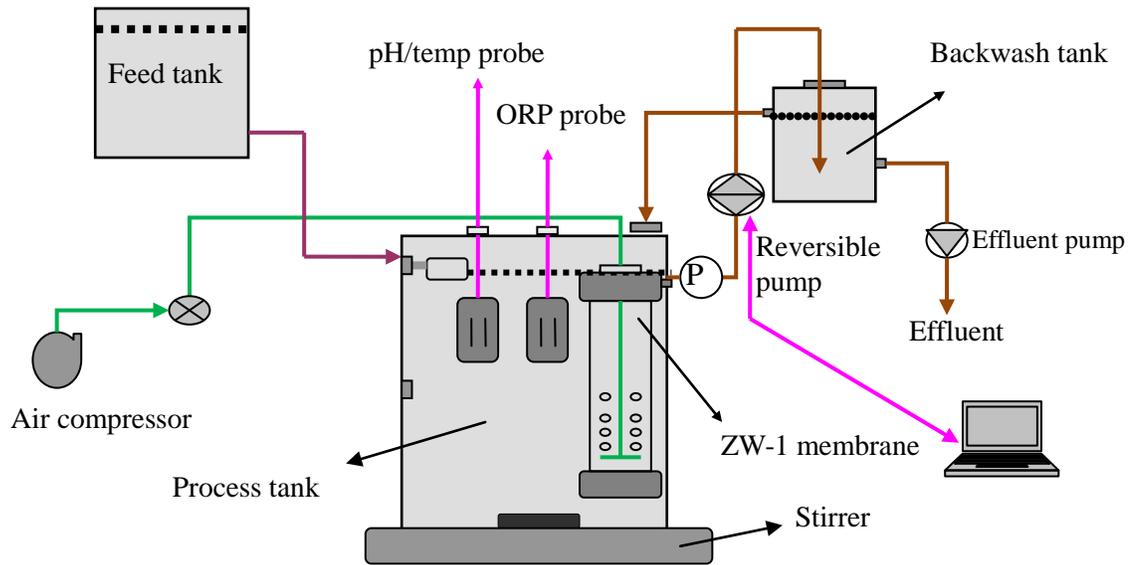
Figure 3.1 Schematic diagram of laboratory scale membrane bioreactor

3.3.2 Composition of swine wastewater

Raw swine wastewater was collected from the Animal Science Research Unit at the University of Manitoba. The raw swine wastewater was settled in a storage tank. The supernatant was screened by cloth filter and used as feed. The characteristics of swine wastewater used in this study were shown in Table 3.1. As outlined in Table 3.1, concentrations of selected parameters varied widely during the experimental period.

Table 3.1 Characteristics of the swine wastewater

Parameter	Mean value	Minimum value	Maximum value
pH	7.14	6.95	7.31
Alkalinity (mg/L)	2364	2167	2538
TCOD (mg/L)	3840	2268	5316
sCOD (mg/L)	2506	1260	3528
TSS (mg/L)	494	230	940
VSS (mg/L)	436	220	830
NH_4^+-N (mg/L)	553	410	679

3.3.3 Analytical methods

Conventional parameters such as pH, alkalinity, total suspended solids (TSS), volatile suspended solids (VSS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), total COD, soluble COD, NH_3-N, NO_2-N and NO_3-N were measured according to standard methods (APHA-AWWA-WEF, 1998). The estrogenic activity (EA) of samples was determined using a sample preparation and extraction step followed by the yeast estrogen screen (YES) assay. During the process of sample preparation, influent and sludge samples were first centrifuged at 10000g for 15 min. The supernatant was decanted and filtered through 0.45 µm GF/C filters. The filtrates and effluents were extracted as liquid samples. The unfiltered influents and waste activated sludge (WAS) were extracted to measure the total EA. During extraction, a 20 ml liquid or unfiltered sample was placed in 125 ml conical flasks along with 10 ml cyclohexane. The flasks were shaken for 4 h on a vertical shaker. A portion of cyclohexane (5 ml) was extracted from each conical flask, dried down under N_2 stream,

and reconstituted in 0.5 ml absolute ethanol for the YES assay. Details of the YES assay were described in Chapter 2. The efficiency of the extraction using cyclohexane was evaluated by spiking 17β-estradiol (E2) into water to a final concentration 1000 ng/L. Average recoveries from the extraction procedure were $74 \pm 12\%$ (n=4).

3.4 Results and Discussion

3.4.1 Biomass concentration and organic matter removal

The reactor was seeded with activated sludge from a full-scale pollution control center in Winnipeg, Manitoba, Canada. After 72 days of start-up and acclimation, consistent performance was achieved in the investigated MBR system. During the stable operation period, biomass concentrations were maintained at 3,800-4,070 mg MLSS/L with MLVSS/MLSS ≥ 0.85 (Figure 3.2).
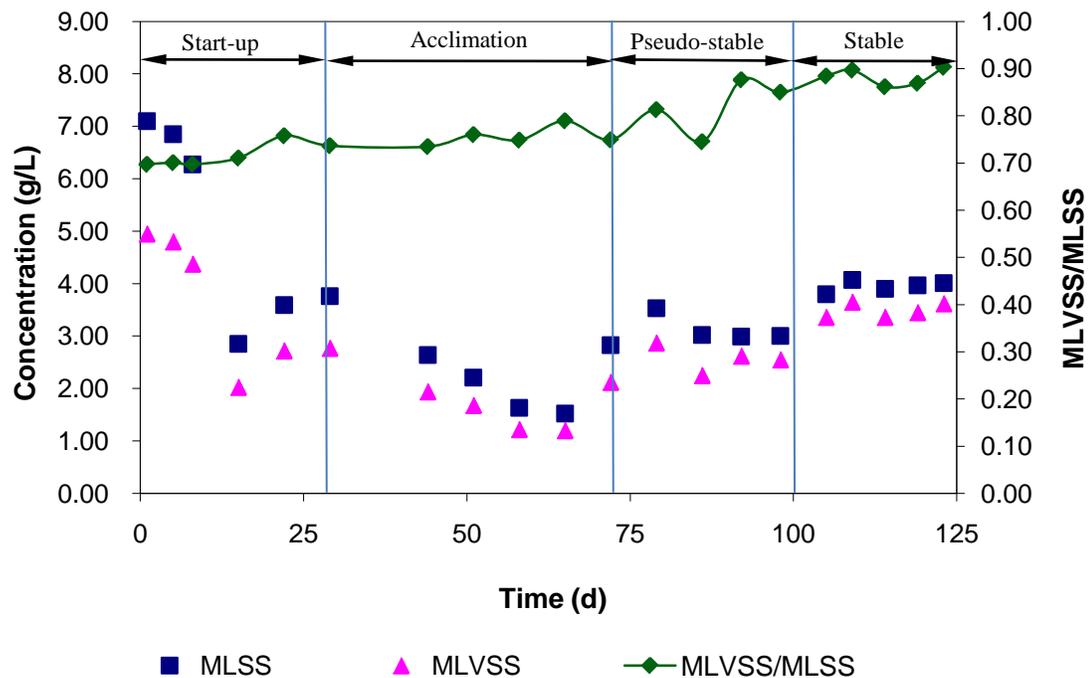
Figure 3.2 Mixed liquor suspended solids concentrations in the bioreactor

Due to high COD concentrations in the influent, the bioreactor was run at a long HRT of 6.0 d with an average COD loading rate of 0.18 kgCOD/kgMLSS·d. Figure 3.3 shows the COD concentrations of the influent and effluent, soluble COD concentrations of the mixed liquor, and COD removal rates. During the pseudo-stable and stable operation period, the total COD removal efficiencies ranged from 68.5 to 82.7%. Also, the soluble COD of mixed liquor was measured. Since the difference between the soluble COD of mixed liquor and membrane permeate COD accounted for a small portion of the total COD removed, it was concluded that the COD removal in the MBR system was primarily due to biodegradation rather than membrane retention.
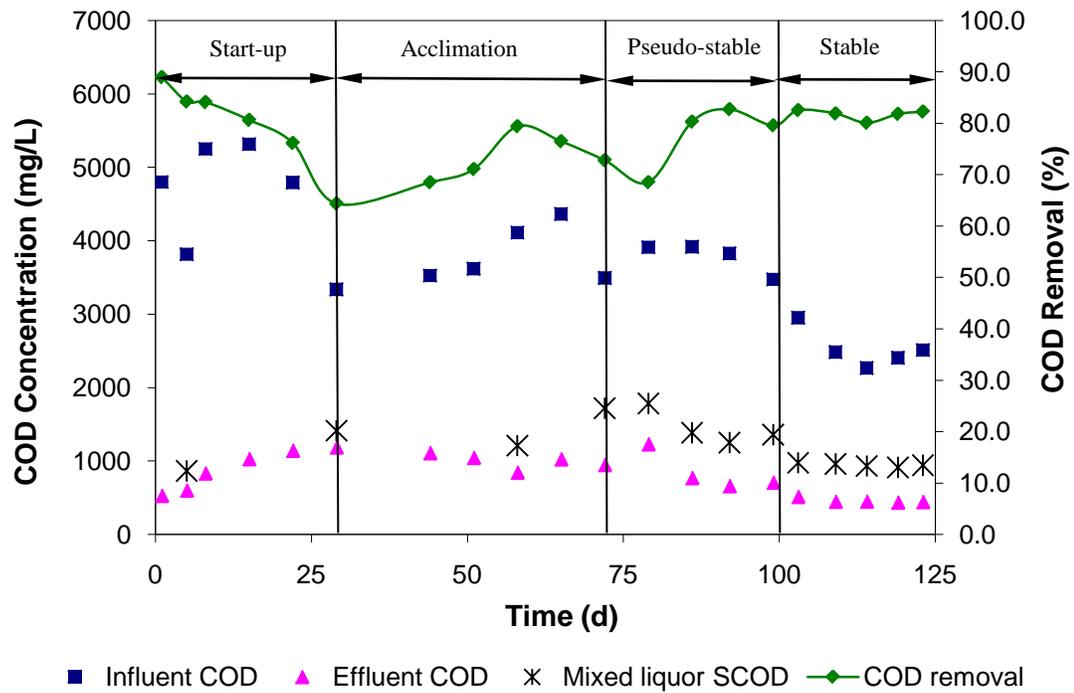
Figure 3.3 COD removal in the membrane bioreactor

3.4.2 Biological nitrogen removal

The seed activated sludge from the wastewater treatment plant being operated at SRT of

3-4 days. After a 45 day acclimation period, the NH_3-N removal rate reached 84.2%. In

the following 4 weeks, the NH_3-N removal rates dropped to 52.9%, as shown in Table

3.2. During the experimental period without pH control, the pH dropped to 6.2. Another

possible reason of poor ammonia removal was the low ratio of alkalinity to NH_3-N in

the influent. The average ratio was 4.27, which is much lower than 7.14 g alkalinity

required for the conversion of each gram of ammonium nitrogen to nitrate nitrogen

(Metcalf & Eddy, 2003). In order to achieve better ammonia removal, sodium carbonate

solution was added to the bioreactor to control both alkalinity and pH. With pH

controlled at 7.3-7.6, the removal rate of NH_3-N increased up to 99.9%. The effluent

NH_3-N concentrations were lower than 5 mg/L during the stable operation period with pH control.

Table 3.2 Summary of nitrogen removal at pseudo-stable and stable stages

Condition	Without pH control	With pH control
Time (day)	72--99	103--123
pH	6.9-6.2	7.3-7.6
Influent NH_3-N (mg/L)	588.1 ± 63.7	540.3 ± 7.8
Effluent NH_3-N (mg/L)	183.0 ± 82.1	2.4 ± 1.2
NH_3-N removal (%)	69.4 ± 12.7	99.6 ± 0.2
Effluent NO_2-N (mg/L)	72.9 ± 72.0	0
Effluent NO_3-N (mg/L)	220.4 ± 126.0	432.8 ± 18.7

Table 3.2 also presents the concentrations of nitrite and nitrate in the effluent. In the first two weeks of stable operation without pH control more nitrite than nitrate was present in the effluent. In the following period, as nitrification became more complete, nitrite produced from ammonia was further oxidized to nitrate. The total amounts of nitrite and nitrate produced were lower than the amounts of NH_3-N removed, which indicates that certain extent of denitrification might occur in some parts of the bioreactor where anoxic conditions occur.

3.4.3 Estrogenic activity (EA) removal

In this study, EA was measured by YES assay and represented as E2-Eq concentrations.

The YES assay detects both the parent compounds and any intermediate degradation products that activate estrogen receptors, regardless of their identity. Thus, this approach provides a real indication of the ability of an MBR to reduce EA of the investigated wastewater; such information cannot be obtained by chemical analysis alone, because some of the intermediate degradation products that might possess a certain extent of EA cannot be measured by chemical analysis of the parent compound.

EA in the solid phase of WAS and influent samples was calculated by subtracting the EA in the liquid samples from the EA in the unfiltered samples. The EA in the solid phase of the influent accounted for 31.2% of total EA. As shown in Table 3.3, the average EA of raw swine wastewater was 1576.3 ng/L, which was much higher than sewage EA. Holbrook et al. (2002) reported that the EA of influents from various municipal wastewater treatment plants varied from 17.4 to 58.6 ng/L.

Table 3.3 Estrogenic activity (EA) and TSS concentration of influent, effluent and WAS

Stream	Total EA (ng/L)	Liquid EA (ng/L)	Solid EA (ng/g)	TSS (g/L)
Influent	1576.3 ± 128.1	1301.3 ± 123.5	1195.6 ± 154.3	0.23 ± 0.03
Effluent	84.7 ± 20.5	84.7 ± 20.5	--	--
WAS	1482.6 ± 31.0	175.3 ± 21.3	321.2 ± 30.2	4.07 ± 0.32

The average removal rate was 93.5% in terms of EA of the soluble phase of dilute swine wastewater, and 94.5% in terms of total EA. These removal rates are high. However, it is not practical to compare the removal rates in this study with those in the previous

studies. Most of studies with respect to EDCs removal in MBR dealt with municipal wastewater, not high-strength wastewater such as swine wastewater. It was reported that degradation activity for natural estrogens in the MBR sludge increased by a factor of 2-3 with comparison of the conventional activated sludge (Joss et al., 2004). The increase might be due to the difference in sludge age between the MBR (SRT=30 d) and the conventional activated sludge process (SRT=11 d). In addition, the EA in the liquid phase of WAS was more than double that of the effluent EA. This indicates that membrane filtration could retain some estrogenic compounds, resulting in a lower EA in the membrane permeate. The bio-cake, which accumulated on the membrane surface might play an important role in reducing estrogenic compounds (Chang et al., 2006).

A mass balance was performed by multiplying average EA concentrations by daily flow rates, as shown in Figure 3.4. This revealed that an average of 5.4% of the EA in the influent was detected in the treated effluent, whereas 9.4% of the influent EA was removed from the MBR system in the form of WAS. This leaves over 85% of the influent EA, which was reduced through the MBR process by degradation or volatilization. This observation is inconsistent with the results in a previous study that more EA was found in the liquid effluent than in the WAS (Holbrook et al., 2002). However, the extraction method used in this study only recovered about 70% EA of the samples. It is possible that the non-extracted portion was mainly associated with the solid phase, which could change the proportions in the mass balance. More effective and reliable extraction methods, especially for solid samples, need to be developed to confirm the mass balance established on the basis of current data in this study.

Biodegradation and volatilization

1621.1 ng/d   (85.2%)

1891.6 ng/d   **Membrane**   101.6 ng/d
Influent   **Bioreactor**   Effluent
(100%)*   (5.4%)

177.9 ng/d   (9.4%)

WAS
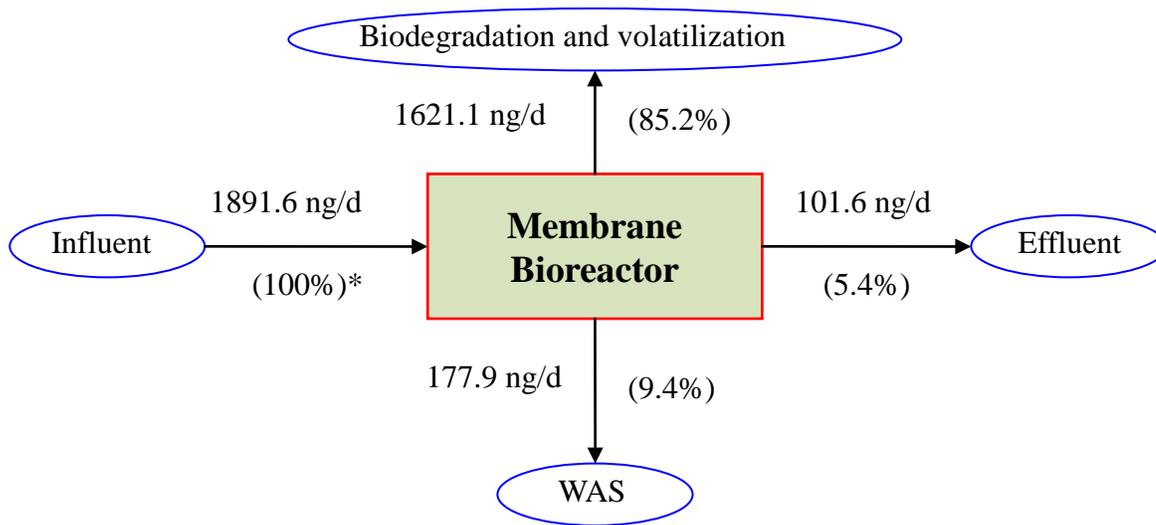
* Parenthetic values represent percentage of EA from influent

Figure 3.4 Mass balance of estrogenic activity in the membrane bioreactor

3.4.4 Overall performance of the pilot-scale membrane bioreactor

Table 3.4 presents the overall performance of the pilot-scale MBR in comparison with the bench-scale MBR. Overall, the performance of these two MBR systems was consistent with each other in terms of COD removal and EA removal. The poor NH_3-N removal efficiency by the pilot-scale MBR was attributed to the absence of pH control as similar results were observed in the laboratory scale MBR during the period without pH control.

Table 3.4 Overall performance of the pilot-scale MBR in comparison with the bench-scale MBR

	Pilot-scale MBR	Bench-scale MBR
COD removal (%)	85.7	78.9
NH$_4$-N removal (%)	51.2	69.4 (99.6*)
EA removal (%)	>90.0	94.6

* Mean values under the condition with pH control

3.5 Conclusions

Both laboratory and pilot scale MBRs were demonstrated as effective systems for EA removal from swine wastewater. The average removal rate was 93.5% in terms of EA in the soluble phase of dilute swine wastewater, and 94.5 % in terms of total EA. During the pseudo-stable and stable operation periods, the total COD removal efficiencies ranged from 68.5 to 82.7%, and the removal rate of NH$_3$-N reached up to 99.9% with proper pH control.

A model mass balance revealed that over 85% of the influent EA was reduced through the MBR process by degradation or volatilization. An average of 9.4% of the influent EA was removed from the MBR system in the form of WAS, whereas 5.4% of the EA contained in the influent was found in the treated effluent. Membrane filtration can retain some estrogenic compounds. In the future, more effective extraction methods are needed to formulate a more reliable mass balance and fully elucidate the fate of EDCs in the wastewater treatment process.

CHAPTER 4: EFFECTS OF POWDERED ACTIVATED CARBON DOSING ON MEMBRANE BIOREACTOR PERFORMANCE AND ESTROGEN REMOVAL

4.1 Abstract

Sludge characteristics associated with filterability as well as the removal of a natural estrogen 17β-estradiol (E2) and a synthetic estrogen 17α-ethinylestradiol (EE2) were investigated in submerged membrane bioreactors (MBR) with and without the addition of powdered activated carbon (PAC) under the same feed and operating conditions. Positive impacts of PAC dosing on membrane fouling and the removal of E2 and EE2 were demonstrated over a six months stable operational period. Experimental results showed that PAC dosing resulted in a slower rate of trans-membrane pressure (TMP) increase by reducing the concentrations of soluble extracellular polymeric substances (EPS) and colloidal total organic carbon (TOC) in the PAC-MBR sludge. A slower rate of TMP increase will reduce the maintenance and operating costs associated with membrane cleaning and/or membrane replacement. The average soluble EPS and colloidal TOC concentrations in the PAC-MBR sludge was 60.1 and 61.8% lower than the control MBR sludge, respectively. Regardless of PAC dosing, concentrations of colloidal TOC were strongly related to concentrations of soluble EPS and soluble carbohydrates in the sludge. In addition, the mean flocs size of the sludge with PAC

dosing was shifted from 49.4 to 60.3 µm. PAC dosing in the MBR increased the removal rates of E2 and EE2 by 3.4 and 15.8%, respectively. The greater impact of PAC dosing on EE2 removal was due to its more hydrophobic property and greater affinity to adsorption.

4.2 Introduction

Membrane bioreactor (MBR) technology has been advancing rapidly around the world both in research and commercial applications in the last decades (Yang et al., 2006). Membrane fouling, which has been widely recognized to be one of the major limitations to faster commercialization of MBRs, has been investigated from various perspectives in many studies including the causes, characteristics, mechanisms of fouling, and methods to prevent or reduce membrane fouling. Flux improvement can be achieved with powdered activated carbon (PAC) addition because PAC plays an important role in reducing the biomass cake resistance and changing the filtration characteristics of the sludge in bioreactors (Li et al., 2005). In addition, PAC might have a scouring effect for removing the cake layer from membrane surfaces. The application of PAC dosing in MBRs dates back to 1998 (Ohnishi et al., 1998). In their system, PAC was added primarily to reduce the color of treated wastewater for reuse. It was also found that the activated sludge dewaterability could be improved by adding PAC in the bioreactor. The first application of PAC addition for flux enhancement in a membrane anaerobic bioreactor was published in 1999 (Park et al., 1999). In their study, a flux improvement with PAC addition was achieved, especially with a higher PAC dosage of up to 5 g/L.

From then on, a number of studies were conducted, aiming to use PAC dosing as a membrane fouling control method (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Kim and Lee, 2003; Li et al., 2005; Liu et al., 2005; Ng et al., 2006; Remy et al., 2009; Satyawali and Balakrishnan, 2009; Vyrides and Stuckey, 2009).

The occurrence and fate of endocrine disrupting compounds (EDCs) including estrogens in wastewater treatment plants (WWTPs) has been of major concern to the aquatic environment all over the world. Some scientists concluded that discharge from WWTPs is one of the largest sources of EDCs in the aquatic environment (Liu et al., 2009; Servos et al., 2005; Tan et al., 2007). Steroid estrogens, especially 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are major EDCs of particular concern for the aquatic environment. Despite controversial opinions on the impact of EDCs on human beings and overall ecology, the adverse effects on aquatic species such as fish have been well documented. The negative impacts include the feminization due to hormonal imbalance and reduced reproductive success in fish and avian (Rodgers-Gray et al., 2000). Although a number of studies have been done to understand the fate of EDCs in wastewater treatment processes, further research is needed to develop effective ways to reduce them to environmentally acceptable levels.

The removal mechanisms of estrogens in WWTPs and their transport into the environment have been shown to depend on the design and operational characteristics of the treatment plant. Conventional biological treatment processes, such as the activated sludge process, have been reported effective in reducing levels of some EDCs

in wastewater and sewage. The principal removal mechanisms of steroid estrogens in the activated sludge process are likely to be sorption and biodegradation (Liu et al., 2009; Johnson and Sumpter, 2001). Most of previous studies on EDCs in wastewater treatment have been done using activated sludge processes. Field data suggested that conventional activated sludge processes could remove some EDCs with a wide range of removal efficiencies (Fernandez et al., 2007; Johnson and Sumpter, 2001; Liu et al., 2009; Servos et al., 2005).

MBRs have been proven quite effective in removing both organic and inorganic pollutants as well as biological entities from water or wastewater streams. Due to their advantages such as high sludge age, high biomass concentration and complete particle retention, MBRs are expected to achieve enhanced performance in removing EDCs. This hypothesis has been supported by a number of previous studies on the removal of EDCs in MBR process (Chang et al., 2006; De Gusseme et al., 2009; Joss et al., 2004; Lee et al., 2008). By dosing PAC in the bioreactor, the characteristics of the sludge (PAC-sludge) can be expected to vary. PAC-sludge is hypothesized to possess higher adsorption capacity, which would increase its capacity to adsorb substances, including estrogens, to its surface. A couple of studies have been conducted to test the efficiency of PAC in further eliminating estrogens in MBR. Wintgens et al. (2003) investigated the removal efficiencies of EDCs with different process configurations in landfill leachate treatment plants. The results showed that the final activated carbon adsorption following membrane bioreactor was able to remove half of the remaining micro-pollutants. In another study (Korner et al., 2001), it was reported that additional activated carbon

filtration was very efficient in further eliminating estrogenic activity from WWPTs effluents. On the contrary, other investigators have shown that the estrogenic activity in aqueous phase leaving the MBR system was not further reduced by polishing with either granular activated carbon (GAC) or PAC (Holbrook et al., 2002).

With the expansion of MBR installations for wastewater treatment and water reuse, the removal behaviour and mechanisms of estrogens in MBRs need to be further studied. Meanwhile, whether PAC dosing is able to enhance the overall MBR performance including the removal of estrogens needs be assessed. The objectives of this study were to assess the effects of PAC dosing on MBR performance and estrogens removal in bench-scale MBRs.

4.3 Materials and Methods

4.3.1 Experimental set-up

Two bench-scale MBRs, one without PAC dosing and the other with PAC dosing, were operated in parallel at similar experimental conditions (Figure 4.1). A hollow fiber membrane module with a surface area of 0.08 m^2 from Korea Membrane Separation Ltd. was submerged in each bioreactor. The specifications of the membrane modules are summarized in Table 4.1. The working volume of each bioreactor was 3.0 L. Coarse bubble air-diffusers at the bottom of the membrane module were used to provide dissolved oxygen (DO) for biomass growth and to introduce vigorous shear force on the

membrane surface to reduce membrane fouling.
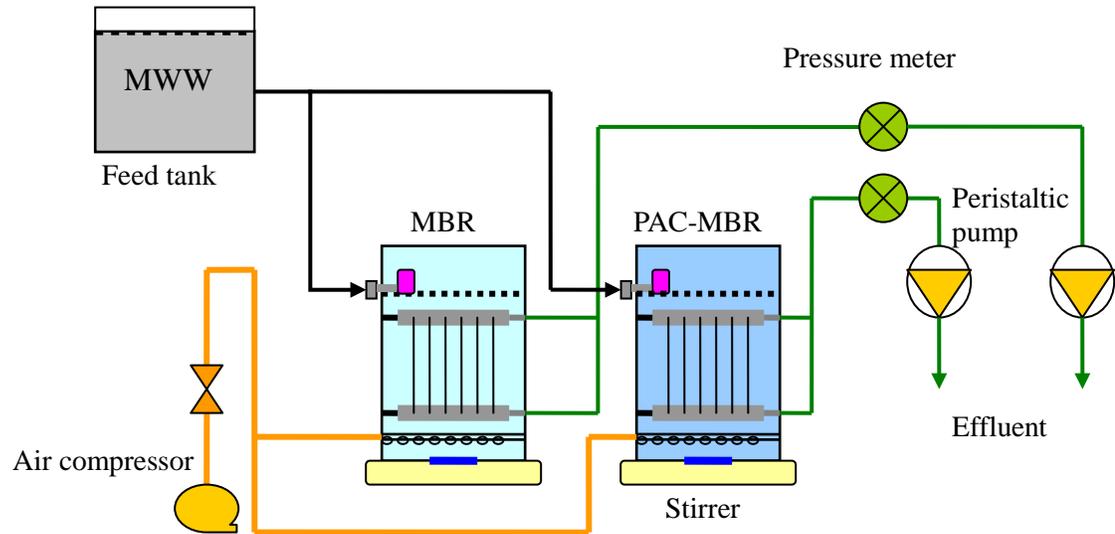
Figure 4.1 Schematic of the bench-scale MBR and PAC-MBR

Table 4.1 Specifications of membrane modules

No.	Parameter	Specification/value
1	Membrane type	Hollow fiber
2	Pore size	0.4 μm
3	Surface area	0.08 m^2/module
4	Material	Polyethylene
5	Module shape	Thinly spread
6	Configuration	Vertically submerged
7	Cleaning method	Relaxation + chemical cleaning

4.3.2 Operation of the MBRs

The two MBR systems were operated at constant flux by withdrawing the permeate

through peristaltic pumps from the outside-to-inside hollow membrane fibers. The two MBR systems were fed with identical synthetic wastewater by gravity. The components of the synthetic wastewater are shown in Table 4.2. The water levels of the bioreactors were controlled by floating valves. To control membrane fouling, a cyclic pumping mode with 10 min ON and 2 min OFF was used. Solids retention time (SRT) of both MBR and PAC-MBR remained 25 d by wasting a portion of the mix liquor from bioreactors daily. During the experimental period, trans-membrane pressures (TMP) of the two MBR systems were monitored every day. Temperature, DO, and pH in the bioreactors were measured on a regular basis. Table 4.3 summarizes the main operating conditions of the control MBR and PAC-MBR during the experimental period.

Table 4.2 Components of the synthetic wastewater

Component	Unit	Concentration
$FeCl_3 \cdot 6H_2O$	mg/L	7.0
KH_2PO_4	mg/L	60
$NaHCO_3$	mg/L	200
$(NH_4)_2SO_4$	mg/L	120
$CaCl_2 \cdot 2H_2O$	mg/L	12
$MgSO_4$	mg/L	38
Glucose	mg/L	400
Yeast extract	mg/L	10
E2 or EE2	ng/L	500

Table 4.3 Operational conditions of the MBR and PAC-MBR

Parameter	Unit	Value
SRT	day	25
HRT	hour	5.8-6.0
DO	mg/L	>4.0
pH	--	6.2-7.1
Temperature	°C	20.4-22.6

In the PAC-MBR, PAC with particle size ranging from 50 to 150 µm was added into the bio-reactor at a dosage of 2.0 g/L. The PAC dosage remained at the same level by re-supplementing the bioreactor with 0.24g PAC every other day. The selection of the particle size of PAC was based on previous studies. Smaller particles provide quicker rates of adsorption, but might tend to increase the small particle population of the activated sludge and shift the flocs size to lower ranges (Ng et al., 2006). However, PAC with an average size of 100 µm was reported to shift the particle size distribution of the mixed liquor to a relatively higher range (Park et al., 1999). Based on these results, the PAC with a relatively higher particle size range would be suitable for dosing into MBR systems

4.3.3 Analytical methods

For wastewater and sludge samples, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD), color, soluble COD, ammonia-nitrogen (NH_3-N) and nitrate concentrations were measured according to standard methods (APHA-AWWA-WEF, 1998). The nitrification rates

were measured in two batch test reactors with the MBR sludge and PAC-MBR sludge, respectively. Non-limiting oxygen and ammonia concentrations conditions (DO > 5 mg/L and initial NH_3-N = 20 mg/L) were supplied in the batch tests. The ammonia and nitrate concentrations were monitored every 15 min for 3 h. The nitrification rate was calculated as the slope of the increase of nitrate concentrations.

Total organic carbon (TOC) was measured by a TOC analyzer (Phoenix 8000, USA). The difference of TOC between the filtrate passing through a 1.2 µm filtration paper (GF/C, Whatman, USA) and the permeate directly collected from the MBRs was referred to as colloidal TOC. Soluble extracellular polymeric substances (EPS) were measured as supernatant after the centrifugation of the sludge samples at 10,000g for 30 min and calculated by summing the contents of the carbohydrate and protein fractions. Carbohydrate was measured according to Dubois *et al*. (1956) and protein was measured according to modified Lowry's method (Lowry et al., 1951). The bound EPS was measured as extract after 1 h heat extraction at 100°C (Chang and Lee, 1998). The particle size of sludge was measured by a laser particle counter (Spectrex PC-2000, USA).

A yeast estrogen screen (YES) assay was used to quantify estrogenic activity associated with E2 or EE2 in samples. Prior to the YES assay, samples were extracted by using cyclohexane extraction for the enrichment of EA. The method of cyclohexane extraction has been reported in a previous study (Yang and Cicek, 2008). The details of the YES assay were described in Chapter 2. In the tests of extraction recovery, some

sludge samples were spiked with 500 ng/L E2 or EE2; extracted by cyclohexane extraction; and analyzed by the YES assay. The average extraction recoveries for E2 from MBR and PAC-MBR sludge were 82.3 and 71.0%, respectively. The average extraction recoveries for EE2 from the MBR and PAC-MBR sludge were 76.7 and 64.2%, respectively.

4.4 Results and Discussion

Two identical MBRs were seeded with activated sludge from a full-scale water pollution control center in Winnipeg, Manitoba, Canada. After 80 d of start-up and acclimation, stable operation and consistent performance was achieved in both MBR systems. One of the MBRs was then dosed with PAC, and the other MBR was operated as a control. The control MBR and PAC-MBR were then run in parallel for over 6 months.

4.4.1 Biomass growth

Figure 4.2 shows MLVSS concentrations of the two investigated MBRs during the whole experimental period. It should be pointed out that, in Figure 4.2, no PAC was added in PAC-MBR during the start-up and acclimation stage (before 80 d operation). Before PAC was added in one of two bioreactors, the biomass concentrations were nearly identical at all sampling points. The MLVSS concentrations remained stable in the two bioreactors at 5.5 ± 0.2 g/L for one SRT before PAC was added into one of

bioreactors on day 80.
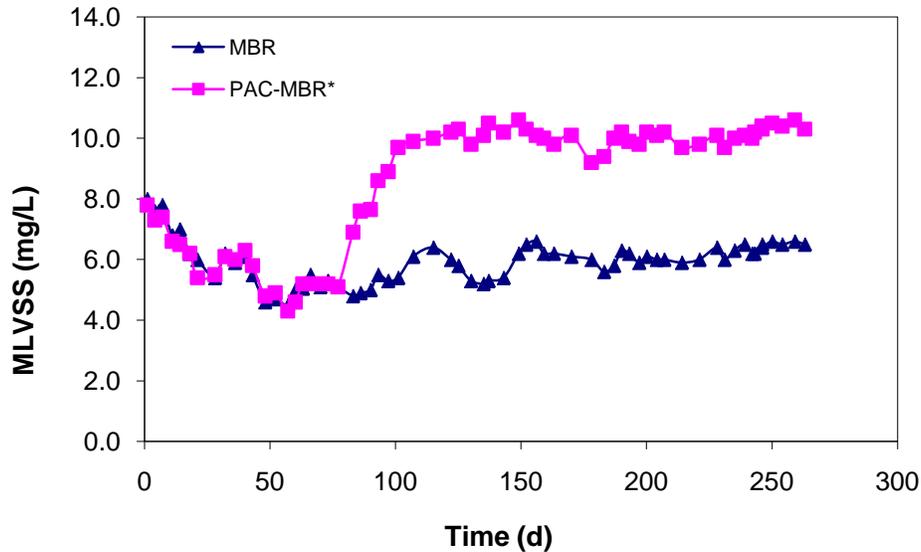
Figure 4.2 Comparisons of MLVSS between the MBR and PAC-MBR

During the first SRT (25 d) period after PAC was added into PAC-MBR reactor, the MLSS and MLVSS concentrations in the PAC-MBR increased gradually whereas the MLSS and MLVSS concentrations in the control MBR remained quite stable. The average MLVSS concentration of MBR and PAC-MBR after 105 d operation was 6.1±0.4 and 10.1±0.3 g/L, respectively. As the dosage of PAC in PAC-MBR remained at 2.0 g/L and the volatile content at 550°C of PAC is 96.7%, the MLVSS concentration in the PAC-MBR was expected to be 1.93 g/L (2.0 g/L × 96.7%) higher than that in MBR provided the biomass growth in the control MBR and PAC-MBR were the same. However, the average MLVSS concentration in the PAC-MBR after 105 d operation was 4.0 g/L higher than that in MBR. To examine whether PAC dosing imposed any impact on the biomass growth, the protein content and nitrification rate of the sludge

from MBR and PAC-MBR bioreactors were compared. As shown in Figure 4.3, there was no considerable difference in either protein content or nitrification rate of the sludge from the control MBR and PAC-MBR. This indicates the biomass content in MBR and PAC-MBR sludge was not significantly different. It has been reported that PAC addition in a submerged MBR did not show enhancement in microbial activity (Satyawali and Balakrishnan, 2009). Some of the higher MLVSS concentration of PAC-MBR sludge can be attributed to the adsorption and accumulation of incoming (or generated) soluble organic substances on the PAC surface. Results on soluble COD data show a 31mg/L difference between the control MBR and the PAC-MBR. It is also possible that more PAC was added than extracted from the MBR on a daily basis due to settling of the PAC in the reactor. This could have resulted in a new equilibrium at the MLVSS levels observed in the PAC-MBR.
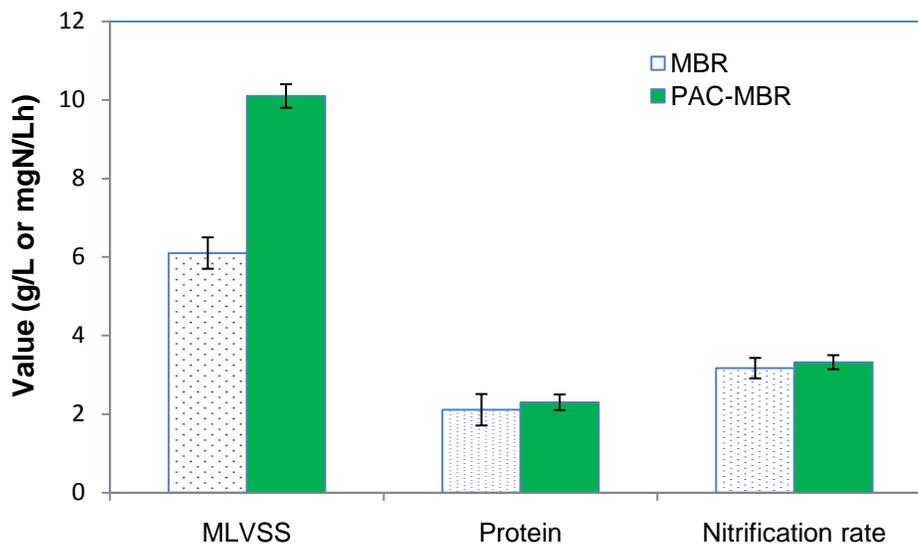
Figure 4.3 Comparison of biomass characteristics between the MBR and PAC-MBR

4.4.2 TMP

Trans-membrane pressure (TMP) is an important parameter to control the MBR operation as TMP is an indicator of when membrane modules need be cleaned. To ensure the membrane modules in the two MBR systems had identical filtration performances, the two investigated MBRs were operated for three membrane-cleaning periods before PAC was added into one of the bioreactors. As shown in Figure 4.4, the daily TMPs in the two MBR systems before PAC dosing were nearly the same and the increasing pathway followed the same pattern. The membrane provider recommended a normal operation pressure range for the membranes of 7 to 40 kPa. However, during the start-up and acclimation period, the permeate flux dropped greatly when TMPs reached 15-20 kPa. The membranes were cleaned by soaking in 0.3% NaOCl for 3 h when permeate flux decreased by more than 20%, even though the TMPs were still relatively low. It is difficult to explain why the permeate flux decreased at low TMPs because the conditions of biomass and membrane fibers at the start-up and acclimation period were changing and different from the stable operation.
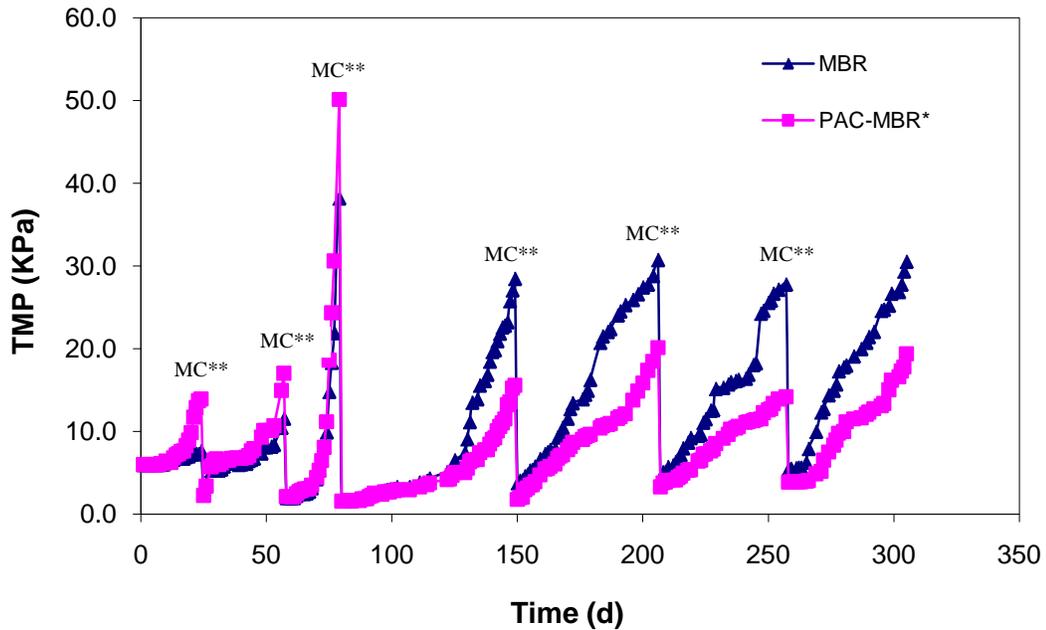
*No PAC dosing before 80 d
** MC – Membrane cleaning

Figure 4.4 Changes of TMPs in the MBR and PAC-MBR

After PAC was added in one of the two MBRs, the profile of TMPs in PAC-MBR was quite different from the control MBR. Generally speaking, PAC dosing resulted in a slower rate of TMP increase in the PAC-MBR. Based on the results during four membrane cleaning cycles, the average daily TMP increasing rates in the control MBR and PAC-MBR were 0.467 and 0.269 kPa/d, respectively. In practice, a slower TMP increase rate means a longer operating period between membrane cleaning events. Consequently, the maintenance cost for membrane cleaning could be reduced.

In addition, PAC dosing was found being able to recover TMPs to a lower level after chemical cleanings. Figure 4.5 plots the initial TMPs in the control MBR and PAC-

MBR after each membrane cleaning at the same cleaning frequency. It can be found that the initial TMPs in PAC-MBR were consistently lower than those in the control MBR. This could result in a longer lifespan of membrane fibers because the membranes can be cleaned more often without compromising their performance. Consequently, the operating cost for membrane replacement could also be reduced.
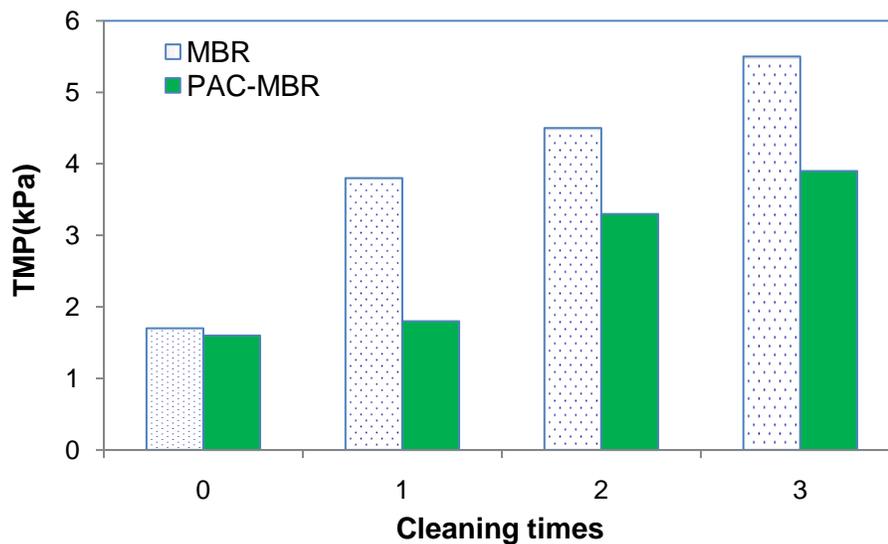
Figure 4.5 Initial TMPs of the MBR and PAC-MBR after membrane cleanings

4.4.3 Effect of PAC dosing on MBR performance

The comparison of overall performance in the MBR and PAC-MBR at steady state conditions was summarized in Table 4.4. The soluble COD of the mixed liquor in the PAC-MBR bioreactor was 47±3 mg/L, which was much lower than in the control MBR (78±5 mg/L). This indicates that PAC is capable of enhancing the adsorption of soluble COD to the PAC-sludge. On average, 31 mg/L more sCOD was adsorbed by the PAC onto PAC-sludge, provided that the biodegradation was the same in the two reactors. However, there was no much difference in effluent COD between the control MBR and

PAC-MBR. This indicates that PAC dosing did not remove the refractory COD that could not be removed by biological degradation or membrane filtration.

Table 4.4 Summary of the performance of the MBR and PAC-MBR

	MBR	PAC-MBR
Influent COD (mg/L)	475.6±50.7	475.6±50.7
Mixed liquor sCOD (mg/L)	78±5	47±3
Effluent COD (mg/L)	21.4±2.6	20.7±3.3
COD removal rate (%)	95.5±1.1	95.6±1.2
Effluent NH_4-N (mg/L)	<0.1	< 0.1
NH_4-N removal rate (%)	>99.5	>99.5
Effluent color (TCU)	15±5	<5
Effluent E2 (ng/L)	56.6±6.2	39.3±8.8
E2 removal rate (%)	89.0±0.7	92.4±1.4
Effluent EE2 (ng/L)	139.1±15.2	63.8±9.8
EE2 removal rate (%)	70.9±1.2	86.7±1.1

For nitrogen removal, both the control MBR and PAC-MBR had complete nitrification with ammonia removal rates higher than 99.5%. Ohnishi et al. (1998) found PAC dosed in a wastewater reclamation system at a dosage of 50 mg/L could stably obtain treated water with a color of less than 5 true color unit (TCU). This observation was confirmed in this study. The color of the effluent from PAC-MBR was consistently lower than 5 TCU, which was lower than the average color of the effluent from the control MBR of 15 TCU. The enhanced color removal is beneficial for the application of MBR for direct water reuse.

A natural estrogen E2 and a synthetic estrogen EE2 were selected as target contaminants to demonstrate the removal mechanisms of estrogen in MBR and PAC-MBR, because E2 and EE2 have shown to be discharged from a wide variety of wastewater treatment plants and processes relatively high estrogenic activity (Cicek et al., 2007). The average removal rate of E2 and EE2 by the control MBR was 89.0 and 70.9%, respectively. These removal efficiencies were consistent with the results from previous studies in activated sludge processes (Cicek et al., 2007; Liu et al., 2009). The synthetic estrogen EE2 appeared to be removed to a lesser extent than E2, which is reasonable because of its more stable chemical structure. PAC dosing increased the removal efficiencies of both E2 and EE2 in the MBR system. In comparison with the control MBR, the removal rate of E2 and EE2 in the PAC-MBR was increased by 3.4 and 15.8%, respectively. The impact of PAC dosing on EE2 removal was found to be greater than on E2 removal. The mechanisms will be discussed in the following section.

4.4.4 EPS in the MBR and PAC-MBR

There are a number of studies which have shown the main foulants with regard to membrane fouling in MBRs are EPS excreted from cells (Chang and Lee, 1998; Fan et al., 2006; Li et al., 2005; Liu et al., 2005). To reveal whether the slower rate TMP increase in the PAC-MBR was associated with the concentration of EPS in the sludge, EPS was analyzed in the MBR and PAC-MBR. Figure 4.6 and Figure 4.7 present the concentrations of soluble EPS and bound EPS in the sludge of MBR and PAC-MBR. As shown in Figure 4.6, soluble carbohydrate, protein and the total soluble EPS of PAC-MBR sludge were greatly lower than the MBR sludge. The average soluble EPS of

PAC-MBR sludge was 60.1% lower than the control MBR sludge. Lower amounts of EPS in the MBR with the addition of PAC have been reported in previous studies (Kim and Lee, 2003; Ying and Ping, 2006). In contrast, there was no much difference in bound EPS including carbohydrate and protein between PAC-MBR sludge and MBR sludge (Figure 4.7). This observation was consistent with the conclusion in a previous study that soluble EPS would have greater impacts on membrane fouling than would bound EPS (Fan et al., 2006). This might be due to the fact that the bound EPS are mainly present within the MLSS flocs while the soluble EPS are the main components of colloidal substances in the sludge. Colloidal particles have been widely recognized as being more responsible for membrane fouling than MLSS flocs (Stephenson et al., 2000).
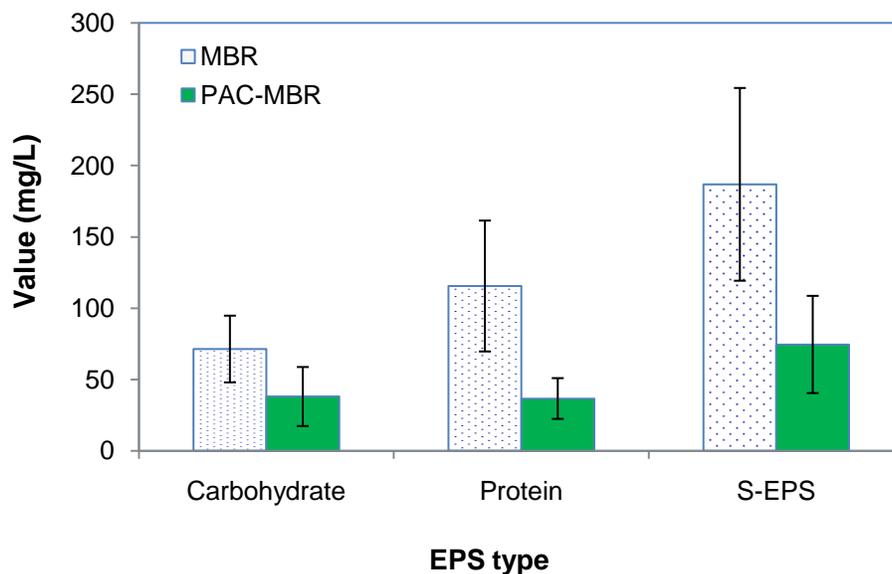
Figure 4.6 Comparison of soluble EPS between the MBR and PAC-MBR sludge
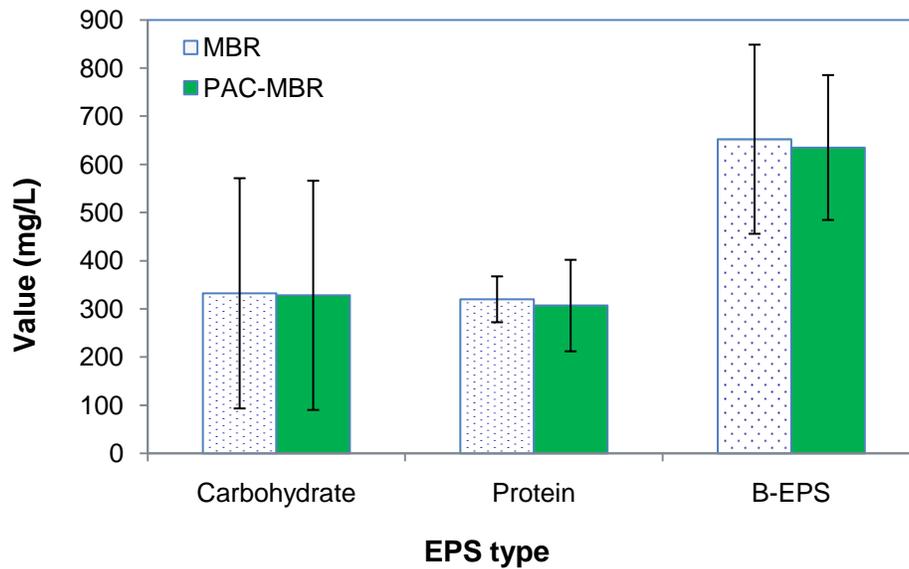
Figure 4.7 Comparison of bound EPS between the MBR and PAC-MBR sludge

It should be pointed out that bound EPS mentioned in this study referred to the portion of carbohydrate and protein in sludge that could be extracted by the method described in the previous section. It is possible that more EPS substances were accumulated in the PAC-MBR sludge but could not be extracted by current extraction method due to the high adsorption ability of PAC.

4.4.5 Colloidal particles in the MBR and PAC-MBR

The size of colloidal particles in wastewater treatment is typically classified in the range from 0.01 to 1.0 µm (Metcalf & Eddy, 2003). In this study, the difference between filtrate from a 1.2 µm filter and the permeate from MBR systems was used to measure colloidal TOC. This is in line with other studies, which used similar methods to represent the concentration of colloidal particles in activated sludge (Fan et al., 2006).

Although the nominal pore size of membrane fibers used in MBRs is 0.4 µm, the fouled membrane fibers were able to retain smaller particles due to the additional filtration provided by the cake formed on the membrane surface. This was supported by the fact that the TOC of the filtrate passing through a 0.22µm filter was higher than the TOC of the permeate from the MBRs.

The results of colloidal TOC measurements showed that the average of colloidal TOC of the PAC-MBR sludge and MBR sludge was 6.5 and 17.0 mg/L, respectively. At each sample point, the colloidal TOC of the PAC-MBR sludge was consistently lower than the MBR sludge. The lower colloidal TOC in PAC-MBR sludge might be associated with the slower rate of TMP increase in the PAC-MBR. Fan et al. (2006) found a good correlation between colloidal TOC and soluble EPS in a pilot-scale MBR system. Figure 4.8 plots the soluble carbohydrate, soluble protein and soluble EPS as a function of colloidal TOC, whether or not the sample was obtained from the MBR or PAC-MBR. Consistently, a higher soluble EPS in the sludge resulted in a higher colloidal TOC. It is reasonable because the soluble EPS in the sludge liquor excluding that in the permeate could constitute the main components contributing to colloidal TOC.
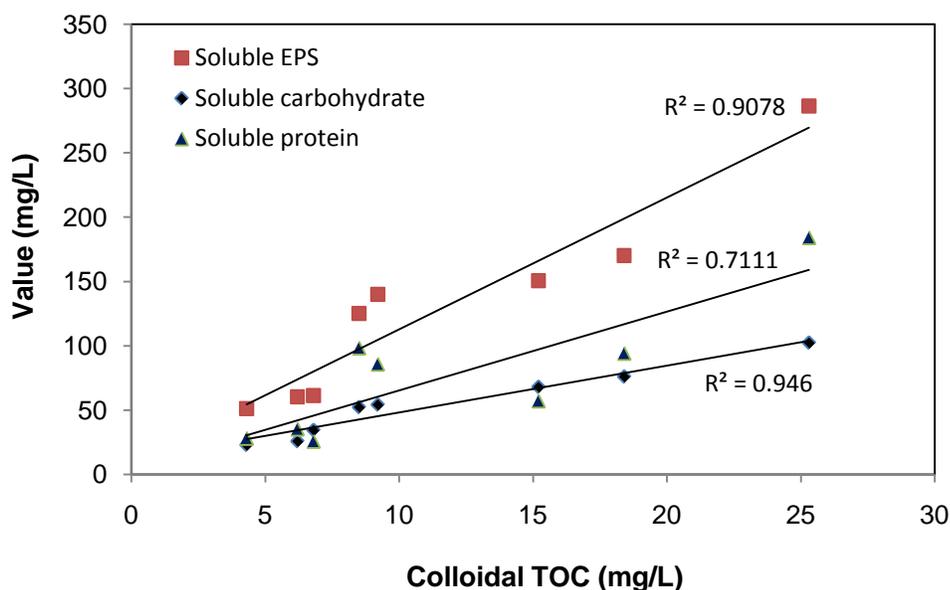
Figure 4.8 Correlation between colloidal TOC and soluble EPS

As shown in Figure 4.8, the linear regression between soluble carbohydrate and colloidal TOC had the highest coefficient of correlation. However, little correlation existed between the soluble protein and colloidal TOC with the coefficient of correlation of 0.7111. Fan et al. (2006) strongly suggested colloidal TOC as a reliable indicator for membrane fouling in MBRs. Therefore, soluble carbohydrates would have greater impact on membrane fouling than would soluble proteins.

4.4.6 Effect of PAC dosing on particle size distribution

PAC dosing could affect the flocs size of the activated sludge in the bioreactor (Li et al., 2005; Park et al., 1999). Figure 4.9 shows the floc size distribution in both the PAC-MBR and the control MBR systems. In comparison with the MBR sludge, the particle size distribution of the PAC-MBR sludge was shifted to a relatively higher range. The

mean floc size of the PAC sludge was 60.3 µm, higher than that of the control MBR sludge 49.4 µm. This could be explained by the fact that the dosed PAC could adsorb dissolved organics, colloidal particles and free bacteria onto the PAC-MBR sludge surface. Thus the overall particle distribution with PAC dosing was changed to a greater particle size range. Lim and Bai (2003) suggested that smaller particles could cause more severe membrane fouling than larger particles. Therefore, the larger particle sizes of the PAC-MBR sludge would be beneficial to alleviate membrane fouling during MBR operation.
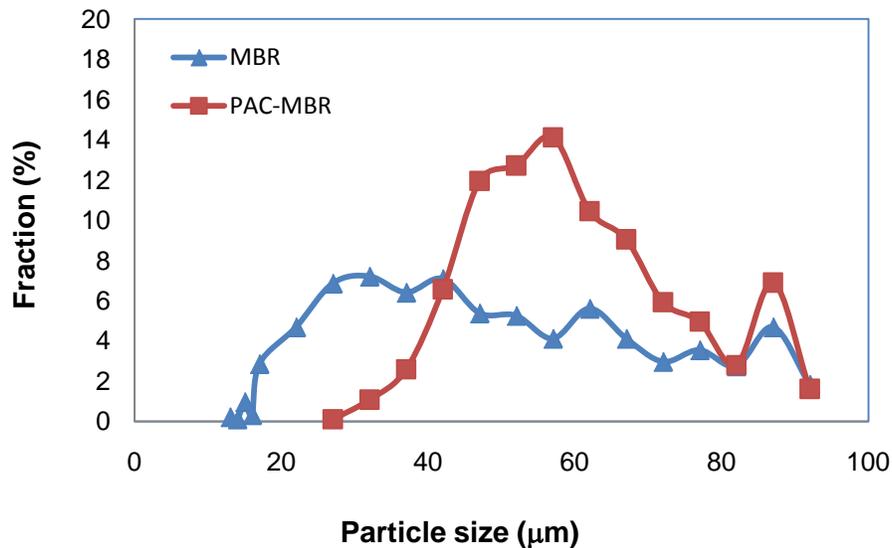
Figure 4.9 Comparison of particle size distribution between the MBR and PAC-MBR sludge

4.4.7 Effects of PAC dosing on estrogen removal

In order to further understand the effects of PAC dosing on the estrogen removal in MBR systems, mass balances of E2 and EE2 in the control MBR and PAC-MBR were performed by multiplying average concentrations of E2 and EE2 with daily flow rates.

Figure 4.10 shows the results of the mass balance for E2 in the MBR and PAC-MBR. It was found that an average of 11.0 and 7.6% of E2 in the influent was detected in the MBR effluent and PAC-MBR effluent, respectively. By wasting sludge every day, 1.3 and 3.0% of E2 in the influent was removed from the MBR and PAC-MBR, respectively. This resulted in that about 88% of E2 were removed by biodegradation or volatilization in both the MBR and PAC-MBR systems.

As shown in Figure 4.11, an average of 29.1% and 13.3% of EE2 in the influent was detected in the MBR effluent and PAC-MBR effluent, respectively. By wasting sludge every day, an average of 4.1 and 17.1% of EE2 in the influent was removed from the MBR and PAC-MBR, respectively. This indicates that PAC dosing increased the adsorption of EE2 to the wasted sludge by 13.0%, much higher than the increase rate of 1.7% for E2 by PAC dosing. It was also found that about 68% of the EE2 was removed by the mechanisms of biodegradation or volatilization in the MBRs regardless of PAC dosing. Because estrogens have very low Henry's law constants, the removal of estrogens from wastewater by volatilization was likely to be negligible (Khanal et al., 2006). Therefore, biological degradation was the dominant mechanism for the removal of estrogens in MBRs.The similar removal rates of E2 and EE2 by biological degradation in the control MBR and PAC-MBR indicated that PAC dosing had little impact on the biomass biodegradation ability.
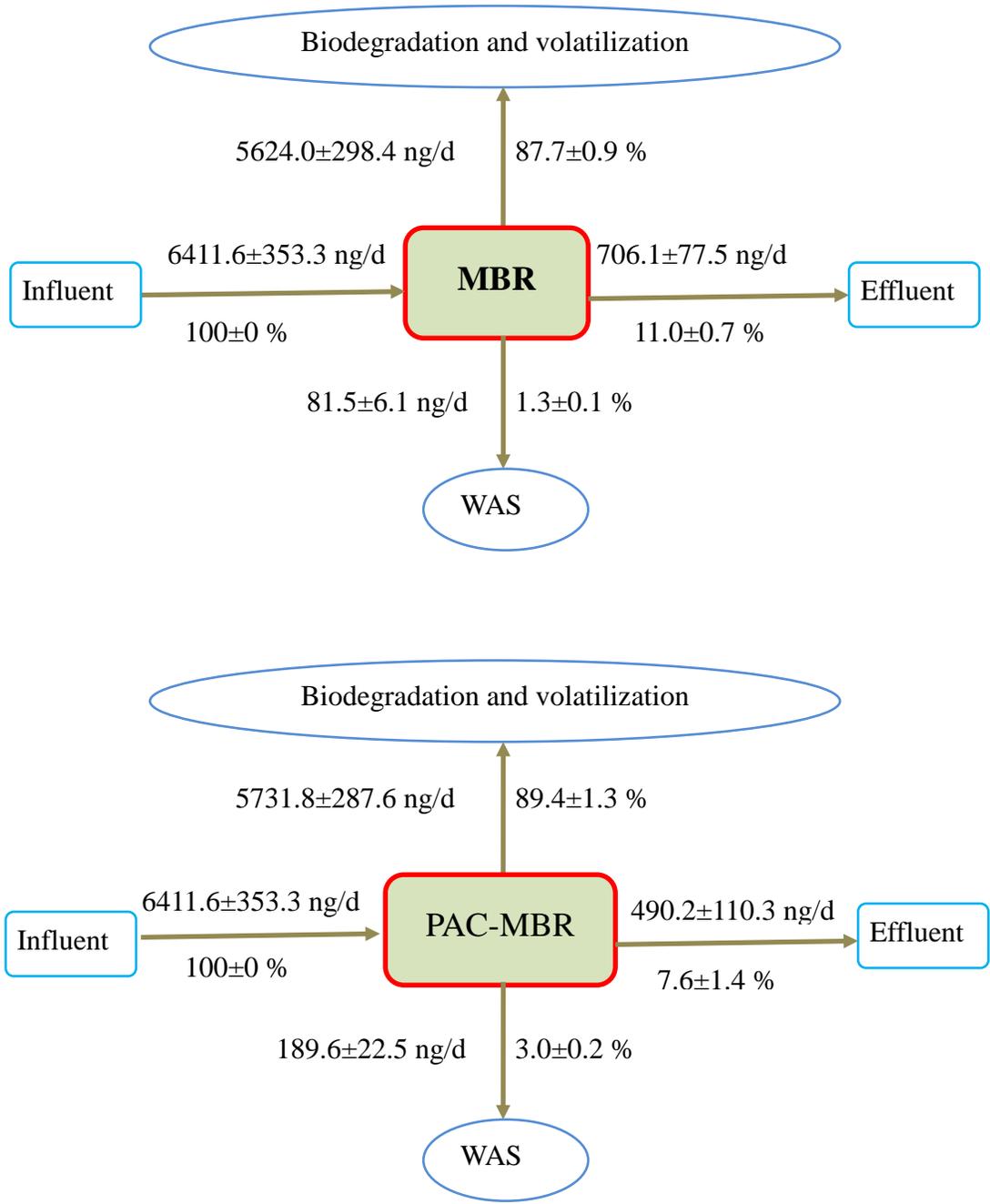
Figure 4.10 Mass balance of E2 in the MBR and PAC-MBR
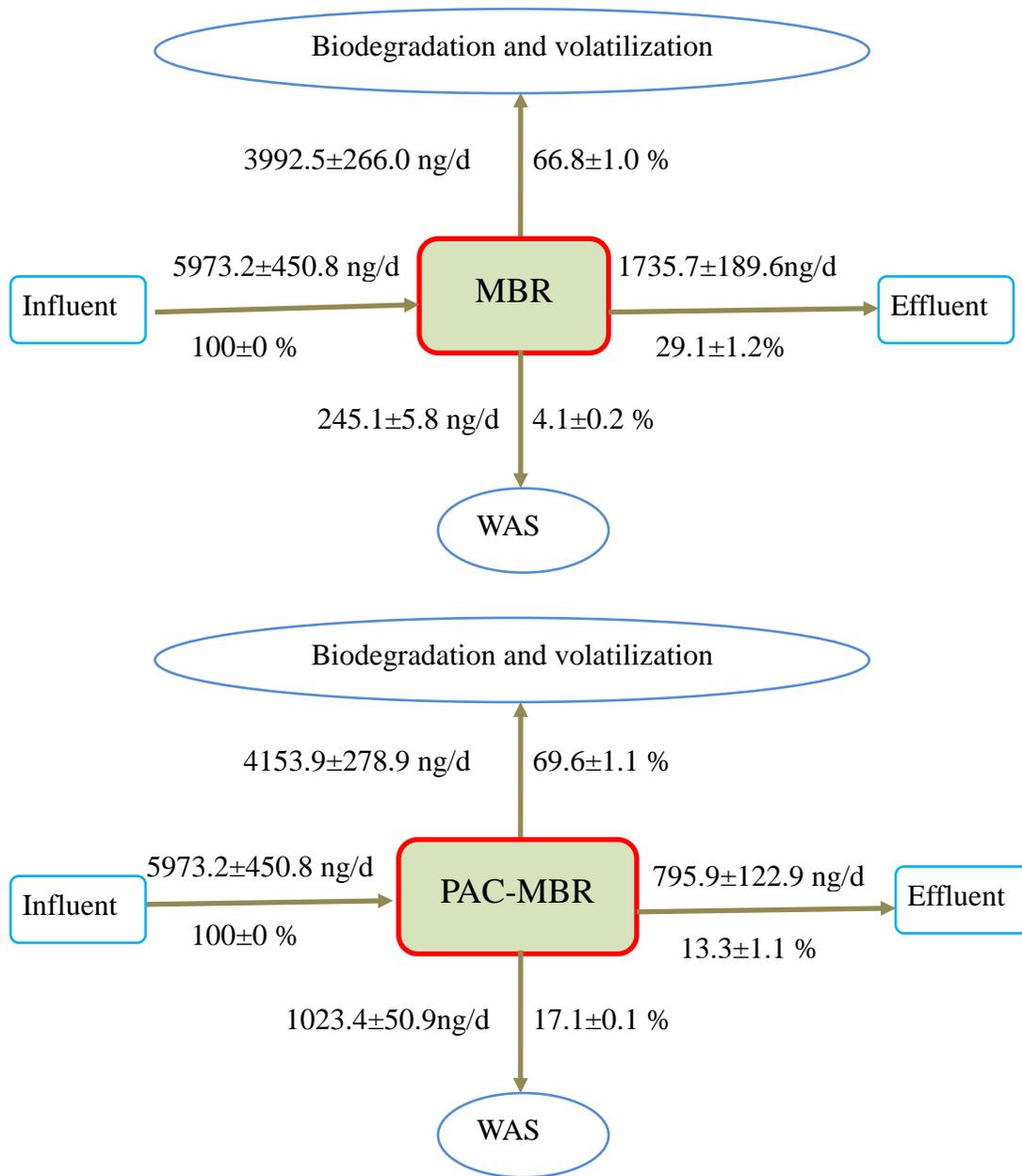
Figure 4.11 Mass balance of EE2 in the MBR and PAC-MBR

Assuming that the removal of estrogens in MBRs by volatilization was negligible, an average of 87.7% E2 and 66.8% EE2 would be removed by biological degradation in the control MBR. Joss et al. (2004) have confirmed that biodegradation of estrogens at

low concentrations followed up pseudo-first-order kinetics. By fitting the experimental data into the pseudo-first-order model, the biodegradation constant k_{bio} for E2 and EE2 was calculated to be 8.38 and 4.41 d^{-1}, respectively. Those values were consistent with the results of Urase and Kikuta (2005) who studied the contribution of adsorption and degradation to the removal of estrogens in activated sludge process. In comparison with the biodegradation rates between E2 and EE2, higher biodegradation rates of E2 were found than EE2. This might be due to the more complicated chemical structure of EE2. Ternes et al. (1999) investigated the persistence of natural estrogens and contraceptives under aerobic conditions using an experimental activated sludge system. The results showed that while in contact with activated sludge, E2 was degraded by the transformation of E2 to a much less estrogenic product E1. On the other hand, EE2 was principally persistent under the selected aerobic conditions in the batch experiments.

It was also found that PAC dosing had a greater impact on the removal of EE2 by wasting sludge than E2. Based on the concentrations of E2 and EE2 in liquid and solid phase, the observed adsorption coefficients k_D for E2 and EE2 to the MBR and PC-MBR sludge were calculated. The results were presented in Table 4.5. The values of observed k_D for E2 and EE2 to the MBR sludge were in good agreement with the results of Clara et al. (2005b). The difference of k_D between E2 and EE2 might be due to their difference in hydrophobic properties, which significantly affect their adsorption capacity to the sludge and PAC. Natural estrogen E2 is considered to be weakly hydrophobic. The hydrophobicity of EE2 is approximately 10 times greater than E2 (Lai, et al., 2000). Therefore, removal of EE2 by sorption to sludge could play a more

important role in the removal mechanisms of estrogens in biological wastewater treatment processes. The enhanced adsorption capacity of PAC-MBR sludge could increase the amount of EE2 being adsorbed onto the sludge.

Table 4.5 Results of the concentrations of E2 and EE2 in liquid and solid phase and their observed adsorption coefficients k_D in the MBR and PAC-MBR sludge

	MBR Sludge			PAC-MBR Sludge		
	Liquid (ng/L)	Solid (ng/gTSS)	Observed k_D (L/gTSS)	Liquid (ng/L)	Solid (ng/gTSS)	Observed k_D (L/gTSS)
E2	92.8±9.0	85.0±4.8	0.916	81.5±13.6	136.2±9.9	1.671
EE2	187.8±21.6	268.8±4.3	1.431	184.0±17.9	758.6±37.0	4.123

It was reported that activated carbon (AC) had very high adsorption capacities for estrogens, with a maximum adsorption constant 12.2 L/g for E2 (Zhang and Zhou, 2005). Assuming that the PAC dosed in the MBR reached its maximum adsorption capacity 12.2 L/g-AC for E2, the amount of E2 removed by the adsorption to the PAC in the wasted PAC-MBR sludge should be 238.6 ng/d (81.5 ng/L × 0.24 g/d × 12.2 L/g). The calculated amount was much higher than the observed amount 108.1 ng/d, which was the difference of the removal amounts of E2 by WAS between the MBR and PAC-MBR (Figure 4.10). The lower observed value was likely because AC had lower adsorption capacity for E2 in mixed liquors than in water. The maximum adsorption capacities of activated carbon were obtained from the experiments using aqueous solution of E2 in water. The complex composition of wastewater and mixed liquor could reduce the AC's adsorption capacity (Ifelebuegu et al., 2006).

4.5 Conclusions

Two bench-scale MBRs, one without PAC dosing and the other with PAC dosing, were operated in parallel at similar experimental conditions to examine the effects of PAC dosing on the MBR performance and estrogen removal. Positive impacts of PAC dosing on membrane fouling and the removal of the investigated estrogens E2 and EE2 were demonstrated. The following conclusions can be drawn from the study:

(1) Biodegradation was the dominant mechanism for the removal of estrogens in MBRs. PAC dosing in the MBR increased the removal rate of E2 and EE2 by 3.4 and 15.8%, respectively. The greater impact of PAC dosing on EE2 removal was due to its greater hydrophobic property.

(2) PAC dosing could improve the color removal in MBR system. However, PAC dosing could not remove the refractory soluble COD that could not be removed by biodegradation or membrane filtration.

(3) PAC dosing significantly reduced the concentrations of soluble EPS and colloidal TOC in the PAC-MBR sludge. Also, the overall particle size distribution of the sludge with PAC dosing was shifted to a greater particle size range. Those effects resulted in a slower rate of TMP increase in the PAC-MBR, which could prolong the operating period between membrane cleaning events and/or increase the lifespan of membrane modules.

(4) PAC dosing had little impact on the biomass content and the nitrification rate of the sludge in MBRs.

(5) Regardless of PAC dosing, concentrations of colloidal TOC were strongly correlated to soluble EPS, especially to soluble carbohydrate.

CHAPTER 5: UV DEGRADATION KINETICS OF ESTROGENS BY DIRECT PHOTOLYSIS

5.1 Abstract

The fate and behavior of endocrine disrupting compounds (EDCs) in the aquatic environment have gained great interests in past decades. In this study, the degradation kinetics of three estrogens, estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) in de-ionized water by ultraviolet (UV) irradiation was investigated. The experimental results showed both the apparent concentration and estrogenic activity of all three investigated estrogens in water decreased with direct UV irradiation. At the dosage similar to those used in UV disinfection of wastewater effluents for water reuse, significant E1 removal was observed. The removal of E1 was much faster than E2 and EE2 with initial concentrations at both mg/L and μg/L ranges. With 2 min exposure of UV light, at the initial concentration of 1 mg/L, 76% of E1 was removed, while less than 20% of E2 and EE2 were removed. The degradation of the three estrogens in water by UV irradiation followed pseudo-first-order reaction kinetics. The degradation rate constants of E1 were approximately 5-10 times higher than those of E2 and EE2 at various initial concentrations. The estrogenic activities of the intermediate products are negligible and there is no secondary risk in increasing the estrogenic activity as a result of photolysis of estrogens in water under the experimental conditions in this study.

5.2 Introduction

Since the mid-1990s, occurrence and behavior of endocrine disrupting compounds (EDCs) in the aquatic environment, especially in wastewater treatment plants (WWTPs), have gained considerable global attention. Most studies in this field focused on investigating the occurrence or removal efficiencies of EDCs in WWPTs (Baronti et al., 2000; Servos et al., 2005). However, definitive understanding of the fate and removal mechanisms of EDCs in wastewater treatment unit operations has not been established. Efforts are now ongoing to comprehensively understand the fate of EDCs within individual treatment processes and to develop effective ways to reduce them to satisfactory levels (Cicek et al., 2007; Lee et al., 2003b).

The EDCs in the environment include a large variety of chemicals, of which steroid estrogens and alkylphenol polyethoxylates (APEs) and their associated metabolites have gained particular concerns for the aquatic environment. Steroid estrogens include natural estrogens (mainly 17-β-estradiol E2 and estrone E1) and the synthetic estrogen 17-α-ethinylestradiol EE2, which is one of the main components of birth control pills. Although estrogens are present at very low concentrations in the environment (ng/L range), they are responsible for a significant part of the endocrine-disrupting effects seen in the aquatic environment due to their high endocrine-disrupting potency.

Ultraviolet (UV) irradiation has been widely used for microbial disinfection both in drinking water and wastewater treatment due to its disinfection effectiveness in

inactivating a wide range of waterborne pathogens including most viruses, spores and cysts (US EPA, 1999). One of the advantages of UV disinfection over the use of chlorine is that UV irradiation does not generate disinfection by-products that can be harmful to humans or aquatic life. In addition to its effectiveness through inactivating microorganisms, UV irradiation can also cause degradation of organic compounds by direct or indirect photolysis as a consequence of UV light absorption. Recently, a limited number of researchers have investigated the degradation of estrogens by UV irradiation. Liu and Liu (2004) studied the direct photolysis of natural estrogens E1 and E2 in aqueous solutions irradiated by UV-light and UV-Vis-light in a batch laboratory-scale UV-irradiation system. The results showed that both E1 and E2 concentrations decreased after irradiation with the UV lamp. However, the doses used in this study were from 900 to 5400 mJ/cm^2, which were much higher than those commonly used for WWTP effluent disinfection (i.e., 20 to 120 mJ/cm^2). In another bench-scale study conducted by Rosenfeldt and Linden (2004), it was reported that UV treatment alone for EDC removal might not be effective. However, UV-based advanced oxidation processes, including the photo-Fenton system and TiO_2 photo-catalysis, were reported to degrade EDCs more effectively than direct UV photolysis alone (Benotti et al., 2009; Chen et al., 2006; Ohko et al., 2002). In a study by Cicek et al. (2007), the fates of E1, E2, and EE2 were evaluated in each treatment process within a full-scale WWTP. No statistically significant impact of the UV disinfection process on E1, E2, and EE2 was observed.

Although considerable work has been done to investigate the occurrence and removal of estrogens in the activated sludge process, the fate of specific estrogens and overall

estrogenic activity in the UV irradiation process has not been definitely understood. Since UV has been more widely used as an option for disinfection of drinking water and wastewater, further study in this area is required. The objectives of this study were to establish degradation kinetics of three estrogens E1, E2 and EE2 in water by UV irradiation.

5.3 Materials and Methods

5.3.1 Estrogen solutions

Stock solutions of E1, E2 and EE2 (Sigma-Aldrich) were prepared in methanol at the concentration of 1000 mg/L. The working solutions of estrogens (E1, E2 and EE2) at designed concentrations in de-ionized water were prepared at desired concentrations shortly before each irradiation experiment.

5.3.2 UV irradiation and sample preparation

The UV irradiation system (Trojan UVMax B), which consisted of a stainless steel reactor and a high-output UV lamp, was obtained from Trojan Technologies (Canada). For each irradiation experiment, 1.2 L of freshly prepared working solution was transferred to the reactor and irradiated for a given time period. The effect of initial concentration of estrogen on the degradation was investigated by repeating the procedure with estrogen solutions at various concentrations. Samples were taken out

from the reactor at time 0 min (prior to UV irradiation) and then regularly at designed intervals up to 8 min throughout the experiments. Estrogen solutions at two different concentration ranges were tested by using two separate analytical tools. The samples at mg/L range in water were mixed with methanol in 1:1 (v/v) and measured by high performance liquid chromatography (HPLC) analysis. The samples with an initial concentration of 1 µg/l in de-ionized water were analyzed in terms of estrogenic activity (EA) by the yeast estrogen screen (YES) assay. Details of the YES assay were described in Chapter 2.

5.3.3 HPLC analysis

The concentrations of E1, E2 and EE2 in water were measured at the mg/L range by a HPLC workstation (DX500, Dionex) equipped with an AD20 absorbance detector. A RP-C18 reverse phase column served as the stationary phase, with 1 ml/min of eluent consisting of an acetonitrile to Milli-Q water ratio of 65 to 35%. Samples were injected in 0.5 ml methanol: water (1:1, v/v) with a 100 µL loop.

5.4 Results and Discussion

5.4.1 Direct photolysis of estrogens E1, E2 and EE2 in water

Figure 5.1 and Figure 5.2 present normalized concentrations of E1, E2 and EE2 with 0-8 min UV irradiation at initial concentration of 1 mg/L and 1µg/L, respectively. The

error bars in the figures represent standard deviations of the results from triplicate irradiation experiments.

As shown in the figures, both the apparent concentration and estrogenic activity of all three investigated estrogens E1, E2 and EE2 in aqueous solutions decreased with direct UV irradiation. The removal of E1 was faster than the other two estrogens E2 and EE2 with initial concentrations at 1.0, 3.0, 5.0 mg/L and 1.0 μg/L. With 2 min exposure of UV light, at the initial concentration of 1 mg/L, 76% of E1 was removed while less than 20% E2 and EE2 were removed. In the case of the starting concentration of 1 μg/L, removal rates of E1, E2, and EE2 with 2 mins exposure of UV light were 62, 23 and 25% respectively. This indicates UV irradiation will be more effective for water streams containing primarily E1, which is beneficial for irradiation of wastewater treatment plant effluents as a greater quantity of E1 than E2 is commonly found in WWTP effluents (Desbrow et al., 1998). The degradation trends of E2 and EE2 by direct UV irradiation were very similar, which might be due to their similar phenolic structures. This finding is in agreement with the result in a previous study that no statistically significant difference between the removal rates of E2 and EE2 by UV photolysis was observed (Rosenfeldt et al., 2007). Even at irradiation times less than 0.5 min, at which the dosage is close to those widely used in UV disinfection systems for water reuse, considerable E1 removal can be expected.
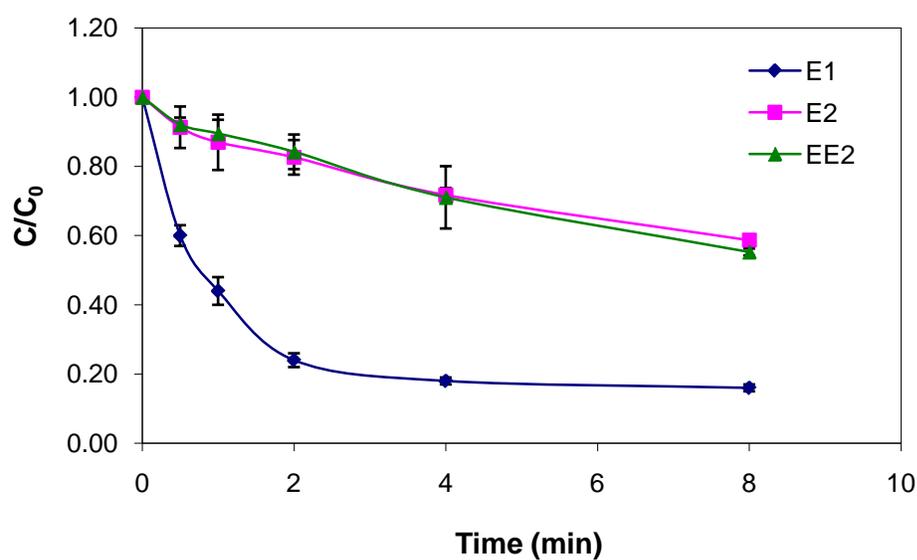
Figure 5.1 Photolysis of E1, E2 and EE2 at the initial concentration of 1 mg/L
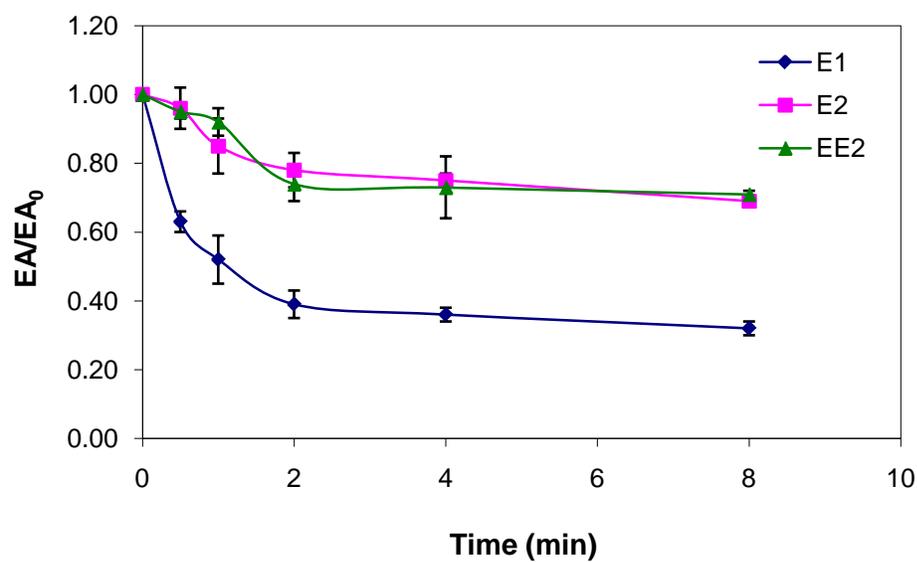
Figure 5.2 Photolysis of E1, E2 and EE2 at the initial concentration of 1 µg/L

The mechanisms of degradation of selected EDCs by UV irradiation are hypothesized as the breakage of benzene rings and the oxidation of the radical groups (-OH) to produce compounds containing carbonyl groups (Liu and Liu, 2004). It was reported that the phenol moiety of aromatic compounds might play a key role in their estrogenic activity (Ohko et al., 2002). In practice, most EDCs with potent estrogenic activity, including the three estrogens investigated in this paper, have phenol groups. On the contrary, other compounds having similar steroid structure but without phenol groups generally exhibit lower or no estrogenic activity. The reduction of EA by UV irradiation indicates that UV light might be capable of destroying the phenol moiety of estrogens.

5.4.2 Degradation kinetics of estrogens in water by UV irradiation

The photolysis results were plotted as time t against $\ln(C/C_0)$ for each experiment carried out, where C is the concentration of estrogens at time t and C_0 is the initial concentration of estrogens. The best fit line was drawn. Rate constants k were determined from the negative slope and shown in Table 5.1.

The linear relationship between $\ln(C/C_0)$ and t indicates the degradation of three estrogens by UV irradiation follows pseudo-first-order reactions. The photolysis rate constant k decreased with increasing initial concentration C_0 of estrogens, especially for E1. This is consistent with most cases of UV photolysis of organic compounds reported previously (Liu and Liu, 2004; Pereira et al., 2007). The mechanisms of photo-degradation of organic compounds are usually complex. The first order kinetics is

apparently tenable, where photolysis rate constants vary with initial concentrations. The results also show that the degradation rate constants of E1 were approximately 5-10 times higher than those of E2 and EE2 at various initial concentrations.

Table 5.1 Values of rate constant k of E1, E2 and EE2 at various initial concentrations

Estrogen	C_0 (mg/L)	k (min^{-1})	R^2
E1	1.0	0.69	0.984
	3.0	0.51	0.981
	5.0	0.37	0.995
E2	1.0	0.063	0.973
	3.0	0.06	0.995
	5.0	0.053	0.995
EE2	1.0	0.072	0.992
	3.0	0.062	0.996
	5.0	0.059	0.991

5.4.3 Inhibition of the photolysis of E1 in water

Since direct UV irradiation is much more effective in reducing E1 than E2 and EE2 in water, the effects of initial concentration and occurrence of the other two estrogens on the photolysis of E1 by UV irradiation were investigated. Figure 5.3 and Figure 5.4 present concentration profiles of E1 only in water and E1 present along with E2 and EE2 at the same initial concentrations during UV irradiation. It is shown that the removal efficiencies of E1 by UV irradiation decreased with increased initial concentration regardless of E1 being present alone in water or co-present with other estrogens.

When E1 was present in solution along with E2 and EE2, the removal efficiencies of E1 by UV direct irradiation were lower than those when E1 was in water alone. This indicates the occurrence of other compounds will impact the photolysis of a specific estrogen. The irradiation of E2 or EE2 alone in water did not produce any detectable E1 (data not shown), which indicates that inhibition might be caused by E2 or EE2 competing with E1 for absorbing UV light. This is more apparent at higher concentrations of estrogens. At the initial concentration of 5.0 mg/L, 52% of E1 was removed from its pure solution with 2 min exposure of UV light; while only 29% of E1 was removed when the same initial concentration of E2 and EE2 were co-present in the solution. In the case of treating actual wastewater, the presence of a variety of components in wastewater might make the competing absorption of UV light more complex. These components might have effects on the extent of estrogens removal by UV irradiation.
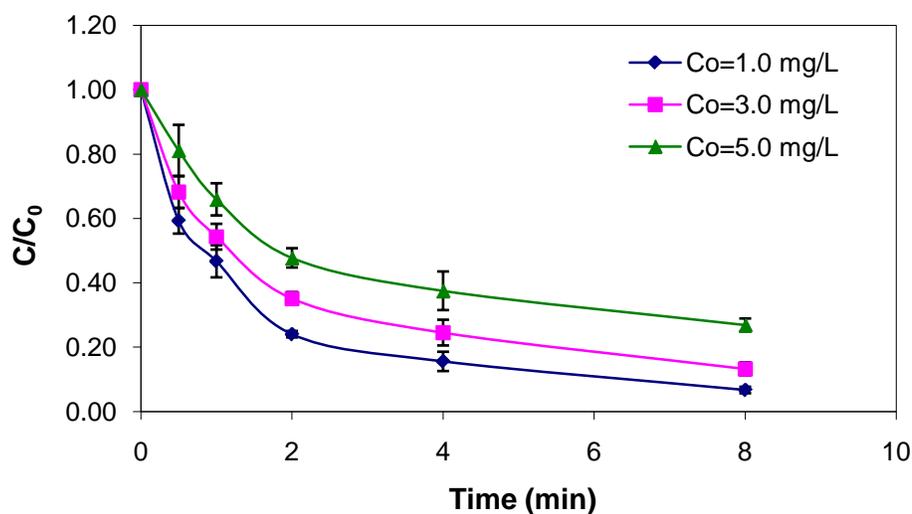
Figure 5.3 Normalized concentration profile of E1 only in water irradiated by UV
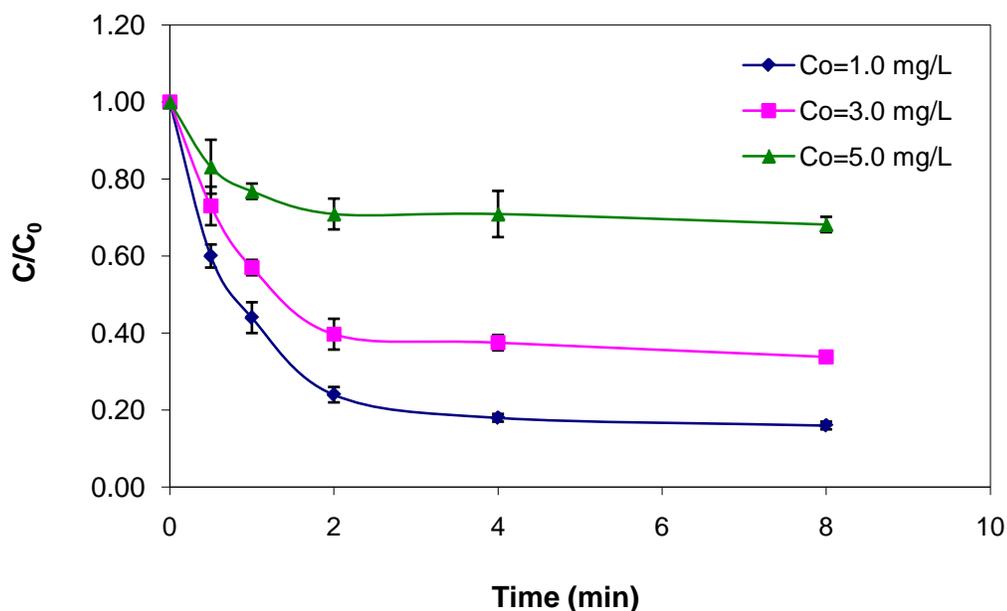
Figure 5.4 Normalized concentration profile of E1 present along with the same initial concentration of E2 and EE2 irradiated by UV

5.4.4 Estrogenic activity of photolysis products

To investigate if the products of UV photolysis of estrogens in water possess any estrogenic activity, the solutions of individual estrogens irradiated after 8 min irradiation of UV light were measured both in terms of concentration by HPLC analysis and in terms of EA by YES assay (Figure 5.5). The initial concentration of estrogens was 3 mg/L. The samples before and after UV photolysis were diluted in de-ionized water for the YES assay.
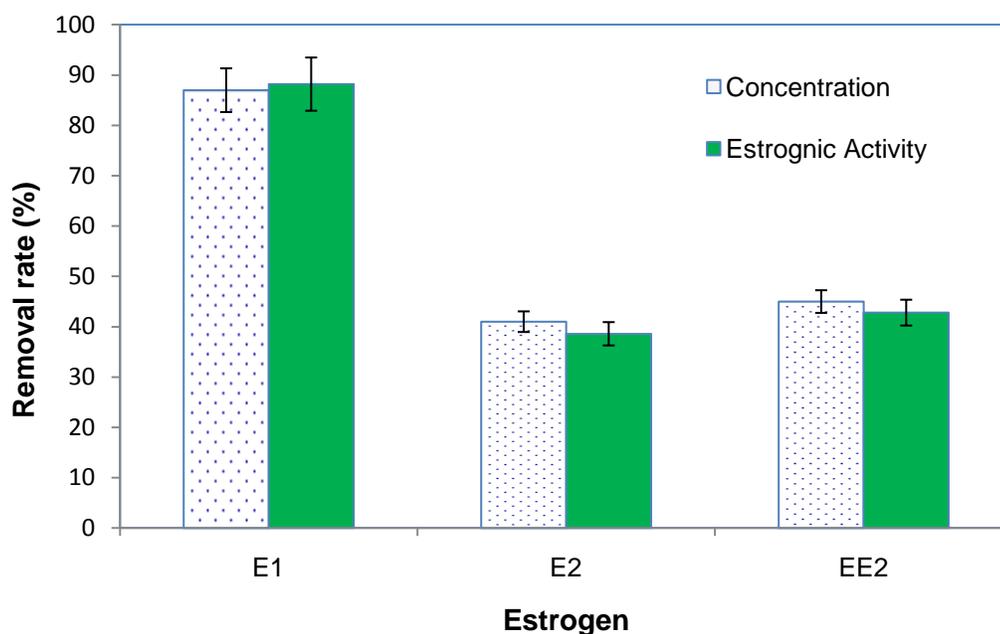
Figure 5.5 Comparison of removal rates in terms of concentration and estrogenic activity

With 8 min exposure of UV light, the removal rates of three estrogens in terms of concentration and estrogenic activity were similar for all three investigated estrogens. This indicates that the estrogenic activities of the products are negligible and there is no secondary risk in increasing the estrogenic activity as a result of photolysis of estrogens in water under the experimental conditions in this study. If the intermediate products have appreciable estrogenic activities, the removal rates in terms of estrogenic activity should be lower than those in terms of concentration. It should be pointed out that the irradiation experiments of individual estrogens were conducted separately. The interference between the estrogens (estrogens co-present in water) was not investigated in this research.

5.5 Conclusions

Direct UV irradiation was effective in reducing all three investigated estrogens E1, E2 and EE2 in water both in terms of apparent concentration and in terms of EA. At initial concentrations of both mg/L and μg/L ranges, the removal of E1 was much quicker and more effective than E2 and EE2. With 2 min exposure of UV light, at the initial concentration of 1 mg/L, 76% of E1 was removed while less than 20% E2 and EE2 were removed. At the initial concentration of 1 μg/l, the removal rates of E1, E2, and EE2 after 2 min of UV irradiation were 62, 23 and 25%, respectively.

The degradation of three estrogens by UV direct irradiation followed pseudo first order reaction kinetics. The degradation rate constants of E1 were approximately 5-10 times higher than those of E2 and EE2 at various initial concentrations. The estrogenic activities of UV photolysis products of individual estrogens E1, E2 and EE2 in water by UV irradiation under the experimental conditions were negligible. The co-presence of estrogens in solution could inhibit the photolysis of certain estrogens by UV irradiation.

CHAPTER 6: INVESTIGATION OF ESTROGENIC ACTIVITY OF WASTEWATER THROUGH UV DISINFECTION IN WASTEWATER TREATMENT PLANTS

6.1 Abstract

Endocrine disrupting compounds (EDCs) in the environment is presently of great concern all over the world. In this study, The estrogenic activity (EA) of pre-UV and post-UV samples from five wastewater treatment plants were measured in both liquid and solid phase by Yeast Estrogen Screen assay. Over 95% EA of wastewater, both in pre-UV and post-UV samples, is associated with liquid phase. In liquid phase, the EA of filtered wastewater decreased slightly through UV disinfection in three plants NE, SE and ML; whereas the EA of filtered wastewater increased through UV disinfection in the other two plants PT and BD. In the plant BD, the EA in liquid phase increased by 41.3% through UV disinfection, with an average 96.0 ng/L in pre-UV samples and 135.6 ng/L in post-UV samples, respectively. In solid phase, EA of wastewater decreased after UV disinfection in all five investigated plants.

6.2 Introduction

Endocrine disrupting compounds (EDCs) in the environment have recently emerged as one of the major environmental issues around the globe. EDCs in the environment could have long-term impacts on the health of wildlife and human beings. Despite controversial opinions on the impact of EDCs on human beings and overall ecology, the adverse effects on aquatic species such as fish have been well documented. The negative impacts include the feminization due to hormonal imbalance and reduced reproductive success in fish and avian (Jobling et al., 1998; Rodgers-Gray et al., 2000). Some researchers have attributed decreases in human sperm quality and quantity over the past 5 decades to EDCs in the environment. Likewise, it has been suggested that sharp increases in breast, testicular, and prostate cancers reported over the past 40 years are related to EDCs in the environment. However, this view is not well supported by experimental data. Other scientists believe that it is unlikely that these effects would be caused by EDCs in water due to their extremely low concentrations and small doses compared to estrogenic compounds present in food sources (Snyder et al., 2003).

It has been widely accepted by scientists that the most likely source of EDCs in the aquatic environment is from discharge of municipal and industrial effluents along with runoff from agricultural production (Hewitt and Servos, 2001). EDC discharge through wastewater treatment effluent has been well-documented in many countries around the world. Efforts are now ongoing to comprehensively understand the fate of EDCs in the aquatic environment, especially in the wastewater treatment processes, and to develop effective ways in reducing them to environmentally satisfactory levels (Cicek et al.,

2007; Lee et al., 2003b).

Ultraviolet (UV) irradiation has been widely used for microbial disinfection both in drinking water and wastewater treatment due to its disinfection effectiveness in inactivating a wide range of waterborne pathogens. One of the advantages of UV disinfection over the use of chlorine is that UV irradiation does not generate disinfection by- products that can be harmful to humans or aquatic life. In addition to its effectiveness through inactivating microorganisms, UV irradiation can also cause degradation of organic compounds, including some EDCs, by direct photolysis as a consequence of UV light absorption. Recently, a limited number of researchers have investigated the degradation of estrogens by direct UV irradiation or UV-based advanced oxidation processes (Liu and Liu, 2004; Rosenfeldt et al., 2007). A conclusion has been drawn that UV irradiation can somehow decrease concentrations of EDCs, even though the doses in these studies for EDCs reduction were much higher than those commonly used for wastewater effluent disinfection.

However, all of above studies were based on EDCs in water solutions. Little is known about the fate of specific estrogens and overall estrogenic activity (EA) in wastewater in the UV irradiation process. In a research conducted on the removal of estrogens during lab-scale UV disinfection studies, two doses were investigated with multiple replicates. In each case, some results showed removal occurred while others showed an increase in concentration during UV process (Birkette and Lester, 2003). Since the use of UV has been expanding as an option for disinfection of drinking water and wastewater, further

study in this area is required. In a study conducted by Cicek et al. (2007), the fates of three estrogens were evaluated around each treatment process within a full-scale wastewater treatment plant (WWTP); it was found impact of the UV disinfection process on the investigated estrogens was not statistically significant. The objective of this study was to investigate the impact of UV disinfection on the overall EA of secondary effluents from WWTPs.

6.3 Materials and Methods

6.3.1 Description of the investigated WWTPs

The investigated five WWTPs, all located in Manitoba, include four municipal and one industrial WWTP. The secondary treatment methods are activated sludge process in three plants and sequencing batch reactor in the other two plants. All five plants are using Trojan UV systems at similar UV dosages. Four of them are using polychromatic light UV lamps, whereas the plant PT is using monochromatic light lamps. The specifications of the five WWTPs and UV systems are summarized in Table 6.1.

Table 6.1 Specification of the five investigated WWTPs and UV systems

WWTP	NE	SE	PT	BD	ML
Flow (MLD)	250	65.0	15.5	16	5.5
Wastewater sources	55% residential+ 45% others*	70% residential+ 30% others*	36% municipal+64 % industrial	Mixed**	Industrial (Slaughter ing)
Secondary treatment	Activated Sludge	Activated Sludge	Sequencing Batch Reactor	Sequencing Batch Reactor	Activated Sludge
UV Manufacturer	Trojan	Trojan	Trojan	Trojan	Trojan
UV product	UV4000 Plus	UV4000	UV3000	UV4000	UV4000
UV lamp type	Medium pressure	Medium pressure	Low pressure	Medium pressure	Medium pressure
UV dose (mJ/cm^2)	35	35	30	28.9	29.8

* Including commercial, industrial and infiltration

** Mixture of municipal and industrial wastewater, but the proportion is not known.

6.3.2 Sample preparation and analysis

Pre-UV and post-UV samples were collected from five WWTPs in grab fashion. All containers used during sampling and storage in this study were glass bottles and were acid-washed and rinsed with 50% methanol before use. Sampling from all five WWTPs took place three times, and pre-UV and post-UV samples from each sampling were analyzed in duplicate.

Portion of samples were filtrated by GF/C filters. The GF/C filters were pre-treated by heating in a furnace at 550ºC for 1h to avoid any EDCs in filters. The filtrates and filters with solids from samples were then extracted by cyclohexane extraction for the

enrichment of EA. During extraction, a 20 ml filtrate or filter with solids from samples was placed in a 125 ml conical flask along with 10 ml cyclohexane. The flasks were shaken for 3 h on a vertical shaker. After shaking, 4 ml of cyclohexane were extracted from each conical flask, dried down under N_2 stream, and reconstituted in 0.2 ml absolute ethanol for the determination of EA by yeast estrogen screen (YES) assay. Details of the YES assay were described in Chapter 2.

6.4 Results

6.4.1 Characteristics of pre-UV samples

Some characteristics of pre-UV samples from the five investigated WWTPs, including temperature, pH, total suspended solids (TSS), UV transmittance and chemical oxygen demand (COD), were presented in Table 6.2. UV transmittances of pre-UV wastewater in the investigated plants were in the range of 49.3-70.8%. TSS of pre-UV samples from the plant NE was higher than those from other plants.

Table 6.2 Characteristics of pre-UV samples from the five investigated WWTPs

WWTP	NE	SE	PT	BD	ML
Temperature (ºC)	16.5	17.0	18.0	12.0	26.0
pH	6.8	7.1	7.0	7.2	7.7
TSS (mg/L)	13.0	3.3	4.3	5.4	3.3
UV Transmittance (%)	49.3	51.6	58.5	61.5	70.8
COD (mg/L)	57	65	25	40	22

6.4.2 Estrogenic activities of wastewater in liquid phase

The EA of pre-UV and post-UV filtered samples from five WWTPs are comparatively presented in Figure 6.1. Except much higher EA of wastewater from the plant BD, the EA of wastewater from the other four plants were comparable with the results reported in previous studies (Holbrook et at., 2002). As shown in Figure 6.1, EA of filtered wastewater slightly decreased through UV disinfection in three plants NE, SE and ML; whereas EA of filtered wastewater increased through UV disinfection in the other two plants PT and BD. The increase of EA through UV disinfection was slight in the plant PT. However, the increase of EA through UV disinfection was significant in the plant BD. The EA of filtered wastewater in the plant BD increased from an average 96.0 ng/L in pre-UV samples to an average 135.6 ng/L in post-UV samples.
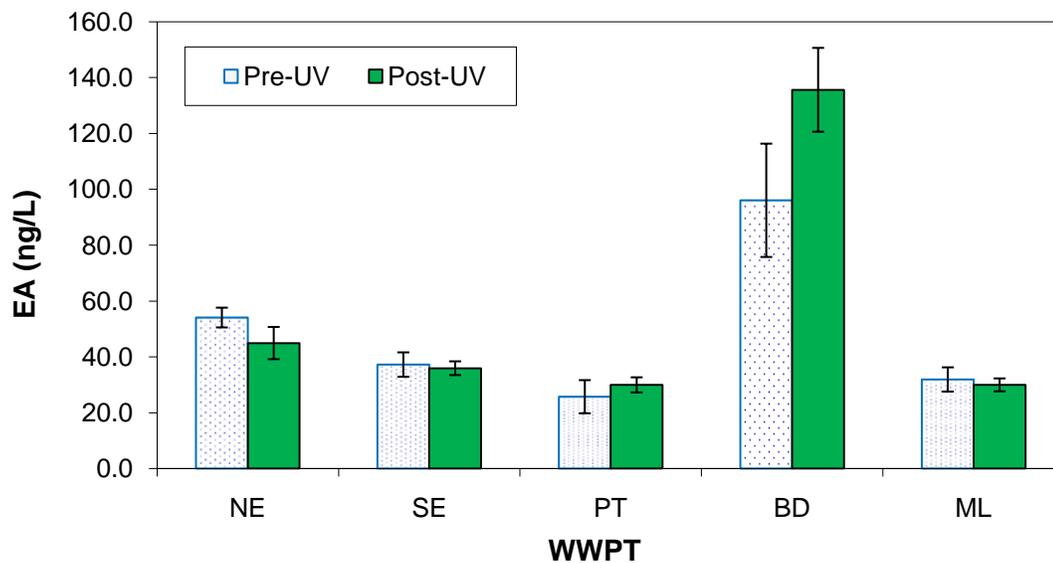
Figure 6.1 Estrogenic activities in liquid phase of wastewater from the five investigated WWPTs

6.4.3 Estrogenic activities of wastewater in solid phase

Unlike the inconsistency of wastewater EA in liquid phase during UV disinfection observed between the investigated WWTPs, the EA of wastewater in solid phase decreased after UV disinfection in all five plants (Figure 6.2). It is also shown that the EA of wastewater in solid phase, for both pre-UV and post-UV samples, were much lower than that in liquid phase.
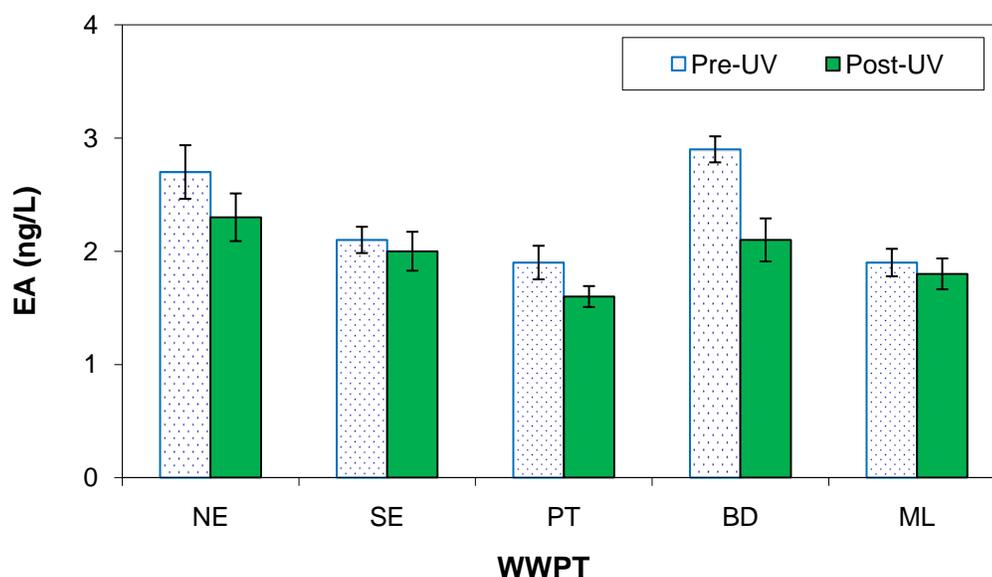
Figure 6.2 Estrogenic activities in solid phase of wastewater from the five investigated WWPTs

6.4.4 Change of estrogenic activity by UV disinfection

Change of wastewater EA in liquid phase, solid phase and total through UV disinfection at five WWPTs were presented in Figure 6.3. Positive values indicate the EA of wastewater increased, whereas negative values indicate the EA of wastewater decreased through UV disinfection. The total EA of wastewater from the plants NE, SE and ML

decreased 3.6-16.9% through UV disinfection. As over 95% EA of wastewater is associated in liquid phase, the total EA of wastewater from the plants PT and BD increased through UV disinfection although the EA of wastewater in solid phase from those plants decreased after UV disinfection.
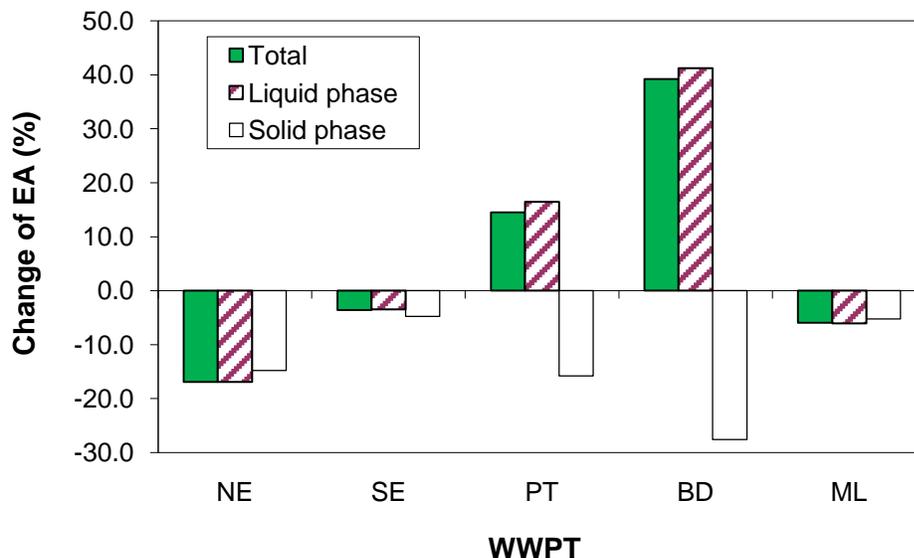
Figure 6.3 Change of estrogenic activity of wastewater in liquid phase, solid phase and total through UV disinfection

6.5 Discussion

In this study, EA was used to represent the estrogenic effects of wastewater and measured by YES assay. The YES assay detects both the parent compounds and any intermediate degradation products that activate estrogen receptors, regardless of their identity. Thus, this approach provides a real indication of the estrogenic effects of UV disinfection on the investigated wastewater; such information cannot be obtained by chemical analysis alone, because some of the intermediate photolysis products that

might possess a certain extent of EA cannot be measured by chemical analysis of their parent compounds. And it is impossible to measure concentrations of all parent estrogenic compounds in wastewater by chemical analysis. However, one of the disadvantages of YES assay is that the identity of compounds which might contribute to EA of wastewater can't be identified.

Among the five investigated WWTPs, two plants showed an increased EA in post-UV samples. This observation is completely unexpected and contradictory with the results of estrogens in water solution by UV irradiation. One possibility for the increase in EA of wastewater effluents after UV irradiation could be the transformation of certain compounds into photolysis products with higher estrogenic potency. It was shown in a previous study (Yang and Cicek, 2007) that the photolysis products of individual estrogens in water did not possess any significant estrogenic activity. Therefore, some compounds other than estrogens might play an important role in the UV disinfection process of wastewater. It was reported that the products from the oxidation of Bisphenol A had estrogenic effect on the aquatic wildlife (Nomiyana et al., 2007). In another research, the experimental results showed UV irradiation increased the toxicity of chlorinated Bisphenol A (Mutou et al., 2006). Further tests are still required in order to determine the fate of individual EDCs in wastewater during UV disinfection process. The wide range of potential photolysis products would make analysis more complicated.

The higher EA and an increase of EA after UV disinfection in the plant BD might be associated with a high industrial wastewater discharge to the sewer system in the city of

BD. Although this hypothesis hasn't been confirmed by data in this study, it is suggested that the test of UV on the estrogenic effects of wastewater should be performed before implementing UV disinfection for wastewater treatment with a high contribution of industrial discharges.

6.6 Conclusions

The EA of pre-UV and post-UV samples from five WWTPs were measured in both liquid and solid phase by YES assay. In liquid phase, EA of filtered wastewater decreased through UV disinfection in three plants NE, SE and ML; whereas the EA of filtered wastewater increased through UV disinfection in the other two plants PT and BD. The increase of EA through UV disinfection is slight in the plant PT. However, the increase of EA through UV disinfection is significant in the plant BD, from an average 96.0 ng/L in pre-UV samples to an average 135.6 ng/L in post-UV samples. In solid phase, EA of wastewater decreased after UV disinfection in all five plants. As over 95% EA of wastewater is associated in liquid phase, the total EA of wastewater from the plants PT and BD increased although the EA of wastewater in solid phase from those plants decreased after UV disinfection.

The reason for the increase in EA of wastewater effluents after UV disinfection is still not clear. It could be related to high industrial wastewater inputs into the wastewater collecting system. Further research is needed to determine the impact of UV disinfection on wastewater quality from WWPTs in terms of estrogenic effects.

CHAPTER 7: ENGINEERING SIGNIFICANCE

EDCs in the environment have recently emerged as a major issue internationally and in Canada. Scientific studies on the impacts of EDCs on wildlife in Canada and around the globe have brought this issue to the forefront. Among the sites and sources identified for potential endocrine disruption in the Canadian aquatic ecosystem were municipal effluents, intensive livestock production areas, and agricultural activities involving pesticides and herbicides. Industrial and municipal sewage effluents constitute a major source of EDCs in aquatic bodies. Thus, increasing attention is now being focused on the role played by industrial and municipal wastewater treatment works in preventing the release of EDCs to receiving bodies. The studies in previous chapters showed MBRs could effectively remove EDCs in wastewater steams and PAC dosing could improve the MBR performance including the enhancement of estrogens removal. It was also found that the impacts of UV on the fate of EDCs in water and wastewater were different, depending on the experimental or field conditions. The objective of this Chapter was to evaluate the cost effectiveness of PAC dosing in MBRs and to provide recommendations for UV system with consideration of EDCs removal.

7.1 Evaluation of Cost Effectiveness of PAC dosing in MBRs

7.1.1 Cost of PAC dosing in MBRs

Membrane bioreactor technology has gained wide appeal both for municipal and industrial authorities in the past two decades. This research investigated the overall performance and specific mechanisms involved in treating EDCs by MBR and MBR-based hybrid systems. It has been found that the MBR, especially the PAC-MBR hybrid process, was effective in removing EDCs from wastewater streams. This may extend the MBR application areas and provide an alternative way for eliminating the adverse effects of EDCs on the environment. Effectively removing EDCs can strengthen the safety of wastewater reuse. If EDCs were regulated in the future, MBR technology and/or PAC-MBR hybrid process would become more competitive in the application field of wastewater reclamation and reuse. Beyond the enhanced EDCs removal, PAC dosing in the MBR also resulted in a slower rate of TMP increase. In practice, a slower rate of TMP increase may significantly reduce the operating cost for membrane cleaning and/or membrane replacement.

Table 7.1 presents operational characteristics of a typical MBR with a capacity of 1000 m^3/d and an operating cost analysis of PAC dosing in such a MBR system at a dosage of 2.0 g/L. The majority of the cost for PAC dosing is the cost for PAC replenishment. The labour cost for PAC dosing is negligible because the PAC replenishment can be done within half an hour every other day, even every five days. Previous studies (Ng et al., 2006; Park et al., 1999) have shown PAC dosing could achieve significant flux

enhancement at a dosage as low as 1.0 g/L. If the dosage of 1.0 g/L was applied for PAC dosing in MBRs, the cost for PAC replenishment would drop by 50% down to 9,235 US$/year.

Table 7.1 Cost analysis of PAC dosing in MBR

Parameter	Value	Comments
MBR Capacity (m^3/day)	1000	Municipal wastewater treatment
HRT (hour)	6	
SRT (day)	25	
PAC dosage (g/L)	2.0	Same with the bench-scale experiment
Cost for initial PAC dosing (US$)	1,265	2.53 US$/kg *
PAC replenishment (kg/day)	20	
Cost for PAC supplement (US$/year)	18,469	2.53 US$/kg *

* CMR Online, http://www.the-innovation-group.com/chemprofile.htm, (March 9, 2009)

7.1.2 Benefits of PAC dosing in MBRs operation

Membrane fouling has been widely considered to be one of the major limitations to faster commercialization of MBRs. Although a number of techniques have been developed to prevent membrane fouling to certain extents, the decrease of membrane permeability is inevitable due to pore clogging, sludge cake formation and bio-fouling. When membrane flux decreases dramatically below the design value, membrane cleaning is required to recover the membrane permeability. Chemical cleaning has been extensively used for recovering membrane permeability. The choice of chemical agents

for recovering membrane flux depends on the nature of the foulants at different MBR systems. There are no generic guidelines for this technique. Operators tend to rely on their experience and technical support from MBR providers or manufacturers. Acids, bases, oxidants and surfactants are typically used agents for membrane cleaning in MBR systems. Sodium hypochlorite solution has been commonly used in commercial MBR systems (Yang et al., 2006). Even with effective membrane cleaning, the membrane modules will need to be replaced somewhere between five and ten years with the current technology (DeCarolis et al., 2007; Fletcher et at., 2007; Gander et al., 2000). While the membrane price has significantly decreased over the past years, these modules can still be classified as expensive (Yoon et al., 2004).

The TMP profile in Figure 4.4 showed a slower rate of TMP increase in the PAC-MBR than the control MBR. This means PAC dosing could prolong the operating period between membrane cleaning events. The membrane manufacturer recommends a membrane cleaning event by soaking membrane modules in 0.3% NaOCl solution for 3-5 hours when TMP reaches 60 kPa. Table 7.2 presents the time (T) for TMP reaching to 60 kPa in the MBR and PAC-MBR in the four membrane cleaning cycles. The data of T were obtained by exponential regression using the daily TMPs in the MBR and PAC-MBR. The R-squared value (R^2) of each regression was shown in Table 7.2 as well.

Table 7.2 Comparison of T between the MBR and PAC-MBR

Cycle of membrane cleanings	MBR		PAC-MBR	
	T (d)	R^2	T (d)	R^2
I	90	0.9663	122	0.9691
II	66	0.9547	84	0.9011
III	67	0.9662	94	0.958
IV	61	0.9105	77	0.9453

As shown in Table 7.2, the average T in the control MBR and PAC-MBR was 71 and 94 d, respectively. This indicates that the time for TMP reaching 60 kPa in a membrane cleaning cycle in the PAC-MBR could be 32.3% longer than the control MBR. Assuming the lifespan of membranes in a MBR is 7 years, the lifespan of membranes in a PAC-MBR would be prolonged to 9.3 years.

The cost for membrane maintenance primarily consists of costs for membrane cleaning and membrane replacement. Table 7.3 summarizes a cost assessment of membrane maintenance in the MBR and PAC-MBR for municipal wastewater treatment with a capacity of 1000 m^3/d. As shown in Table 7.3, the annual depreciation for membrane replacement accounts for over 97% of the cost for membrane maintenance in MBRs. PAC dosing could retard TMP increase in MBRS operation and result in a longer lifespan of membranes, which can reduce annual cost for membrane maintenance by about 25%. The benefit from reducing the operating cost for membrane maintenance by PAC dosing at a dosage of 2.0 g/L in a 1000 m^3/d MBR is 14, 320 US$ per year.

Table 7.3 Cost analysis of membrane maintenance in the MBR and PAC-MBR

Parameter	MBR	PAC-MBR
Treatment Capacity (m^3/day)	1,000	
Operating flux (m^3/m^{2}d)	0.25	
Surface area of membrane (m^2)	4,000	
Capital cost for membrane [a](US$)	400, 000	
Cost for each membrane cleaning [b] (US$)	369	
Membrane lifespan (year)	7	9.3
Cleaning frequency (times/year)	4	3
Cost for membrane cleaning ($/year)	1,476	1,107
Depreciation for membrane replacement ($/year)	57,143	43,192
Total cost for membrane maintenance ($/year)	58,619	44,299

[a] Based on 100 US$/m^2 (Yoon et al, 2004)
[b] Based on labour 2×20US$/h for 8h, NaOCl (12.5%) price 0.64 US$/gallon, 2×2×3m tank for membrane cleaning

7.1.3 Cost effectiveness of PAC dosing in MBRs.

As the discharge of EDCs has not been regulated so far, it is not practical to assess the economic benefits of PAC dosing in improving EDCs removal in MBRs. Therefore, the cost effectiveness of PAC dosing in MBRs was evaluated in terms of the impacts of PAC dosing in MBRs operation.

As shown in Table 7.1 and 7.3, the benefit from reducing the operating cost for membrane maintenance by PAC dosing can offset 77.5% operating cost for PAC replenishment at a dosage of 2.0 g/L. If 1.0 g/L PAC were applied in such a MBR

system and it could achieve similar performance, the benefit of PAC dosing would exceed the operating cost for PAC dosing. It should be pointed out that the cost assessment in this Chapter was based on the bench-scale experimental data. In real applications, the additional costs generated by PAC dosing, e.g. higher aeration cost due to higher concentration of MLVSS, should be considered in order to evaluate the economic feasibility of PAC dosing in MBRs.

Addition of PAC in MBRs could increase the sludge production. The impact of PAC dosing on the cost for sludge processing and disposal need be taken into account. The cost for sludge management normally accounts for approximately 30% of the operating cost of MBRs (Judd, 2006). Assuming an addition of 1 g/L PAC in a MBR with a MLSS of 10 g/L, the mass of sludge produced from the PAC-MBR would increase by 10%. Therefore, the operating cost of a PAC-MBR would increase by about 3.0% due to the management of the increased amount of sludge.

7.2 Recommendations for UV System Design with Consideration of EDCs Removal

An important finding of this research was that the EA of secondary effluents increased after UV disinfection at two of the five investigated WWTPs. In the past decades, UV has been more widely used as an option for disinfection of wastewater effluents due to its advantage of not generating disinfection by-products. However, the observation of the increase of EA after UV disinfection in this research could drive engineers to take

this issue into consideration when they design UV systems for certain wastewater disinfection. Particularly, the two WWTPs associated with EA increases after UV disinfection are both using sequencing batch reactor (SBR) as secondary treatment. The correlation between the change of EA through UV disinfection and biological treatment processes in WWTPs has not been established. Further research is needed to determine the consequent effects of secondary treatment process on the fate of wastewater EA through UV disinfection. In addition, the two WWTPs associated with EA increases after UV disinfection are both located in areas where high percentages of industrial wastewater discharge into the sewer systems. It is suggested for design engineers that the test of UV on the estrogenic effects of wastewater should be performed before implementing UV disinfection for wastewater treatment with a high contribution of industrial discharges, such as pharmaceutical wastewater, food processing wastewater, plastics producing wastewater etc.

The kinetics study of estrogens by UV irradiation may provide valuable information for engineers to design UV systems treating certain kinds of wastewater. With 2 min exposure of UV light, apparent removal of estrogens (particularly E1) was observed in the kinetics study. Although the UV fluence with 2 min irradiation is much higher than the commonly used UV fluence in UV systems for wastewater disinfection, it could be feasible for treating certain kinds of wastewater, e.g. pharmaceutical wastewater with high concentrations of estrogens. In small systems, a longer UV retention time (e.g. 0.5-2 min) would be practical when estrogens are of particular concern.

CHAPTER 8: GENERAL CONCLUSION

8.1 The Context of the Research

The primary objective of this research was to investigate the fate of EDCs in two wastewater treatment processes, i.e. MBR and UV processes. One of the reasons for selecting those two processes is because of the fact that the application of MBR and UV disinfection for wastewater treatment has been expanding rapidly around the world. The investigation was conducted in various experimental facilities at three scale levels, i.e. bench-scale, pilot-scale and full-scale.

Following an introduction (Chapter 1) and description of methodology (Chapter 2), four studies were conducted and presented in four chapters in this thesis. In Chapter 3, the removal of EDCs in terms of overall EA at high concentrations was studied in a laboratory scale MBR for the treatment of swine wastewater. The results were confirmed in a pilot-scale MBR at similar conditions. To reveal specific removal mechanisms of EDCs in terms of individual estrogens at lower concentrations, Chapter 4 showed the study on estrogens removal in submerged MBRs using membrane modules with a thin-spread shape. Synthetic wastewater spiked with two estrogens E2 and EE2 was used in this study. Meanwhile, the feasibility of PAC dosing in MBR to enhance EDCs removal was evaluated by long term operation of the two MBRs, one with PAC addition and the other one without PAC addition.

The studies on EDCs in UV process were presented in the following two chapters. In Chapter 5, a basic study on UV degradation kinetics of estrogens was introduced. The findings from the kinetics were the basis of the further research on the fate of EDCs through the UV process. In order to gain a better understanding of the impacts of UV disinfection on the fate of EDCs in wastewater, a survey on estrogenic activity in UV disinfection at five full-scale WWTPs was conducted and presented in Chapter 6. This survey revealed that the fate of EDCs in terms of estrogenic activity through UV disinfection depended on the field conditions at certain WWTPs.

8.2 Major Findings

Both laboratory and pilot scale MBRs were demonstrated as effective systems for EA removal from swine wastewater. The average removal rate was 93.5% in terms of EA in the soluble phase of dilute swine wastewater, and 94.5 % in terms of total EA. A model mass balance revealed that biodegradation was the dominant removal mechanism of EDCs by MBR and over 85% of the influent EA was reduced through the MBR process by degradation or volatilization. It was also found that membrane filtration could retain some estrogenic compounds.

The hybrid PAC-MBR process was demonstrated to be an effective way in enhancing the removal of E2 and EE2 in MBR and improving the MBR overall performance. PAC dosing in MBR increased the removal rates of E2 and EE2 by 3.4 and 15.8%, respectively. The greater impact of PAC dosing on EE2 removal was due to its more

hydrophobic property. It was also found that PAC dosing resulted in a slower rate of TMP increase by reducing the concentrations of soluble EPS and colloidal TOC in the PAC-MBR sludge. The average soluble EPS and colloidal TOC of PAC-MBR sludge was 60.1 and 61.8% lower than the control MBR sludge, respectively. Regardless of PAC dosing, concentrations of colloidal TOC were strongly related to concentrations of soluble EPS and soluble carbohydrate in sludge. In practice, a slower rate of TMP increase in the PAC-MBR may significantly reduce the operating cost for membrane cleaning and/or membrane replacement. The operating cost for PAC dosing can be offset by the benefit from its reducing the cost for membrane maintenance.

Direct UV irradiation was effective in reducing all three investigated estrogens E1, E2 and EE2 in water both in terms of apparent concentration and in terms of EA. The removal of E1 was much quicker and more effective than E2 and EE2 with initial concentrations both at mg/L and μg/L ranges. The degradation of three estrogens by UV direct irradiation followed pseudo first order reaction kinetics. The degradation rate constants of E1 were approximately 5-10 times higher than those of E2 and EE2 at various initial concentrations. The occurrence of other compounds could inhibit the photolysis of certain estrogens. The estrogenic activities of photolysis products of individual estrogens in water were negligible.

The impacts of UV disinfection on EDCs in wastewater, however, depend on certain field conditions in WWTPs. The EA of pre-UV and post-UV samples from five wastewater treatment plants were measured in both liquid and solid phase by the YES

assay. Over 95% EA of wastewater, both in pre-UV and post-UV samples, was associated in liquid phase. In solid phase, EA of wastewater decreased after UV disinfection in all five investigated plants. However, the total EA of secondary effluents decreased after UV disinfection in three of the five investigated plants but increased in the other two plants. Tests of UV on the estrogenic effects of wastewater should be performed before implementing UV disinfection for wastewater treatment with a high contribution of industrial discharges, such as pharmaceutical wastewater, food processing wastewater, plastics producing wastewater etc.

8.3 Recommendations for Future Research

As mentioned above, the increase of wastewater EA after UV disinfection under certain field conditions and with certain wastewaters was observed in this research. The reasons for the increase of EA of wastewater effluents after UV disinfection are still not clear. It could be related to high industrial wastewater inputs into the wastewater collecting system or related to the EA of the photolysis byproducts. Further research is needed to determine the impact of UV disinfection on wastewater quality from WWPTs in terms of estrogenic effects. Particularly, further study should be conducted to identify the mechanisms of the transformation of EDCs in wastewater during UV disinfection.
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In this research, liquid extraction was used to enrich and extract EDCs from wastewater and mixed liquor. However, the effectiveness of the liquid extraction of EDCs, especially from solid matrix i.e. sludge in this research, need to be evaluated and

improved further. The concentrations of the investigated EDCs in sludge are crucial to illustrate the removal mechanism in biological wastewater treatment processes. Solvent screen experiments can be carried out to determine which solvent is able to achieve the highest extraction efficiency. Ultrasonication may be used to further improve the overall extraction efficiencies of the liquid extraction.

Further research is also needed to investigate the impacts of PAC dosing on the microbial characteristics of the sludge in MBRs. It was found that PAC dosing resulted in a higher MLSS and MLVSS than was expected. The results from the measurements of the protein content and nitrification rates of MBR and PAC-MBR indicated that PAC dosing did not greatly affect the biomass activity of the sludge. Full microbiological analysis and other biomass speciation and quantifying methods should be used to assess the impacts of PAC dosing on the microbial characteristics of the sludge in MBRs.

To address the cost issue of PAC dosing in MBRs, pilot-scale study is recommended to test the effects of lower dosage of PAC addition on EDCs removal and MBRs performance in terms of mitigating membrane fouling. PAC dosing will result in a larger amount of wasted sludge with the mixture of PAC. The options for PAC-sludge management and cost assessment may also be studied in pilot-scale tests.

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APPENDICES

Appendix A: Protocol of Yeast Estrogen Screen (YES) Assay

PREPARATION AND STORAGE OF MINIMAL MEDIUM AND MEDIUM COMPONENTS

Minimal medium and medium components prepared in glassware contaminated with an oestrogenic chemical will lead to elevated background expression. Glassware, spatulas, stirring bars, etc., must be scrupulously cleaned, and should not have had prior contact with steroids.

Rinse glassware, spatulas, stirring bars twice with absolute ethanol, and leave to dry. Alternatively, wash twice with methanol, and once with ethanol.

Minimal Medium (pH 7.1)

Add 13.61 g KH_2PO_4, 1.98 g $(NH_4)_2SO_4$, 4.2 g KOH pellets, 0.2 g $MgSO_4$, 1 ml $Fe_2(SO_4)_3$ solution (40 mg/50 ml H_2O), 50 mg L-leucine, 50 mg L-histidine, 50 mg adenine, 20 mg L-arginine-HCl, 20 mg L-methionine, 30 mg L-tyrosine, 30 mg L-isoleucine, 30 mg L-lysine-HCl, 25 mg L-phenylalanine, 100 mg L-glutamic acid, 150 mg L-valine, and 375 mg L-serine to 1 L Milli-Q water. Place on heated stirrer to dissolve.

Dispense 45 ml aliquots into glass bottles.

Sterilise at 121˚C for 10 min, and store at room temperature.

<u>D-(+)-Glucose</u>

Prepare a 20% w/v solution.

Sterilise in 20 ml aliquots at 121˚C for 10 min.

Store at room temperature.

<u>L-Aspartic Acid</u>

Make a stock solution of 4 mg/ml.

Sterilise in 20 ml aliquots at 121˚C for 10 min.

Store at room temperature.

<u>Vitamin Solution</u>

Add 8 mg thiamine, 8 mg pyridoxine, 8 mg pantothenic acid, 40 mg inositol, and 20 ml

biotin solution (2 mg/100 ml H_2O) to 180 ml Milli-Q water.

Sterilise by filtering through a 0.2-µm pore size disposable filter, in a laminar air flow

cabinet. Filter into sterile glass bottles in 10 ml aliquots.

Store at 4˚C.

<u>L-Threonine</u>

Prepare a solution of 24 mg/ml.

Sterilise in 10 ml aliquots at 121˚C for 10 min.

Store at 4˚C.

Copper (II) Sulfate

Prepare a 3.2 mg/ml solution.

Sterilise by filtering through a 0.2-µm pore size filter, in a laminar flow cabinet. Filter into sterile glass bottles in 5 ml aliquots.

Store at room temperature.

Chlorophenol red-ß-D-galactopyranoside (CPRG)

Make a 10 mg/ml stock solution. Sterilise by filtering through a 0.2-µm pore size filter into sterile glass bottles, in a laminar flow cabinet.

Store at 4˚C.

PREPARATION AND STORAGE OF 10-X CONCENTRATED YEAST STOCKS

Carry out all yeast work in a type II laminar flow cabinet.

Day 1

Prepare growth medium by adding 5 ml glucose solution, 1.25 ml L-aspartic acid solution, 0.5 ml vitamin solution, 0.4 ml L-threonine solution, and 125 µl copper (II) sulfate solution to 45 ml minimal medium. Transfer to a sterile conical flask (final volume of approximately 50 ml). Add 125 µl of 10X concentrated yeast stock from cryogenic vial stored at -20˚C. Incubate at 28˚C for approximately 24 hour on an orbital shaker, or until turbid.

<u>Day 2</u>

Add growth medium to two conical flasks (each with a final volume of approximately

50 ml). Add 1 ml yeast from 24-h culture to each flask.

Incubate at 28˚C for approximately 24 hour on an orbital shaker, or until turbid.

<u>Day 3</u>

Transfer each 24-h culture to a sterile 50-ml centrifuge tube.

Centrifuge the cultures at 4˚C for 10 min at 2,000 g.

Decant the supernatant, and resuspend each culture in 5 ml of minimal medium with 15%

glycerol (add 8 ml sterile glycerol to 45 ml minimal medium).

Transfer 0.5 ml aliquots of the 10X concentrated stock culture to 1.2-ml sterile cryovials.

Store at -20˚C for a maximum of 4 months.

PREPARATION OF STANDARD SOLUTIONS

Glassware must be scrupulously cleaned since contamination may give rise to false

positives. Rinse all glass bottles twice with absolute ethanol (or twice with methanol,

and once with ethanol), and leave to dry.

Prepare the 17ß-estradiol (E2) stock solution in absolute ethanol, at 50. µg/L

Serially dilute solutions at concentrations of 50 ng/L to 50 µg/L

ASSAY PROCEDURE

Carry out all yeast work in a type II laminar flow cabinet.

Day 0

Prepare growth medium by adding 5 ml glucose solution, 1.25 ml L-aspartic acid solution, 0.5 ml vitamin solution, 0.4 ml L-threonine solution, and 125 µl copper (II) sulfate solution to 45 ml minimal medium. Transfer to a sterile conical flask (final volume of approximately 50 ml). Add 125 µl of 10X concentrated yeast stock from cryogenic vial.

Incubate at 28˚C for approximately 24 hour on an orbital shaker, or until turbid.

Day 1

Prepare assay medium by adding 0.5 ml CPRG to 50 ml fresh growth medium. Seed this medium with 2 ml yeast from the 24-h culture prepared on Day 0. For every 2.5 assay plates, prepare 50 ml assay medium.

Transfer 10 µl water samples to a 96-well optically flat bottom microtitre plate. Add 190 µl of the seeded assay medium (growth medium containing CPRG and yeast) to wells using a multichannel pipette.

Each plate should contain at least one row of blanks (solvent and assay medium only), and each assay should have a 17ß-estradiol standard curve.

Seal the plates with autoclave tape and shake vigorously for 2 min on a titre plate shaker.

Incubate at 32˚C in a naturally ventilated heating cabinet for 72 h.

Day 4

After incubating for 3 days, shake plates (2 min) and leave for approximately 1 hour to allow the yeast to settle. Read the plates at an absorbance of 540 nm (optimum absorbance for CPRG ~575 nm) and 620 nm (for turbidity) using a plate reader.

Leave the plates at room temperature and read later if necessary.

CALCULATIONS

To correct for turbidity the following equation needs to be applied to the data:

Corrected value = chem. abs. (540 nm) - [chem. abs. (620 nm)-blank abs. (620 nm)]

MATERIALS AND CHEMICALS

Apparatus and glassware:

1 L beaker (1)

weighing paper

balance

stirrer with heating

stirring bars

60 ml glass bottles (22)

200ml beakers (5)

20 ml glass bottles

oven (121 °C)

0.2 μm pore size filter

conical flasks

pipets

50 ml graduated cylinder

orbital shaker

incubator

centrifuge

50 ml centrifuge tubes

96-well microtitre plate

microtitre plate shaker

plate reader

Chemicals:

Potassium phosphate monobasic anhydrous

Ammonium sulphate

Potassium hydroxide

Magnesium sulfate

Iron (III) sulfate

L-Leucine

L-Histidine

Adenine

L-Argenine

L-Methionine

L-Tyrosine

L-Isoleucine

L-Lysine

L-Phenylalanine

L-Glutamic acid

L-Valine

L-Serine

Thiamine (hydrochloride)

Pyridoxine

D-Pantothenic acid (hemicalcium salt)

Inositol

d-Biotin

D-(+)-Glucose (anhydrous; mixed anomers)

L-Aspartic acid (free acid)

L-Threonine

Copper (II) sulfate (anhydrous)

Chlorophenolred--D galactopyranoside (CPRG)

Glycerol

β-Estradiol

Ethanol (absolute)
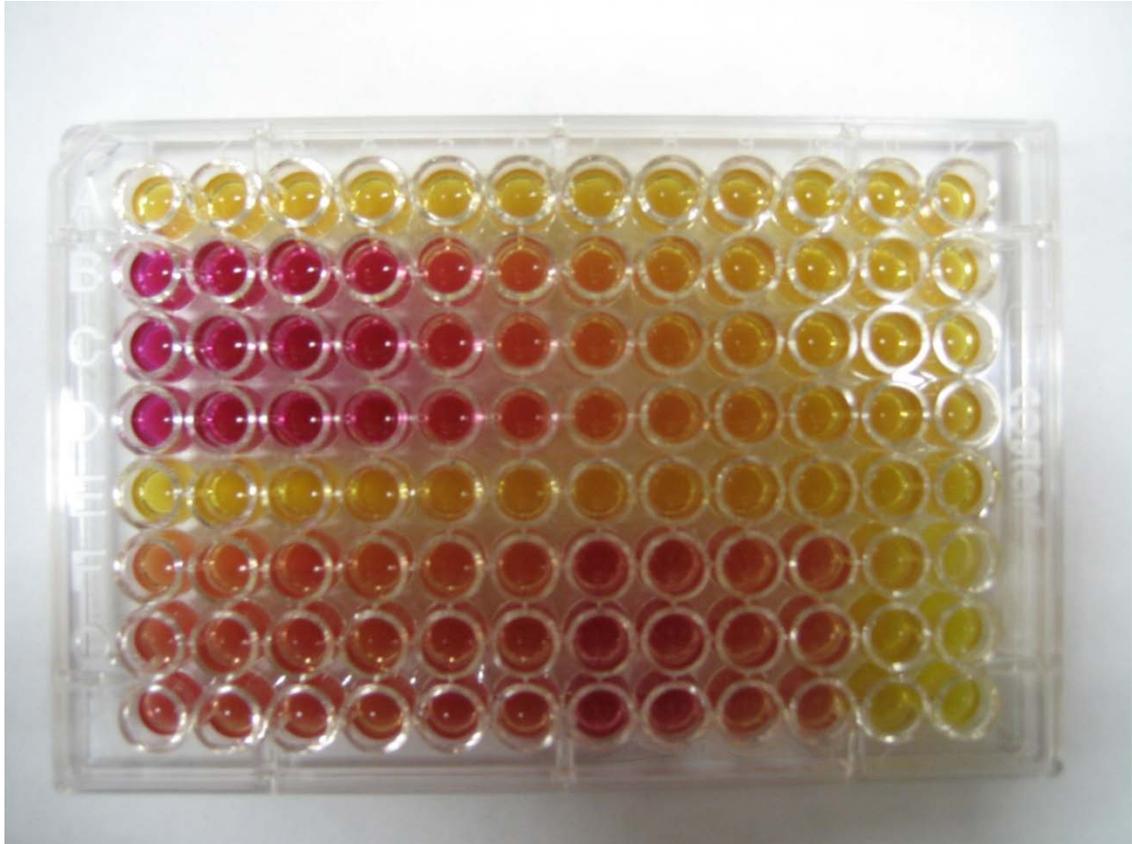
Appendix B: A Picture of YES Assay Plate

Figure A-1 A picture of YES assay plate

Appendix C: A Representative Standard Curve of YES Assay

The chart shows Absorbance (y-axis, 0.000 to 3.500) versus Concentration (ng/L) (x-axis, logarithmic scale from 1.0 to 100000.0).

$$y = 0.8124\ln(x) - 4.0927$$
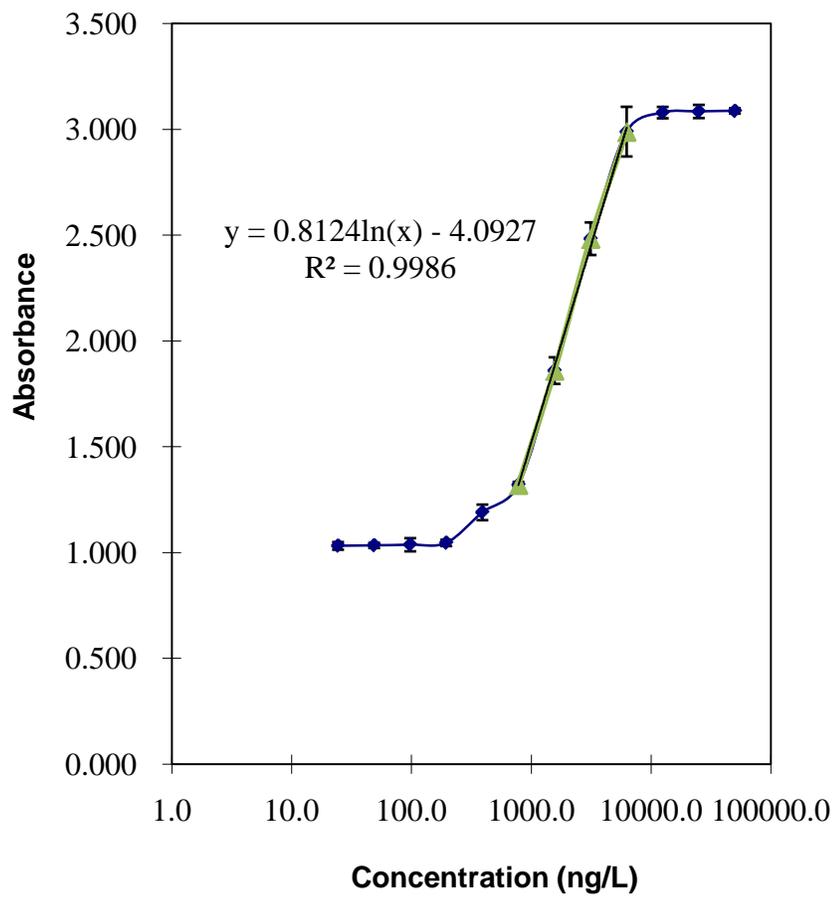$$R^2 = 0.9986$$

Figure A-2 Standard curve of YES assay

Appendix D: ZW-10 Demonstration MBR

System Description

The ZeeWeed[®]-10 (ZW-10) demonstration MBR is a simple, compact ZeeWeed (ZW) membrane filtration system that can be used to generate preliminary performance data prior to further evaluation or pilot scale testing. It uses the same membrane as the full-scale ZeeWeed[®]-500 modules and hence the effluent quality is representative of full-scale modules.   Similarly, the unit can be used to evaluate or compare alternative membrane chemistries.   However, as fibers are shorter and as aeration pattern is different, ZW-10 module cannot be used to study filtration variables such as flux, pressure and energy requirements.

The system is supplied as a kit, that is, the components are provided loose and some assembly of the piping is required prior to commissioning (Figure A-3). The unit requires a space of approximately 1 m x 1.5 m (3' x 5'). The main elements of the system are a process tank, a small control panel, a process pump and a blower.

Figure A-3 A picture of ZW-10 MBR

Capacity: 100 – 400 USgpd (380 Lpd – 1500 Lpd), varies with water being treated

Dimension: 26 in W x 42 in L x 62 in H (66 cm W x 107 cm L x 158 cm H)

Weight: 467 lbs (212 Kg)

Power: 110 V / 1 ∅ / 60 Hz or 220 V / 1 ∅ / 60 Hz

Materials: Frame: Epoxy coated carbon steel

               Piping: PVC

               Tubing: Polypropylene

               Tanks: HDPE

System Assembly

1. Unpack components and familiarize yourself with them. Note: do not remove ZW-10 module from package. It will remain sealed until startup.

2. Identify nozzles on the process and back-pulse tanks and understand their function.

3. Identify a suitable location for the tank, control panel, reject/effluent pump and blower. The drain valve should be near an area floor drain. The control panel should be on a table or shelf; it can be either vertically or horizontally mounted. If mounted on a shelf, an opening in the shelf is necessary for the process pump.

4. Assemble spools according to drawings in Appendix A. Install drain valve, temperature indicator, pressure indicator, feed valve and overflow on process tank. Install spools for both process tank and back-pulse tank. Do not connect to ZW-10 module (it is still in package). Note: feed flow should be turned off upstream of float valve. This valve will only be opened when system is ready for operation on process water.

5. Fill backpulse tank with potable water to overflow level and conduct leak test on tank fittings. Tighten if required. Drain water to float valve level.

6. Put steel support bracket on top of process tank and orient so back-pulse tank is above stem of float valve. Fasten to tank with hardware supplied.

7. Fit back-pulse tank between white plastic holders and connect spools to process and reject pumps.

8. Plug reject pump into upper outlet on panel.

9.   Plug blower into lower outlet on panel.

10.   Ensure that all switches on panel and on reject pump are in the off position, and then plug power cord into suitable receptacle.

11.   Set V1 to ½ open. Test blower by turning on. Adjust V1 until air flow is 2 scfm (3.6 m^3/h). Check for air leaks at connections with dilute liquid soap or similar product. Turn off blower when flow is confirmed; do not close V1.

12.   Set timer for 60 second production and 15 second back-pulse interval. With this interval, initial setup will be quicker as you will be able to set flows faster.

13.   Test process pump operation by simply drawing water from process tank, fill up back-pulse tank and let it overflow to process tank. Check for vacuum leaks in tubing. Understand the subtleties of the pump design and learn how to adjust flow direction and motor speed.

System Startup

1.   Unpack ZW-10 module and spray module thoroughly with clean warm (maximum 40°C) water.

2.   Fasten ZW-10 module to support bracket with 2 clamps per module directly above one another. Set height so liquid level is 2 – 5 cm above top header (with float valve just closed). Connect air, permeate and pressure indicator spools to module.

3.   Turn on air and pump in auto mode and confirm flow rates. Check for leaks. Adjust airflow to 2 scfm if required. Confirm that airflow distribution around the module is uniform; adjust module orientation if required.

4. Direct permeate to drain at 1 L/min for five minutes without back-pulsing.

5. Drain the process tank.

6. Refill process tank with potable water; add sufficient NaOCl to produce a 200 ppm solution in the process tank.

7. Soak the module in 200 ppm NaOCl solution at room temperature for a minimum of 5 hours. Dump process tank contents and rinse; refill with potable water.

8. Start system in auto mode. Determine clean water permeability: measure flux at 35 kPa (5 psi) trans-membrane pressure (TMP) to calculate permeability as lmh/bar. Save this value for future use to determine cleaning efficiency.

9. Reset timer according to desired protocol and unit is ready for testing on process fluid. Use 9.75 minutes (585 seconds) permeation and 0.25 minutes (15 seconds) back pulse as a typical value. This must be optimized for each application.

10. Measure flow rate and TMP immediately before (~60 seconds), during and immediately after (~20 seconds) back wash.

Routine Operation

The ZW-10 unit is intended to operate without continuous supervision. It is though a simple design, without interlocks so if a problem develops, other problems may arise. The unit should be periodically inspected to confirm that it is running properly, i.e. tank level is at the float level, the pump is permeating, back-pulse is occurring correctly etc.

For maintenance cleaning, sodium hypochlorite should be added to the back-pulse tank

to a concentration of 500 to 1000 ppm. A manual back-pulse of the membranes should be initiated at the control panel. The specific cleaning procedure is application specific; contact Zenon's Corporate Technology group or other technical support staff if assistance in setting protocol is required.

A soak cleaning may also be required. The general protocol requires removing any cake apparent on the membranes by hand or with a gentle spray and soaking the module in 200 ppm NaOCl at room temperature for a minimum of 5 hours. If there is inorganic fouling, rinse system thoroughly and perform an additional soak in HCl solution at pH of 2.0 for a minimum of 5 hours. Note: if hypochlorite is not rinsed out well, the acid and the hypochlorite will react to form highly toxic chlorine gas. If, during cleaning, chlorine gas vapors are detected, clear the area immediately and advise appropriate plant safety official or other supervisor.