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# Anaerobic Membrane Bioreactor (AnMBR) for Treatment of Landfill Leachate and Removal of Micropollutants

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# Anaerobic Membrane Bioreactor (AnMBR) for Treatment of Landfill Leachate and Removal of Micropollutants

by

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*I would like to dedicate this dissertation*
*to my parents*
*Do Xuan Cuong and Le Thi Lan*
*For their ultimate love and support*
*for me to accomplish my dreams*

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# ABSTRACT

To date, most studies on the fate and removal of endocrine disrupting compounds (EDCs) and pharmaceutical and personal care products (PPCPs) in wastewater focus on their fate in municipal wastewater treatment plants, and mostly under aerobic condition. There are limited studies related to anaerobic condition and (to our knowledge) no study on the removal of EDCs in landfill leachate by AnMBR. Moreover, for most studies under anaerobic condition, the removal of EDCs was only reported in the liquid phase; solid phase extraction was not reported, thereby preventing mass balance in the studies.

This research was conducted to investigate the potential of AnMBR for reduction of organic strength and removal of EDCs in landfill leachate. A novel lab-scale upflow anaerobic sludge blanket (UASB) reactor equipped with dual-flat sheet ultrafiltration and microfiltration membrane modules was designed and constructed to test the potential to remove EDCs and traditional landfill leachate constituents (COD, turbidity). The target EDC was 17β-estradiol (E2), a prevalent female hormone used for contraceptives and hormone replacement therapy. Due to the nature of packaging and widespread use in households, the entry of E2 into landfills is highly likely, and has been reported. The quantification of E2 from liquid phase in this project is performed by the use of solid-phase microextraction (SPME) with GC/MS.

Batch assays were conducted to determine the anaerobic biodegradability of E2 as well as to measure the respective distribution coefficients of E2 to PAC, colloids and

anaerobic sludge biomass. In the adsorption batch assays, it was found that the PAC has stronger adsorption potential than anaerobic sludge. The adsorption potential of E2, E1 and EE2 on sludge follows the order E2>EE2>E1 which correlates to the $K_{ow}$ values (4.01, 3.67, 3.1, respectively). However, all three compounds showed the same adsorption potential to the Norit 20B PAC. The biodegradability of E2 was investigated in both liquid and solid phase and under several conditions such as methanogenesis, methanogenesis with aid from PAC, and methanogenesis with additional alternative electron acceptors added (sulfate and nitrate). E2 was found to transform to E1 under all tested conditions. The compounds are present in both liquid and solid phase. E2 and E1 were not detected (< 4ng/L and <10ng/L, respectively) in the liquid phase after 25 days in most cases except the case of adding additional sulfate.

The AnMBR was designed, fabricated and operated for 2 years. During the stable condition period of the AnMBR, the high removal efficiencies of COD and E2 achieved were around 92% and 98%, respectively. However, E2 was still detected in the effluent at average concentrations of 30-40 µg/L range. To expand hormone retention and removal by the AnMBR, as well as to control membrane fouling, powder activated carbon (PAC) was added to the reactor. After the PAC was added, the concentration of E2 was reduced to less than the detection limit (4ng/L) in both MF and UF effluents. The log removal of E2 in the AnMBR system increased immediately from 1.7 without PAC to 5.2 after PAC was added. This study demonstrated that the AnMBR has high potential for removal of E2, and with aid from PAC, the AnMBR can remove E2 from landfill leachate to levels below detection limit.

# CHAPTER 1: INTRODUCTION

In recent years, increased attention has been given to the presence of xenobiotics in the environment, especially pharmaceutical and personal care products (PPCPs), many of which exhibit traits as potential endocrine disrupting compounds (EDCs). To date, the majority of the studies on PPCPs have focused on their fate in sewage treatment plants. However, PPCPs can also enter the municipal landfill via several routes, including household solid wastes and sludge from municipal wastewater treatment plants (WWTPs) (Bound and Voulvoulis, 2005; Slack et al., 2005). For example, in light of the general ineffectiveness of conventional wastewater treatment systems to completely remove these contaminants, the public is increasingly instructed to dispose of PPCPs in household trash (e.g., in Michigan, Minnesota and New Hampshire). Also, in an effort to protect the environment, on February 20, 2007, the White House Office of National Drug Control Policy (ONDCP), the Department of Health and Human Services (HHS), and the Environmental Protection Agency (EPA) jointly released official guidelines on the proper disposal of unused, unneeded, or expired prescription drugs through household trash (WHONDCP, 2007). In a recent survey conducted in the United Kingdom, two-thirds of the subjects disposed of unwanted or expired medication through household trash (Bound and Voulvoulis, 2005). With the maturing of the Baby Boom Generation and our society's increasing reliance on medication, there is good reason to anticipate that states with high populations of the elderly, such as Florida, will receive high loadings of PPCPs

to landfills in years to come. Even if the PPCPs are disposed in bags or containers (e.g., prescription bottles), it is likely that they will be released once they enter the general trash stream, either through mechanical compaction and breakage in the garbage trucks or at the landfill. Additionally, containers, e.g. plastic bottles and bags, can lose integrity in the landfill from degradation, thereby enabling the contents to enter the general contents of the landfill.

Perhaps most importantly, in many cases, landfill operators collect leachate onsite and transport the leachate to local WWTPs for discharge. Hence, even if the PPCPs are diverted to landfills, they can still find their way back to municipal WWTPs. The fee that landfill operators pay for discharge depends on both quantity and strength (COD and nutrients) of the leachate, so there is an incentive for pretreatment to reduce costs of disposal. However, with conventional treatment methods, it is unlikely that PPCPs are significantly removed before they are transferred to the municipal WWTP. In short, landfills can serve as a long-term source of these xenobiotics for soil and groundwater contamination, as well as surface water contamination in cases where leachate is brought to municipal WWTPs.

To prevent environmental contamination and to comply with state and local regulations, an effective pretreatment method is needed for treating and removing xenobiotic compounds from landfill leachate. Landfill leachates are among the most difficult waste streams to treat, as they typically contain high concentrations of dissolved and colloidal organics (much of which may be recalcitrant and hard to degrade), inorganics (e.g., ammonium), heavy metals (e.g., arsenic, mercury, cadmium, copper) and xenobiotic organic pollutants (e.g. chlorinated organics) (Kjeldsen et al., 2002). Further,

constituents of the effluent can be toxic or inhibitory to many conventional biological treatment processes. Although there is a growing trend to operate landfills themselves as biological reactors, young landfills (i.e., less than 5 years) will rely most heavily on an external leachate treatment system while the biological activity establishes within the landfill itself.

The membrane bioreactor (MBR), in which biological waste treatment and membrane separation (typically micro- or ultrafiltration) are synergistically-coupled, is a technology that has gained growing popularity in the past fifteen years. MBRs are an attractive option for wastewater treatment because they can offer efficient treatment, a particle-free effluent, small footprint, and the potential for remote monitoring and control. Solids retention time (SRT) can be adjusted independently of hydraulic residence time (HRT), allows biomass wasting rates to be chosen as to favor desirable microorganisms and select against undesirable organisms. To date, MBRs are used primarily for the treatment of municipal and some industrial wastewaters. While MBRs have been used with success for the treatment of landfill leachate in Europe (more than 30 installations in Europe during the 1990's), there have been relatively few applications of such in the United States, with only one full-scale plant commissioned in North America to date (Yang et al., 2005).

The combination of membrane retention, longer SRT, and dense and diverse microbial populations make the MBR a better system for degrading recalcitrant contaminants such as EDCs than conventional activated sludge processes (Cicek et al., 1999; Clara et al., 2005). Furthermore, because MBRs are also better at handling shock loadings and toxicity in the influent, they may be especially suitable for the treatment of

landfill leachate. For example, when combined with a post-treatment stage such as nanofiltration or activated carbon adsorption, more effective removal of EDCs (nonylphenol and bisphenol-A) was obtained than when using reverse osmosis alone (Wintgens et al., 2003).

Anaerobic processes have the potential to degrade or transform xenobiotic organic compounds, including polychlorinated organics, surfactants (Yeh et al., 1998; Yeh and Pavlostathis, 2005) and pesticides. Further, biological treatment processes have enhanced capability of removing hormonal compounds from wastewater when both anaerobic and aerobic conditions are imposed (Joss et al., 2004). Combining anaerobic waste conversion with membrane filtration, anaerobic MBRs (AnMBRs) have a great potential for treating a variety of waste streams previously deemed too difficult to treat biologically. Further, if sorbents such as powdered activated carbon (PAC) are added to the system to decouple the chemical retention time (CRT) from the hydraulic retention time (HRT), the potential to further retain and remove recalcitrant target pollutants may be increased.

The fate of EDCs in an AnMBR system treating landfill leachate was the major focus of this research. Because of its high endocrine disruption potential to human and other organisms, and because there is limited information on its fate under anaerobic conditions, 17β-Estradiol (E2) was chosen as the target compound. A lab-scale AnMBR was built and operated in order to study the potential for removing organic strength and EDCs from landfill leachate. The research began with the operation of the AnMBR system to treat young landfill leachate where the removal of chemical oxygen demand (COD) and production of biogas was tested (chapter 5). The research then focused on the

phase distribution of E2 in a hybrid bioreactor system containing PAC (Chapter 6). The biological fate of E2 under different anaerobic conditions was invested in Chapter 7. Finally, the fate of E2 (due to biotransformed/biodegraded and sorption to biomass and PAC) in the AnMBR system (with and without PAC) was investigated (Chapter 8). Results of this research not only further the existing knowledge about the removal of E2 and other estrogenic compounds in an AnMBR system but also provide the proof of concept on a new approach to remove organic nutrients and a variety of xenobiotic compounds from young landfill leachate. Furthermore, the decoupling of CRT from HRT can provide useful design information for reactor systems treating recalcitrant compounds. In addition, this research can serve as an educational tool for the general public, providing information on the effects of micropollutants on human and environmental health. This research may help encourage the public to avoid disposing of their medicine or pharmacy products into their household trash.

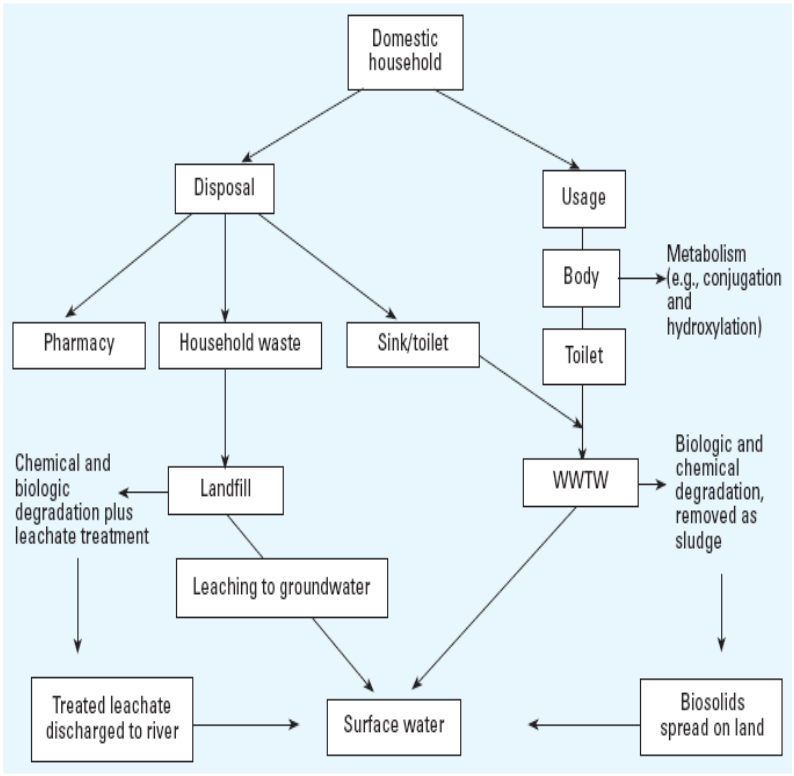



**Figure 1.1:** Pathways of drug fate from domestic households to the environment. (From Bound and Voulvoulis, 2005). While not shown in the diagram, landfills also receive sludge from wastewater treatment works (WWTW) which may likely contain PPCPs. Landfills may also transport leachate to WWTWs, providing another exposure pathway into the environment.

# CHAPTER 2: BACKGROUND

## 2.1. Landfill Leachate

Today, landfilling has become the most common and socially accepted way to dispose of municipal wastes. Growth of cities results in both increased amount and diversity of solid wastes. In landfills, the liquid (generated internally during waste stabilization or externally from rainwater) percolates through the wastes known as leachate. Owing to the diversity of the solid wastes deposited in the landfills, landfill leachate content is also very complex due to high concentrations of dissolved and colloidal organics (much of which may be recalcitrant and hard to degrade), inorganics (e.g., ammonium, phosphate and salts), heavy metals (e.g., arsenic, mercury, cadmium, and copper) and xenobiotic organic pollutants (e.g., chlorinated organics and PPCPs) (Kjeldsen et al., 2002). The characteristic of each landfill leachate depends on many factors such as rainfall level, type of wastes on each landfill, and age of leachate. Due to the high chemical oxygen demand (COD) of the waste organics and limitations in oxygen mass transfer, the biological activity within the landfill is predominantly anaerobic. Throughout the depth of a landfill, multiple processes affect the solid wastes, such as physical compaction, disintegration, hydrolysis and fermentation of organic compounds. The leachate which migrates through the landfill will contain reactants, intermediates and

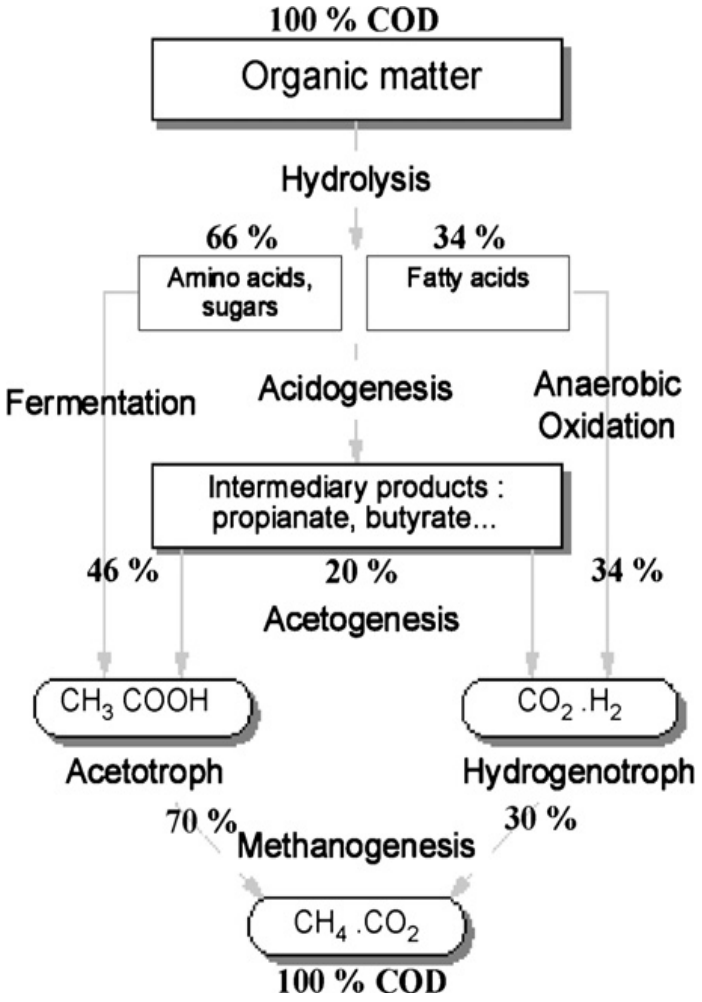



**Figure 2.1:** The anaerobic degradation pathway of organic matter in landfills. (From Renou et al. 2008).

products from all of these processes. The anaerobic degradation pathway of organic compounds in the landfill is described as in Figure 2.1.

Hydrolysis dominates the degradation processes during the first few years in the landfill. The leachate in the initial period therefore contains many byproducts of the hydrolysis which are mainly monomers and acids. The leachate can be classified based on the age of the landfill: young (less than 5 years), middle age (5 years to 10 years) and old (more than 10 years). Because most of the biodegradable organic compounds are degraded by anaerobic oxidation, acidogenesis and fermentation in the early period, old leachate contains mostly recalcitrant compounds. Characteristics of the leachate classified by age, as adapted from Alvarez-Vazquez et al., 2004, are shown in Table 2.1.

EDCs and PPCPs have been detected in landfill leachate. The presence of these micropollutants in landfill leachate is because municipal landfills are essentially the final resting ground of most of society's wastes and contain a variety of potential sources of micropollutants such as pesticides, plastics, industrial chemicals, pharmaceuticals and personal care products. When these wastes are buried in the landfill, hydrolytic processes occur and release the micropollutants into leachate. Concentrations of Bisphenol-A (a plasticizer) in landfill have been reported between 0.3-17,200 μg/L (median: 269 μg/L) by Yamamoto et al. (2001) and between 0.15-2980 μg/L by Yasuhara et al. (1997). The concentration of nonylphenol in leachate was detected at 2.8μg/L by Behnisch et al. (2001). Landfill leachate can potentially reach the environment through two routes: 1) leaks into groundwater if landfill liner integrity is compromised, or 2) wastewater treatment plant effluent if leachate is collected and transferred to WWTPs, diluted with

**Table 2.1:** Characteristics of landfill leachates. (From Alvarez-Vazquez et al., 2004).

| **Parameter** | **Landfill 1** | | **Landfill 2** | | **Landfill 3** | | **Landfill 4** | |
|---|---|---|---|---|---|---|---|---|
| | Young Leachate (<2 yrs) | Old Leachate (> 6.5yrs) | Young Leachate (< 2 yrs) | Old Leachate (> 10 yrs) | Young Leachate | Old Leachate | Medium Leachate | Old Leachate |
| BOD (mg/L) | 24000 | 150 | 2500-3000 | 10-20 | 11900 | 260 | 1600 | 160 |
| COD (mg/L) | 62000 | 300 | 3000-60000 | 100-500 | 23800 | 1160 | 6610 | 1700 |
| TOC (mg/L) | NG | NG | 1500-20000 | 80-160 | 8000 | 465 | 1565 | 625 |
| BOD/COD | 0.39 | 0.05 | 0.05-0.67 | 0.04-0.10 | 0.5 | 0.2 | 0.24 | 0.09 |
| $NH_4$-N (mg/L) | 1400 | 350 | 10-800 | 20-40 | 790 | 370 | 1500 | 2300 |
| pH | 5.8 | 8 | 4.5-7.5 | 6.6-7.5 | 6.2 | 7.5 | 5.6-7.3 | 7.9-8.1 |

domestic sewage, and not sufficiently removed in conventional wastewater treatment processes. Once in the environment, micropollutants are further diluted and will be extremely difficult to remove. Therefore, it is critical that landfill leachate be properly collected, and subjected to a pretreatment process, which is capable of removing trace contaminants while they are still present at relatively higher concentrations in the leachate.

## 2.2. Membrane Processes for Landfill Leachate Treatment

Current technologies for landfill leachate and wastewater treatment can be classified as chemical, physical and biological treatment. While advanced oxidation methods (such as UV, ozone, peroxide) are effective at removing micropollutants in drinking water or municipal wastewater effluent, they are not effective at all when targeting micropollutants in raw leachate (due to the high turbidity, oxidant demand, color and UV absorbance associated with high concentrations of organic matter in leachate). Clearly, a treatment method, capable of removing micropollutants from a background matrix containing high concentrations of organic matter, is needed. According to 157 case studies investigated by Alvarez- Vazquez (2006), less than 30% of treatment systems use chemical methods, less than 10% use physical treatment and 60% use biological treatment. Conventional biological treatment utilizing activated sludge is used in most of wastewater treatment plants and landfill leachate treatment applications. However, the high COD content of leachate also means high energy input (for aeration) and high sludge generation (due to high cell yield for aerobic microorganisms) when aerobic systems are used. Alternatively, anaerobic systems, which have a lower energy

footprint (no aeration requirement and can convert COD into methane for use as fuel) and generate less sludge (lower cell yield), may be more appropriate for high strength wastes such as leachate.

In recent decades, membrane processes have emerged as alternatives for landfill leachate treatment. In the water/wastewater treatment industry, membranes are pressure-driven absolute barriers used to separate constituents from water. Membranes separate the treatment stream (feed) into a stream containing rejected constituents (retentate or concentrate) and a stream of relatively clean water (permeate). The quality of the permeate depends on the membrane pore size or molecular weight cut-off (MWCO). In order of decreasing pore size or MWCO, membranes are classified as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) (Figure 2.2). As the pore size or MWCO decreases, membranes can reject particles, bacteria, viruses, organic molecules, and even ions. However, increasing rejection capability comes at a cost of increasing pressure (hence energy) and also ease of fouling (fouling propensity). Nonetheless, owing to its high performance, membrane technology is one of the best available tools for water and wastewater treatment. The remainder of this literature review examines the current status of the application of membrane processes for landfill leachate treatment.

### 2.2.1. Reverse Osmosis (RO) Membrane

Reverse osmosis is a high pressure driven membrane process which is used for water treatment and wastewater treatment. Recently, RO has been applied for landfill leachate treatment at both the lab and industrial scale. With its exceptional filtration

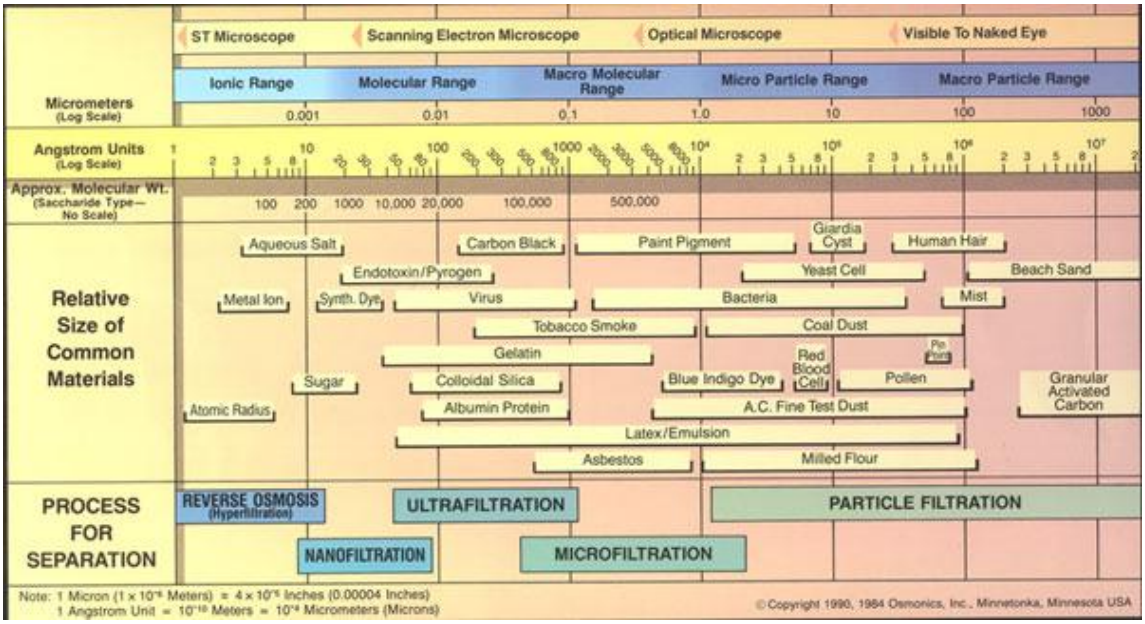



**Figure 2.2:** Constituents removed by varying types of membranes classified by pore size. (From website: http://www.liquidfiltration-products.com).

ability to separate ions and small molecular weight compounds from water, RO has demonstrated impressive results in term of removal of COD, $NH_4^+$, heavy metals and other contaminants from leachate. According to Renou et al., (2008), tubular and spiral-wound RO systems were used for leachate treatment since 1984. And in 1988, the Disc Tube RO (DT-RO) model was invented and applied successfully in Germany (Renou et al., 2008; Liu et al., 2008). Since then, the DT-RO has received popularly worldwide. According to Liu, there were more than 200 sites using DT-RO membrane for leachate treatment. Renou et al., (2008) showed that the removal of COD and heavy metals from leachate can be achieved in excess of 98 and 99%, respectively, by RO process. Linde et al., (1995) and Liu et al., (2008) also reported similar result as Renou (2008) for COD and $NH_4^+$ removal from leachate. For a full-scale application of DT-RO for leachate treatment, Liu demonstrated that the removal of total dissolved solids (measured through electrical conductivity) and metals can also reach to 99.6% and 99.9%, respectively. The

high removal efficiency is one of the major advantages of using RO for leachate treatment, especially with old leachate, since it contains less biodegradable organic compounds and high concentration of ammonia and salts.

The benefits of high treatment efficiency of RO membrane process for landfill leachate are clear. But some major disadvantages of RO membrane have limited this process from being applied more widely in leachate treatment. The first major disadvantage of the RO process is the huge consumption of energy. Because RO is a tight membrane which rejects all salts (contributing to osmotic pressure), higher total dissolved solids (TDS) concentration in the leachate mandates more transmembrane pressure, hence energy costs. The second disadvantage is membrane fouling. Fouling is a problem for all membrane types, but ROs are especially intolerant of fouling due to the high resulting operational pressure and low permeate flux when fouling occurs. As mentioned above, landfill leachate contains a multitude of contaminants (bacterial, particulate, organic, colloidal, and ionic) in high concentrations. Therefore, the fouling of RO membrane when applied for landfill leachate treatment is faster and more severe than when used for cleaner applications, such as drinking water treatment. The fouling of the RO membrane increases both operational costs (higher operating pressure, chemicals and downtime for cleaning, labor to mitigate fouling), and also reduces the lifetime of the membrane. Another disadvantage of a tight membrane like RO is the generation and management of the concentrate stream. The concentrate stream can be brought back to landfills in some cases. However, ultimately the concentrate still ends up in leachate.

For the above reasons, although RO is a very promising technology for landfill leachate treatment, it is necessary to first solve the aforementioned limits before the

process finds greater application for landfill leachate treatment. In order to reduce the load of contaminants on the RO membrane, which results in decreased membrane fouling, pretreatment methods such as coagulation, MF, UF membrane techniques, membrane cleaning or searching for new membrane materials have been applied and studied. However, there is not any technique which can solve the membrane fouling issue completely. In truth, RO membranes may not be the most appropriate technology for treating raw leachate, but rather as a tertiary polishing step after the landfill leachate is treated by another technology such as looser membrane processes, as described below.

### 2.2.2. Nanofiltration (NF) Membrane

Nanofiltration is an alternative membrane technology for landfill leachate treatment. Nanofiltration membrane is also a high pressure filtration application. But the operation pressure of nanofiltration is lower than RO membrane process. However, NF membrane only can remove divalent ions and molecules which are larger than 0.2 kD in molecular weight. Therefore, the removal efficiency of NF membrane to $NH_4^+$ which has been reported is very low. According to the literature review of Renou (2008), the $NH_4^+$ removal by NF can only reach 50%. In the same review, the COD removal by NF membrane was also reported much lower than the RO membrane's which is around 60-70%. Kwon et al., (2008) conducted a study on using NF-Rotary Disk Membrane (NF-RDM). The research also showed the poor removal performance of NF membrane to $NH_4^+$ and COD. The removal of $NH_4^+$ and COD were achieved only around 13.9% and 51.9%, respectively. This limitation results in the application of direct treatment by NF membrane for landfill leachate is not widely applied compare to RO membrane.

Moreover, NF membrane has the same issue as RO membrane which is fouling of the membrane. When applied to highly contaminated water such as leachate, the fouling of NF membrane can be severe. Thus, there are often pretreatment methods used before the NF membrane treatment step. The pretreatment steps will help reduce the fouling on the membrane and also enhance the removal efficiency of the membrane. The fouling of the NF membrane and RO membrane is because of the constituents such as dissolved organic compounds, inorganic compounds, colloidal and suspended particles. Similar to RO membrane, energy consumption is also a factor that should be considered when using NF membrane. Although NF membrane is operated at lower pressure than RO membrane, NF is still a high pressure driven membrane application. Thus the energy consumption of NF is one of the big factors when considering using this application.

### 2.2.3. Ultrafiltration (UF) and Microfiltration (MF) Membranes

UF and MF membranes are rarely used directly for leachate treatment. The rejection size of UF and MF are 0.01 and 0.1 μm, respectively. Therefore, the removals of dissolved organic and inorganic contaminants in leachate by UF and MF are not significant. However, UF and MF are quite capable of removing colloidal matter, suspended particles and macromolecules. Therefore, UF and MF membranes are often not used alone for landfill leachate treatment, but rather as pretreatment methods for NF and RO membrane. The pretreatment by MF and UF helps to reduce the fouling on NF/RO membrane.

Tabet and colleagues (2004) reported a study on the purification of landfill leachate by different types of membranes. In the research, the range of membrane size

was tested from 20,000Da (UF size) to 100 Da (RO size), and the leachate which was chosen for the study has the COD of 1300 mg/L. COD removal efficiency of UF sizes of 20,000, 10,000 and 3000 Da are around 15%, 30% and 46%, respectively. The removal efficiency of the UF membranes was much lower than the RO membrane. The COD removal efficiency achieved by RO was higher than 93%. The result of RO membrane in this study once again confirmed that the COD removal efficiency of RO membrane can be achieved at very high percentage.

Recently, studies have applied the combination of adsorption or coagulation methods, and UF/MF membrane to treat landfill leachate. The results are very promising. Adsorption and coagulation will enhance the size rejection process of the membranes. Moreover, in the adsorption process, $NH_4^+$ can sorb to the adsorbent, followed by rejection of the adsorbent by the membranes. This concept was tested and reported with promising results. Pi et al., 2009, introduced a combined process of air stripping and coagulation/ultrafiltration for leachate treatment. The study showed that with this combined process helped to increase the BOD/COD ratio (a measure of biodegradability), likely by removing the more recalcitrant fractions of leachate organic material. The COD removal efficiencies for single coagulation process, single UF process (3kDa) are 38% and 84.2%. The combination of these two processes was achieved at 84.6% which is an insignificant difference from a single UF process. However, the BOD/COD of this combined process was higher than each single one which is 0.43 compared to 0.31 of UF and 0.124 of coagulation process. This means the combined process can removes more recalcitrant compounds, thereby increasing the biodegradability of the permeate. Nevertheless, the COD remained after this application

was still very high, more than 2000 mg/L. Therefore, this application can only be used as a pretreatment for biological processes or NF/RO membrane processes.

## 2.3. Membrane Bioreactor for Landfill Leachate Treatment

The membrane bioreactor technology has been applied widely recently for wastewater treatment. The coupling between two processes, biological processes and physical processes has helped to increase the removal efficiency of contaminants in the leachate. The biological process (aerobic or anaerobic) helps to degrade or transform organic compounds and the physical process (membrane) helps to retain the sludge in the reactor and remove solids, bacteria, colloidal particles and macromolecules from the wastewater. Typically, MF and UF membranes are used in MBR applications. Compared to conventional method, the advantages of MBR can be listed as below:

- Very compact design because of high biomass concentration
- Suspended solids, microorganism and trace contaminants can be removed by MBR
- Low excess-sludge production (for anaerobic systems)
- Very stable process operation
- High sludge age has the potential of degrading more recalcitrant compounds.
- Higher effluent quality

Due to the many advantages, the MBR has emerged as a new focus area in the fields of wastewater and landfill leachate treatment. Many authors have reported their studies on applying MBRs for landfill leachate treatment. However, most of the attention has been on aerobic processes such as variations of activated sludge. One of the

advantages of MBR for landfill leachate treatment is reduced operator attention concerning bulking problems of the sludge. In the report of Laitinen et al., 2006, the authors showed that the bulking problem was absent in the aerobic MBR system. Moreover, in the same report, the total suspended solids (TSS), COD, BOD, total P removal efficiencies, 99%, 97%, 84% and 88% respectively, were much higher compared to the SBR which was fed with the same leachate. These high results were also proved by other studies such as study by Bodzek et al., 2006 and study of using airlift-aerobic-MBR system for landfill leachate treatment in Beijing, China by Chen and Liu Junxin, 2006.The removal of soluble COD varied from 70-96% and the BOD's was about 99%. In the study, Chen and Liu, 2006, also showed the performances of some other MBR systems as in the Table 2.2.

The high removal efficiency of MBR was also demonstrated for anaerobic thermophilic bioreactor which was studied by Visvanathan and colleagues in 2007. The efficiencies of 62%-79%, 97%-99% were observed in the study for COD and BOD, respectively. Depending on the strength and characteristics of the leachate treated, the COD removal efficiency in MBR systems generally vary from 31%- 90%, with residual COD depending on concentration and characteristics of the influent. Some modifications to the MBR system have been tested, which may help to further increase the COD removal. One of that is a hybrid MBR system. For instance, Pirbazari et al., 1996, studied the combination of using microorganism (activated sludge), powdered activated carbon (PAC) and cross flow ultrafiltration membrane to remove TOC, COD, BOD in landfill

**Table 2.2:** MBRs performance for landfill leachate treatment. (After Chen and Liu, 2006).

| | | COD | | | BOD | | | $NH_4^+$-N | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Scale | HRT (h) | In (mg/L) | Out (mg/L) | Removal (%) | In (mg/L) | Out (mg/L) | Removal | In (mg/L) | Out (mg/L) | Removal |
| Full | 96 | 3000 | - | - | <0.1 ( c) | - | - | 1200 | 29(a) | 96 (b) |
| Full | - | 400-1500 | 211-856 | - | 100-500 | 4.3-29 | - | 200-1400 | 100-408 | - |
| Lab | 24 | 8000-9000 | 1800-2400 | - | 0.4-0.45 (c) | 60-100 | - | 340-360 (d) | 120-150 | |
| Lab | 24 | 1800 | - | 31.3 | 267.5 | - | 98 | 114.8 | - | 66 |

Note: (a) Inorganic nitrogen; (b) total nitrogen removal; (c) $BOD_5$/COD; (d) after ammonia stripping.

leachate. The PAC provided the mechanism of adsorption organic carbon, then exposed to the microorganism and acclimation of the microorganism cultures. In the meantime, the microorganism degraded the organic carbon. The UF membrane has the function of size removal of organic carbon which adsorbed to sludge and PAC and retained the sludge in the reactor. This system is known as activated sludge MBR combined with PAC. Pirbazari et al., showed that the removal efficiencies of TOC, COD, BOD in this system were 96-97%, 96-97% and 96-98% respectively. Additionally, the removal efficiencies of some micro-pollutants such as phenol and benzoic acid were over 99.7%. It should be noted this is a hybrid aerobic MBR process with PAC. To our knowledge, hybrid anaerobic MBR with PAC addition for leachate treatment and micropollutant removal, the subject of this study, has not been reported in the literature.

Anaerobic processes have been used for the treatment of young landfill leachate, which generally has high COD and high BOD/COD ratio (an indication of % biodegradability). With wastewater having high organic loading, such as landfill leachate, anaerobic processes show more advantage because of low energy requirement. Bohdziewicz et al., 2008 showed that the removal of COD in their AnMBR could reach as high as 90% at HRT of 2 days. Yiping et al., showed approximately the same removal efficiency of COD in leachate which is 89%, and the BOD and TOC removal efficiencies were also reported very high, 99% and 89% respectively. The organic loading rate was achieved at a highest level of 2.5 kg/m3d.The initial COD concentrations of both above studies were lower than 10,000 mg/L. Jia et al., from Ninja, China reported at the International Conference on Energy and Environment Technology, 2009 that the COD removal could reached 83% after almost 3 start-up months when they treated the leachate

which have COD average values of 59,000 mg/L. Although the AnMBR can achieve over 80% of removal of COD, the COD remaining in the effluent is still too high for surface discharge. Therefore, post treatment methods applied for polishing the MBR effluent may be appropriate. Although there are some studies reported on the performance of AnMBRs for removal of micropollutants in wastewater, the research for potential of micropollutants removal by AnMBR is still largely unknown.

## 2.4. Micropollutants: Endocrine Disrupting Compounds (EDCs) and Pharmaceutical and Personal Care Products (PPCPs)

Recently, micropollutants such as endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) have increasingly attracted attention from scientists and the public. Their concern over EDCs and PPCPs stems from the significant ecological and health consequences at trace levels. Richard et al., 2004 showed that at a medium and high concentrations of PPCPs mixtures (combination of three compounds: ibuprofen, floucetin and ciprofloxacin) (from 60 μg/l-1000 μg/l) fish mortality occurs in time of 35 days and 4 days, at medium and high concentration, respectively. EDCs are hypothesized to cause alterations for endocrine system of wildlife. Sumpter (2005) showed that EDCs caused feminization of male fish and affected the fecundity of female fish (Diniz et al., 2005). There have been several investigations on the effects of EDCs on animals other than fish such as birds, amphibians, and panthers (Nghiem, 2002; Nghiem, 2004). There has also been speculation in recent years of potential negative impacts to human health, such as decreases in male sperm counts and increases in testicular, prostate, and ovarian cancers.

**Table 2.3:** Characteristics of some EDCs.

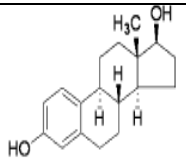
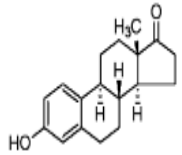
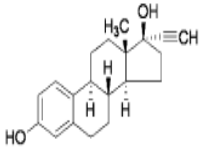
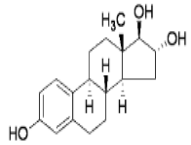
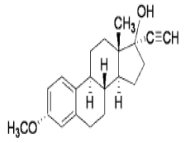
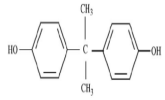
| **Compound** | **Structure** | **M.W. (g/mol)** | **Water Solubility (mg/L)** | **Log $K_{ow}$** | **$pK_a$** | **Vapor Press. (mmHg)** | **Henry's Law Constant ($atm.m^3/mole$)** | **Typical Uses** |
|---|---|---|---|---|---|---|---|---|
| 17 β-Estradiol (E2) |  | 272.4 | 3.9-13[1] 5.4-13.3[2] | 4.01[3] 3.8-4.0[2] | 10.46[4] | $1.26*10^{-8}$[3] | $3.64*10^{-11}$[3] | Hormone replacement therapy.[5](birth control, drug, etc) |
| Estrone (E1) |  | 270.4 | 13[3] 0.8-12.4[2] | 3.13[3] 3.1-3.4[2] | 10.34[4] | $1.42*10^{-7}$[3] | $3.80*10^{-10}$[3] | Natural hormones in human body, pharmaceutical |
| 17α-Ethynylestradiol (EE2) |  | 296.4 | 4.8[3] | 3.67[3] | 10.4[4] | $2.67*10^{-9}$[3] | $7.94*10^{-12}$[3] | Oral Contraceptive[5] |

**Table 2.3** (Continued).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Estriol ($E_3$) |  | 288.4 | $13_{[3]}$ 3.2- $13.3_{[2}$ | $2.45_{[3]}$ 2.6- $2.8_{[2]}$ | 10.38 | $1.97*10^{-10}{}_{[3]}$ | $1.33*10^{-12}{}_{[3]}$ | Natural hormones in human body, Pharmaceuticals |
| Mestranol |  | 310.4 | $0.32_{[3]}$ | $4.8_{[3]}$ | 10.26 | $7.5*10^{-10}{}_{[3]}$ | ----- | Oral contraceptives |
| Bisphenol A |  | 228.29 | $3_{[6]}$ | $3.40_{[6]}$ | 9.59- $11.3_{[6]}$ | $3.975*10^{-8}{}_{[6]}$ | $1*10^{-11}{}_{[3]}$ | Polycarbonate plastic container and food cans. |

Among the EDCs, estrogenic compounds are of higher concern than other compounds. At very low concentrations (i.e., ng/l range), estrogenic compounds can stillhave negative effects on fish (Arcan-Hoy et al., 1998; Panter et al., 1998). The changes in fish reproduction can be measured when fish are exposed to 17β-estradiol (E2) and 17α-ethinyl estradiol (EE2) with concentration of 2ng/l in laboratory conditions. Gadal et al., (2005) indicated that natural estrogen E2 plays a very important role in breast cancer initiators. Estrogenic compounds can enter the environment via different sources. However, it is reported that a significant source for E2 in the environment is via improper or inadequate disposal of medicine. Therefore, in order to prevent harm to the environment and human health, estrogenic compounds should be removed from any source which may have the potential for releasing them into the environment.

## 2.5. Current Findings of Behaviors of EDCs in Conventional Wastewater Treatment Under Aerobic and Anaerobic Condition

There are many studies on the behaviors of EDCs under aerobic and anaerobic processes. The behaviors of EDCs also vary considerably, depending on the characteristic of each compound. The behaviors could be sorption of the compounds to the solid phase, transformation to another compound or completely degradation (mineralization) to$H_2O$ and $CO_2$ (aerobic system) or $CH_4$ and $CO_2$ (anaerobic system).

### 2.5.1. Adsorption of EDCs into Sludge

Adsorption behavior of EDCs to solid phase is found in most of the studies and is one of the important mechanisms for EDCs removal (Birkett and Lester, 2002; Ren et al., 2007). The adsorption rate of EDCs in sludge systems are listed in Table 2.4.

The adsorption rate of the EDCs to sludge was found as higher for those compounds with higher $K_{ow}$ (Urase et al., 2005). Birkett and Lester (2002) stated that for compounds with log $K_{ow}$ higher than 4, adsorption to the sludge is dominated, while there is low adsorption potential and dissolved organic species are more important if log $K_{ow}$ is less than 2.5. The reason for that is because higher $K_{ow}$ means lower solubility in water and higher distribution to organic matter. Most of the EDCs have their $K_{ow}$> 2.5, therefore adsorption mechanism is always found in sludge system.

### 2.5.2. Biotransformation and Biodegradation

Beside the adsorption mechanism found for removal of EDCs, biodegradation and biotransformation of EDCs were also found. Under aerobic condition, many EDCs such as nonylphenol and bisphenol A were reported to be able to degrade under aerobic condition (Tanghe et al., 1998; Staples et al., 1998; Ike et al., 2006; Zhao et al., 2008). Zhao et al. (2008) found that 98% of BPA can be degraded in activated sludge in their research and only small amount still stayed in the sludge phase after 5hour of experiment operation. Ying and Koona (2003) conducted the experiment to treat 5 EDCs such as BPA, E2, EE2,4-*tert*-octyl phenol (4-*t*-OP), and 4-*n*-nonyl phenol (4-*n*-NP) in seawater by marine sediments. The results showed that under aerobic condition all 5 compounds were degraded within 56 days.

The EDCs were also found degradable under anaerobic condition (Ike et al., 2006; Kang and Kondo, 2002; Ying and Koona, 2003). However, most studies report that EDCs degraded better under aerobic condition than under anaerobic condition (Yi et al., 2008; Ying and Koona, 2003; Kang and Kondo, 2002a). Kang and Kondo (2002a) showed that

**Table 2.4:** Adsorption of EDCs compound to sludge system.

| EDCs | System | $K_d$ ($L.Kg^{-1}$) | Adsorption rate | Reference |
|---|---|---|---|---|
| BPA | Anaerobic digester sewage sludge | 123-199 | 75% | Ivashechkin et al., (2005)<br>Clara et al., (2004) |
| 17β-estradiol, | Sewage granule matrix | | >90% | Keenan et al., (2008) |
| 17α- estradiol, | Sewage granule matrix | | >90% | Keenan et al., (2008) |
| 17a-ethinyl-oestradiol-3-methyl ether | Sewage granule matrix | | >90% | Keenan et al., (2008 |
| E2 | Activated sludge | 245-604<br>691.83 | 87.2% | Ifelebuegu et al., (2010)<br>Clara et al., (2004) |
| EE2 | Activated sludge | 267-631<br>691.83 | 92.5% | Ifelebuegu et al., (2010)<br>Clara et al., (2004) |
| Nonylphenol | Activated sludge | | 99 % | Bouki et al., (2010) |

BPA degraded better under aerobic condition (>90%) than under anaerobic condition (only < 10%). Several reports also found that the EDCs can be degraded under denitrification and sulfate reducing conditions (Czajka and Londry, 2006; Ying et. al., 2008). Czajka and Londry et al., 2006 studied the biotransformation of estrogenic compounds such E2, E1 and EE2 under the conditions of methanogenesis, sulfate-reducing, and nitrate-reducing by using lake sediment. The author stated that EE2 was not degraded in any of anaerobic condition and E2 was oxidized to E1 under all four conditions. The conversion back and forth from E1 to E2 was also reported from some conditions (Shi et al., 2010).

## 2.6. MBR for Removal of Micropollutants

Wastewater treatment has continuously developed over the years both in terms of treatment objectives and technologies. According to Lyko et al., in the 1950's, the wastewater treatment system only had a conventional activated sludge (CAS) tank and a clarifier for the main purpose of BOD treatment. Then the processes of biological nutrient removal (nitrification, denitrification and phosphorous removal) were developed over the following decades for the objective of controlling eutrophication in surface waters. Coming to the first decade of the 21$^{st}$ century, a question which has been raised for wastewater treatment is how to enable and enhance the removal of micropollutants.

With the arrival of the 21$^{st}$century, the industry also saw the rapidly increasing application of MBRs in wastewater treatment. MBRs exhibit numerous advantages compared to conventional treatment, and is considered the state of the arts in wastewater

**Table 2.5:** MBR performance for EDCs removal. (Lyko et al., 2005).

| Compound | MBR rejection [%] | CAS rejection [%] | Reference |
|---|---|---|---|
| NPEOs [a] | 91 - 97 | < 86 | Schröder et al. 2000 [6] |
| $NP_2EO$ [b] | 97.8 | 98.5 | Hegemann et al. 2002 [8] |
| NP | 81.2 | | Hegemann et al. 2002 [8] |
| E1 [c] | 96.3 | 91.2 | Hegemann et al. 2002 [8] |
| E2 [d] | 100 | 91.0 | Hegemann et al. 2002 [8] |
| EE2 [e] | 92.4 | 100 | Hegemann et al. 2002 [8] |
| E1 | 93.8 – 99.7 | 87.8 – 97.5 | Zühlke et al. 2003 [9] |
| E2 | 95.7 – 98.5 | 94 – 97.5 | Zühlke et al. 2003 [9] |
| EE2 | 81.9 – 93.6 | 59.4 – 81.5 | Zühlke et al. 2003 [9] |
| E2-Eq [f] | 75 | 58 | Love et al. 2002 [7] |
| BPA | 92.7-99.9 | 82.6 | Clara et al. 2004a [10] |

[a] NPEOs = nonylphenol ethoxylates
[b] $NP_2EO$ = nonylphenol diethoxylate
[c] E1 = estrone
[d] E2 = 17β-estradiol
[e] EE2 = 17α-ethinylestradiol

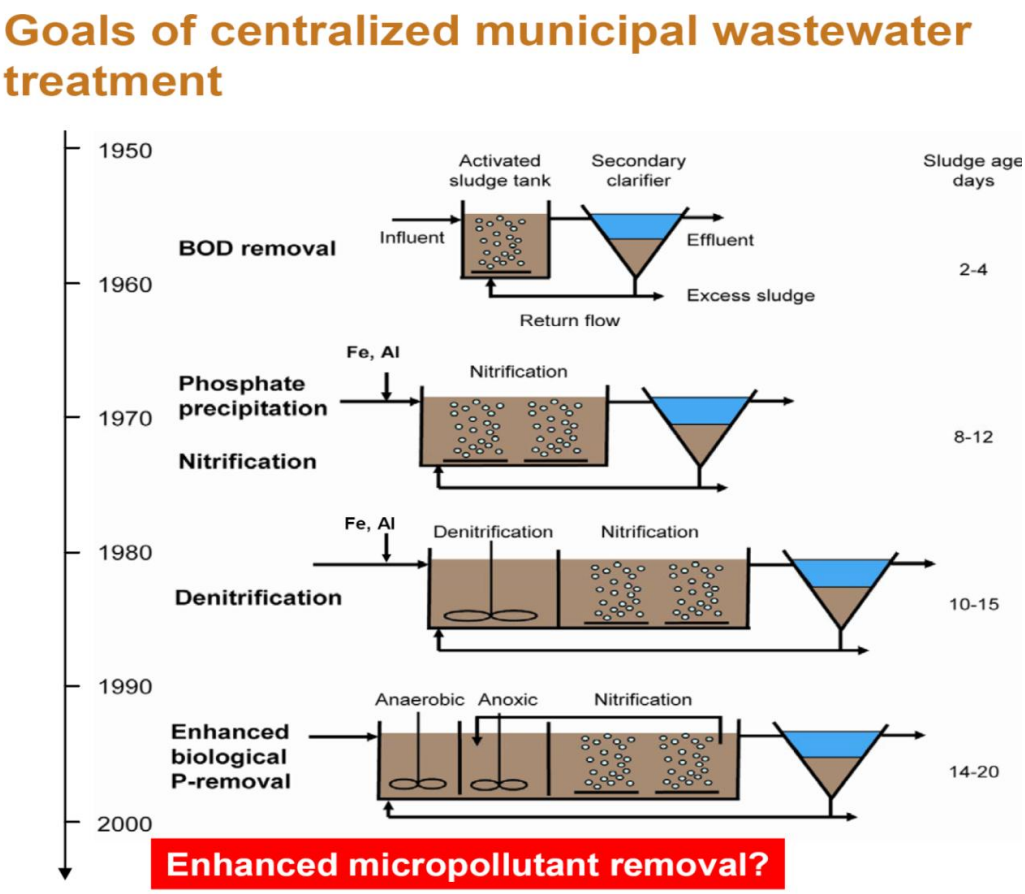



**Figure 2.3:** Evolution of municipal wastewater treatment. (From Lyko et al., 1998).

**Table 2.6:** Comparison between conventional activated sludge system (CAS) and MBR.

| **Mechanisms** | **CAS** | **MBR** |
|---|---|---|
| Adsorption to sludge and Adsorbents (e.g., PAC) | Yes + Retention by clarifier ( + ) | Yes + Retention by membrane ( + + ) |
| Biotransformation and Biodegradation | Yes ( + ) | Yes ( + + ) |
| Adsorption to membrane Surface | NA | Yes ( + ) |
| Solid retention time | ( + ) | ( + + ) |

treatment. Recently, the potential of removal of micropollutants in the wastewater by MBR technology has attracted a lot of attention in the research community. Several studies were implemented to answer the question of whether the MBR can enhance the removal of micropollutants. Lyko et al., 2005, summarized the comparison of MBRs and conventional activated sludge (CAS) for removal of micropollutants in wastewater (Table 2.5). The results in Table 2.5 showed that, compared to CAS's, the MBR's removal efficiencies were higher for some micropollutants, yet equal or lower for some others. Factors which affect the behaviors of micropollutants in the biological reactor are related to rates of degradation and transformation of micropollutants and adsorption to particles and biomass. For the scenario where micropollutants are degradable, the MBR removal efficiency can be higher or equal to the CAS. This is because the MBR has much higher biomass activities than the CAS, so the removal of the MBRs should be at least equal to CAS. This hypothesis was supported by the results of Clara et al., (2005), who showed that the compounds which were considered as degradable during wastewater treatment processes, such as BPA, IBP, BZF, had enhanced removals in MBRs compared to CAS. Dewever et al., 2007 also reported similar findings, that the degradation of Benzothiazole-2-sulfonate (BTSA) was more extensive in the MBR system than the CAS. Another scenario of removal is that some micropollutants only sorb to the sludge and are not degraded. Under this scenario, the removal efficiencies might be higher for the MBRs than the CAS at the beginning because the sorption of the micropollutants to higher concentration biomass in MBRs. However, when sorption to biomass reaches saturation, the removal efficiency of the MBR becomes similar to CAS because of the accumulation of the micropollutants in the reactor. The third scenario is if the

micropollutants can neither be degraded nor adsorbed, as is the case for certain recalcitrant chemicals which only sorb weakly to sludge. In this case, the micropollutants may come out in the effluent at the same concentration as the influent. If the membrane in the MBR can retain or adsorb this compound to its surface, then the removal efficiency in the MBR system can be higher than the CAS. Otherwise, it would be at the same efficiency if the compound neither adsorb nor degraded in the reactor. Based on the three scenarios, it can say that MBR system has generally enhanced the potential in removal of micropollutants over the CAS process. The higher removal efficiencies of MBR to micropollutants compared to CAS were reported in many reports. Besides the studies mentioned above, Radjenovic et al., 2007, also showed their results when compared the removal of many micropollutants such as carbamazepine, ibuprofen, clofibric acid, Bezafibrate, etc, in MBRs and CAS. Most of the cases proved that the MBRs removed more than the CAS. The summary of the potential of MBRs in removal of micropollutants can be summarized in Table 2.6.

## 2.7. Theoretical Framework Related to Effect of Retention Time on Fate of Recalcitrant Compounds in Wastewater Treatment

Solids retention time (SRT), hydraulic retention time (HRT) and chemical retention time (CRT) are three important parameters related to operating a biological reactor for wastewater treatment. The ideal wastewater treatment system would have high SRT, high CRT and low HRT value. Longer SRT means giving more time for biomass (microorganism) to remain in the system, an important consideration for anaerobic processes which have many slow growth microorganisms. Thus, the microorganisms in

the system not only have enough to grow but also more contact time with the contaminants or micropollutants to break and consume them. Longer CRT would give the contaminants or micropollutants more contact time in the bioreactor, so as to facilitate their degradation (especially important for those which are recalcitrant). Low HRT means more effluent throughput from the reactor, which is desirable. Unfortunately, most systems are unable to decouple HRT and CRT. Hence, greater effluent throughput also means less contact time for chemicals of concern, and vice versa. Over the years, the development of the wastewater treatment system has aimed to decouple more the SRT, CRT with HRT. Figure 2.4 shows the evolution of wastewater treatment along with the effort to further decouple those parameters.

For a conventional biological reactor with just a completely stirred tank reactor (CSTR), the SRT, CRT and HRT are equal since there is no mechanism to separate solids, colloids and dissolved chemicals from water (Figure 2.4a). The contaminants and biomass come out the system at the same time as the water. Obviously, the treatment efficiency is not high at all with this system. With the addition of a gravity-driven settling tank (clarifier) to the CSTR, biomass and other suspended solids are mostly retained within the system (Figure 2.4b). Hence, the system is capable of mostly decoupling the SRT to HRT (SRT $\geq$ HRT). However, with the exception of some sorption to biomass, dissolved chemicals and those associated with colloidal matter still travel with the bulk liquid and there is no separation of CRT and SRT (CRT = HRT). An improvement for the system happens when a sand filter is used for tertiary filtration following the clarifier (Figure 2.4c). The sand filter helps to remove fugitive solids and traps colloidal matter, which assists in removing chemicals sorbed to colloids and solids.

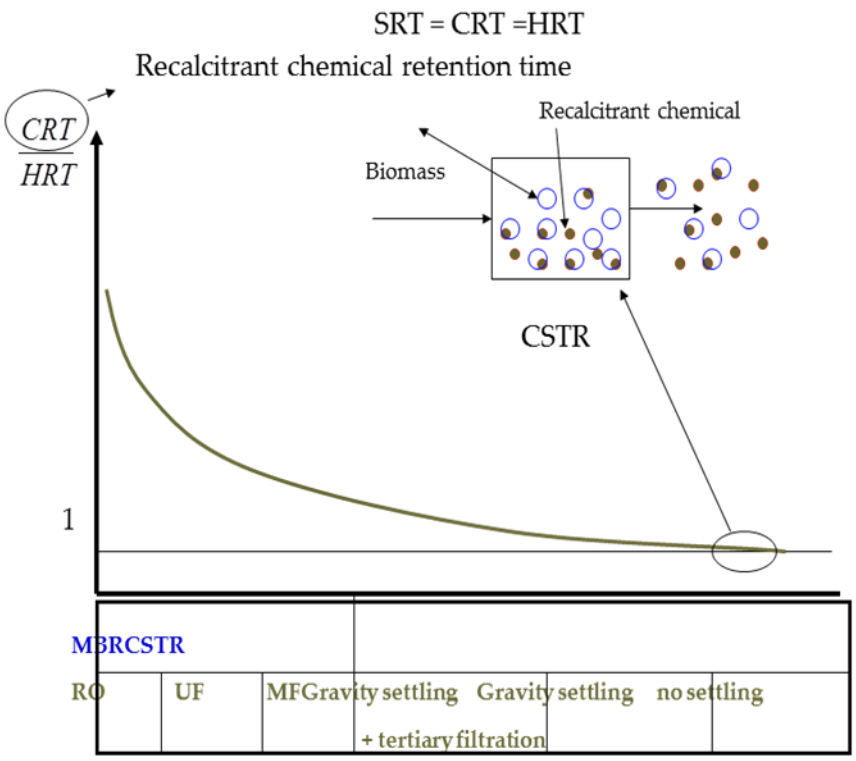
With this system, the CRT is improved to be greater than the HRT. A technological breakthrough occurred with the invention and acceptance of the MBR. The membrane in MBR system can retain 100% of suspended solids and colloids. Because this system uses absolute barrier separation (based on size) rather than gravity separation (based on specific gravity), the SRT can be completely decoupled from the HRT and be increased significantly (SRT >>HRT). As mention in previous session, the biomass concentration and activity in the bioreactor are much higher than the CAS because of the biomass retention capability of the MBR system. However, the CRT is only slightly improved from the previous tertiary filtration system (CRT>HRT) because the MF or UF membranes used in MBR, owing to their pore size, are not capable of directly rejecting organic compounds (although some rejection occurs for organic compounds when they are associated with colloidal matter which do get rejected by UF or some MF membranes).

A significant research question is whether and how can the MBR system be improved to significantly decouple the CRT and the HRT. As previously noted, some micropollutant removal can occur when the chemicals are sorbed to biomass. If a strong adsorbent (with capacity much greater than biomass) is added to the reactor and it is of a size large enough to be rejected by membranes used in MBR systems, then the micropollutant will be retained in the system separate from the membrane permeate, resulting in significant decoupling of CRT and HRT (CRT >> HRT) and low micropollutant concentration in the effluent. The micropollutants retained in the system would then be provided longer incubation time (equal to SRT) and be subjected to more extensive biotransformation or biodegradation, possibly overcoming recalcitrance. This

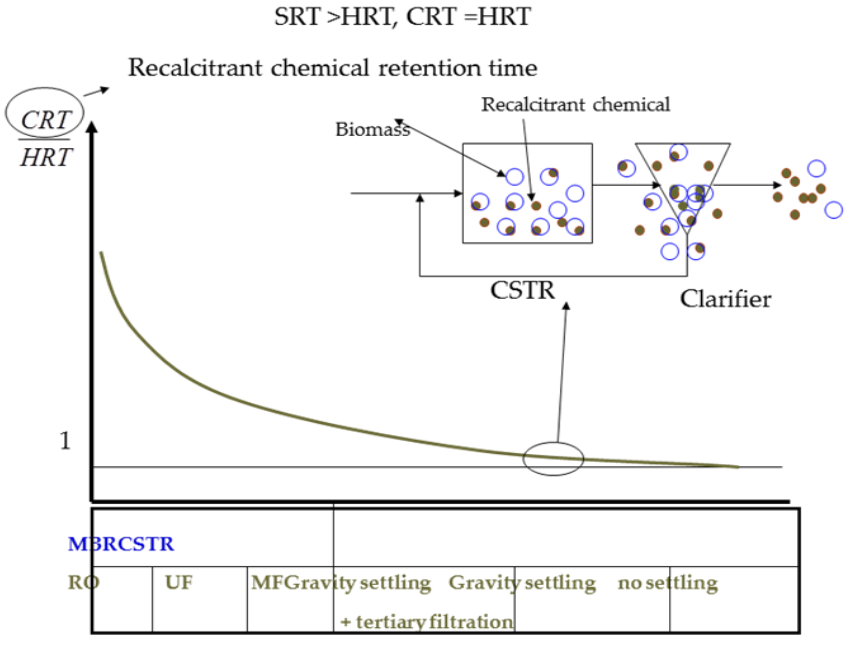
hypothesis will be tested in this study. The above discussion on effect of technology on SRT, CRT and micropollutant removal is summarized in Table 2.7.

**Table 2.7:** Effect of bioreactor system configuration on biomass and chemical retention in wastewater treatment system.

| **Technology** | **Ratio of SRT/HRT** | **Ratio of CRT/HRT** | **Removal of micropollutants** |
|---|---|---|---|
| CSTR | 1 | 1 | Poor |
| CSTR + clarifier | >1 | ~1 | Fair |
| CSTR + clarifier + sand filtration | >1 | >1 | Good |
| MBR | >>1 | >1 | Very good |
| MBR + PAC | >>1 | >>1 | Excellent |



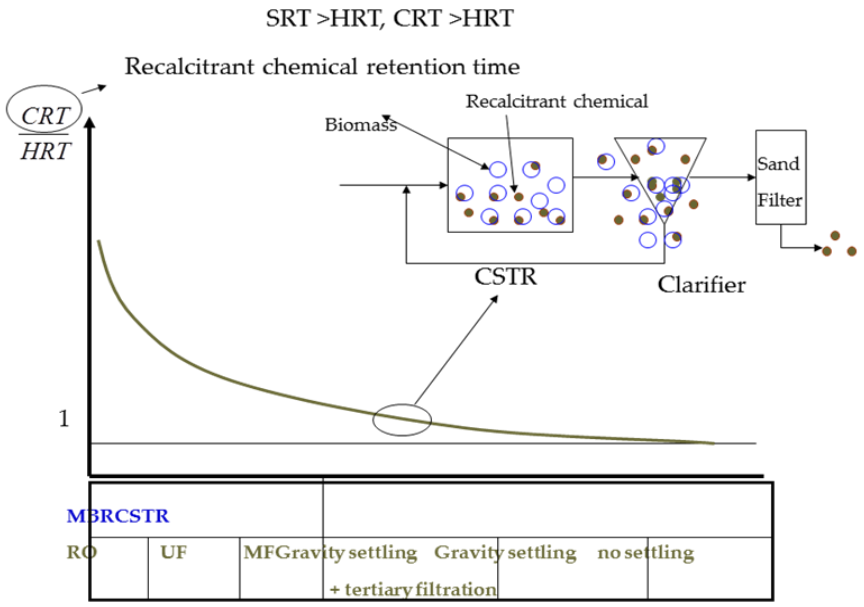

a.




b.

**Figure 2.4:** The evolution of wastewater treatment for micropollutants removal. a. CSTR, b. CSTR + clarifier, c. CSTR + clarifier + sand filter system as tertiary treatment, d. CSTR + UF membrane (MBR system), e. CSTR + UF membrane + adsorbent (e.g., PAC ) added (hybrid MBR system).

c.




d.

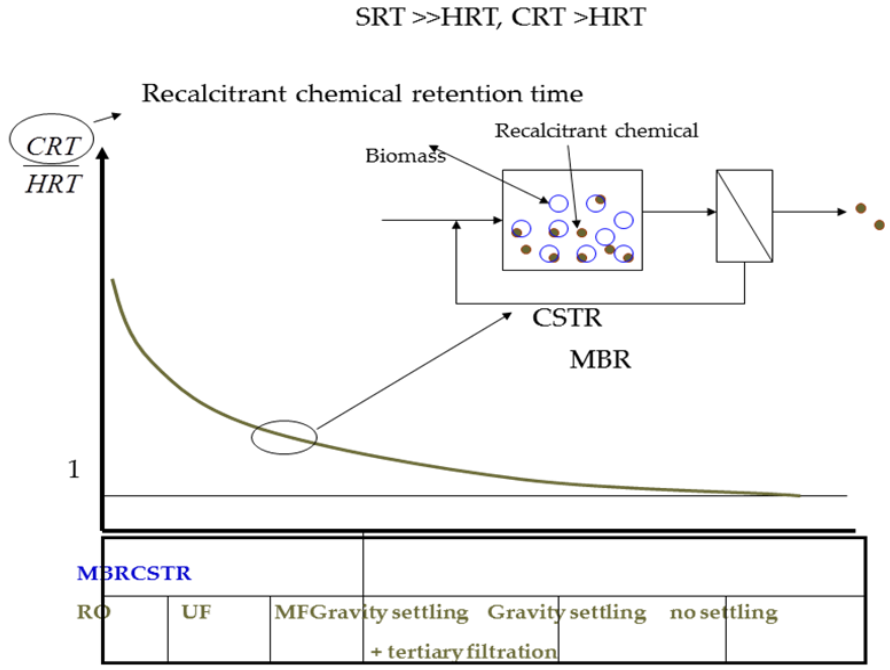



**Figure 2.4** (Continued).

e.

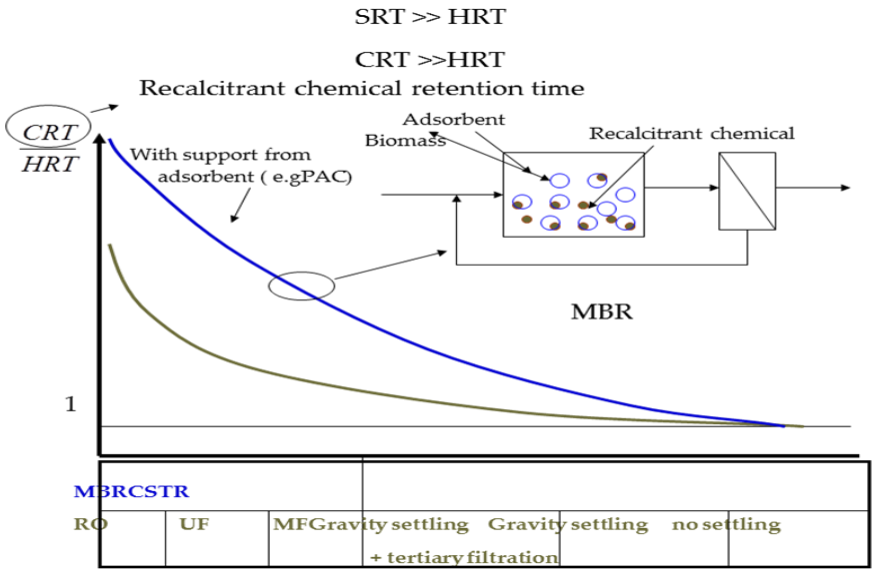



**Figure 2.4** (Continued).

# CHAPTER 3: RESEARCH APPROACH AND PHASES OF STUDY

## 3.1. Research Motivation

Currently, one of the most common ways to treat landfill leachate is to transport the leachate to a WWTP. However, it would not only consume a lot of chemicals, but also be a financial burden to treat the young landfill leachate without any pretreatment in a WWTP, because young landfill leachate contains a lot of COD, BOD. Moreover, according to our literature review, WWTP cannot completely remove micropollutants such as PPCPs or EDCs. Therefore, it is necessary to look for a pretreatment method which is able to reduce COD and remove completely micropollutants in young landfill leachate.

According to existing literature, there are some gaps in studies of landfill leachate treatment which need further research to investigate:

- There is no treatment methods which can both reduce efficiently COD and completely remove micropollutants such as PPCPs and EDCs in landfill leachate.
- The AnMBR has not been commonly applied to landfill leachate treatment due to membrane fouling issues.
- There is no study to investigate the potential of AnMBR for removal of EDCs in a high organic compound background wastewater such as young landfill leachate.

- The decoupling potential of CRT and HRT by adding a strong adsorbent such as PAC into AnMBR for removal of micropollutants has not been fully investigated.

## 3.2. Methodology

A novel hybrid AnMBR process was tested on its ability to significantly reduce organic strength (COD) and remove micropollutant from landfill leachate. A hormone prevalent in the environment and household trash, 17β-estradiol (E2), was used as the model compound for determining the efficiency of the treatment process. Methods were developed for the extraction and quantification of E2 from liquid and solid phases. The phase distribution and biodegradability of E2 were assessed in separate batch assays. A laboratory-scale system was developed to treat first synthetic leachate, then actual landfill leachate. The objectives are to demonstrate proof of concept and determine important operational parameters.

## 3.3. Hypotheses

As mentioned, the concentrations of micropollutants in landfill leachate can be quite high. Few research have reported on the removal of micropollutants in landfill leachate by MBR system in general; and fewer by AnMBR systems. The mechanisms on how anaerobic processes may biotransform or biodegrade estrogens such as E2 have not been sufficiently studied. It is necessary to have these understanding in order to develop a hybrid AnMBR system for effectively removing micropollutants in landfill leachate. This research will focus on studying the mechanisms in AnMBRs system for removing

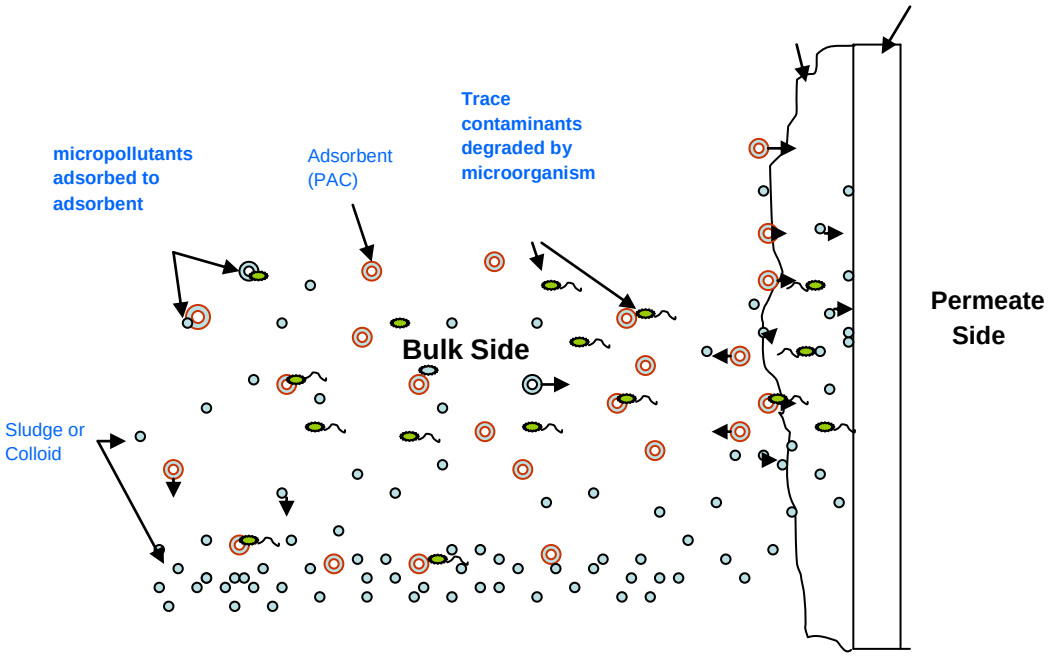



**Figure 3.1**: Hypothesized mechanisms for removal of micropollutants in MBR system.

micropollutants in landfill leachate. The success of research will help to emerging issues, that of negative impacts of micropollutants on environmental and human health. The hypotheses which will be tested in the research are as follows.

- E2 can be biotransformed or biodegraded under anaerobic condition, when given adequate reaction time (chemical residence time, CRT).
- While ultrafiltration (UF) membranes are not expected to significantly reject E2 due to the size of the molecule, the presence of colloidal matters or added adsorbent in leachate can improve the rejection of E2 by UF membrane, thereby decoupling CRT and the hydraulic residence time (HRT).
- Longer CRT can result in increasing removal of E2 due to increase retention and reaction in the system.

- The hybrid AnMBR process (with sorbent addition) can be effective for removing recalcitrant micropollutants such as E2 from leachate.

## 3.4. Phase of the Study

Research on using AnMBRs to remove estrogenic compounds in landfill leachate was conducted in five phases.

- Phase I: Develop analytical methods for measuring E2 and other estrogenic compounds (both liquid and solid phases) in landfill leachate and bioreactor system.
- Phase II: Design, build and start up, an AnMBR system treating leachate, and quantify COD removal.
- Phase III: Determine phase distribution of E2 in a hybrid membrane bioreactor system with PAC addition.
- Phase IV: Conduct batch experiments to determine anaerobic biotransformation or biodegradation rate of E2.
- Phase V: Operate hybrid AnMBR for removal of COD and E2 from leachate.

Phase I focused on developing the analytical methods to quantify estrogenic compounds in water, wastewater and solid phases. Estrogenic compounds are among the most difficult chemicals to analyze at low concentrations. Therefore, success in developing in the analyzing those chemical was critical to the success of this research. Three estrogenic compounds chosen to test are E2, estrone (E1) and 17 α-ethynyl estradiol (EE2). Methods evaluated were UV/Vis spectrophotometry, GC/MS with direct injection of analyte, and GC/MS with solid phase micro-extraction (SPME) of analyte.

Factors which may affect the analysis of the estrogenic compounds were also tested such as pH, concentration of salt, temperature, mixing speed in order to optimize the analytical methods for those compounds. A solid phase extraction method was developed to enable mass balance determinations.

Phase II focused on design, fabrication and start-up of the AnMBR system. The AnMBR system was designed and built under an original design, then the system was tested and modifications were made during the operation of the MBR. Performance data such as transmembrane pressure, temperature, pH, permeate flux and biogas production were set up to be collected by a data acquisition station. The stages of reactor operations are indicated in Table 3.1

Phase III focused on the phase distribution of E2 in a hybrid AnMBR system with PAC. In order to understand the behavior of EDCs in AnMBR processes, it is important to find out what process in AnMBR are involved in removing of EDCs out of landfill leachate. AnMBRs processes include two major processes, which are anaerobic biological process and separation process by membrane. However, for EDCs, adsorption can also be an important process for removal of EDCs, especially with the addition of PAC. Therefore, determination of the fate of EDCs –through sorption to anaerobic sludge or PAC –was made through a series of isotherm batch experiments. Sorption coefficients such as sludge adsorption coefficient ($K_d$) and carbon adsorption coefficient ($K_s$) are determined.

Phase IV focused on determining the biological fate of E2 in an anaerobic system, using a series of batch experiments. The studies were conducted under varying anaerobic conditions such as methanogenesis, nitrate reduction and sulfate reduction. The

methanogenesis condition containing ideal anaerobic media was tested and served as reference for comparison with series which contained leachate. In this study, PAC was also added to determine whether PAC can enhance (or deter) the methanogenesis and E2 biotransformation in anaerobic cultures.

Phase V was a culmination of all the previous phases. It focused on study of the hybrid An-MBR for the removal of E2, using capabilities and knowledge acquired from the previous phases, to test the hypotheses stated earlier. PAC was added to the An-MBR system to facilitate the removal of E2. The stages of reactor operations are indicated in Table 3.1.

**Table 3.1:** Stages of operation of the AnMBR reactor.

| Chap. | Days | Stage | Feed | OLR ($kg/m^3day$) | HRT (d) | membrane | E2 | PAC | Note |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 36-53 | Ia | Dextrose | 3.9 | 4.16 | MTR UF | -- | -- | |
| 5 | 55-80 | Ib | Synthetic Leachate | 3.9 -5 | 4.16 | MTR UF | -- | -- | |
| 5 | 0-207 | IIa | OR Leachate | 3.6-4.8 | 4.4 | Orelis UF/MF | -- | -- | After Reactor modification |
| 5 | 207 -288 | IIb | OR Leachate | 7.3-9.3 | 2.3 | Orelis UF/MF | -- | -- | HRT change |
| 8 | 289 -385 | IIc | OR Leachate | 8.9-13 | 2.3 | Orelis UF/MF | 600 mg/L | -- | E2 added to leachate |
| 8 | 386-428 | IId | OR Leachate | 9-9.7 | 2.3 | Orelis UF/MF | 600 mg/L | 1g/L | PAC added to reactor |

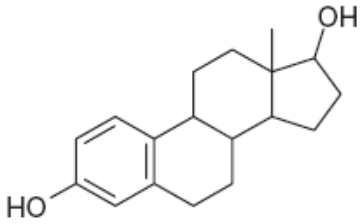
Note: Stage I was operated for 80 days (the first 34 days was sludge acclimation). Stage II was started after reactor modification at end of Stage I and operated for 425 days. The initial day of Stage II was reset at 0. Please repeat this for Table 5.2 and Table 8.1.

# CHAPTER 4: MATERIALS AND METHODS

## 4.1. Materials

### 4.1.1. Estrogenic Compounds

17β-estradiol (E2), one of the most environmentally prevalent estrogenic compounds, has been the focus of numerous studies. However, most of the studies related to the removal of E2 are for water treatment (such as study of Yoon et. al, 2003) and for aerobic processes for wastewater treatment (such as study of Zuhkle et al., 2003). According to our literature review, there has not been research on the removal of E2 in landfill leachate by AnMBR system. Therefore, E2 was chosen as the target compound for this study.




17β-Estradiol (E2)

MW: 272 ; pKa:10.46; Log $K_{ow}$: 4.01

**Figure 4.1:** 17β-Estradiol molecular structure.

In few studies, it was demonstrated that under anaerobic conditions, E2 transformed to E1; and under certain condition, E1 convert back to E2 (see Chapter 2). Therefore, it was important to be able to measure E1, and the analytical methods for E1

were developed accordingly. EE2 also was selected to develop the analysis method because EE2 is one of most common compounds in the estrogenic family.

E1, E2, and EE2 were purchased in grade of 98% from Sigma Aldrich (St. Louis, MO, USA). Deuterated E2-d4 (Cambridge Isotope Laboratories) was chosen as an internal standard for analyzing the target hormone compounds. The stock solutions of individual hormone compounds (E1, E2, and EE2) were prepared in methanol at 400mg.$L^{-1}$ and stored in a refrigerator at 5$^{o}$C. The methanol was purchased in HPLC grade from Fisher Scientific (New Jersey, USA). The stock solutions were diluted into proper portion with MilliQ water when used.

### 4.1.2. Anaerobic Sludge

Anaerobic sludge was collected from Howard F. Curren Advanced Wastewater Treatment in Tampa, Florida, was used for seeding the AnMBR, representing biomass in sorption studies, and providing biological activity in batch anaerobic bioassays. The sludge was stored at room temperature and used less than one week after collection. Prior to use, the sludge was filtered by a 20 mm sieve to remove large particles of waste which were retained in the WWTP. The sludge was characterized before used.

### 4.1.3. Synthetic Leachate

In Stage I of AnMBR reactor operations (see Table 3.1), a synthetic leachate was used. The recipe for adding chemicals was referenced from Rowe et al., 2002 and also used as synthetic leachate (Table 4.1). All the chemicals added in the landfill leachate were purchased as lab grade.

**Table 4.1:** Recipe for synthetic leachate. (After Rowe et al., 2002).

| Kelly Valley Synthetic Leachate | |
|---|---|
| **Compound** | **Quantity (mg/L)** |
| Acetic acid | 7 mL |
| Propionic acid | 5 mL |
| Butyric acid | 1 mL |
| NaOH | Titrate to a desired pH (5.8-6)<br>pH adjustement |
| $Na_2S$ x $9H_2O$ | Titrate to and Eh -120-180 mV<br>Eh adjustment and reducing medium preparation |
| $K_2HPO_4$ | 30 |
| $NH_4HCO_3$ | 2439 |
| $NaHCO_3$ | 3012 |
| $KHCO_3$ | 312 |
| $K_2CO_3$ | 324 |
| $MgSO_4$ | 156 |
| NaCl | 1440 |
| $CaCl_2$ | 2882 |
| $MgCl_2$ x $6H_2O$ | 3114 |
| $NaNO_3$ | 50 |
| $CO(NH_2)_2$ | 695 |
| TMS | 1 mL/L |
| Distilled water | to make 1L |
| **TMS** | |
| $FeSO_4$ | 2000 |
| $H_3BO_3$ | 50 |
| $ZnSO_4$ x $7H_2O$ | 50 |
| $CuSO_4$ x $5H_2O$ | 40 |
| $MnSO_4$ x $H_2O$ | 500 |
| $(NH_4)_6Mo_7O_{24}$ x $4H_2O$ | 50 |
| $Al_2(SO_4)_3$ x $16H_2O$ | 30 |
| $CoSO_4$ x $7H_2O$ | 150 |
| $NiSO_4$ x $6H_2O$ | 500 |
| 96% conc. $H_2SO_4$ | 1 |
| Distilled water | 1 L |

### 4.1.4. Organically-Replenished (OR) Leachate

In Stage II of AnMBR reactor operations (see Table 3.1), actual landfill leachate was used. The actual leachate was collected from Polk-County Landfill, Winter Haven, Florida. Polk-County Landfill has landfill cells in three phases. The leachate used in this research is a combination of phase I and phase II. Phase I is the 20 year old landfill which was opened in 1989 and closed in 2000, and phase II is a 10 year old landfill which was opened in 2000 and closed at 2008. Our original intent was to use a young leachate with high COD and background inorganics. However, because Phase III was mostly uncovered and exposed to chronic rain, the leachate was relatively dilute (similar to storm water) and did not represent a typical young leachate.

Consequently, the combined Phases I/II old leachate was collected (to provide background matrix of inorganic constituents) and later combined with additional VFAs for added organic strength. As collected, the Phases I/II leachate had total solid (TS) of 6494 mg/L, total suspended solid (TSS) of 1331 mg/L, COD of 1630 mg/L, turbidity of 26.6 NTU, pH of 7.4, ammonium of 454 mg-N/L, total phosphate and total nitrogen at 8.1 and 640 mg/L, respectively. The leachate was stored in a refrigerator at $5^{o}$C. Before using for experiments, lab grade acetic acid, butyric acid, and propionic acid were added into the leachate in ratio of 7mL: 5mL: 1mL per 1L of actual leachate, in order to raise the COD of the leachate to roughly 20,000 mg/L. Sodium hydroxide (NaOH) was also added to raise the pH of the OR leachate to 5.7. The resulting leachate has a similar composition as most young leachate described in literature, and is referred to as organically-replenished (OR) leachate. In summary, the OR leachate is primarily actual

leachate (to provide the background matrix of inorganic constituents), but received additional volatile fatty acids to increase the organic strength.

## 4.2. Analytical Methods and General Procedures

### 4.2.1. Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS)

TSS and VSS were analyzed by following the procedures from *Standard Method, 21st Edition*. Sludge samples and other liquid samples such as leachate samples were filtered by micro-glass fiber filter AH-934. The filter was then weighted in an aluminum plate (Fisher Scientific, Pittsburg, PA, US). Then the sample was dried out in the oven at 103-105$^{o}$C for more than 1 hour. After that, the aluminum plate was transferred from the oven to a desiccator until cool, and the weight after drying was determined. After the VSS was determined, the sample was transferred to a furnace and ignited at 550$^{o}$C for 20 min. The sample then was transferred to a desiccator for cooling down and subsequent weighing.

### 4.2.2. Chemical Oxygen Demand (COD)

Methods to determine COD were based on the *Standard Method, 21st Edition*. The liquid and sludge samples were centrifuged at 3000 rpm for 45 min in a Beckman Coulter Allegra 6 Centrifuge, to separate the supernatant for determining soluble COD. Then the samples were measured by the HACH test kits with range of 0-1500 mg/L.

4.2.3. Total Organic Carbon (TOC) and Total Nitrogen (TN)

TOC and TN samples were determined by a Shimadzu TOC-$V_{CSH}$ Total Organic Carbon Analyzer equipped with TNM-1 Total Nitrogen Measuring Unit and the SSM-5000A Solid Sample Module (Columbia, MD). Before measuring by instrument, the samples were centrifuged at 3000 rpm for 45 minutes and then diluted in proper ratios. Internal acid addition was set up in the instrument to automatically add 2M HCl to acidify the sample (pH<3) in order to strip out dissolved inorganic carbon and measure non-purgeable organic carbon (NPOC), which was used to represent dissolved organic carbon (DOC). The instrument also was set up to triplicate the injection (by an autosampler attached with the system) for each sample.

4.2.4. pH and Ammonia Measurement

pH and $NH_4$ were determined by using a gel-filled combination pH electrode (Model 2411-10, Cole Palmer, Vernon Hills, IL) and an ammonium ion-selective electrode (Vernier, Beaverton, OR), respectively. The meters were calibrated before each analysis. The electrodes were rinsed with Milli-Q or distilled water and dried with a Kimwipe tissue before and after each measurement.

4.2.5. Methane and Carbon Dioxide Analysis

The equipment used for gas measurement was a gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID) (Agilent Technology, US). The gas samples were collected from the serum bottles or from gas sampling ports of the MBR system by a 0.5 mL PTFE tipped gas tight

syringe (Hamilton, Reno, NV, US) and 0.2 mL of gas were injected directly to the GC injector which is connected to the TCD detector. The temperature of the oven was isothermally operated at 40$^{o}$C. The injector and detector temperature were set at 185$^{o}$C and 160$^{o}$C, respectively. The column used to determine carbon dioxide and methane was a 30m GS-Carbon PLOT column with 0.32 mm ID (Agilent Technology, US). Ultra-pure helium was used as a carrier gas. All gases used for making calibration curves were ultra-high purity (UHP).

4.2.6. VFAs Analysis

The liquid samples were filtered through a 0.2 µm glass fiber filter. The filtrates were acidified with a ratio 1:1 to 2.5% $H_3PO_4$. Then the samples were put in 2mL amber HPLC vials. The GC/FID was used to determine the VFAs in the samples. The oven temperature program was operated as follow: initial temperature at 90$^{o}$C and hold for 2 minutes, then ramp 2$^{o}$C/min to 100$^{o}$C, continue ramping 6$^{o}$C/min to 120$^{o}$C, after that 30$^{o}$/min to 230$^{o}$C and hold for 5 min. Total run time is 17.5 min. Temperature of the injector was set at 250$^{o}$C, and the carrier gas was helium set at 4.5 mL/min. The detector was set as follows: 250$^{o}$C, 450mL/min air flow rate and 40mL/min Hydrogen flow rate. The VFAs used for making calibration curves such as acetic acid, propionic acid, butyric acid, iso-butyric acid and valeric acid were purchased at HPLC grade from Fisher Scientific (Pittsburg, PA, US).

## 4.3. Development of Solid Phase Micro-Extraction Coupled with GC/MS Method for Analysis of Hormone Compounds in Liquid

The analysis procedure of 17β-Estradiol (same for other hormone compounds) by GC/MS is described as in the figure 4.2. First, the liquid samples were filtered by a 0.7μm glass fiber filter. Then the hormone compounds in the samples were extracted by a solid phase microextraction (SPME) fiber which has high affinity to sorb and concentrate the compounds. Next, the compounds which already adsorbed to the surface of the fiber were then derivatized with a derivatization agent (such as BSTFA or MSTFA). After reaction with the derivatization agent, the hormone compounds on the fiber transform to a compound with higher volatility, which facilitates the GC/MS analysis. Then, the fiber is inserted (manually or with autosampler) into the GC/MS injector, where the analytes thermally desorb and are loaded onto the GC column.

### 4.3.1. GC/MS Set Up

The instrument used for the EDCs analysis in this research was a Gas Chromatograph (GC) 3800 coupled with Mass Spectroscopy Saturn 2000 (from Varian Inc., US) and also equipped with an 1179 split/splitless injector. According to our literature review, DB-5MS was one of the most popular columns used for analysis of trace contaminants by GC/MS due to its low bleed, non-polar characteristic and excellent inertness for active compounds. Therefore, DB-5MS was selected as the column for the GC/MS in the project. In this research, because HP-5MS has the same characteristics as DB-5M, HP-5MS were also used to replace DB-5MS sometimes. The size of the capillary HP-5MS and DB 5MS columns in the research was 30m×0.25mm×0.25m.

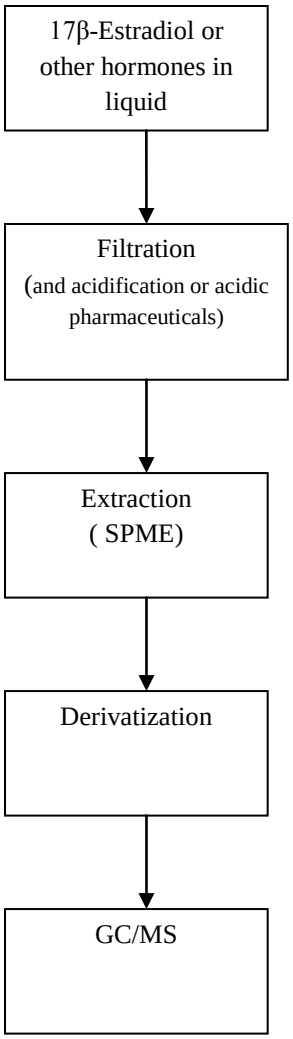



**Figure 4.2:** Flow chart of GC/MS method for hormonal compounds analysis.

Many temperature programs were assembled and evaluated. Several programs were selected and tested. The best temperature program was chosen as: Column oven at 80$^{o}$C initial, hold for 1 min, ramp 15$^{o}$C/min to 180$^{o}$C, hold for 1 min. then ramp 10$^{o}$C/min to 280 and hold at this temperature for 3 min. Multiplier offset: ± 70V.

4.3.2. SPME (Solid Phase Micro-Extraction) Method Development

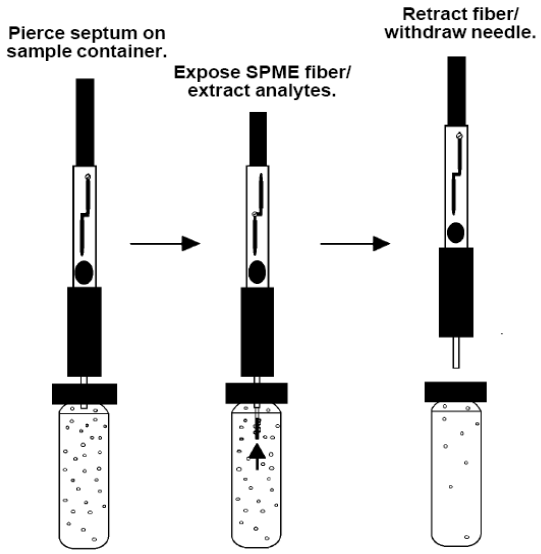



**Figure 4.3**: SPME method. (From Ormsby, 2005).

The purpose of SPME method is to enhance the detection limit for analysis of the hormone compounds by GC/MS. Figure 4.3 describes how the SPME method works.

For fiber selection, two popular SPME fibers were tested, which are Polydimethylsiloxane (PDMS) and Poly Acrylate (PA). PA is a type of fibers which is always used for polar compounds as phenols and semi-volatile compounds. In the other way, PDMS is for non-polar compounds. Therefore, for polar compounds such as E2 and other estrogen compounds, PA will have more affinity than PDMS. The results from the

experiment supported this. Both PDMS and PA fibers used in this experiment are from Supelco, Inc. (Bellefonte, PA, USA). The PA fibers were 0.85 µm PA fibers used for manual or automation. Figure 4.4 shows the test result of PA fiber for E2 analysis.

The autosampler for GC/MS selected in this study was Combi-PAL. The Combi-PAL autosampler is an application which is specifically designed for SPME work. The Combi PAL was equipped with a heated six position incubator which is very useful for doing extraction or derivatization.

For fiber extraction, the fiber was submerged directly in liquid samples which were contained in 10 mL vials as showed in figure 4.5. These vials purchased from Variance Inc., US were designed for use by the CompiPAL autosampler. Then liquid samples were mixed at a certain speed and kept isothermal until equilibrium conditions of the target compounds were achieved between fiber and liquid phase.

In order to increase the sensitivity and the selectivity, a derivatization step is necessary. The fiber after extraction was inserted into a headspace of a 10mL vial which contained a derivatization agent such as BSTFA or MSTFA. The derivatization would need a certain time for the compound to fully react with the derivatization agent. Then the fiber was located to the injector of the GC/MS.

Table 4.2 shows the mass spectra of some estrogen compounds when using the SPME with derivatization method for analysis, and the chromatography of these compounds is shown in figure 4.7 by using SPME with derivatization coupled with GC-3800/MS-2000. The retention time of all tested compounds was quite close to each other. However, each compound was still able to be recognized by different mass spectrum.

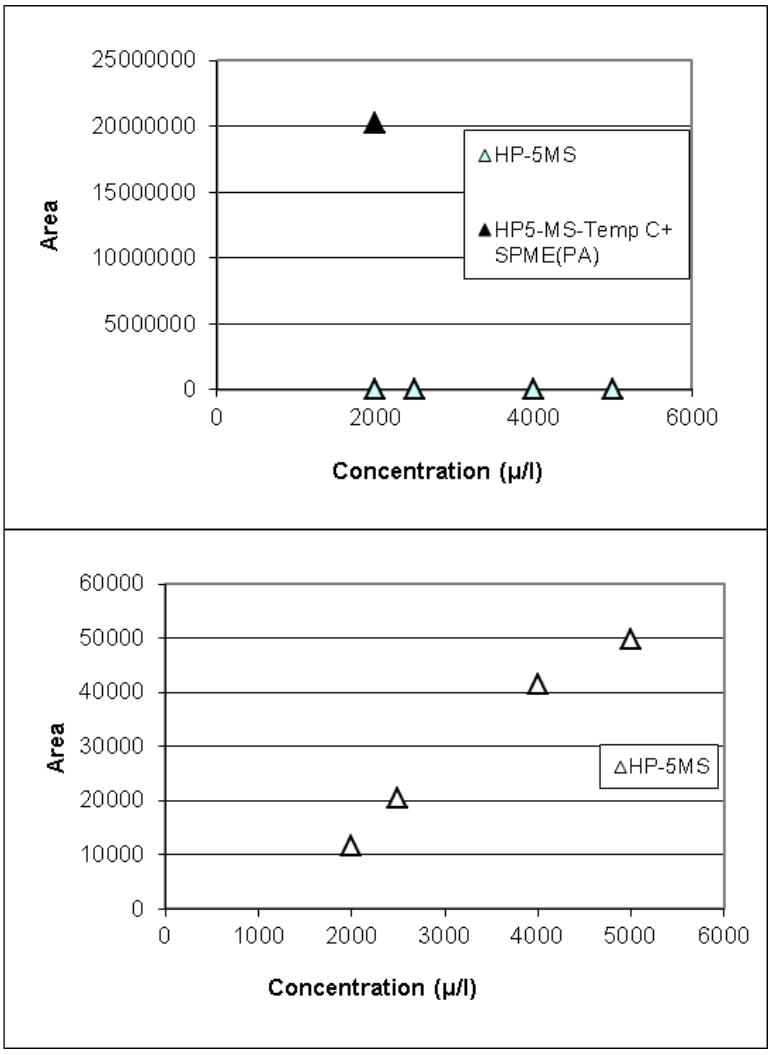



**Figure 4.4**: SPME method for E2 detection with 0.85µm poly acrylate fiber without derivatization.

### 4.3.3. Factor Effects on SPME Method

There are several factors which can affect the SPME method such as extraction conditions (temperature, speed), sample condition (pH, salt), and derivatization conditions (type of derivatization agent, derivatization time or temperature). All the experiments were conducted with an extraction time of 45 minutes. This length of time was enough for the estrogen compounds to reach equilibrium. In each factor testing experiment, the other factors (exclude the testing factor) were default at salt concentration of 30% (w/v), pH 7, temperature of extraction 35$^{o}$C, extraction speed of 500 rpm, and derivatization temperature of 70$^{o}$C.

#### 4.3.3.1. Effect of pH

In order to understand the SPME method and to have more effectiveness for estrogenic compounds measurement, it is necessary to test the effect of pH. pH may have an effect on the affinity of each compound to the fiber. It might be because at some certain pH, the targeted compounds can be dissociated and make them less effective for attaching to the fiber. Thus, it will reduce the ability to measure estrogenic compounds by GC/MS. The first experiment was run on effect of pH on E2 by SPME without derivatization. The purpose was to understand, with and without derivatization, whether the pH affects the extraction of E2 in liquid or not. The result from Figure 4.8 showed that pH 6.8 has the most effectiveness on extraction of E2. Meanwhile, pH 3 and pH 5 showed lower potential for E2 extraction. pH 8.6 showed lowest ability on attachment of E2 to the fiber.

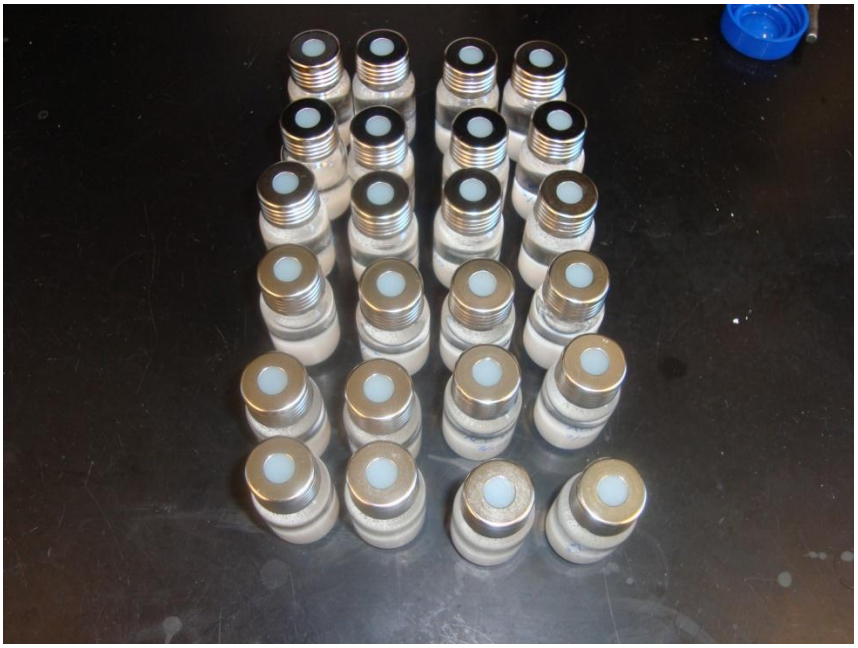


**Figure 4.5:** 10 mL SPME vials.


**Figure 4.6:** GC-3800/MS Saturn 2000 equipped with a Combi PAL autosampler.

**Table 4.2:** Information of quantitative ions and retention time of target compounds with SPME derivatization.

| Compounds | CAS | Molecular Mass | Retention time (min) | Quantitative ion | Confirmation ion |
|---|---|---|---|---|---|
| E2 | 50-28-2 | 272 | 17.2 | 285 | 416 (100%), 326 |
| E1 | 53-16-7 | 270 | 16.85 | 342 | 257 (100%) |
| EE2 | 57-63-6 | 296 | 18.0 | 285 | 425 (100%) |
| E2-d4 | NA | | 17.0 | | |
| BPA | 80-05-7 | 213 | 13.7 | 357 | 372 |

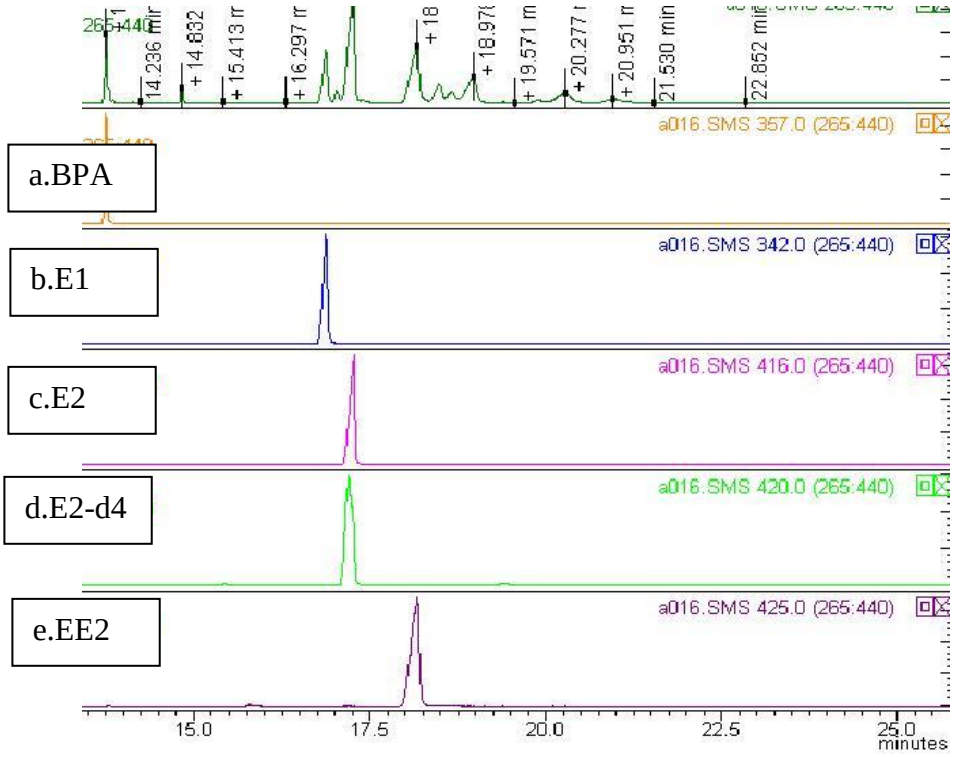



**Figure 4.7:** Chromatography of estrogen compounds with GC3800 and MS 2000 with SPME + derivatization.

**Table 4.3:** Characteristics of some EDCs.

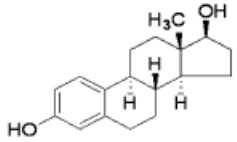
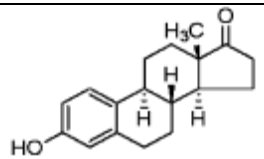
| **Compound** | **Structure** | **M.W. (mg)** | **Water Solubility (mg/L)** | **Log $K_{ow}$** | **$pK_a$** | **Vapor Press. (mmHg)** | **Henry's Law Constant ($atm.m^3/mole$)** | **Typical Uses** |
|---|---|---|---|---|---|---|---|---|
| 17 β-Estradiol (E2) |  | 272.4 | 3.9-13[1] 5.4-13.3[2] | 4.01[3] 3.8-4.0[2] | 10.46[4] | $1.26*10^{-8}$[3] | $3.64*10^{-11}$[3] | Hormone replacement therapy.[5] (birth control, drug, ect) |
| Estrone (E1) |  | 270.4 | 13[3] 0.8-12.4[2] | 3.13[3] 3.1-3.4[2] | 10.34[4] | $1.42*10^{-7}$[3] | $3.80*10^{-10}$[3] | Natural hormones in human body, pharmaceutical |

**Table 4.3** (Continued).

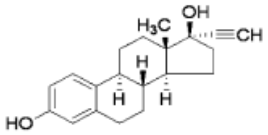
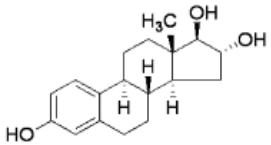
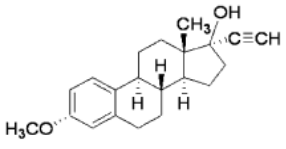
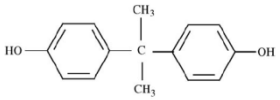
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17α-Ethynylestradiol (EE2) |  | 296.4 | 4.8[3] | 3.67[3] | 10.4[4] | $2.67*10^{-9}$[3] | $7.94*10^{-12}$[3] | Oral Contraceptive[5] |
| Estriol ($E_3$) |  | 288.4 | 13[3] 3.2-13.3[2 | 2.45[3] 2.6-2.8[2] | 10.38 | $1.97*10^{-10}$[3] | $1.33*10^{-12}$[3] | Natural hormones in human body, Pharmaceuticals |

**Table 4.3** (Continued).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mestranol |  | 310.4 | 0.32[3] | 4.8[3] | 10.26 | $7.5*10^{-10}$[3] | ----- | Oral contraceptives |
| Bisphenol A |  | 228.29 | 3[6] | 3.40[6] | 9.59-11.3[6] | $3.975*10^{-8}$[6] | $1*10^{-11}$[3] | Polycarbonate plastic container and food cans. |

Note: [1]: Stumpe et al., 2007 , [2]:Khana et al., 2006, [3]:Bodzek et al., 2006 , [4]: De Mes et al., 2005, [5]:Arcand-Hoy et al., 1998, [6]: Cousin et al., 2002.

In the second set of experiments, several estrogen compounds such as E2, EE2, E1, E2-d4, plus BPA, were selected to test with SPME plus derivatization. The pH range was chosen from 3-10. The results showed on the figure. 4.9 were normalized to have better understanding of pH effects on all those compounds. The results showed that pH from 3-7 showed better potential on extraction of the compounds than pH 8-10. In that pH 7 showed the best result for extraction of E2 and most of other compounds except EE2. The best extraction of EE2 showed at pH 3. But at pH 7, it showed about 90% of its best extraction under different pH conditions. Therefore, pH 7 is considered as the optimum pH for extraction of our targeted compounds. This result is also similar to the result received from non-derivatization results.

4.3.3.2. Effect of Ionic Strength

Beside pH, ionic strength might also play an important role on extraction of E2 by SPME. Similarly to pH, the experiment of salt effect on extraction of E2 was also done without derivatization. The ionic strength of the sample is controlled by adding salt into sample. With additional salt (NaCl) or increasing ionic strength, the extraction of E2 andother estrogen compounds into the fiber might change. The reason might be with higher ionic strength, the solubility of the targeted compounds increases. Therefore, experiments on the effect of ionic strength were implemented. The experiment of E2 extraction was Figure 4.10 showed at salt 20% (w/v), the E2 extraction by SPME had the best result compared to other tested salt concentrations. The results showed that even without salt, the extration of E2 still can achieve around 80%.

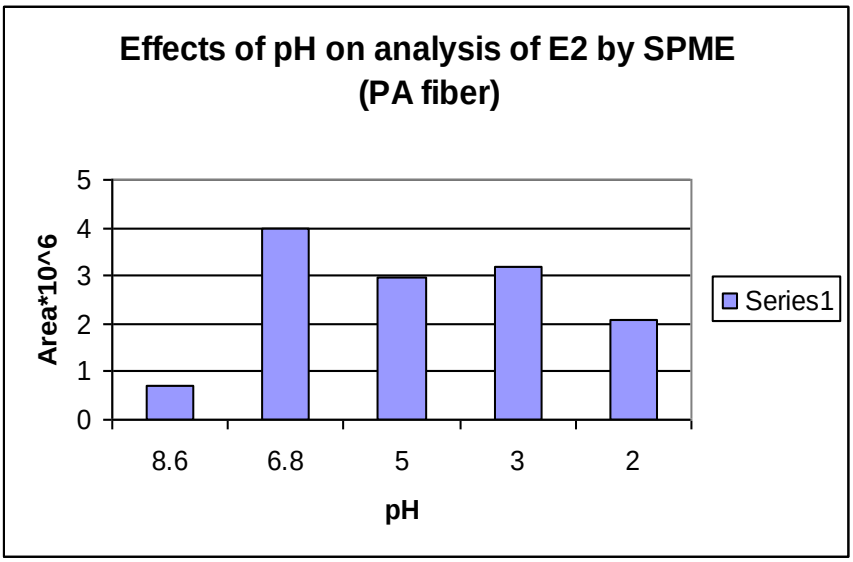



**Figure 4.8:** Effect of pH on SPME for E2 without derivatization, experiment conducted with 80 µg/L of E2.

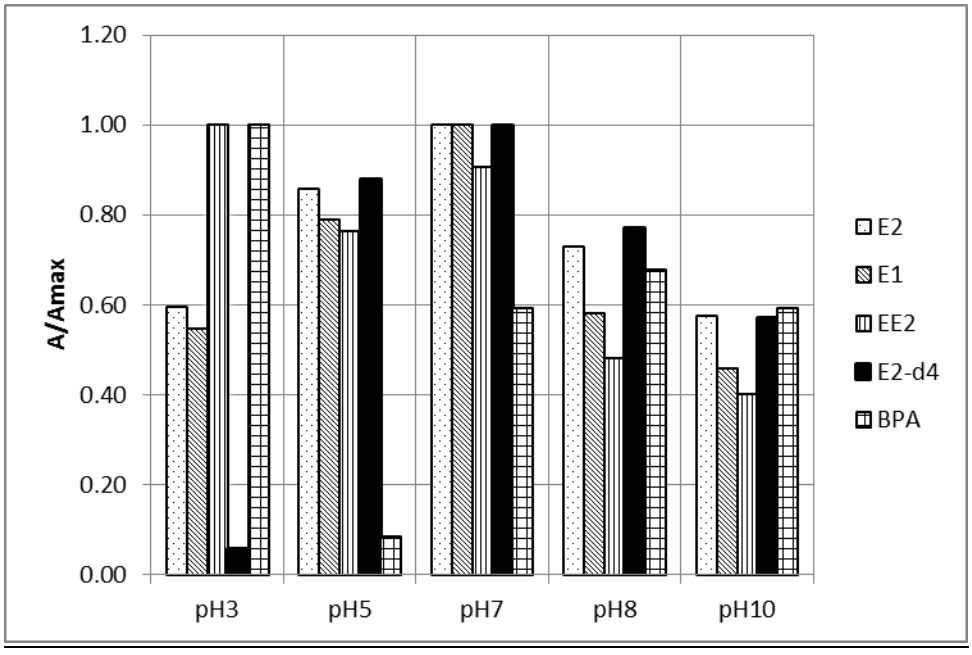



**Figure 4.9:** Effect of pH on SPME with derivatization detected by GC/MS.

Note: A is area of the peak at a certain pH of each compound. $A_{max}$ : maximum area peak of each compound obtained from pH range 3-10 conducted with salt concentrations from 0-30% (weight/volume).

Another set of experiments on all target compounds was conducted with salt concentrations from 0-50% (w/v) and with derivatization. Figure 4.11 showed slightly different results from experiments without derivatization with E2. At concentration of salt 30%, almost every compound showed the best extration. BPA showed the best extraction condition at a salt concentration of 20% and E1 showed its best at a salt concentration of 40%. However, compared to the other salt concentration, 30% salt still showed better extraction to target compounds compared to others. Without salt, there is almost no extraction or the estrogen compounds except BPA. BPA still can attach to the fiber even without salt present, but the extraction was very low. From the result, it is determined that a salt concentration of 30% is the best condition of ionic strength for the extraction of the compounds.

4.3.3.3. Effect of Extraction Speed

The extraction speed is also a potential factor which can affect on the extraction effectiveness. The extraction speed can either make the equilibrium of the target compound in fiber slow or fast. Figure 4.12 showed the normalized results base on the area peaks produced in the GC/MS of all target compounds at different extraction speeds per area peak collected at a speed of 500 rounds per minute (rpm). It is shown in Figure4.12 that at 500 (rpm), the extraction achieved the best result for all target compounds. At 250 rpm, the extraction was very low for all target compounds and it was higher at 400 rpm. At 400 rpm, BPA can receive the best extraction while others were not. At 600 rpm, the extraction was lower for all compound compared to that at 500 rpm except E2-D4. It maybe because when the speed is too high, additional phenomena

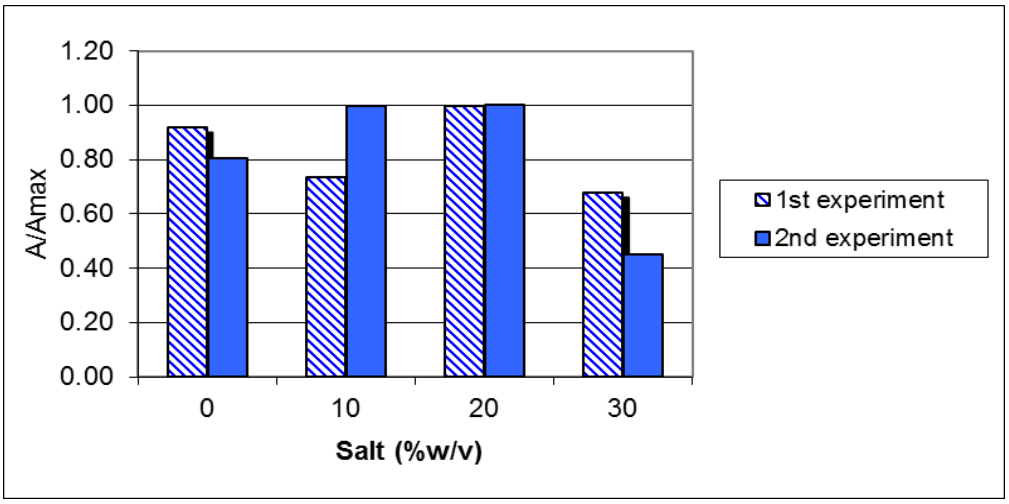



**Figure 4.10:** Effect of ionic strength (NaCl) on E2 detection by SPME without derivatization-GC/MS.

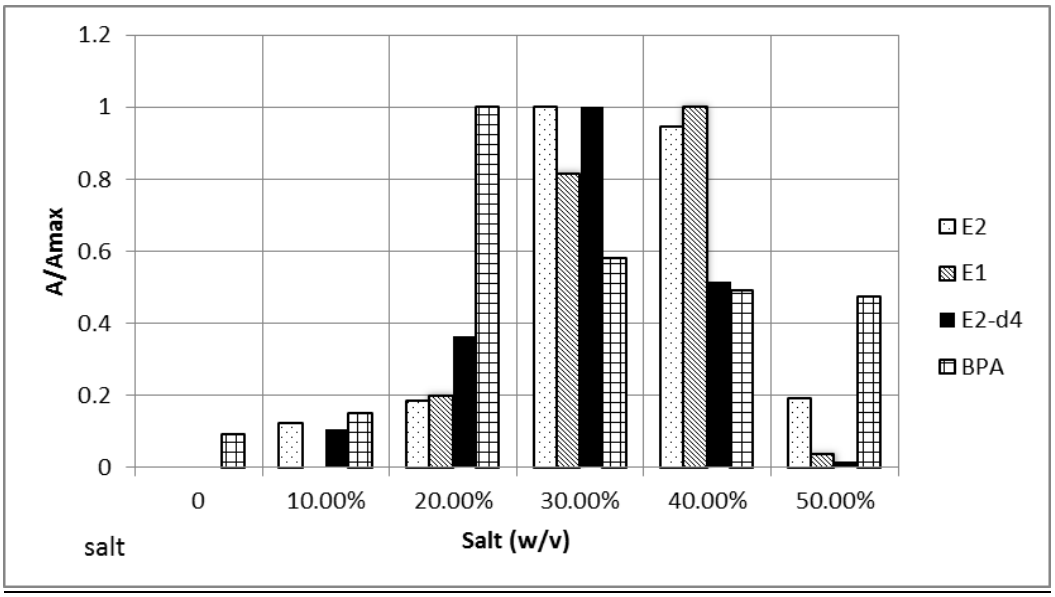



**Figure 4.11:** Effect of ionic strength on target compounds by SPME with derivatization.

Note: A is area of the peak at a certain salt concentration of each compound. $A_{max}$: maximum area peak of each compound obtained from salt concentration range 0-50% (w/v)

occur (such as boundary layer effects) which interfere with extraction. Additionally, when automation operated with high speed, the SPME fiber is easy to be broken. Therefore, 500 rpm is determined as the best extraction speed for all target compounds.

4.3.3.4. Effect of Extraction Temperature

The temperature experiment was conducted from 35-85 $^{o}$C. The results as in Figure 4.13 showed that at 35$^{o}$C, E2 and E1 have the best extraction. However, BPA and E2-d4 only achieved the best extraction at 45$^{o}$C. However, at 35$^{o}$C, BPA and E2-d4 can reach more than 90% extraction effective. At 45$^{0}$C, E2 and E1 only can reach 30% and 80% of their best, respectively. At 55 $^{o}$C, BPA extraction on fiber still can reach 100 % but other compounds provded less effective. At other temperatures, the extraction of the target compounds showed less effectiency than 35$^{0}$ and 45$^{o}$, 55$^{o}$C. So from the result we achieved, we decided that the best temperature for extraction of our target compounds is 35$^{o}$C.

4.3.3.5. Effect of Derivatization Temperature with MSTFA as a Derivatization Agent

The derivatization process also affects the result of estrogen compound detection. Therefore, the experiment on temperature effect of the derivatization process was implemented in a temperature range of 35-80$^{o}$C. Also, it is shown from Figure 4.14 that higher temperatures achieved better target compound detection. The best temperature for derivatization in the chosen temperature range is 80$^{o}$C. However, when we conducted the derivatization at 80$^{o}$C, the derivatization agent vaporized and disappeared very quickly compared to lower temperature. The temperature 70$^{o}$C, showed a little bit lower

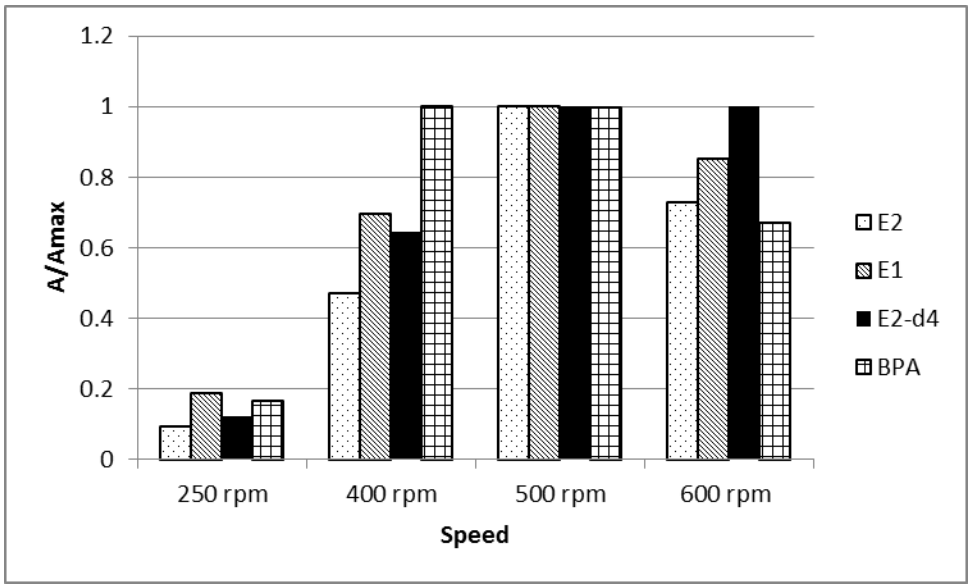



**Figure 4.12:** Effect of extraction speed on detection of the target compounds by SPME-derivatization + GC/MS.

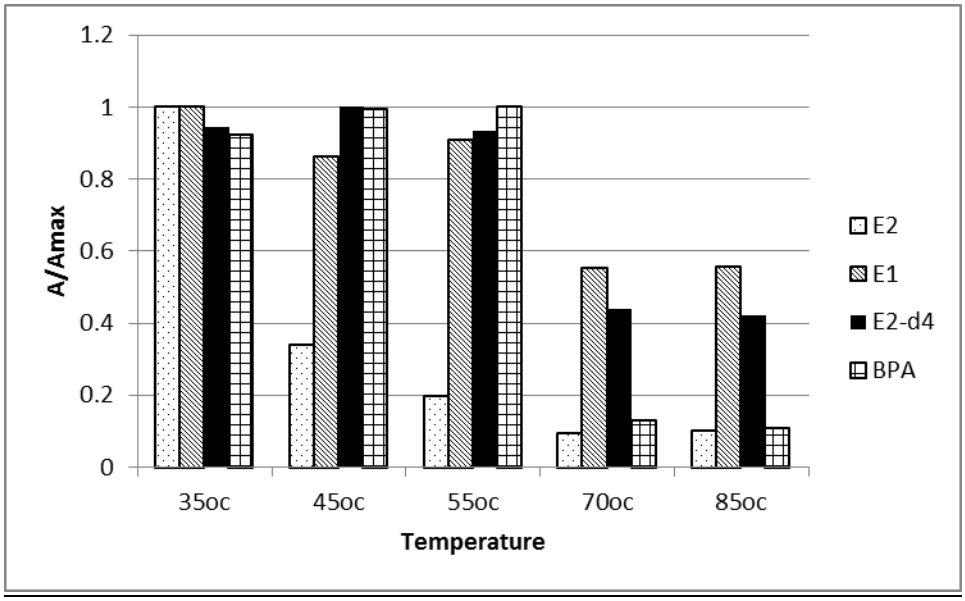



**Figure 4.13:** Effect of extraction temperature on the target compounds.

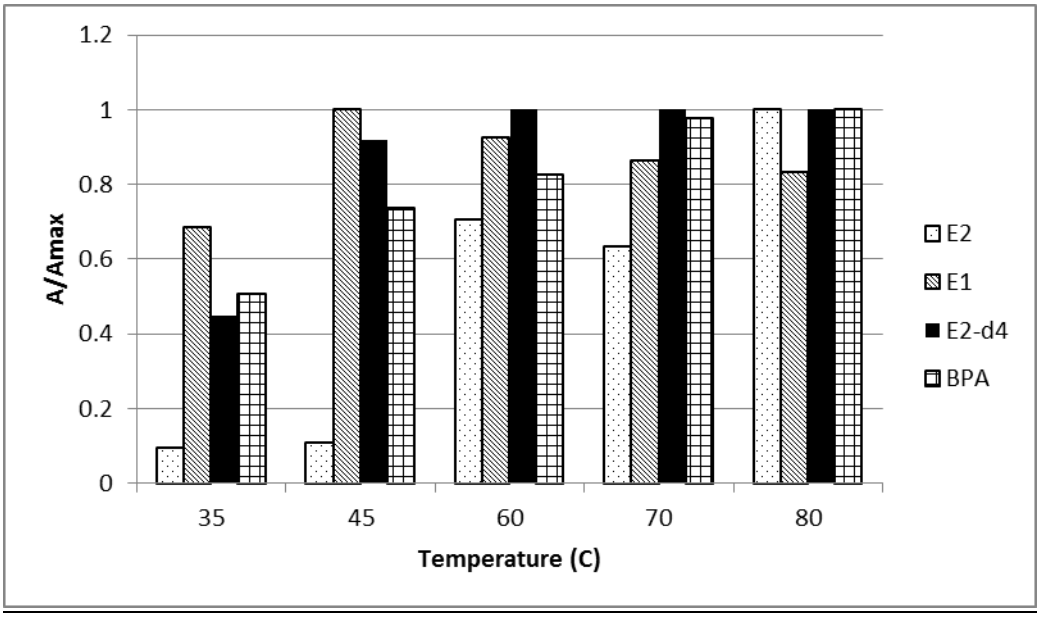



**Figure 4.14:** Effect of derivatization temperature of the target compounds.

effectiveness on detection of the target compounds. However, the derivatization agent lasts longer. Therefore, the optimum temperature for derivatization under our experiment condition is 70$^{o}$C.

4.3.4. Detection Limit and Calibration Curve of Estrogenic Compounds

The detection limit of E2, BPA and EE2 as shown in Table 4.4 is around 4ng/l, 1ng/l and 0.16µg/L, respectively. The minimum concentration of E2 which is assumed to be linear for the calibration curve is 0.16µg/L. The range of E2 in the calibration curve shown in Figure 4.15 is from 0.16 g/l to 120µg/L. The slope is 24771 and the $R^2$ is 0.9937.

4.3.5. Summary for the Final SPME Method and Procedure for Hormone Compound Analysis

Samples were filtered into a GF/F glass microfiber filter (0.7µm). After that, samples are located into 10 mL-vials which are specifically designed for auto-sampler compatible with GC/MS instrument from Varian inc., Palo Alto, California, US. 30% (weight/volume) of NaCl (Fisher scientific, Springfield, NJ, USA) was added into each vial. Then samples were analyzed by a GC- 3800 /MS Saturn 2000 combined with a CombiPAL auto sampler (Varian Inc, Palo Alto, CA, USA). The extraction of the hormone compounds to SPME fiber was set at 40 minutes, 500 rpm and 35$^{o}$C. Next, the fiber with the hormone compounds was derivatized on fiber by MSTFA for 6 minutes at70$^{o}$C. Then the fiber was injected to 1179 injector in GC/MS at splitless mode

**Table 4.4:** Dectection limit for several ECDs compounds.

| Compounds | Detection Limit |
|---|---|
| E2 | 4 ng/l |
| E1 | 0.1 µg/L |
| EE2 | 0.16 µg/L |
| E2-d4 | NA |
| BPA | 1 ng/l |

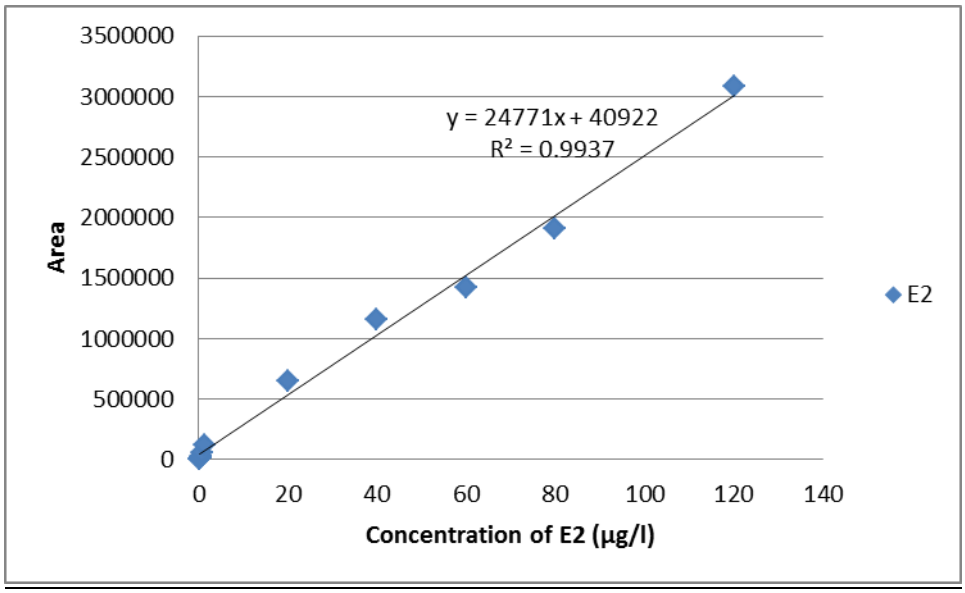



**Figure 4.15:** Calibration curve for E2.

for 5 minutes at 280$^{o}$C. The GC/MS is equipped with a HP-5MS (30m x 0.25mm x 0.25 µm). The GC oven program is set as: initial temperature at 80$^{o}$C, hold for 3 minutes, then ramp 20$^{o}$C/minute to 180$^{o}$C, hold for 1 minute, then ramp 12$^{o}$C/min and hold for 9 minutes. For quantitative purpose, the MS mode is set up for scan from 265-440 m/z.

## 4.4. Solid Phase Extraction

### 4.4.1. Extraction

The solid extraction method for the hormone compounds were developed in order to complete the mass balance of the hormone compounds in the anaerobic system. The method was described Figure 4.16.

15 mL slurry samples were dispensed in 28 mL anaerobic tubes obtained from Bellco glass (New Jersey, USA). The separation of liquid and solid phases in the samples was achieved by spinning the samples at 3000 rpm in 45 min with a Beckman Coulter Allegra 6 Centrifuge. Then, the solid pellets were collected in the same tubes and kept frozen in a fridge at least overnight to increase the extractability. The liquid phase was used for SPME. Once the solid samples were frozen, 5 mL of methanol was added to each sample to extract the EDCs from the solid phase. A vortex was used to break the solid pellet to help better contact between the solid phase and methanol. After that, PTFE-lined stoppers and aluminum crimps were used to seal the tubes. In order to improve the transfer of the EDCs to methanol, the tubes were incubated in an oven at 60$^{o}$C for 24 hours. The level of methanol was marked before locating the tube in the oven and checked after the incubation to determine the loss of the solvent during the extraction. After the incubation and cooled to room temperature, the tubes once again

were centrifuged at 3000 rpm in 45 min. The methanol then was extracted and kept in 20 mL scintillation bottles at 5$^{o}$C until further used for analysis.

### 4.4.2. Analytical Method

The analytical method was developed to determine the EDCs in the methanol solvent after the solid phase extraction. 0.9mL of extracted solution and 0.1 mL of internal standard BPA were added to a 2mL inserted fuse micro-sampling vials (National Scientific, USA). Then the solution was dried in a Multiplevap-118 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA, US). After the samples were completely dried, 0.75 µl of BSTFA +1%TMC was added to the vial to derivatize the EDCs compounds, and the vial was put into an oven at 60$^{o}$C for 30 minutes. Then, the vial was cooled to room temperature and proceeded using the GC/MS to analyze the EDCs. The GC/MS method used for these samples was the same as the GC/MS method used for SPME samples.

### 4.4.3. Solid Extraction Recovery Test

The solid pellets collected from the set of adsorption experiment for EDCs at high initial concentration on anaerobic sludge were used to perform the extraction test in order to determine the recovery potential of the solid extraction method. Figure 4.17 illustrated the mass balance of the E2 in liquid and solid phase from the adsorption tubes. The recovery percentage of E2 was determined from 89%-99%.

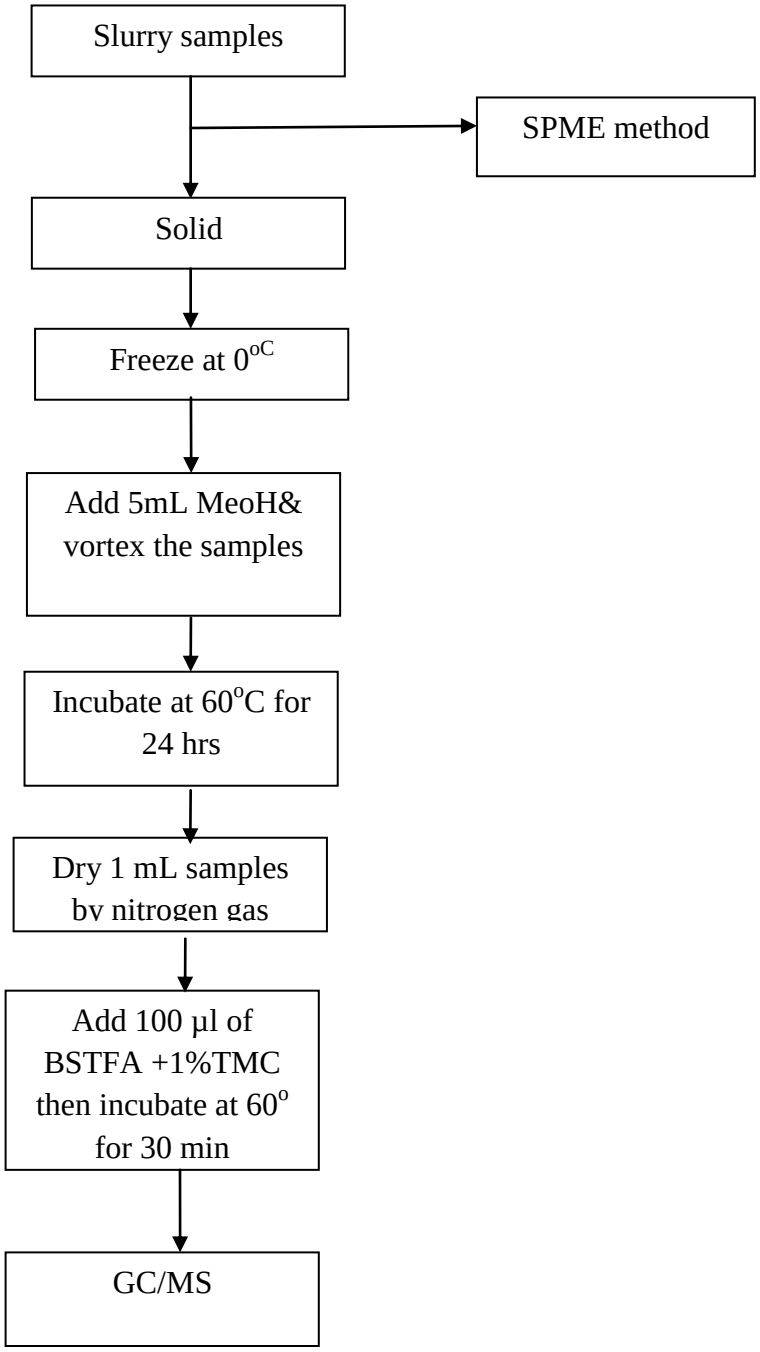



**Figure 4.16:** Solid phase extraction for anaerobic sludge samples.

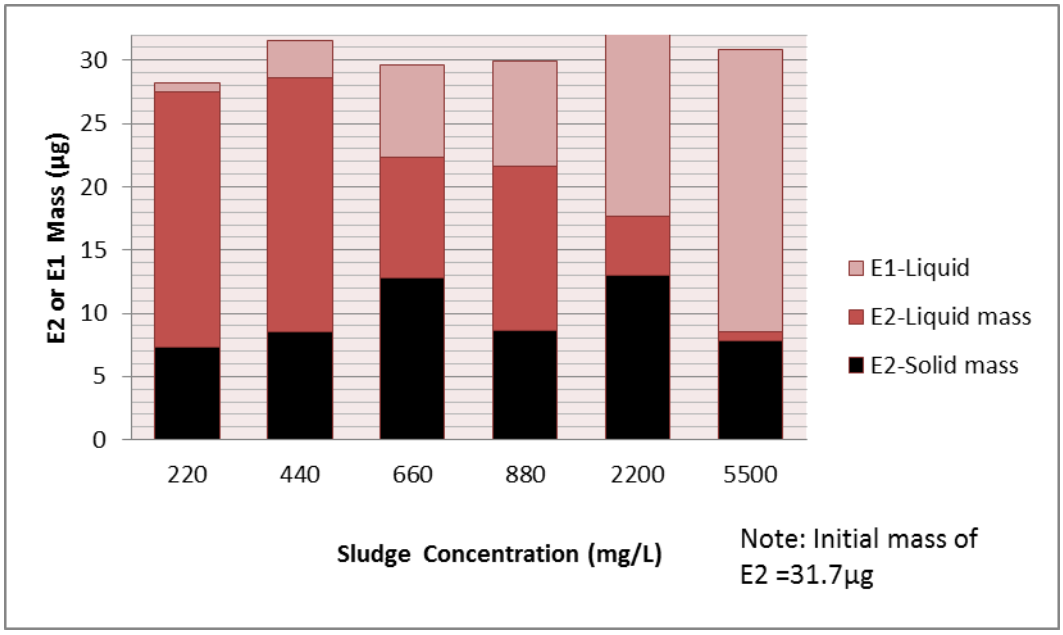



**Figure 4.17:** Mass balance of E2 in solid and liquid phase.

# CHAPTER 5: APPLICATION AND LONG TERM OPERATION OF ANAEROBIC MEMBRANE BIORECTOR FOR REDUCTION OF ORGANIC STRENGTH IN LANDFILL LEACHATE

## 5.1. Abstract

The anaerobic membrane bioreactor (AnMBR) is a promising technology for treating high strength wastewater such as young landfill leachate. A novel lab-scale UASB reactor equipped with dual-flat sheet ultrafiltration (UF) membrane and microfiltration (MF) membrane modules was designed and constructed to test the potential to reduce the organic strength of landfill leachate. Initially, a synthetic leachate was fed to the AnMBR at a concentration of around 20,000 mg/L (about 40x higher than dometic wastwater). During the first 80 days of start up (Stage I), the efficiency of COD removal of the AnMBR was from 40%-60%. The organic loading rate (OLR) was from 3.9-5 kg COD/m3day and the hydraulic retention time (HRT) was 4.16 days. The membranes used in Stage I were two MTR-UF membranes. The average specific flux of the MTR membranes was around 30 LMH. The MLSS of the bioreactor was around 6000-7000 mg/L.

To improve the performance of the system (in terms of membrane flux and COD removal) and to migrate to actual leachate, modifications were made to the reactor (membrane type and configuration and reactor feed), then Stage II was initiated. The

results from stage II showed that the AnMBR can achieve the COD removal efficiency of 81-93% with the highest organic loading rate (OLR) of 80kg COD/m3-day at 35°C and a hydraulic retention time (HRT) of 2.3 days. The highest flux of UF and MF can be achieved at 40 LMH/bar and 80 LMH/bar, respectively when the new membranes were installed or right after the membranes were cleaned. Then, permeate fluxes of MF and UF membranes decreased quickly to stable fluxes of 10 LMH/bar and 8 LMH/bar, respectively. Weekly, the membranes were cleaned in place by applying higher cross flow velocity and close-to-zero transmembrane pressure (TMP).

## 5.2. Introduction

Landfilling has become the most common and socially accepted way to dispose municipal wastes. Landfill leachate is a complex and high-strength waste water because of its high concentrations of dissolved and colloidal organics (much of which may be recalcitrant), inorganics (e.g., ammonium and salt), heavy metals (e.g., arsenic, mercury, cadmium, and copper) and xenobiotic organic pollutants (Kjeldsen et al., 2002; Villar, 2006). The strength and characteristics of individual landfill leachate depend on many factors such as: amount of rainfall, type of wastes in each landfill, and the age of landfill. Throughout the layers of a landfill, there are processes occurring to the solid wastes such as: physical extraction, hydrolytic and fermentative decomposition of organic compounds. Hydrolysis usually dominates in the first few years of the landfill. The leachate during this initial period, called young leachate, contains many of the acidic byproducts of hydrolysis. According to Alvarez-Vazquez et al., 2004, young leachate is formed less than 2 years. Leachate formed 2 years to 5 years after the landfill has started

is considered middle age leachate and the leachate. Leachate from landfills those are older than 5 years are considered old leachate. Old leachate does not contain many biodegradable organic compounds because most were degraded by fermentation, acidogenesis and anaerobic oxidation during the earlier periods. Therefore, the BOD/COD ratio is pretty low which ranges from 0 to 0.3 compared to 0.6-1in young leachate (Alvarez-Vazquez et al., 2004). One of the simplest and most popular methods for dealing with leachate is to send it to the wastewater treatment plant (WWTP). This solution takes advantage of the existing wastewater treatment plants and reduces the cost for onsite treatment of leachate. However, leachate, especially young leachate, is quite complicated and contains high concentration of COD, heavy metals and many xenobiotic contaminants such as endocrine compounds (EDCs) or pharmaceutical and personal care products (PPCPs) (Yamamoto et al., 2001; Yasuhara et al., 1997; Behnisch et al., 2001). WWTPs are not designed to treat waste of this nature, in many cases some of these constituents will pass through the treatment stream. This can result in trace contaminants entering the environment. Therefore, it is essential that pretreatment of leachate occurs before it is sent to the wastewater treatment plant.

Current technologies for landfill leachate and wastewater treatment can be classified as chemical, physical and biological. According to 157 studies conducted by Alvarez- Vazquez et al., (2006), less than 30 % of treatment systems use chemical methods, less than 10% use physical treatment and 60 % use biological treatment. Conventional activated sludge treatment is the most commonly used system for landfill leachate and WWTPs. In recent decades, membrane processes have been emerging as alternatives for landfill leachate treatment.

Recently, membrane bioreactor processes have been widely applied to wastewater treatment. The coupling of a biological and physical process has helped to increase the removal efficiency of contaminants in the leachate. The biological process (aerobic or anaerobic) helps to degrade the organic constituents. The membrane, which serves as the physical process, helps to retain the sludge and to remove solids, bacteria, and small particles from the water. Compared to conventional treatment, the advantages of a MBR can be listed as below (Asatekin et al., 2006; Choi et al., 2002; Daigger et al., 2005):

- Compact design allowed due to high biomass concentration
- Suspended solids, microorganisms and other trace contaminants can be removed by a MBR
- Very stable process operation
- High sludge age can degrade the more complex compounds.
- Higher effluent quality

Due to these advantages, MBRs have emerged as promising technology for wastewater and landfill leachate treatment. Many studies have reported the effectiveness of applying MBRs to leachate treatment. According to our literature review, COD removal efficiency in MBR systems varies from 31.3%- 96% and the remaining COD are always high (Bozek et al., 2006; Bohdziewicz et al., 2008; Laitinen et al., 2006; Shaoshua and Junxin, 2006). The majority of the studies used old leachate with aerobic biological treatment. This study focuses on the potential of Anaerobic MBRs (An-MBRs) for pretreatment of young landfill leachate. The idea is to capture young leachate at high concentrations of contaminants and pre-treat it with an AnMBR before sending it to the wastewater treatment plant. The use of anaerobic digestion has two major benefits, one

being that it converts COD to methane (a valuable energy source) and the second being that it reduces the overall aeration demand required by the WWTP. Additionally, it is hypothesized that the combination of membrane retention, long SRT, and dense and diverse microbial populations make the MBR a more ideal system for degrading recalcitrant contaminants such as EDCs than conventional activated sludge processes (Cicek et al., 1999; Clara et al., 2005). Therefore, it can reduce the risk of trace contaminants released to the environment. In addition, the conventional WWTP usually has to deal with handling of quite high load of sludge waste. So, reduction of contaminants before it goes to the WWTP can help to reduce contaminants in the sludge. We will introduce the potential of AnMBR to remove EDCs and PPCPs in young landfill leachate in another paper.

## 5.3. Materials and Methods

Description of the anaerobic sludge used for the reactor and analytical methods can be found in Chapter 3, Materials and Methods. The reactor was fed dextrose (Stage Ia), synthetic leachate (Ib), then OR leachate (IIa, IIb) as previously described in Chapter 3.

The An-MBR system was designed and fabricated according to Figure 5.1. The system consisted of a 13L glass column aneaerobic reactor. The reactor was continuously fed with leachate from the bottom by a digital peristaltic pump. This pump contaned two identical pump heads (one for influent and one for effluent) driven by the same pump drive. This configuration ensured that the effluent flowrate was equal to the feeding rate. Dual flat-sheet membrane modules were installed within the system. A detailed

characterization of the modules is described in the Table 5.1. Two perilstaltic pumps were used for pumping the water from the anaerobic reactor to the membranes. The membranes's transmembrane pressures were adjusted by pressure valves on the retentate line. The permeate went through flow meters and rain gauges to measure the exact fluxes of the membranes. The permeates were then collected in a permeate distributor box where the effluent was pumped out at a rate which was equal to the feed rate. Excess permeate was pumped back to the anaerobic reactor by a recirculation perilstaltic pump. A HOBO data acquisition box (Onset Computing) was built and attached to the system in order to continuously monitor and collect data of pH, temperature, gas production, membrane pressures and flow rate. Temperature for the anaerobic reactor was kept constant at 37°C.

**Table 5.1:** Rayflow membrane modules.

| | |
|---|---|
| Useful membrane area | 2 x 100 sq. cm. |
| Operating Pressure | 0-3 bars |
| Internal volume of the module | Retentate compartment: 2x 22 mL<br>Filtrate compartment: 2x 8mL |
| Dimension | 240 mm x 220-250mm x 70mm |
| Spacer channel height | 1.5 mm |

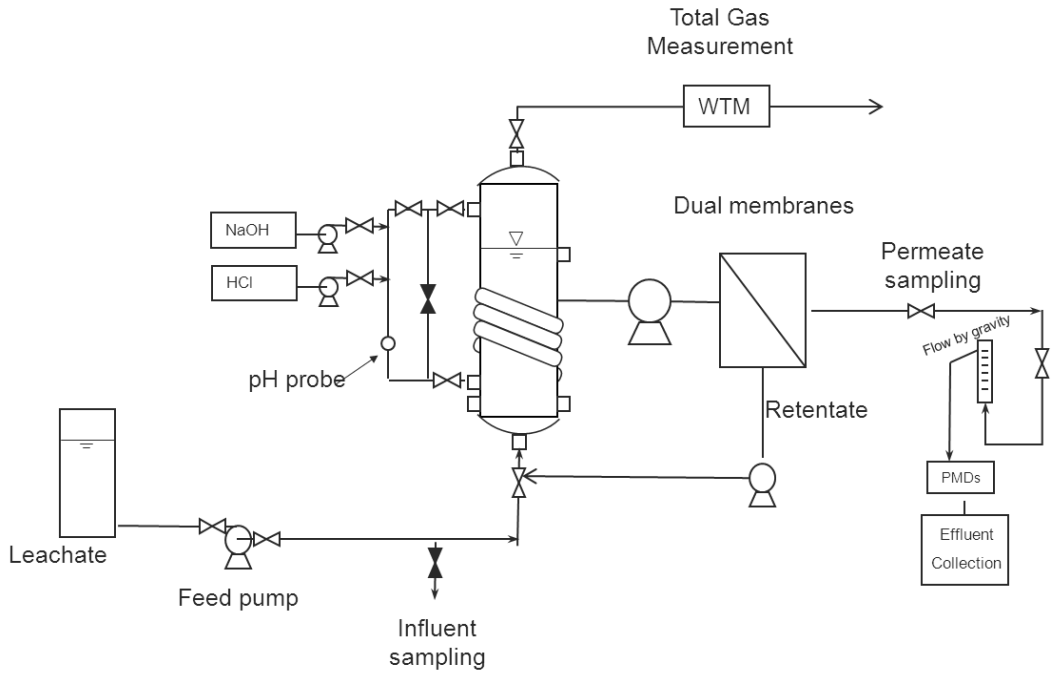



**Figure 5.1:** Schematic diagram of the An-MBR.

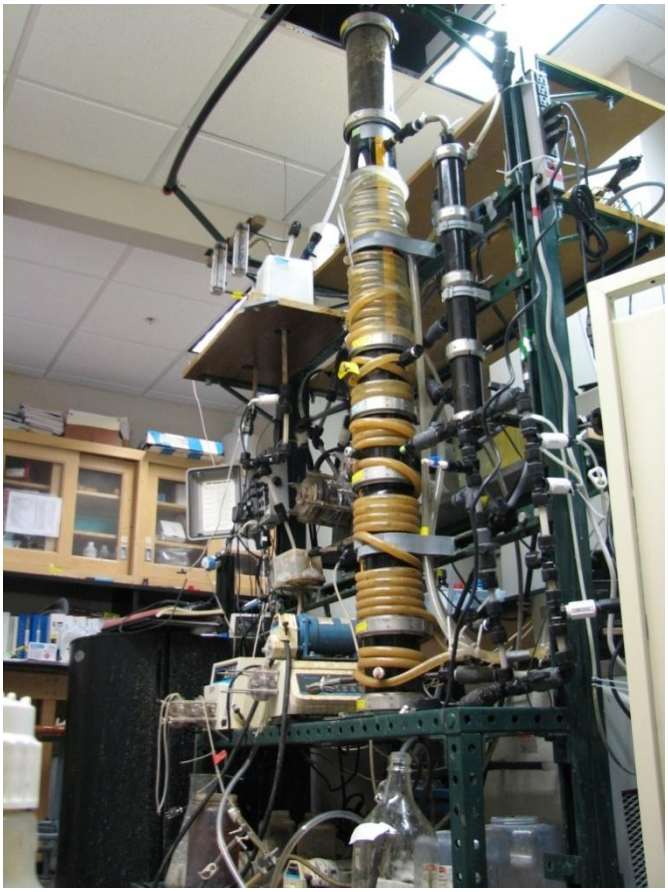


**Figure 5.2:** The lab-scale An-MBR after modification.

Flat sheets membranes were used for the AnMBR system during the experiment. During phase I, a PVDF membrane, obtained from Membrane Technology & Research, Inc. (MTR) in Palo Alto, California, US was selected to test. During phase II, two commercial PVDF membranes, MF and UF, provided from Rhodia Orelis (France) were tested instead of the MTR membrane. The MF membrane has molecular weight cut off (MWCO) of 0.1 µm and the UF membrane has MWCO of 40 KDa.J.

## 5.4. Result and Discussion

### 5.4.1. Stage I-AnMBR Startup Operation

There were two stages of the AnMBR start up. The first stage (Ia and Ib) was to test the performance of the AnMBR system. The second stage (IIa and IIb) was dedicated to modifications and system optimization in terms of performance. The stages are indicated in Table 5.2. At the beginning of the first stage, the reactor was filled with sludge and fed with 2 L of 8.5 g/L-1 dextrose. The reactor was then left for 19 days without feeding. On the $19^{th}$ day, another 2L of 8.5 $g/L^{-1}$ -dextrose was fed again. The purpose was for the sludge to adjust to the environment in the reactor. At day $36^{th}$, the reactor was continuously fed with a dextrose solution (16,700 mg/L COD) at an HRT of 4.16 days. From the $55^{th}$ day onward, the reactor was fed with a synthetic leachate which has the recipe based on Rowe et al., 2002 as presented in the Table 4.1.

**Table 5.2:** Stages of operation of the AnMBR.

| Chap. | Days | Phase | Feed | OLR ($kg/m^3day$) | HRT (d) | Membrane | E2 | PAC | Note |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 36-53 | Ia | Dextrose | 3.9 | 4.16 | MTR UF | -- | -- | |
| 5 | 55-80 | Ib | Synthetic Leachate | 3.9 -5 | 4.16 | MTR UF | -- | -- | |
| 5 | 0-207 | IIa | OR Leachate | 3.6-4.8 | 4.4 | Orelis UF/MF | -- | -- | After Reactor modification |
| 5 | 207 -288 | IIb | OR Leachate | 7.3-9.3 | 2.3 | Orelis UF/MF | -- | -- | HRT change |

Note: Stage I was operated in 80 days and the initial day of Stage I was marked at 0.
Stage II was operated in 425 days and started after Stage I. The initial day of Stage II was marked at 0.

5.4.1.1. Hydraulic Performance

Hydraulic performance of the An-MBR was illustrated as Figure 5.3a. The data of the AnMBR was collected 7 days after the sludge was input into the reactor. The MBRs operated with two membrane modules. At this time, two membrane modules used the same type of membranes which were MTR UF membranes. The VSS in the reactor was around 6000 mg/L during the start up. At the beginning the specific flux had higher value which was 60 LMH/bar and then decreased to a stable value of 30LMH and depleted at day 80. This phenomenon can be explained by fouling of the membranes. It was also observed that there was a sludge layer built up on the surface of the membrane. Over time, the thickness of the sludge layer increased. The sludge layer built up can obviously be considered as the main the reason for the collapse of the fluxed at day 80. The membrane channels were totally blocked by the sludge layers as in Figure 5.4. The buildup of the sludge layer on the surface of the membrane can be explained by several of factors. Firstly, fouling on the membrane surface can play an important role to keep the solid and retain them on the surface of the membrane, and can contribute to channel blockage. Secondly, because the membrane feed was drawn directly from the reactor which had high concentration of VSS, it can results in higher concentration of solid on the surface of the membrane. Moreover, unlike aerobic sludge, anaerobic sludge has a natural trend to form granules. In the study of Jun et al., (2007) for comparison of aerobic granular form and regular aerobic sludge form on membrane fouling, it showed that the granular form could make more severe and irreversible fouling compared to the regular one. Jun et al., (2007) also stated that the fouling mechanism of the granular form on the membrane was pore-blocking mechanism. Therefore, from the observation, it can be

hypothesized for the anaerobic sludge to have the same effects on membrane fouling as the aerobic granular sludge.

5.4.1.2. COD Removal

The COD analyzed and mentioned throughout this paper is soluble COD (which had been centrifuged to remove solids). Thus, for the rest of this document, unless otherwise indicated, the mention of COD refers to soluble COD.

The bioreactor was continuously fed with COD from the 36th day onward. From day 36 through day 56, the bioreactor was fed with a dextrose solution (16,000 mg/L). From day 55 to day 88, the system was fed with synthetic leachate. The range of COD in the synthetic leachate was 18,000 mg/L to 22,000mg/L. The AnMBR was operated with a HRT of 4.16 days for both periods. OLRs of the system were calculated by the equation:

$$\text{OLR (kg/m3day)} = \frac{inputCOD(mg/L)}{1000 * HRT} \qquad (5.1)$$

The OLRs of the reactor ranged from 3.9 -5 kg/m3day.

The COD is expected to be removed by the reactor and the membrane system. Figure 5.2b illustrates that the trend of COD within the reactor is similar to the trend of the COD in the effluent. At the beginning when the reactor was fed with dextrose, the COD in the reactor and COD of the permeate show an increasing trend. This is due to the fact that when the reactor was fed a high COD solution the microbial seed obtained from municipal WWTP anaerobic digester had not yet acclimated to the new food source. However, after a few days, when the microorganism adjusted and became more active, higher amounts of COD were removed which is illustrated in the Figure 5.3. Around 10,000 mg/day COD removed at day $40^{th}$ is much less than the value of 19,000 mg/L at

day 47th. These results can be seen in Figure 5.3. The COD in the reactor and permeate decreased after 6 days of feeding the reactor dextrose. This result corresponds with the biogas production. The biogas production increased just as COD removal increased. The amount of biogas at Day 41 was only 4.5 liters/day, but by Day 47 the biogas had increased up to 7.5 liters/day. This is logical because under anaerobic treatment, part of COD was converted to biogas. By Day 47, the COD in the reactor and permeate had increased to 1000 mg/L but the values were still lower than on Day 41. The reason that after day 47 the COD in the reactor increased slightly may be because the bioreactor was not in stable operation yet.

At day 55, the reactor was fed with synthetic leachate (Table 4.1, after Rowe et al., 2002). At the beginning the COD in both the reactor and permeate were low but increased over time. After around 7 days, the COD in both the reactor and permeate were consistent around 10,000 mg/L and 8000 mg/L respectively. The biogas production during this period was around 6 liters/day. The COD removed was around 10,000 mg/L in the reactor and 12000 mg/L with the whole system. However the COD in the effluent was still higher than 1100 mg/L. This might be because the An-MBR system had not been optimized for leachate treatment. The COD removal efficiency of the system for leachate treatment was only around 50-60 % in this first stage of the startup. This removal efficiency still falls within the range of the values which was reported by other researchers when using MBRs for landfill leachate treatment (Chen and Liu, 2006). However, compared to some reports, this removal efficiency was quite low. The COD removal efficiency of a lab scale An-MBR reported by Jia et al., (2009) and Bohdziewicz et al., (2008) were around 90%-98%. There are two reasons which can explain the low

COD removal efficiency. First, it can be assumed that the startup time was not sufficient for the microbes to acclimate to the high concentration of COD. The second reason is that the convection flow which was created by the pump in the reactor could prevent granular formation of the sludge. It is widely understood that granular sludge has higher methanogenic activity (Hulshoff Pol et al., 2004). Therefore, a lower granular formation rate could result in lower COD removal in the anaerobic sludge system.

### 5.4.2. Stage II of the MBR Start Up and Long Term Performance

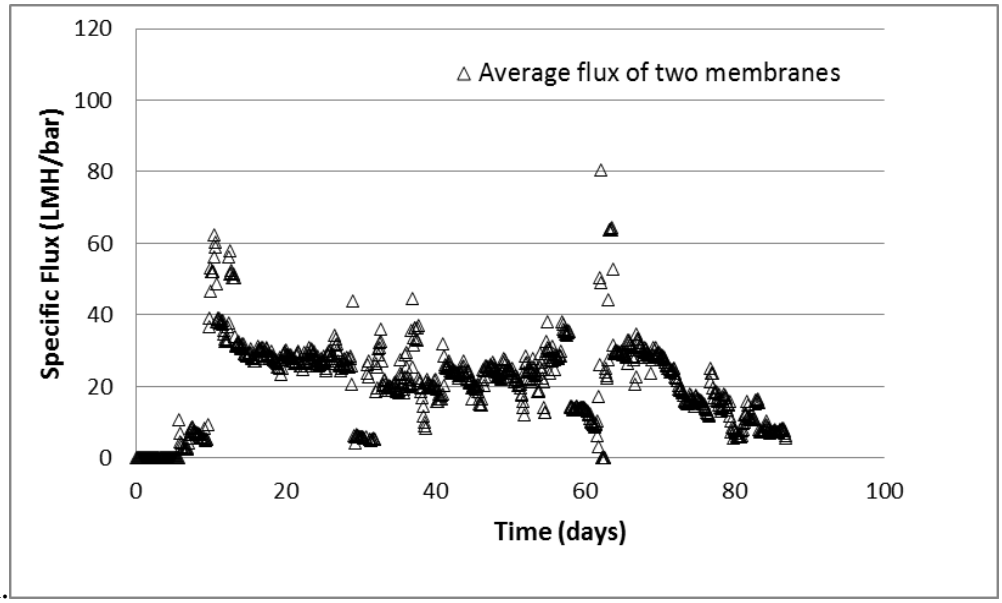
Lessons from Stage I showed that direct sludge withdrawal from the bioreactor to feed the membrane module may result in high solid loading on the surface of the membrane, leading to decreased flux. Another problem was that the membrane was fed by a high speed pump (to enable high cross flow velocity across membrane surface) which returns the retentate directly into the bioreactor and causes quite a bit of mixing within the bioreactor. It was suspected that this excessive mixing reduces anaerobic biological efficiency (by interfering with interspecies hydrogen transfer among fermenters and methanogens), thereby lowering the COD removal rate. Therefore, in the stage II of the startup, the modification of the system was made as described by Figure 5.5. A small side column was created and attached to the main bioreactor. The overflow sludge at the top of the main bioreactor, which had a much lower concentration of solids, flowed to the small side column by gravity. Contents of the side column fed the membrane module and also received membrane retentate. At the bottom of the column, gravity thickened sludge was returned to the main bioreactor through a peristaltic pump. Thus, the side column was dedicated to the membrane recirculation loop (providing feed

and receiving retentate) and sparing the main bioreactor from excessive mixing. This configuration helped to improve the two problems previously described (membrane channel blockage and insufficient COD removal). Additionally, the feed was switched to OR leachate (based on actual landfill leachate) during this stage. Initially, the reactor was filled with sludge and left for 24h without feeding or operation of any pumps. After this 24h period, the OR leachate was fed to the reactor at a rate of 0.5 mL/min (HRT = 13.33 days). A week after running the reactor under these conditions, the feeding rate was increased to 1.5 mL/min (HRT= 4.44 days). The HRT was reduced to 2.3 days after 7 months of continuous operation.

5.4.2.1. Hydraulic Performance

The UF and MF membranes used from the day 0 to day 250 were 2 months old used membranes because these membranes were used previously to test the new modification. The flux profile for the test was included in this paper. However, according to the observation, the initial and also the highest flux for MF and UF membranes were recorded at 40LMH/bar and 36LMH/bar, respectively. These are almost the same as the record for the new membranes tested on day 207$^{th}$. During the first 200days, the flux of the membranes was up at the beginning or after cleaned and decreased by time. The highest flux for UF membrane and MF membrane during this period were 15LMH and 19 LMH respectively. After more than a month, the flux decrease down to 1-2 LMH for both membranes. A major improvement by not drawing sludge directly from the main bioreactor to feed the membrane module, there was no sludge layer which was built up

a.




b.

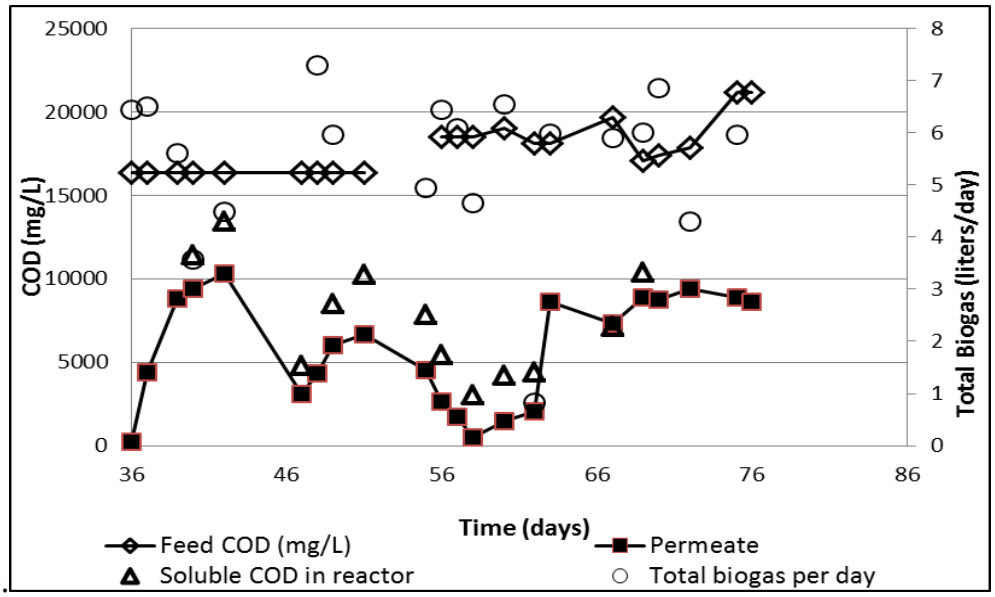
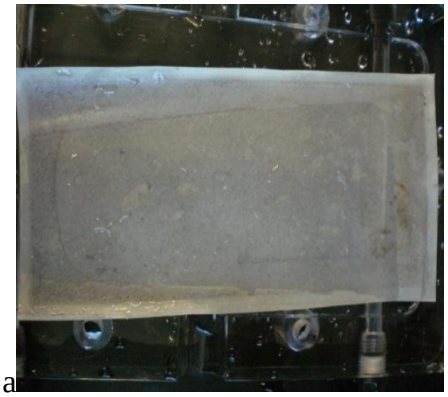



**Figure 5.3:** Performance of the AnMBR in stage I. a.Variation of specific flux with time, b. COD and biogas profile in AnMBR system.

a 

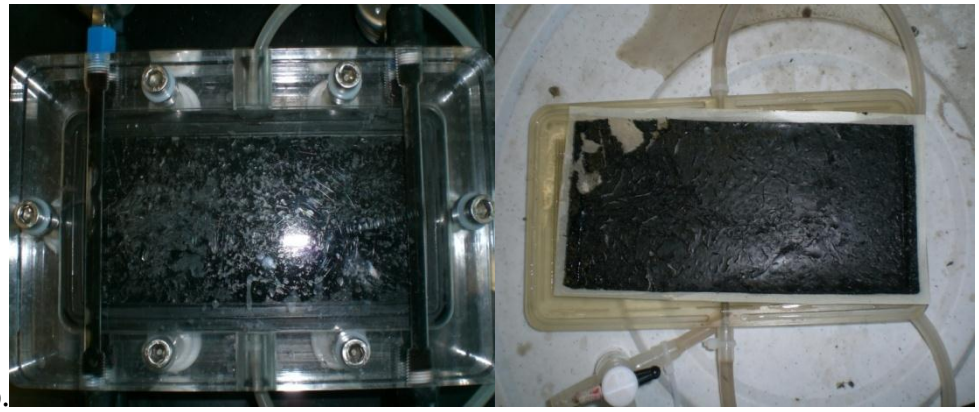
b. 

**Figure 5.4:** Sludge layer attached on membrane surface**.** a. Clean membrane, b. Membrane channel blocked.

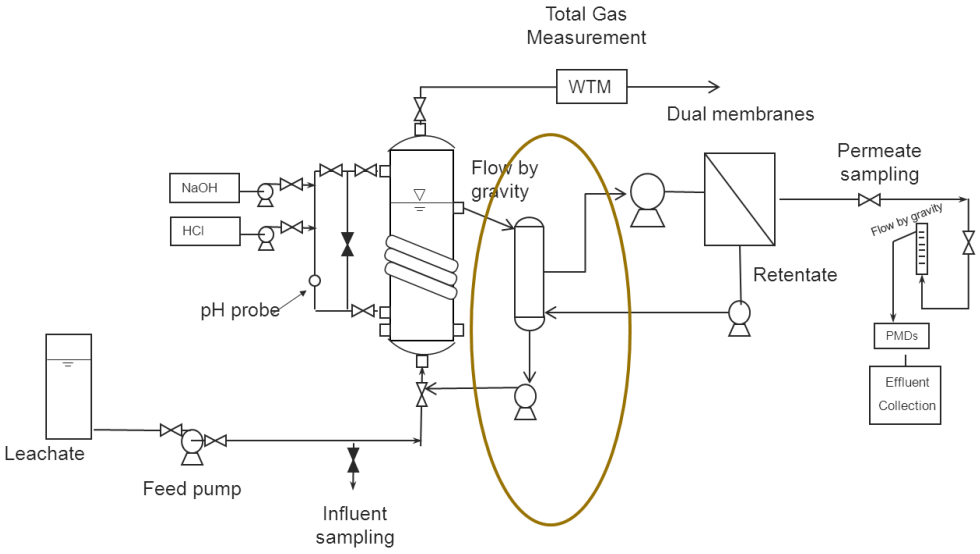



**Figure 5.5:** The scheme flow of the AnMBR system after modification.

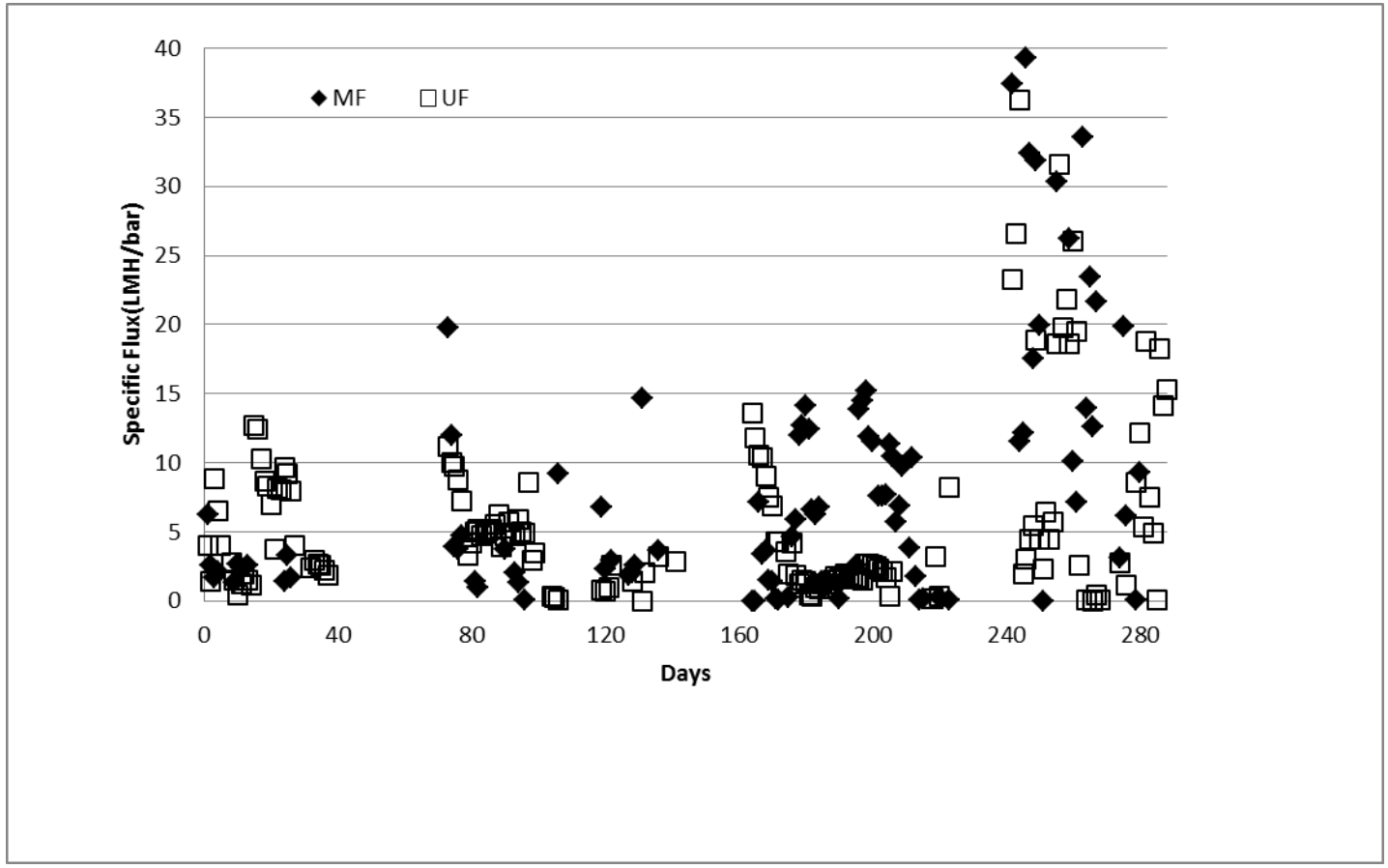



**Figure 5.6:** Flux performance of the UF and MF membranes in the system.

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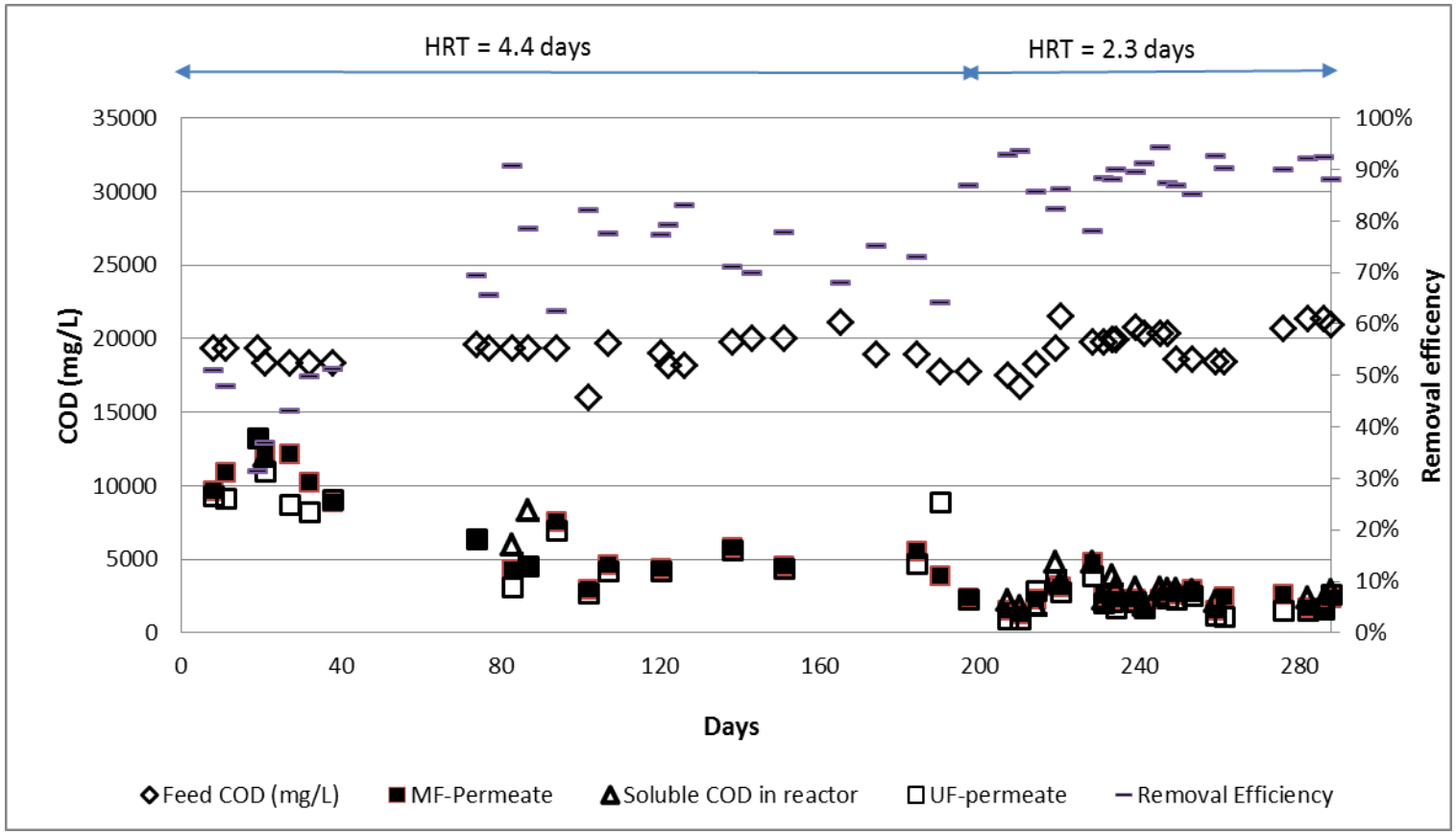



**Figure 5.7:** COD removal profile of the AnMBR after modification.

(a frequent problem during Stage I). Nevertheless, the fouling of the membranes is still one of the main reasons reason for the reduction of the flux. When the membrane was completely fouled which means there is no flux observed, there was not any way that the membranes can be cleaned onsite. Chemicals (different concentration of NaOCl solution and detergents) and water were running cross the surfaces of membranes for few hours. However, the improvement was not significant. Therefore, during this period, the membranes were taken off the membrane modules then soaked overnight in a solution of 20% NaOCl and rinsed by warm water afterward. This cleaning procedure was quite complicated and labor consuming.

On Day 207, the new UF and MF membranes were used to replace the old ones in the system. The cleaning strategy was also changed. Instead of waiting until the membranes were completely fouled, the cleaning was implemented weekly by applying high cross flow velocity and close to zero transmembrane pressure for few hours (or overnight when the membranes were completely fouled). Nevertheless, the decrease of the flux was still observed, even though the fouling process was slower. But the advantage of this strategy is that after the membrane fouled completely, the flux immediately recovered to rates close to the highest flux. The main advantage in applying this strategy was that it was CIP (clean-in-place), so was not necessary to either open up the membranes modules or stop the operation of the system.

Generally, UF and MF membrane, though operated at different pressures, were just slightly different in specific flux performance even MF membrane showed a bit higher flux than UF membrane. Both membranes were fouled at the same time of operation which is around 50-60 days.

5.4.2.2. Solube COD Removal Performance

As mentioned, during the first week, the leachate was fed at a rate of 0.5 mL/min. After that, the feed rate was increased to 1.5mL/min which corresponded to a HRT of 4.4 days. This feed rate was kept until the day 207. The OLR during the first 207 days was around 4.5kg COD/$m^3$day. The COD removal was pretty low at day 8, around 50%. The removal efficiency steadily increased to around 80% after 100days of operation and remained stable from there. The reason for the low COD removal at the beginning was that the microorganisms in the reactor needs time to adapt to the new environment and influent characteristics. After day 207, the feed rate was adjusted to 3 mL/min which resulted in a reduced of HRT to 2.3 days. The OLR of this period was around 8.6 kg COD/$m^3$day. Once the HRT was adjusted, the removal efficiency of the reactor increased by 10%. A higher COD removal efficiency at a HRT of 2 days is supported by Bohdziewicz et al., (2008). The removal efficiency at this HRT in our system can reach as high as 96%. This can be explained by the fact that with a higher feeding rate, the contact between the microorganism and the leachate is higher. Therefore, there is more chance for the microorganism to convert the COD to methane.

Figure 5.7 shows that there is no significant different in the COD concentrations among the MF and UF membranes' permeates. The figure also shows that the COD in the reactor was just slightly higher than MF and UF permeates. It might be because the OR leachate contains most of soluble COD. Therefore, the biofilm layer on the membrane

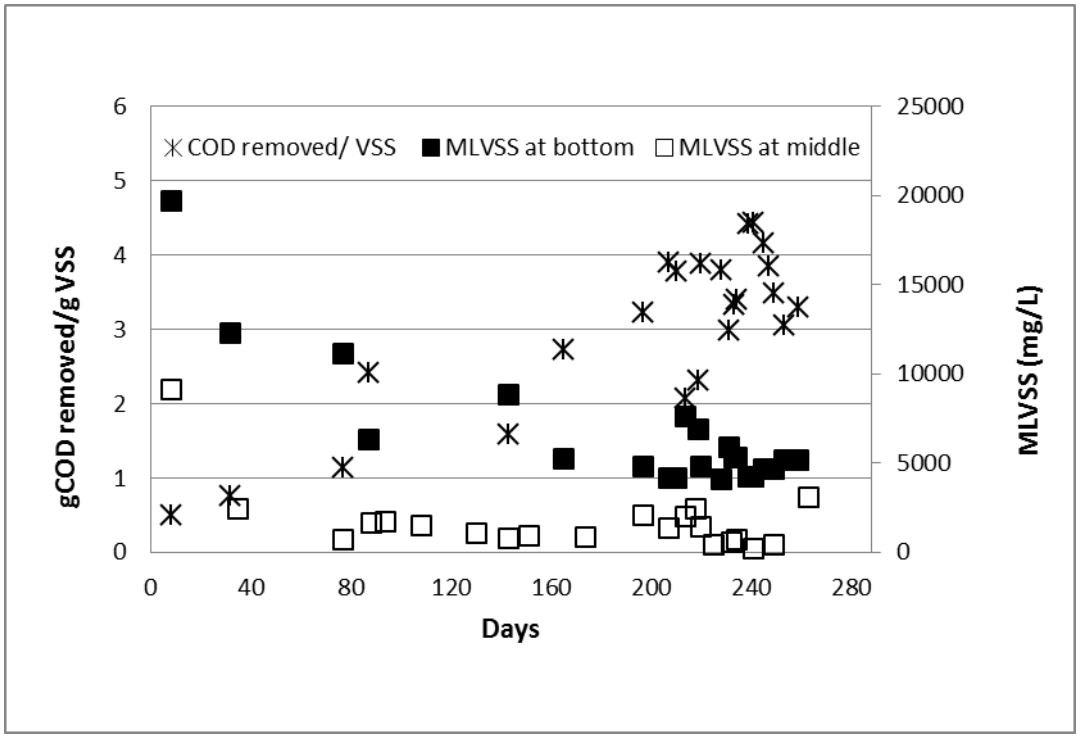



**Figure 5.8:** Specific COD removal.

may play an important role in providing additional COD removal. Another explanation is that COD associated with colloidal organic matter (which normally would be counted as part of soluble COD, separated by centrifugation) was retained and removed by the membranes. Figure 5.8 shows the specific COD removal which is the ratio of COD removed and biomass retained increased during the first 100 days. Near the beginning, the ratio was at 0.32g COD removed/1g VSS. The average VSS used in calculation of the specific COD removal is the VSS at bottom of the reactor. This is because the concentration of the bottom of the reactor is always much higher than the VSS at the middle of the reactor. When the reactor reached steady state conditions, at both HRT =4.44 and HRT =2.3, the ratio between the COD removed and the biomass is almost constant at an average rate of 1.88 g COD removed/gVSS. The COD concentration in the effluent at a HRT of 2.3 was within the range of 1500-2000 mg/L. This range of concentrations is significantly lower than the initial concentration but is still too high to be able to discharge to the natural environment. However, it serves as an effective pretreatment of the leachate for further treatment at a wastewater treatment plant.

5.4.2.3. Biogas Production

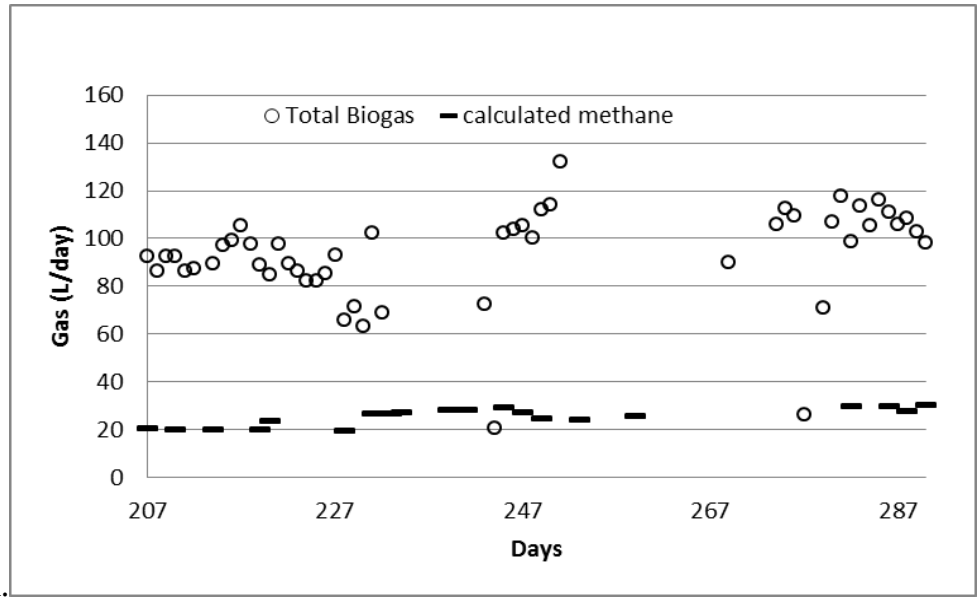
Biogas data was continuously collected by a wet tip meter connected to the acquisition system. Figure 5.9a describes the profile of biogas since the reactor reached stable condition. The rate of biogas production was around 100L/d. Based on the gas collected and the COD removed per day, the average of ratio between the amount biogas (L) per to COD removed (g) was calculated. Due to the high moisture levels within the biogas, direct sampling of the gas was difficult to conduct. A series of batch experiments

were conducted in 50mL serum-bottles as an alternative method to determine the methane content. In the batch experiment, the same type and concentrations of OR leachate was used and the temperature was kept constant at 37$^{o}$C. The gas profile of the batch experiment can be found in Figure 5.9b. Theoretically, 0.35L of $CH_4$ is produced per 1 gram of COD at standard temperature and pressure (STP). The ratio corrected for 37$^{o}$C is 0.39L $CH_4$/1g COD. The percentage between the actual COD produced by batch experiment and the theoretical $CH_4$ at 37$^{o}$C as calculated in Table 5.3 is 89%. The 11% different can be explained by that some COD was converted to biomass. The ratio for the actual methane gas produced and COD removed is 0.35L $CH_4$/1g COD removed. This number was used to apply for determination of the possible amount of methane produced in the anaerobic reactor. The possible amount of methane produced was illustrated in Figure 5.9a by dash line with the average amount around 27 L.

5.4.2.4. Water Quality Performance of the AnMBR

Some water quality parameters such as TN, TOC and Turbidity were also tested as shown in Table 5.4. The TOC and turbidity removal efficiencies for both MF and UF membranes were pretty high which are 77% and 92% and 86 and 96 % respectively. The performance of UF for TOC and turbidity removal was 4-5% higher than the MF. As expected for anaerobic treatment, the amount of total nitrogen was removed from the AnMBR system was not significant. The removal efficiencies for total nitrogen for MF and UF AnMBR system were only 4 and 8%, respectively. Anaerobic digestion

a.

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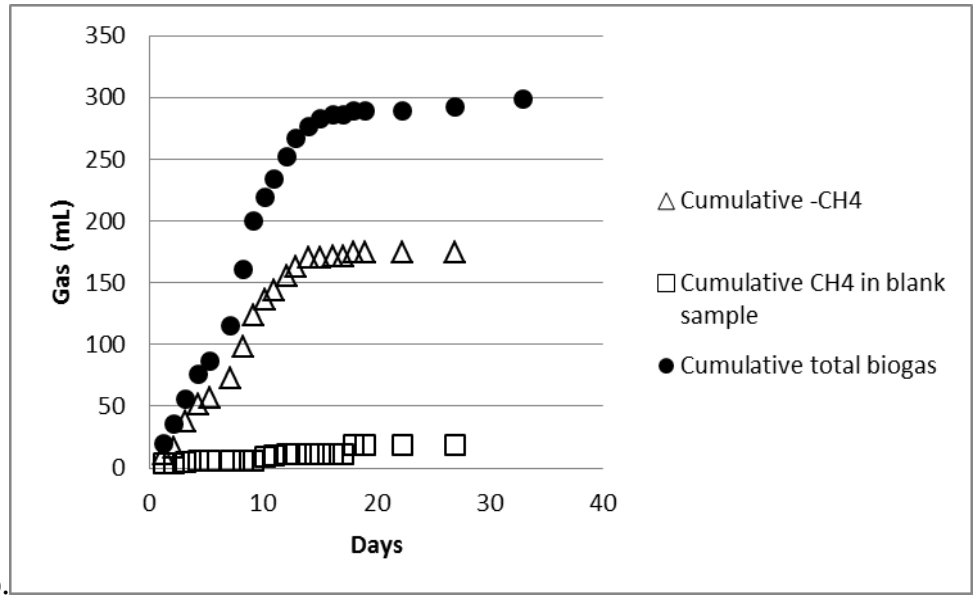



**Figure 5.9:** Gas production performance. a. Profile of gas in the reactor and calculated methane, b. Methane and total gas profile for the batch experiment.

**Table 5.3:** COD and methane data for the batch experiment at day 28.

| Parameter | |
|---|---|
| COD of leachate (mg/L) | 10200 |
| COD final (mg/L) | 2451 |
| COD back ground (mg/L)[a] | 941 |
| COD destroyed (mg/L) [b] | 8690 |
| CH4 gas produced (L/L)[c] | 3.1 |
| Theoretical $CH_4$ at 37C (mL/L)[d] | 3.4 |
| Percentage [e] | 0.907747 |
| Digestibility (%)[f] | 85.19608 |
| SMP (mL)[g] | 357.65 |

Note: [a] COD of the sludge used in the experiment

[b] $COD_{leachate}+COD_{background}-COD_{final}$

[c] Caculatedmethan produced per 1 L of culture based on the methane produced in serum bottle.

[d] Calculated theoretical methane at $37^0$C derived from the COD destroyed and the derivation of the conversion factor at $37^o$C based on the conversion factor 0.35 $mLCH_4$ per 1 g COD destroyed at SMP.

[e] $CH_4$ produced*100%/theoretical $CH_4$

[f] $COD_{destroyed}$/COD of leachate

[g] Specific Methane Prodution ( methane produced per 1g COD destroyed

**Table 5.4:** Water quality performance of the AnMBR system.

| Removal percentage | MF | UF |
|---|---|---|
| Turbidity (%) | 92 | 96 |
| TN (%) | 4 | 8 |
| TOC (%) | 77 | 86 |
| COD removal (%) | 90 | 91 |

Note: All the values in the tables are average values by time when the system reached stable condition

generally does not remove in nitrogen and in fact liberates organic nitrogen to $NH_4^+$ form. The exception is the anaerobic ammonia oxidation (anammox) process, which combines ammonium with nitrite to form nitrogen gas. However, anammox is a specialty process and is not the subject of this research. For this research, it was assumed that the AnMBR process can be followed by a number of either nitrogen recoveries. Struvite precipitation or removal (e.g., BNR at municipal WWTP) processes which are well demonstrated.

## 5.5. Summary and Conclusion

The AnMBR reached steady performance for young OR leachate after 100 days. Lower HRT at 2.3 days showed higher performance than the HRT 4.4 days. The highest COD removal efficiency at HRT of 2.3 days was 96%. The modification to dedicate a simple side column to the membrane loop significantly improved both the COD removal efficiency and membrane performance. In order to prevent the irreversible fouling of the membrane and be able to recover the highest flux of the membranes, weekly cleaning by applying a clean water cross flow with close to zero transmembrane pressure was

necessary but effective. The recovery of methane from the leachate could be at 0.35L/1g COD removed at $37^0$C. The high COD removal efficiency reduced the influent COD of 20,000 mg/L to effluent values around 1500-2000mg/L. Because the effluent COD is still higher than permitted for direct discharge to the natural environment, the AnMBR should be considered an efficient pretreatment method for the landfill leachate, to be followed by a number of possible steps either at the landfill for continued treatment (such as advanced oxidation or RO), or for transport to a municipal WWTP for further treatment.

# CHAPTER 6: PHASE DISTRIBUTION OF ENDOCRINE COMPOUNDS (EDC) IN AN ANAEROBIC SLUDGE SYSTEM WITH POWDERED ACTIVATED CARBON

## 6.1. Abstract

In order to understand the behavior of EDCs in a hybrid anaerobic system with powdered activated carbon (PAC), it is important to find out what process in the system involved in removing of EDCs. The hybrid anaerobic system with PAC includes two major processes which are anaerobic biological process and adsorption process of the EDCs to the solid such as anaerobic sludge and PAC. Therefore, determination of fate of EDCs whether EDCs are adsorbed to anaerobic sludge, colloids are conducted by series of isotherm batch experiments Freundlich model fitted quite well the adsorption data of all three EDCs. The PAC has stronger adsorption potential than anaerobic sludge. At both low (100 µg/L) and high (4mg/L), the adsorption potential of E2, E1 and EE2 on sludge follows the order E2>EE2>E1 which correlates to the $K_{ow}$ values (4.01, 3.67, 3.1, respectively). However, all three compounds showed the same adsorption potential to the Norit 20B PAC.

## 6.2. Introduction

Recently, micropollutants such as endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) have increasingly attracted attention from scientists and the public. Their concern over EDCs and PPCPs stems from the

significant ecological and health consequences at trace levels. Richard et al., 2004 showed that at a medium and high concentrations of PPCPs mixtures (combination of three compounds: ibuprofen, floucetin and ciprofloxacin) (from 60 μg/l-1000 μg/l) fish mortality occurs in time of 35 days and 4 days, at medium and high concentration, respectively. EDCs are hypothesized to cause alterations for endocrine system of wildlife. Sumpter (2005) showed that EDCs caused feminization of male fish and affected the fecundity of female fish (Diniz et al., 2005). There have been several investigations on the effects of EDCs on animals other than fish such as birds, amphibians, and panthers (Nghiem, 2002; Nghiem, 2004). There has also been speculation in recent years of potential negative impacts to human health, such as decreases in male sperm counts and increases in testicular, prostate, and ovarian cancers.

Among the EDCs, estrogenic compounds are of higher concern than other compounds. At very low concentrations (i.e., ng/l range), estrogenic compounds can still have negative effects on fish (Arcan-Hoy et al., 1998; Panter et al., 1998). The changes in fish reproduction can be measured when fish are exposed to 17β-estradiol (E2) and 17α-ethinyl estradiol (EE2) with concentration of 2ng/l in laboratory conditions. Gadal et al., (2005) indicated that natural estrogen E2 plays a very important role in breast cancer initiators. Estrogenic compounds can enter the environment via different sources. However, it is reported that a significant source for E2 in environment is via inadequate medicine disposal.

Reports in recent years have indicated that AnMBRs can be used to remove micropollutants from wastewater. Anaerobic processes have the potential to degrade or transform xenobiotic organic compounds, including polychlorinated organics, surfactants

(Yeh et al., 1998; Yeh et al., 1999; Yeh and Pavlostathis, 2001; Yeh and Pavlostathis, 2005) and pesticides. Further, biological treatment processes have enhanced capability of removing hormonal compounds from wastewater when both anaerobic and aerobic conditions are imposed (Joss et al., 2004). It has also been shown that certain PCPPs are better removed under anaerobic conditions (e.g., antibiotics, naproxen, diatrizoate, estrogens, musk fragrances), while others are suitably-treated aerobically (e.g., ibuprofen, bezafibrate) (Ternes et al., 2005). Limited information is available on the fate of estrogenic compounds such as E2 under anaerobic conditions. While Czajka and Londry (2006) reported that, under a variety of anaerobic electron acceptor conditions, 17β-estradiol (E2) only partially transforms to Estrone (E1) and accumulates as E1 and E2, Fahrbach (2006) recently demonstrated that E2 can completely biodegrade under denitrifying conditions.

PAC was known as a material which can remove organic carbons in water by adsorption mechanism. PAC was reported as an effective additive to anaerobic system to removal of anaerobic inhibitor organic compounds (e.g. 2,4- dicholorophenol, 2-nitrophenol, Nitrobenzene) (Ng. et al., 1988, Widjaja et al., 2010). Combining anaerobic waste conversion with PAC, hybrid anaerobic systems with PAC have a great potential for treating a variety of waste streams previously deemed too difficult to treat biologically. However, limited information is currently available on the potential of hybrid anaerobic MBR system with PAC for removing micropollutants such as hormone compounds. The lack of information on this process with tremendous potential is the motivation of this study which focuses on potential of removing E1, E2, EE2. In this

paper, the operation the phase distribution of the target hormone compounds in a hybrid anaerobic system with PAC will be introduced.

## 6.3. Materials and Methods

### 6.3.1. Materials

Estrogenic compounds (E1, E2, EE2) were purchased in grade of 98% from Sigma Aldrich (St. Louis, MO, USA). Individual hormone compound was prepared in a stock solution of 400 mg/L in methanol. Then the stock was stored in a refrigerator at 5$^{o}$C until used but not less than 3 months. After that, the hormones were diluted by MilliQ water for calibration curves set up. Methanol which is used for stock solution was purchased in HPLC grade from Fisher Scientific (New Jersey, USA).

Deuterated E2-d4 (Cambrige Isotope Laboratories) was chosen as an internal standard for analyzing the target hormone compounds. N-Methyl-N-trimethylsilyltri-fluoroacetamide (MSTFA), a derivatization agent, was purchased from Thermo-scientific (Rockford, IL, USA).

Anaerobic sludge was collected from Howard F. Curren Advanced Wastewater Treatment in Tampa, Florida. Prior to use, the sludge was filtered by a 20 mm sieve to remove large particles of waste which were retained in the waste water treatment plant (WWTP). The sieved sludge was stored in a carboy at room temperature and always well mixed before used. Then in order to inactivate the microorganisms of anaerobic sludge, 1.5g/l of sodium azide was added at least 2 hours before used.

After few days stored in a carboy, the sludge was separated into two phases: liquid phase and condensed biomass phase. The liquid was extract and called as sludge supernatant.

The PAC selected for the experiment was Norit 20B and it was obtained from Norit Americas Inc. (Texas, USA).

TSS was used to indicate the amount of sludge in the experiment. TSS of samples was measured using the Standard Method, 21$^{st}$ edition.

### 6.3.2. Methods

Analysis of hormone compounds was conducted as follows:

- Preparation: Samples were filtered in to a GF/F glass microfiber filter (0.7µm). After that, samples are located into 10 mL-vials which is specifically designed for auto-sampler compatible with GC/MS instrument from Varian inc., Palo Alto, California, US. 30% (weight/volume) of NaCl (Fisher scientific, Springfield, NJ, USA) was added into each vial.
- SPME-GC/MS: After the preparation, samples were analyzed by a GC- 3800 /MS Saturn 2000 combined with a CombiPAL auto sampler (Varian Inc, Palo Alto, CA, USA). The extraction of the hormone compounds to SPME fiber was set at 40 minutes, 500 rpm and 35$^{o}$C. Next, the fiber with the hormone compounds was derivatized on fiber by MSTFA for 6 minutes at 70$^{o}$C. Then the fiber was injected to 1179 injector in GC/MS at splitless mode for 5 minutes at 280$^{o}$C. The GC/MS is equipped with a HP-5MS (30m x 0.25mm x 0.25 µm). The GC oven program is set as: initial temperature at 80$^{o}$C, hold for 3 minutes, then ramp 20$^{o}$C/minute to

180$^{o}$C, hold for 1 minute, then ramp 12$^{o}$C/min and hold for 9 minutes. For quantitative purpose, the MS mode is set up for scan from 265-440 m/z.

The adsorption experiments were set up as follow:

- The hormone compounds adsorption experiments were conducted with PAC, inactivated anaerobic sludge in MilliQ water or sludge supernatant solution. The hormones were prepared in mixture for the adsorption. The initial concentration of each hormone compound in all adsorption experiments were 100µg/L for low concentration experiment and 4mg/L for high concentration experiment. The amounts of PAC and inactivated anaerobic sludge which were used in the sets of adsorption were 5 mg/L-100mg/L and 20-200mg/L, respectively for low concentration test and . All adsorption experiments were implemented in a 40mL vials as in Figure 6.1 under isothermal condition at 35$^{o}$C and with 150 rpm mixing by a lab-line incubator shaker (Chesapeake instrument inc., Columbia, MD, USA).

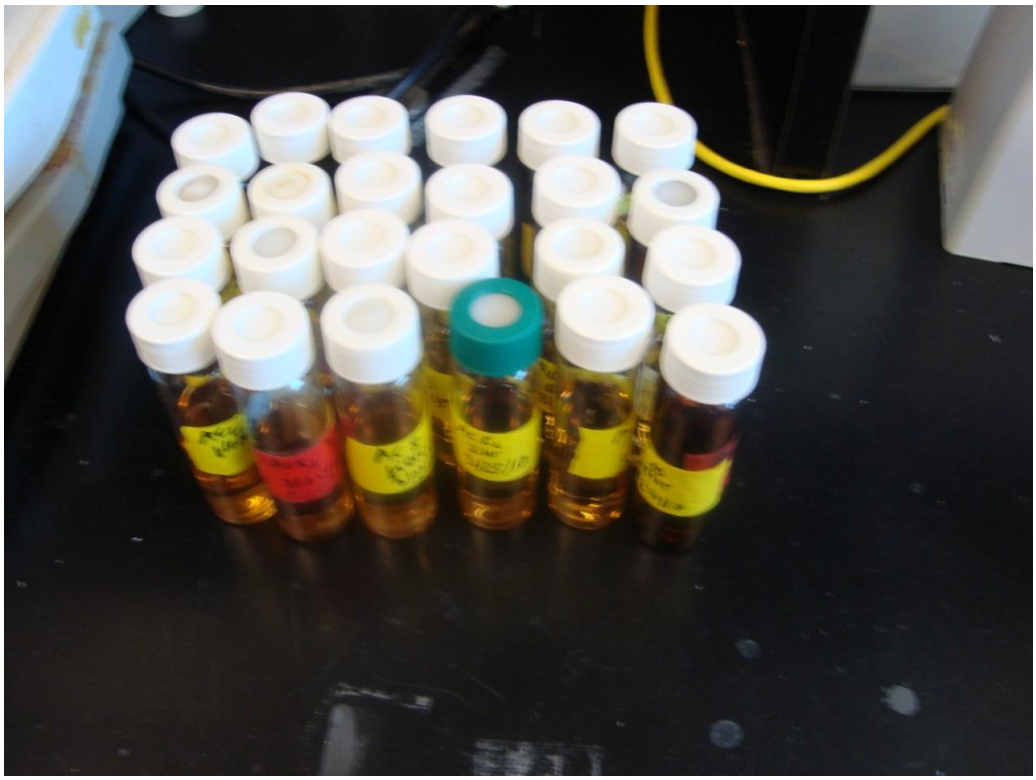


**Figure 6.1:** 40 mL vials for adsorption tests.

## 6.4. Results and Discussion

In a hybrid anaerobic system with PAC, there two phases that the hormones can potentially be delivered into. First, the hormone compounds can be distributed into solid phase which include biomass, colloids, PAC. Second phase for hormones distributed to is liquid phase. Figure 6.2 shows the potentials phases that EDCs can be delivered to. Therefore, the mass balance of each hormone compound in the system can be described as in equation (6.1):

$$M_T = M_{bm} + M_c + M_{PAC} + M_{aq} \quad (6.1)$$

In that, $M_T$ is total mass of a hormone compound in the system

$M_{bm}$, $M_c$, $M_{PAC}$, $M_{aq}$ are the mass of the hormone in biomass, colloids, PAC and aqueous phase respectively.

It is quite difficult to estimate directly the value of each element in the equation (6.1) from the whole hybrid anaerobic system. However, it is possible to determine each value in a single system (i.e. system with only PAC or biomass) by conducting series of adsorption test. Based on some available adsorption models such as Freundlich, Langmuir, the value of those elements can be estimated and predicted.

### 6.4.1. Kinetic Adsorption of the Hormones to Inactivated Anaerobic Sludge and PAC

Two experimental series of kinetic adsorption of the EDCs to inactivated anaerobic sludge and PAC were conducted in 24 hours with each EDCs initial concentration of 100µg/L. The amount of sludge and PAC used in the experiment were 80 mg/L and 33.3 mg/L, respectively. The samples of the experiment were

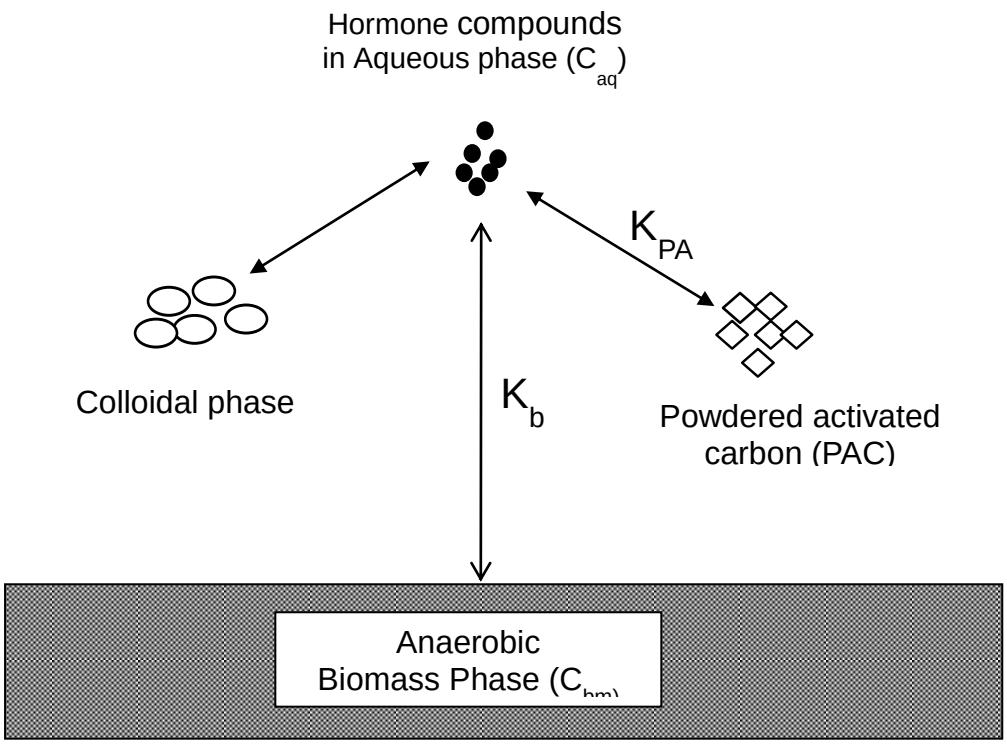



**Figure 6.2:** Distribution of hormone compounds in a hybrid anaerobic system with PAC.

collected by time to test the equilibrium time. It is shown in the Figure 6.2 that after 6 hours the significant amount of three EDCs decreased in both sludge and PAC series and after 8 hours the adsorption of E1 and E2 to both sludge and PAC reached equilibrium. However the equilibrium of EE2 was reached slower compared to the other two. Figure 6.3 showed that the equilibrium of EE2 reached after 24 hours. Therefore, all isotherm adsorption experiments later were conducted in 24hrs.

### 6.4.2. Low Range of Initial Concentration of Each Hormone Compounds (100µg/L)

The adsorption tests were conducted in low and high initial concentration of each hormone in order to see the distribution of the hormone compounds in different concentrations. The concentration of the hormone compounds in the environment can vary from µg/L in surface water to mg/L in landfill leachate as mentioned in chapter 2.

#### 6.4.2.1. Distribution of the Hormones into Inactivated Anaerobic Sludge

The data for adsorption of hormones compound in the biomass at low concentration were illustrated in Figure 6.4a. From several available adsorption models, Freundlich model was found to be able to fit the data as seen in Figure 6.4b. Therefore, the mass of the hormone compounds in the biomass can be expressed by the Freundlich model as follow:

$$M_{bm} = q_{bm}. S_{bm} = K_{bm} S_{bm} (C_{aq})^{1/b} \quad (6.2)$$

$q_{bm:}$ amount of hormone per unit weight of biomass (mg/g)

$K_{bm:}$ Distribution coefficient of hormone compound on biomass

$S_{bm}$: Mass of biomass (MLVSS- mg)

$C_{aq}$: Concentration of hormone compound in aqueous phase (mg/L)

1/b: Freundlich constant

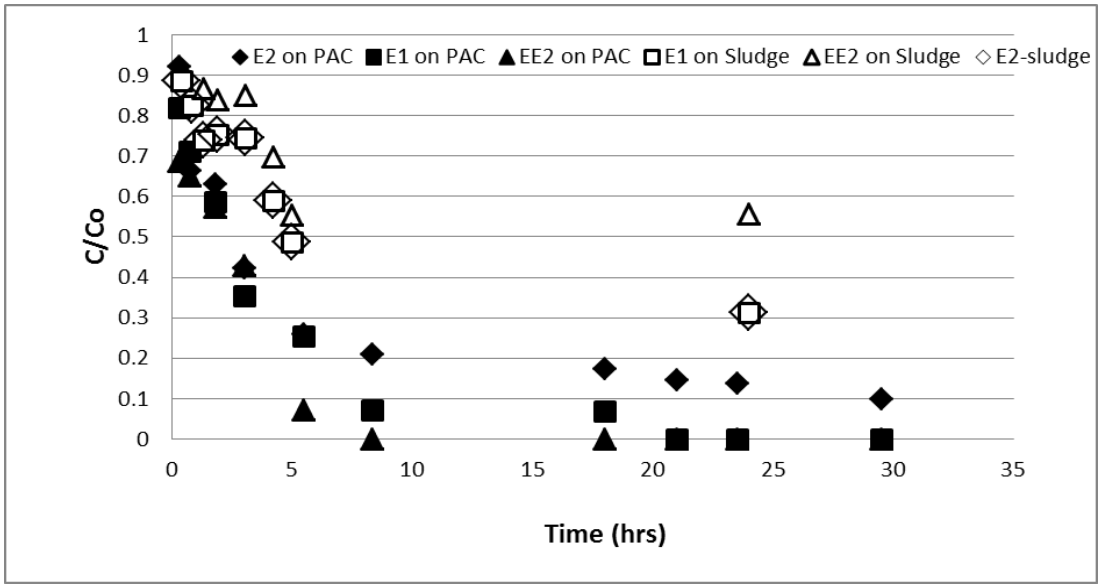



**Figure 6.3:** Adsorption kinetic of EDCs into inactivated anaerobic sludge and PAC at 35$^{o}$C.

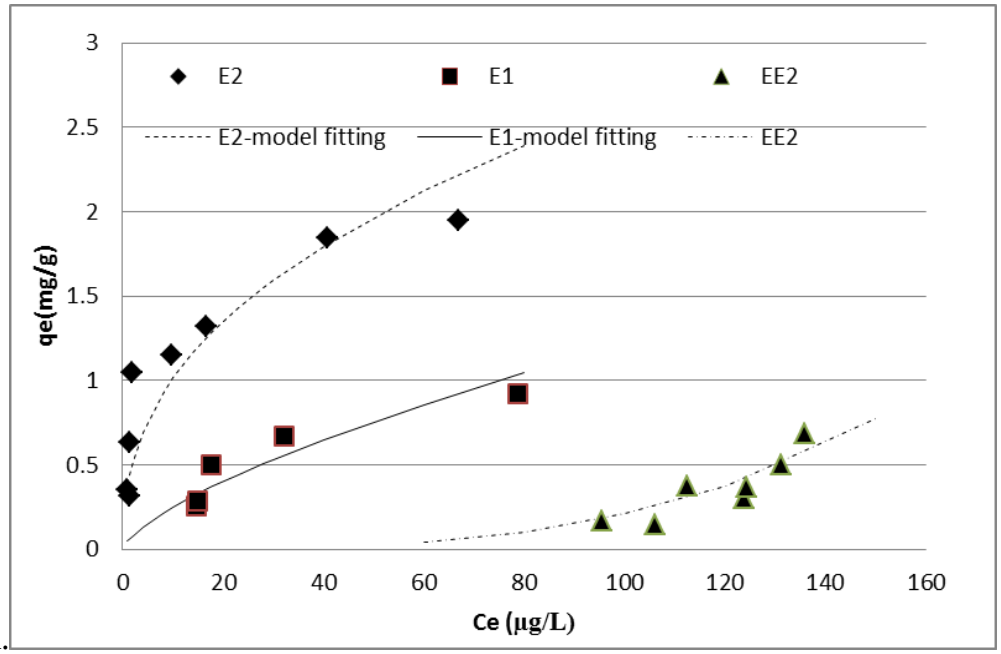
Freundlich constants k and values for the adsorption of E1, E2 and EE2 were determined as in Table 6.1. Based on the values of the constants, the prediction of $q_{bm}$ value look quite fit the data as in Figure 6.4a.

The adsorption potentials of three compounds to the sludge are not equal. Figure 6.3b showed that at qe of E2 is higher than that of E1 and EE2. The adsorption of EE2 is slightly higher than E1. It is hypothesized that the inactivated anaerobic is hydrophobic and the adsorption of sludge to hormone compound is hydrophobic adsorption. Therefore, with higher Kow value which means more hydrophobic than the other two compounds, the E2 can be expected to adsorb more to the sludge. The order of hydrophobicity of the three compounds is E2 > EE2> E1. The results from the Figure 6.4 supported and proved the hypothesis. The adsorption of E2 to the sludge is stronger than EE2's and the EE2's is stronger than E1's. The phase distribution constants were determined for E2, E1, EE2 as in Table 6.1.

6.4.2.2. Distribution of the Hormones into PAC

The results of the distribution of the EDCs to PAC at low initial concentration were shown in Figure 6.5a. Freundlich model was also used to fit the data. Similar to inactivated anaerobic sludge, the model quite fit the PAC data. The K and 1/n constants were also determined as in Table 6.1. Thus, the mass of hormones which adsorbed into the PAC were also determined by Freundlich model:

a.




b.

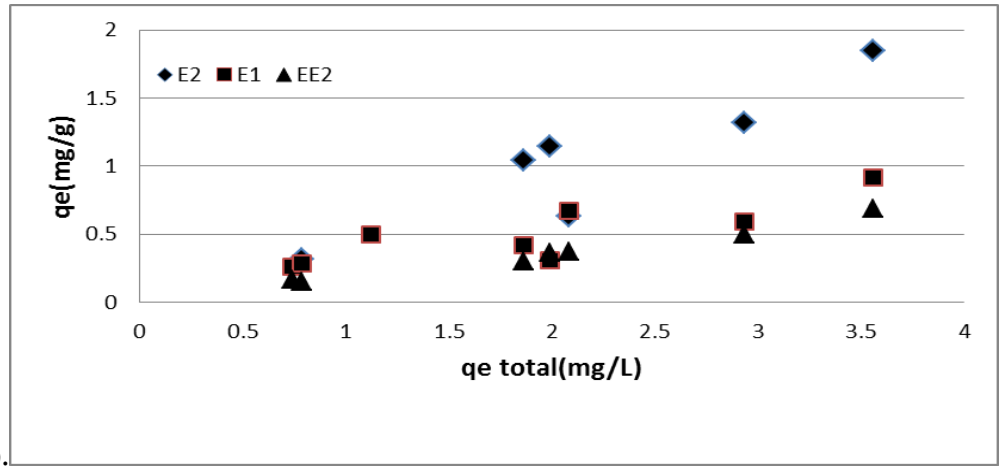



**Figure 6.4:** Adsorption of the EDCs into anaerobic sludge at low initial concentration. a. Adsorption curve of each hormone compound to anaerobic sludge at low initial concentration, b. Comparison of adsorption capability of each hormone compound to anaerobic sludge at low initial concentration.

$$q = K_{PAC.} (C_{aq})^{1/p}$$

$$M_{PAC} = q. S_{PAC} = K_{PAC}. S_{PAC}. (C_{aq})^{1/p} \qquad (6.3)$$

q: amount of hormone compound per amount of PAC (µg/mg)

$M_{PAC}$: Concentration of hormone compound in PAC phase (µg/L)

$K_{PAC:}$ Distribution coefficient of hormone compound on PAC

$S_{PAC}$: Amount of PAC in the system (mg/L)

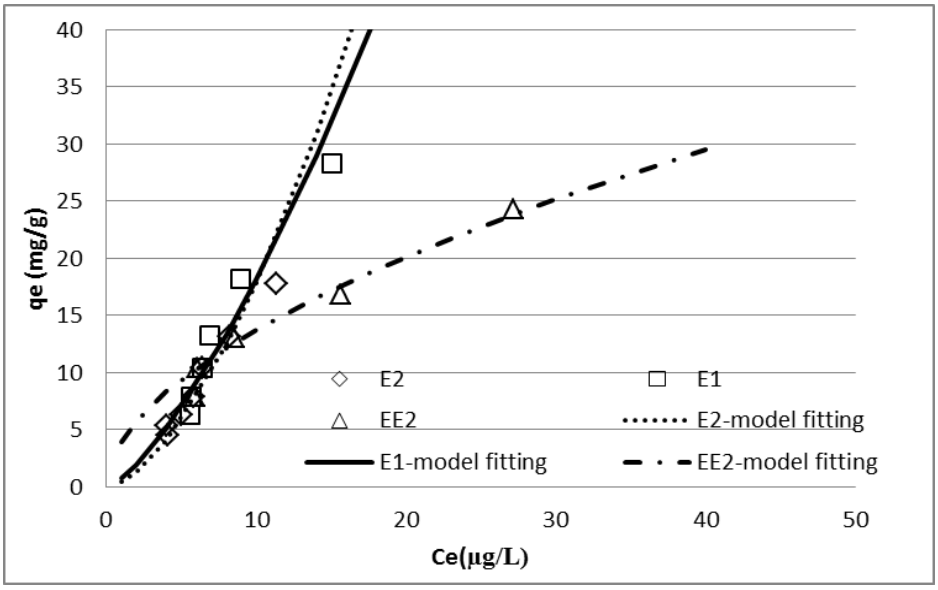
1/p: Freundlich constant

**Table 6.1:** Freundlich constants for adsorption in inactivated anaerobic sludge and PAC at low initial concentration of EDCs.

| | Sludge | | | PAC | | |
|---|---|---|---|---|---|---|
| Compounds | E1 | E2 | EE2 | E1 | E2 | EE2 |
| Log K | -0.14 | -0.93 | 9 | 4.46 | 4.72 | 0.88 |
| 1/n | 0.69 | 0.41 | 3.24 | 1.24 | 1.3 | 0.55 |

The behavior of each hormone adsorbed to PAC is quite similar as observed in Figure 6.5b. At low initial concentration of each hormone compound, the total qe range from 10 mg/L to 60 mg/L. And the amount of each hormone compound adsorbed into the PAC was the same which are shown by overlap points in Figure 6.5b. The linearship can describe the relationship of individual qe to the total qe.

a.




b.

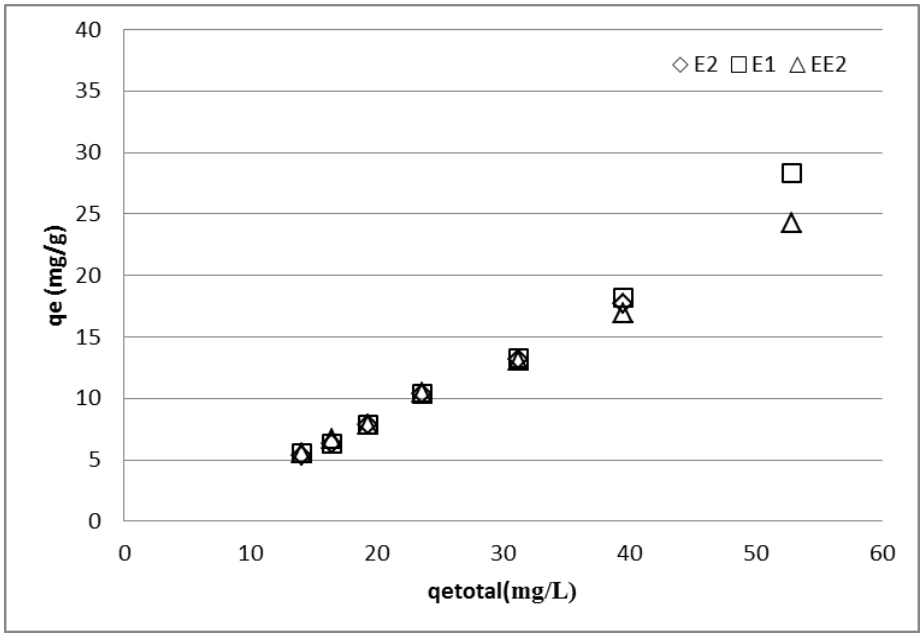



**Figure 6.5:** Adsorption of the EDCs into PAC at low initial concentration.
a. Adsorption curves of each hormone compound to PAC Norit 20B at low initial concentration, b. Comparison of adsorption capability of each hormone compound to PAC at low initial concentration.

### 6.4.3. High Range of Initial Concentration of Each Hormone Compound (4mg/L)

#### 6.4.3.1. Distribution of the Hormones into Inactivated Anaerobic Sludge

Adsorption results of the hormone compounds at high initial concentration to anaerobic sludge were illustrated in Figure 6.6. Similar to the adsorption of the hormone compound at low initial concentration to inactivated anaerobic sludge, the adsorption of the hormones compounds to the inactivated anaerobic sludge at high initial concentration is more favorable to E2 and less with EE2 and E1. This also once again supports the hypothesis that the adsorption of the hormone compounds to anaerobic sludge is caused by hydrophobic adsorption. The Freundlich constants were shown in Table 6.2.

**Table 6.2:** Adsorption coefficient of high concentration range of EDCs on sludge.

| Compounds | E1 | E2 | EE2 |
|---|---|---|---|
| Log K | 11.29 | -1.82 | 1.25 |
| 1/n | 4.72 | 0.43 | 1.42 |

#### 6.4.3.2. Distribution of the Hormones into PAC

The initial concentration of each EDC compound was 4 mg/ L and was prepared in an aqueous background. Two types of background were tested in this set of experiments as well. The first background water was tested was Milli Q water and the second is sludge supernatant. Figure 6.7a illustrated that the adsorption of all three

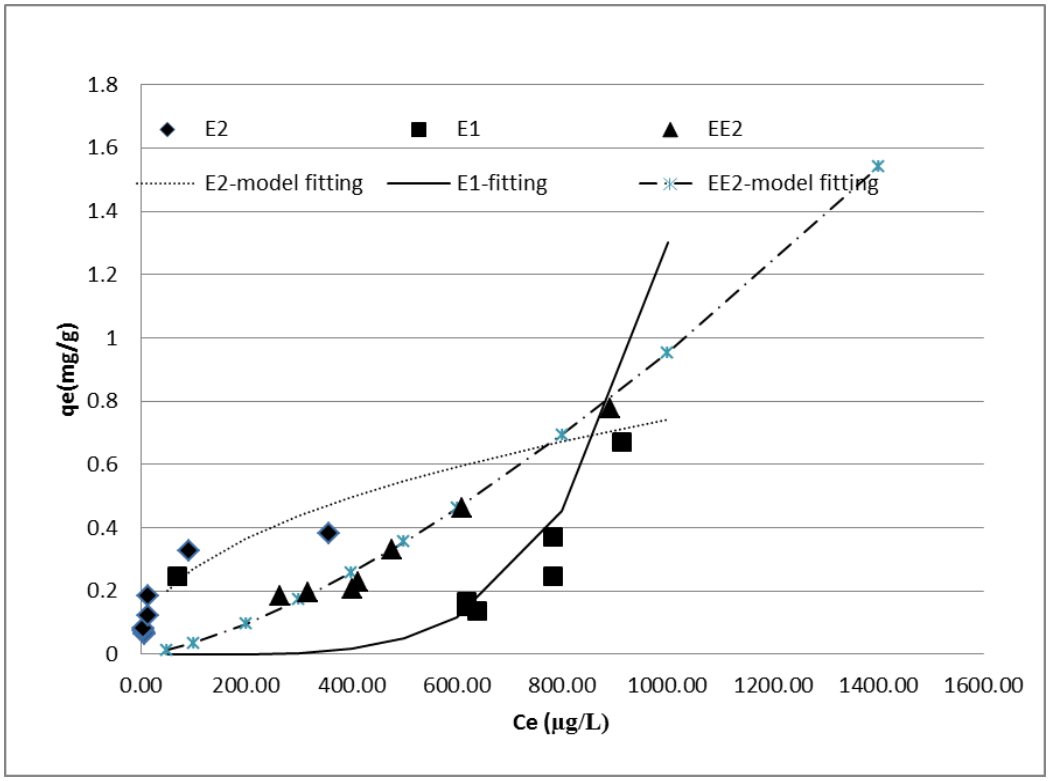
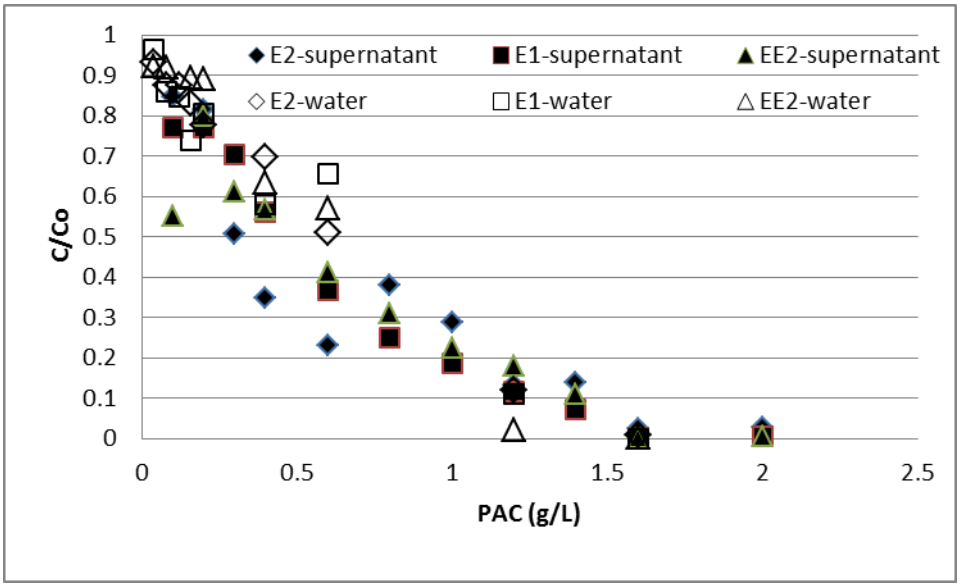



**Figure 6.6:** Adsorption curves of the EDCs to anaerobic sludge at high initial concentration in sludge supernatant matrix.

a.

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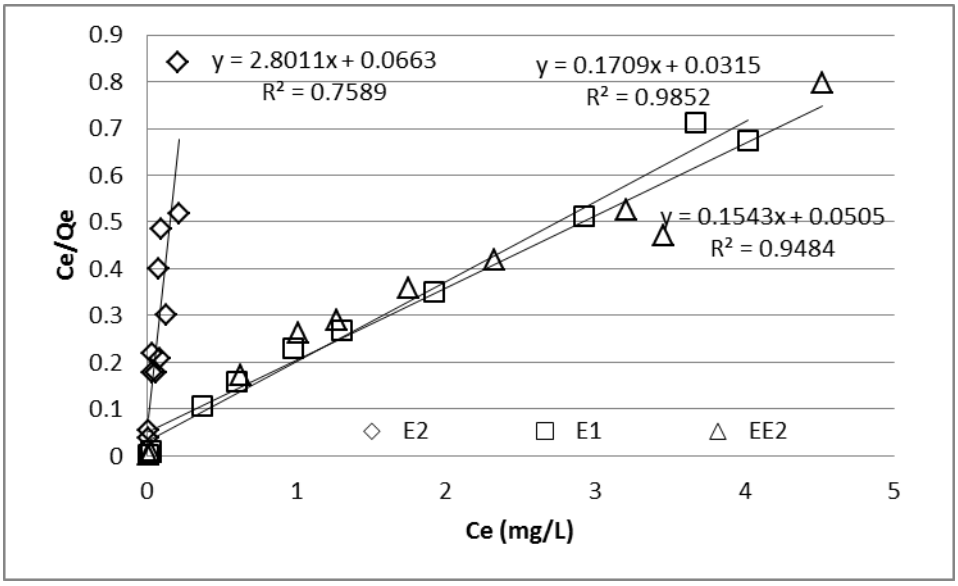



**Figure 6.7:** Adsorption of the EDCs to PAC at high initial concentration.
a. Adsorption curves of the EDCs to PAC Norit 20B at high initial concentration in water and sludge supernatant matrix, b. Langmuir model fit data of hormone compounds adsorbed on PAC in sludge supernatant matrix.

compounds in both background are very similar. There is no significant different for the adsorption of all three compounds to PAC in both background. Thus, it can be hypothesized that the effect of colloids in the sludge supernatant are insignificant compare to the adsorption of the hormones to PAC. Figure 6.7.b showed that the adsorption data of the EDCs to the PAC in the sludge supernatant background can be fitted by Langmuir model. The Freundlich model was also tried to fit the data. However, the Langmuir model fits the data much better than the Freundlich.

$$M_{PAC} = q.\ S_{PAC} = \frac{Q^o m C_{aq}}{1 + m C_{aq}} .S_{PAC} \qquad (6.4)$$

$Q^o$ : is maximum adsorption capacity of the PAC

m: Langmuir constant

The values of $Q^o$ and m for the adsorption of the EDCs to the PAC were determined and shown in Table 6.3.

**Table 6.3:** Langmuir constants in adsorption of EDCs to PAC at high initial concentration.

| | m | $Q^o$ |
|---|---|---|
| E2 | 49.83333 | 0.334448 |
| E1 | 5.428571 | 5.847953 |
| EE2 | 0.327285 | 19.80198 |

## 6.5. Summary and Conclusion

PAC showed higher potential on adsorption of E2, E1 and EE2 than the anaerobic sludge. At both low and high initial concentration, all three compounds show the similar adsorption to PAC. However, the adsorption order to anaerobic sludge of the three compounds follows an order: E2>EE2>E1 which correlated to the order of $K_{ow}$ of three compounds. The Freundlich model fitted most adsorption data, except the adsorption to PAC at high initial concentration.

# CHAPTER 7: ANAEROBIC BIODEGRADATION OF 17β-ESTRADIOL IN LANDFILL LEACHATE

## 7.1. Abstract

A series of serum bottles under anaerobic condition were set up to test the anaerobic biodegradation of E2 in the OR leachate. The biodegradation of E2 achieved under methanogenic condition was 64 ±15 %. PAC was also tested to see if PAC can enhance or deter the biodegradability of E2. However, No significant different was found under methanogenesis without PAC and PAC addition. The addition of different alternative electron acceptors such nitrate and sulfate reduced the rate of degradation of E2. After the nitrate and sulfate depleted, the extent of degradation was comparable to that achieved under methanogenesis. The average contributions of biodegradation to the total removal of E2 under nitrate and sulfate adding condition were 59±23% and 66±19%, respectively. The phenomenon that portion of E2 readily transforms to E1 was observed under all tested conditions, even abiotic. E1 was found in both liquid and solid phases.

## 7.2. Introduction

There are many studies on the behaviors of EDCs under aerobic and anaerobic processes. The behaviors of EDCs compounds are also various upon the characteristic of each compounds. The behaviors could be adsorption of the compounds into the solid phase, transformation to another compound or completely degraded to $H_2O$ and CO2 (aerobic system) or methane and CO2 (anaerobic system). Adsorption behavior of EDCs to solid phase is found in most of the studies and is one of the important mechanisms for EDCs removal (Birkett and Lester, 2002; Ren et al. 2007). The adsorption rate of the EDCs to sludge was found as higher for those compounds with higher $K_{ow}$ (Urase et al., 2005). Birkett and Lester (2002) stated that compounds with log $K_{ow}$ higher than 4, adsorption to the sludge is dominated, with log $K_{ow}$ less than 4, dissolved organic plays more important role and low adsorption potential if less than 2.5. The reason for that may be because higher $K_{ow}$ means lower solubility in water and higher distribution to organic liquid phase. That can result into higher trend adsorbed to the organic solid matter. Most of the EDCs have their $K_{ow}$> 2.5, therefore adsorption mechanism is always found in sludge system.

Beside the adsorption mechanism found for removal of EDCs, biodegradation and biotransformation of EDCs were also found. Under aerobic condition, many EDCs such as nonyphenol and bisphenol A were reported to be able to degrade under aerobic condition (Tanghe et al., 1998; Staples et al., 1998; Ike et al., 2006; Zhao et al., 2008). The EDCs were also found degradable under anaerobic condition (Ike et al., 2006;Kang and Kondo, 2002; Ying and Koona, 2003). Also the several reports also found that the EDCs can be degraded under denitrification, sulfate reducing and anoxic (Czajka and

Londry, 2006; Ying et. al., 2008). Czajka and Londry et al., 2006 studied the biotransformation of Estrogen compounds such E2, E1 and EE2 under four conditions methanogenic, sulfate-reducing, nitrate reducing by using lake sediment. However, according to our literature review, the study of behavior of several EDCs in anaerobic sludge system is still limited. In addition, in most studies, the EDCs were only determined in liquid phase. The behavior of the EDCs in solid phase was not sufficiently concerned. This study will focus on the behavior of EDCs in landfill leachate when treated by anaerobic sludge system. And the behavior of EDCs in both liquid phase and solid phase will be determined. The adding of external adsorption material such PAC or alternative electron acceptors such nitrate or sulfate were also tested in order to determine their effects on the biodegradation of EDCs.

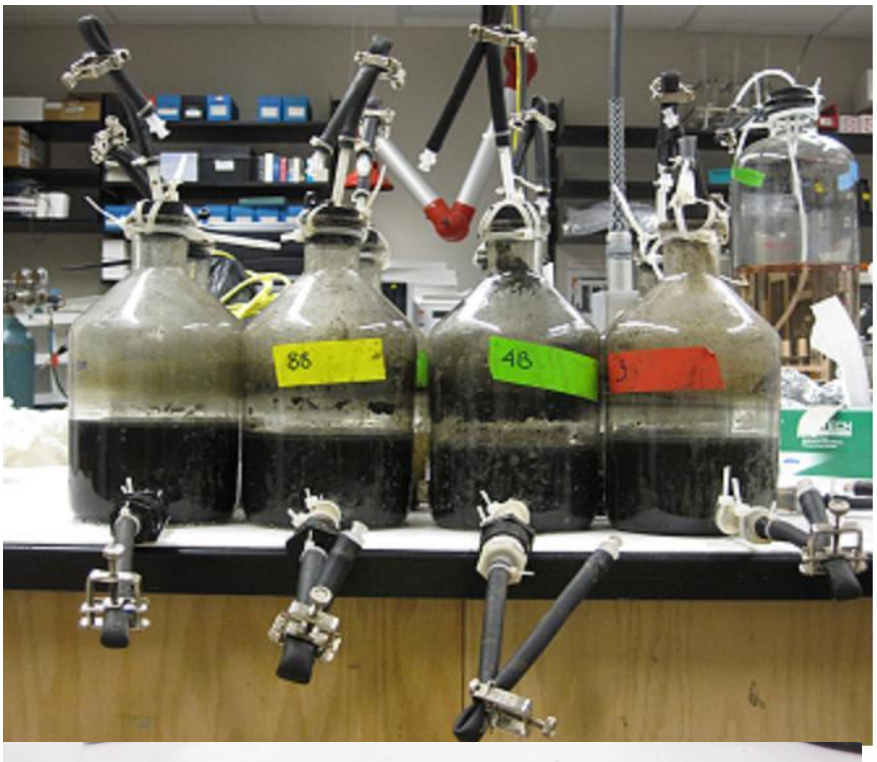
## 7.3. Materials and Methods

Descriptions of the anaerobic sludge used for the bioassays, preparation of the OR leachate, the analytical methods for water quality, and extraction of E2 and E1 from liquid and solid phases can be found in Chapter 3, Materials and Methods.

Anaerobic batch experiment procedures: a series of biotic and abiotic anaerobic batch experiment were set up in order to study the behavior of E2 in anaerobic system. 400mL of anaerobic sludge for biotic experiment or inactivated sludge for abiotic experiment and 400 mL of leachate containing 1mg/L of E2 were added into each 1L Pyrex aspirator bottles with bottom side arm as in Figure 7.1a. Sodium bicarbonate was added into each bottle at concentration of 1.2 g/L for pH buffer during the anaerobic process. PAC was added in each bottle which was used for the effect of PAC study

purpose. Sodium nitrate ($NaNO_3$) and sodium sulfate ($Na_2SO_4$) were added to the bottles, which were used for studying the effect of nitrate and sulfate reducing condition, to have the initial concentration of 20mM. Also, 1g/L of sodium azide was added to the abiotic bottles. The bottles were equipped with an extended tube and a valve at the bottom side arm for liquid and solid sampling purpose. The bottle was sealed with a modified PTFE stopper which has a plastic tube intruded in the middle of the stopper in order to exhaust the flushing gas or the biogas produced. A magnetic bar was put into each bottle in order for mixing purpose. After adding the sludge, leachate and sodium bicarbonate, the bottles was mixed for few minutes. Then a duplicate of 50 mL of the mixed liquor in each bottle was transferred to two 70mL bottles (as in Figure 7.1b) for the biogas measurement purpose. The 70mL was sealed by a PTFE lined stoppers and aluminum crimps. Then bottle 1L bottles set and 70mL bottle set were flushed with helium gas in 5 minutes to provide the anaerobic condition. The bottles were located in an incubator at 37$^{o}$C and were mixed one time per day in 5 minutes on magnetic stir equipment. During the incubation process, the biogas was measured and analyzed from 70mL set bottles and the biogas gas was exhausted for the 1L bottles. 15 mL of slurry of each sample was collected from 1 L bottles over time.

a.




b.


**Figure 7.1:** Experimental vessels for biodegradation tests. a.1L serum bottles with bottom side arm for biodegradation test, b.70 mL serum bottles for gas measurement purpose.

## 7.4. Results and Discussion

### 7.4.1. Methanogenic Condition

Biotic experiment to test the potential of biodegradation of E2 in landfill leachate under methanogenesis processes was conducted. The methanogenesis condition in biotic sample was confirmed by the methane gas product collected during the experiment. The methane gas profiles were showed in Figure 7.2. The abiotic sample was also set up along with the biotic experiment. The differences in amount of E2 adsorbed in abiotic samples and the E2 remained in biotic samples can be considered as the contribution due to biodegradation. In order to determine the total mass of E2 remained in the bottles, mass of E2 was determined in both liquid and solid phases of each sample. Adsorption to solids was the only removal mechanism expected in the abiotic sample. Therefore, 100% of E2 added could be expected to remain in the abiotic bottle after mass balance analysis. However, beside E2, E1 was also found in both biotic and abiotic samples. The conversion of E2 to E1 was reported in several studies under anaerobic condition (Czajka and Londry, 2006; Shi et al., 2010) but the conversion of E2 to E1 in abiotic sample has not been reported in any previous studies. This phenomenon can be explained by several hypotheses. First, in the biotic sample, there might be some chemical enzymes which can convert E2 to E1. The second hypothesis can be that E2 was unstable under anaerobic condition and converted easily into E1. However, these hypotheses should be tested by further experiments.

Because E2 and E1 were present in both samples (even though E2 was the only target compound added), E2 and E1 will be reported together in this study. Figure 7.3a

and 7.3b showed the mass balance of both E2 and E1 in liquid and solid phase under biotic and abiotic condition However, in the abiotic samples, the recoveries of E2 fluctuated around 50-70%. This percentage was much lower than the range of 89-98% of the recovery test which was conducted before. The lower recovery of E2 and E1 may be because some portion of E2 and E1 was adsorbed to the experimental vessel (tubing and the bottle). In addition, the imperfect mixing in the bottles may also contribute to the lower recovery. In the biotic sample, the amount of remaining E2 and E1 decreased very fast on the first day. The percentage between the amount of E2 and E1 remaining and the initial mass of E2 added in biotic sample on Day 1 was just 27% which is much lower than 68% in the abiotic sample. The mechanisms contributing to the loss of E2 are expected to be adsorption or degradation/biotransformation. Therefore the difference of E2 and E1 between the biotic and abiotic sample can be estimated to be amount lost by biodegradation. The total mass of E2 and E1 decreased over time and disappeared after 25 days in the liquid phase of the biotic bottle, and the total mass of E2 and E1 in both phases decreased by time. This showed that the biodegradation of E2 and E1 did occur after E2 converted to E1 at the very beginning. Of the total initial E2 added, biodegradation contributed around 37±13 % removal of E2 (Figure 7.3).

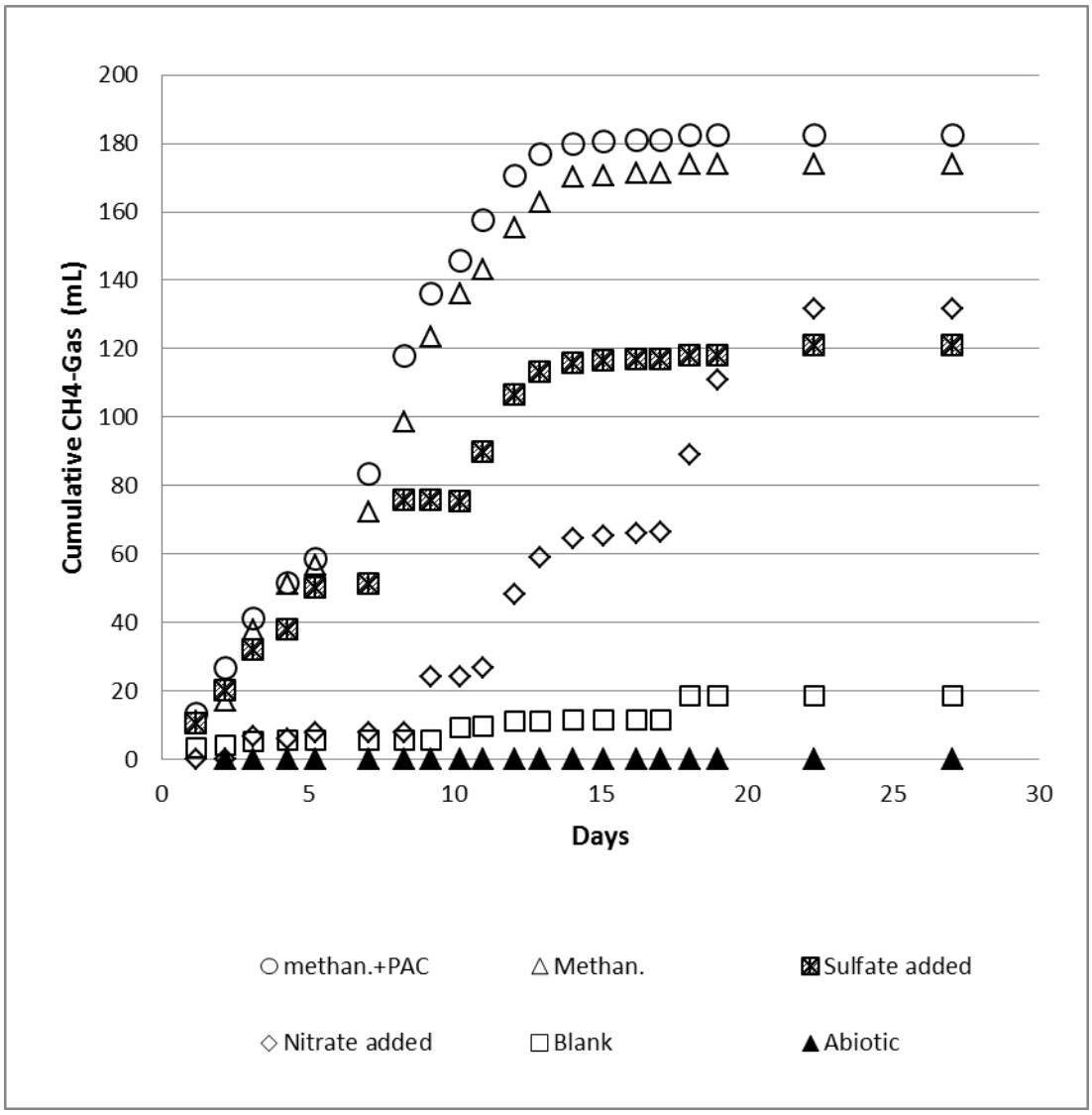
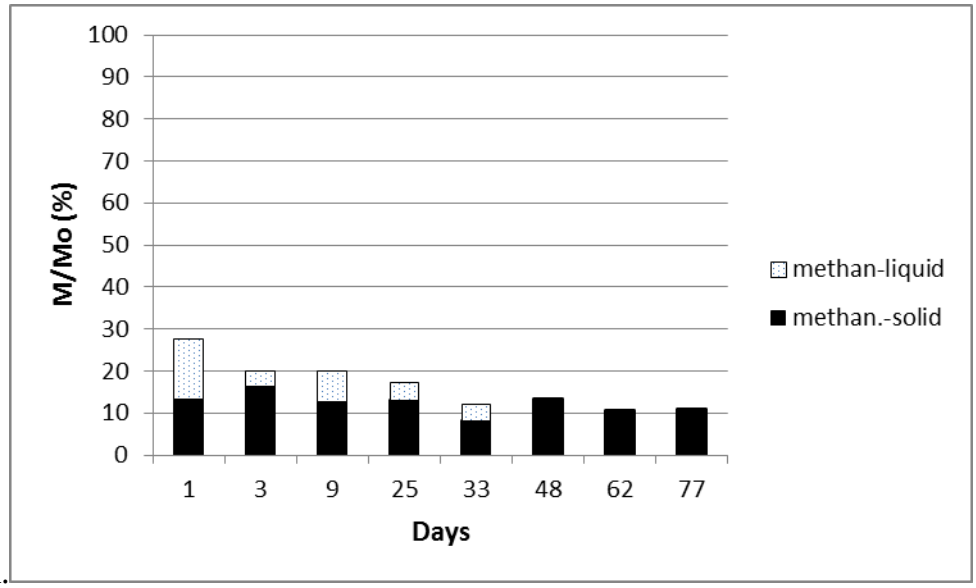



**Figure 7.2:** Methane production in the bioassay series.

### 7.4.2. Methanogenic Series with PAC Addition

The results of the methanogenic+PAC series under biotic and abiotic condition are shown in Figure 7.4. The total mass of E2 and E1 in both liquid and solid phases in the biotic methanogenic plus PAC series showed the general trend of decrease over time. Similar to the methanogenic condition, the E2 and E1 was not detected in the liquid phase after 25 days. The remaining E2 and E1 in the solid phase after 77 days was 6.9 % of the initial mass of E2 added. This value is lower than 11% remaining percentage of E2 and E1 in the methanogenic experiment. The recovery of E2 and E1 in the abiotic +PAC sample was very similar to the abiotic sample which has range of 50-70%. Interestingly, in both abiotic samples (with and without PAC), it was observed that during the first 33 days, E2 and E1 in the liquid phase decreased, but after 33 days, the total mass of E2 and E1 in liquid phase actually increased. This may be due to desorption from biomass as well as experimental vessel, but is unclear. E1 contributed to most of the liquid phase analytes after 33 days. The average biodegradation through 77 days was 31±20%. The biodegradation was calculated by taking the different amount from total mass of E1 and E2 in both liquid and solid phase of abiotic plus PAC sample and total mass in both liquid and solid phase of the biotic plus PAC sample.

a.

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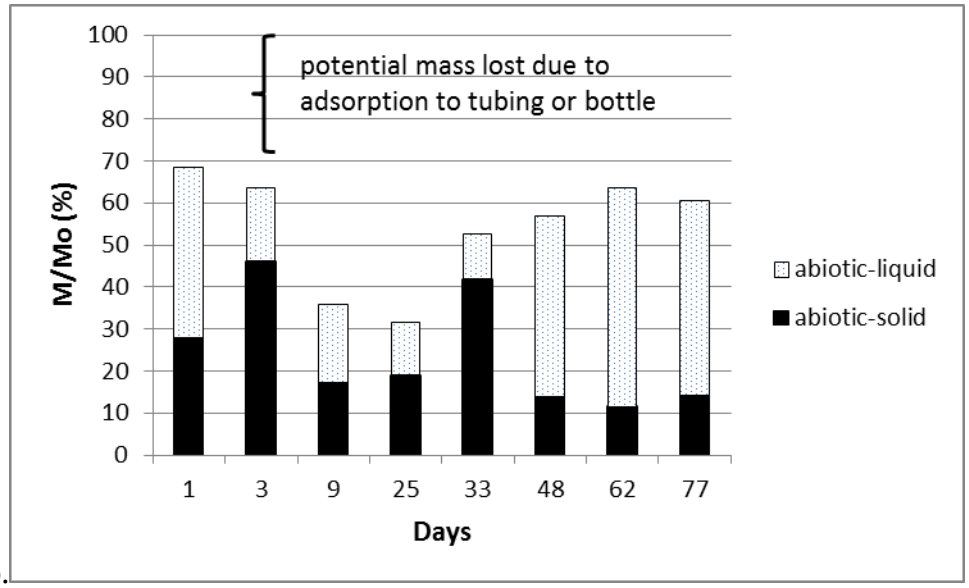
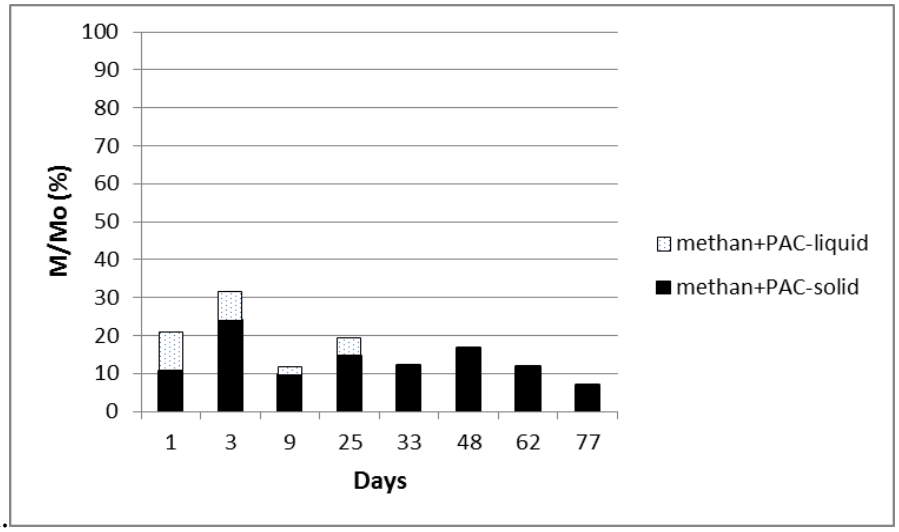



**Figure 7.3:** Remaining percentage of sum of E2+E1 under methanogenic and abiotic conditions. a.Profile of remaining mass of E2+E1 in the methanogenic system, b: Profile of remaining mass of E2+E1 in the abiotic system.

Note: M: remaining mass of E2 + remaining mass of E1 at time t
$M_o$: initial mass of E2 added into the system.

a.

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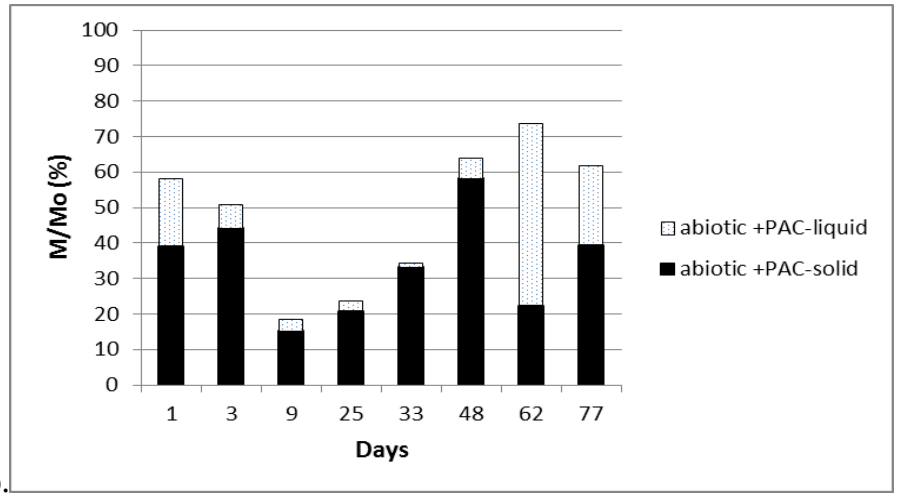


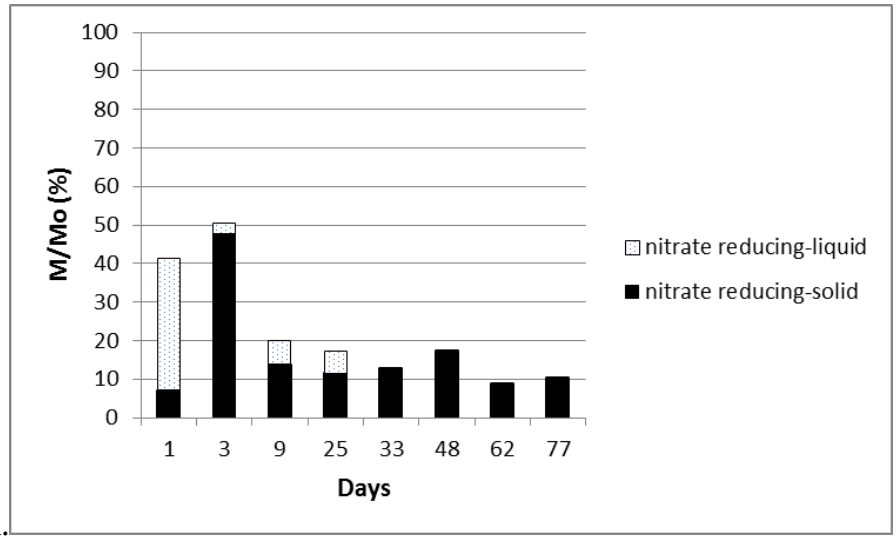

**Figure 7.4:** Study of PAC effect on removal of E2 under anaerobic condition. a. Profile of remaining mass of E2+E1 in the methanogenic +PAC system with standard errors bar, b. Profile of remaining mass of E2+E1 in the abiotic +PAC system standard errors bars.

### 7.4.3. Nitrate Reducing and Sulfate Reducing Condition

In the environment and in bioreactors, a number of anoxic or anaerobic conditions can exist, depending on the predominant electron acceptor. Thus, in addition to methanogenesis, the effect of additional alternative electron acceptor on biodegradation of E2 was also tested. External electron acceptors such nitrate and sulfate were added in order to create nitrate and sulfate reducing conditions, respectively. The experiment results for methane production and E2 removal are shown in Figures 7.4 and 7.5, respectively. As expected, methane production was either delayed or inhibited with the addition of the alternative electron acceptors (Figure 7.4). With nitrate addition, $CH_4$ gas was not produced during the first 10 days. This is because during the first 10 days, denitrification (N2 gas production) competed with methanogenesis. After 10 days, when nitrate was presumably depleted as electron acceptor, methanogenesis was observed, although not at the same level as the reference methanogenic system. Similar delay and inhibition on methanogenesis was observed in the sulfate reduction series, although not as severe. The methane profiles in Figure 7.4 help to substantiate that, indeed, anoxic/anaerobic conditions different from methanogenesis have been created with the addition of nitrate and sulfate.

Similar to methanogenic conditions, it was also observed that E2 transformed to E1 under both denitrification and sulfate reduction conditions (Figure 7.5). The total remaining of E2 and E1 in liquid and solid phase in first 3 day in both conditions was around 50%. This value was higher than the value under methanogenic condition on first 3 day (hence less removal), under methanogenic condition on first 3 day (hence less

a.

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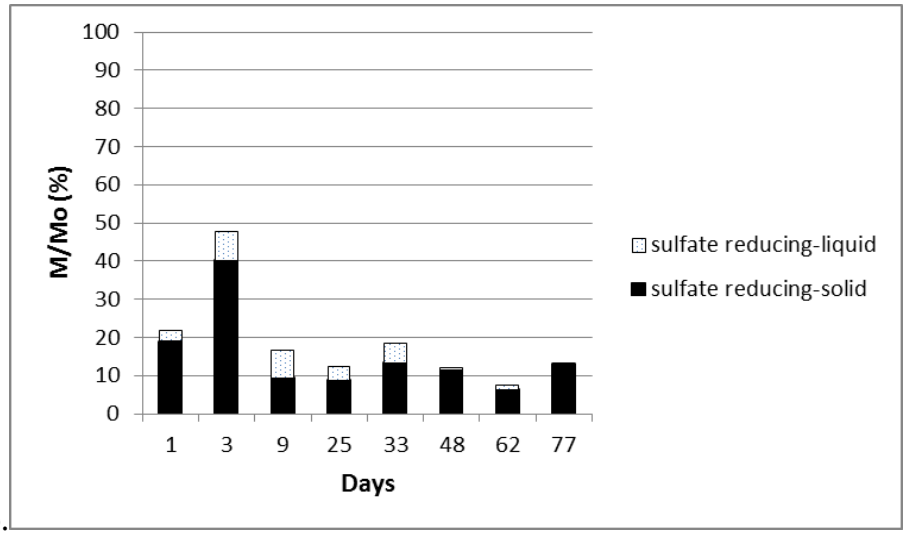



**Figure 7.5:** Profiles of E2 in the presence of additional alternative electron acceptors. a. Profile of remaining mass of E2+E1 in anaerobic condition with Nitrate added as electron acceptor with standard error bars, b. Profile of remaining mass of E2+E1 in anaerobic condition with Sulfate added as electron acceptor with standard error bars.

removal), suggesting that denitrification and sulfate reduction conditions are less conducive to E2 removal than is methanogenesis. Later, with the onset of methane production, the total mass of E2/E1 in the system decreased over time. The trend of E2 and E1 in liquid phase under the nitrate reducing condition was quite similar to the methanogenic condition. After 25 days, the estrogen disappeared from the liquid phase. However, the disappearance of E2 under sulfate reducing conditions took longer (62 days). Under both conditions, the decrease of E2 and E1 in solid phase over time was observed. The final remaining percentages of E1 and E2 under nitrate and sulfate condition were 10% and 13 % respectively. The averaged biodegradation of E2 and E1 (compared to abiotic series) under nitrate and sulfate condition are 31±15% and 35±14%, respectively.

### 7.4.4. Biodegradation Comparison Under Different Conditions

#### 7.4.4.1. Effect of Adding PAC

As discussed, the disappearance of the E2 from the serum bottles can potentially be attributed to three processes: 1. adsorption to experimental vessel (tubing and glass), 2. adsorption to sludge and 3. biotransformation/biodegradation.

$$\% \text{ Actual biodegradation contribution} = \frac{Mabiotic - Mbiotic}{Mabiotic} * 100\% \qquad (7.1)$$

In different system, the loss by adsorption to tubing and glass may be different. Therefore, in order to determine the actual contribution of biodegradation to two major

**Table 7.1:** Biodegradation of E2 under different conditions.

| Condition | Calculation | average biodegradation (%) (mean +/- SD) |
|---|---|---|
| Methanogenesis | $\Sigma[(M_{abiotic}-M_{methan})_t/M_o]/n$ | 37.5±13.7 |
| Methanogenesis +PAC | $\Sigma[(M_{abiotic+PAC}-M_{methan+PAC})_t/M_o]/n$ | 31.5±20.4 |
| Nitrate added | $\Sigma[(M_{abiotic}-M_{nitrate})_t/M_o]/n$ | 31.8±15.5 |
| Sulfate added | $\Sigma[(M_{abiotic}-M_{sulfate})_t/M_o]/n$ | 35.4±14.5 |

Note: M is the remaining mass of E2 and E1 in both liquid and solid phases at time t. Mo is the initial mass of E2 added. n is number of sampling day.

**Table 7.2:** Actual biodegradation contribution based on the availability of the estrogen in the anaerobic system.

| Condition | Calculation | Average Biodegradation (%) (mean +/- SD) |
|---|---|---|
| biotic and abiotic | $\Sigma[(M_{abiotic}-M_{methan})_t/M_{abiotic}]/n$ | 68±15 |
| biotic PAC and abiotic PAC | $\Sigma[ (M_{abiotic+PAC}-M_{methan+PAC})_t/M_{abiotic+PAC}]/n$ | 64±29 |
| nitrate reducing and abiotic | $\Sigma[ (M_{abiotic}-M_{nitrate})_t/M_{abiotic}]/n$ | 59±23 |
| sulfate reducing and abiotic | $\Sigma[ (M_{abiotic}-M_{sulfate})_t/M_{abiotic}]/n$ | 66±19 |
| Solidabiotic+PAC and abiotic | $\Sigma[(M_{abiotic+PAC}-M_{abiotic})_t/M_{abiotic+PAC}]/n$ | 22±35 |

Note: M is the remaining mass of E2 and E1 in both liquid and solid phases at time t. $M_o$ is the initial mass of E2 added. n is number of sampling day. SD is abbreviation of standard deviation. Please see Table 7.1 and 7.2 for comparison of series averages. For more information about standard deviation calculation for solid samples and liquid samples, please see Table 7.3 and Table 7.4.

**Table 7.3:** Standard error and standard deviation calculation for solid samples.

| Type of samples | Standard Deviation* | Standard Error* |
|---|---|---|
| methan.-solid | 2.2 | 0.8 |
| abiotic-solid | 12.5 | 4.4 |
| methan+PAC-solid | 13.1 | 4.6 |
| methan+PAC-solid | 4.9 | 1.7 |
| abiotic +PAC-solid | 13.3 | 4.7 |
| nitrate reducing-solid | 12.3 | 4.4 |
| sulfate reducing-solid | 10.1 | 3.6 |

**Table 7.4:** Standard error and standard deviation calculation for liquid samples.

| Type of samples | Standard Deviation*[1] | Standard Error*[2] |
|---|---|---|
| methan-liquid | 4.53 | 1.60 |
| abiotic-liquid | 15.80 | 5.58 |
| methan+PAC-liquid | 4.03 | 1.42 |
| methan+PAC-liquid | 3.74 | 1.32 |
| abiotic +PAC-liquid | 15.92 | 5.63 |
| nitrate reducing-liquid | 10.88 | 3.85 |
| sulfate reducing-liquid | 2.69 | 0.95 |

Note: *[1]: standard deviation was calculated as:

$$SD = \sqrt{\frac{(M - \overline{M})^2}{n}}$$

n: number of sampling days

M: remaining mass of E2+E1 (in liquid or solid phase) at time t

$\overline{M}$ : average of remaining mass of E1+E2 (in liquid or solid phase) for all sampling days

*[2]: Standard Error: $SE = \frac{SD}{\sqrt{n}}$

removal mechanisms which are adsorption to solid phase (i.e. sludge, colloids, PAC) and biodegradation, the biodegradation will be calculated based on the potential of the estrogen recovery in abiotic bottle as in equation 7.1.

The PAC added to the anaerobic system enhanced the adsorption of both E1 and E2 to the solid phase (Figure 7.4). For the abiotic series, the additional amount of sorption to solid phase due to PAC addition was an average value of 24 %. However, the adsorption enhancement of PAC was not consistent throughout the experiment period. From Day 3 to Day 33, E1/ E2 were distributed much more in the solid phase of abiotic sample than in the solid phase of the abiotic plus PAC sample. The presence of PAC had little effect on biological activities. It neither enhanced nor inhibited methanogenesis and E2/E1 degradation. Figure 7.6 showed that the contributions of biodegradations of E1 and E2 under conditions with and without PAC addition are quite similar. The average values of actual biodegradation contribution with and without PAC are 68±13% and 64 ±15%, respectively.

7.4.4.2. Effect of Adding External Electron Acceptors

As shown in the biogas profile (Figure 7.2), nitrate reducing (denitrification) condition occurred in the first 10 days. After that, methanogenesis occurred. Figure 7.7 showed that in the first 10 days, when the nitrate reducing condition was predominant, the biodegradation of E1 and E2 was lower than the biodegradation under methanogenesis condition. But after the first 10 days, when the nitrate was depleted and methanogenesis was enabled in the nitrate adding experiment, the biodegradation of E2 and E1 was very similar to that observed under methanogenesis condition. This result

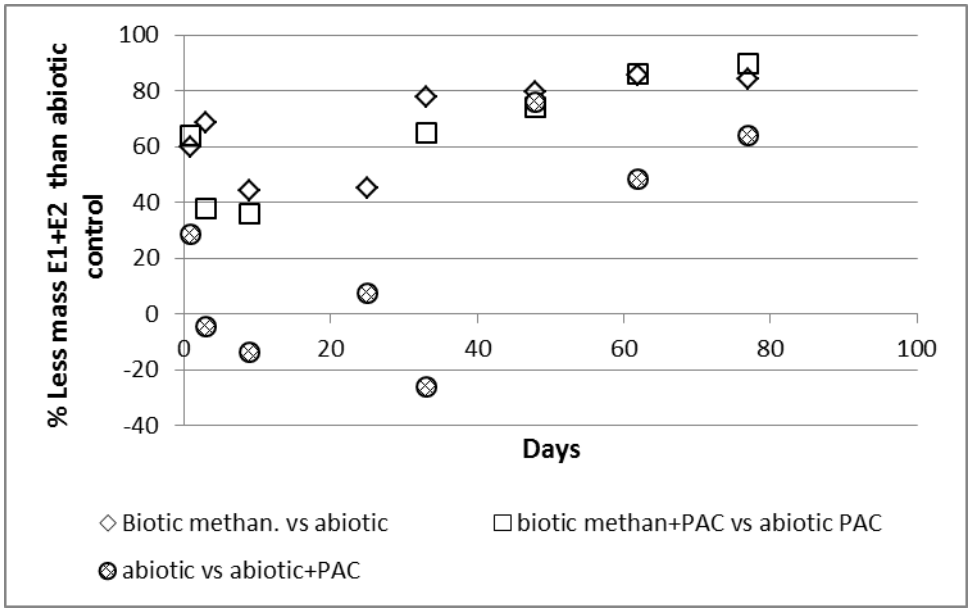



**Figure 7.6:** Effect of adding PAC in the methanogenesis system.

Note: Biotic methan. vs abiotic: % = $(M_{abiotic}-M_{biotic})*100/M_{abiotic}$

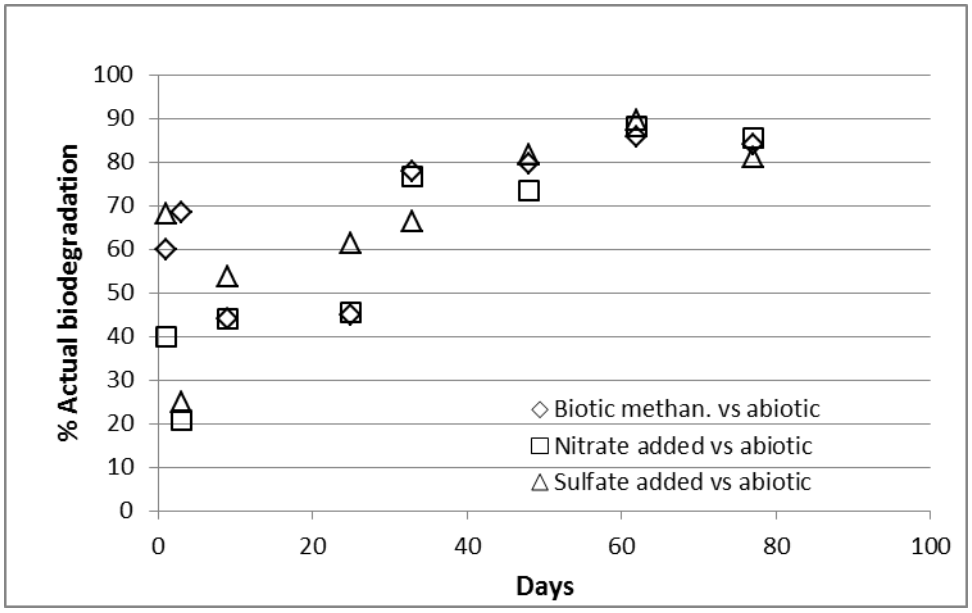



**Figure 7.7:** Effect of adding external electron acceptors in the methanogenesis system.

showed that the methanogenesis condition has higher biodegradation potential than nitrate reducing. The average values of the actual biodegradation contribution in the first 10 days under nitrate reducing condition and whole period in the nitrate amended bottle were respectively 35±10% and 59±23% which are lower than the values under methanogenesis condition, 57±10% and 68±15%.

The methane profile in Figure 7.2 showed that the methane was continuously generated in the sulfate amended bottle though the amount of methane gas was lower than under methanogenesis without adding electron acceptor condition. This suggests that both sulfate reduction and methanogenesis coexisted in the experiment, which is likely given that sulfate reduction is an anaerobic process. Therefore, unlike the denitrification experiment (which is anoxic), the biodegradation in the sulfate adding experiment was just slightly lower than the methanogenesis. The average of actual biodegradation was 66 ±19%. This might be because the concentration of the sulfate added was not enough for the sulfate reducing to dominate the process.

## 7.5. Summary and Conclusion

E2 transformed to E1 under all experiment conditions tested. Biodegradation contributed to the removal of E2 from landfill leachate in anaerobic sludge system. The recovery of E2 in the abiotic samples was around 50-70% because of potential loss to tubing. Two major mechanisms for the removal of E2 in the anaerobic system are adsorption and biodegradation. The biodegradation contribution to the overall two major removal mechanisms under methanogenesis condition was 68±15%. The adding of PAC neither inhibited nor enhanced the biodegradation of E2 in the anaerobic system.

Additional of alternative electron acceptors such as nitrate and sulfate can compete with methanogenesis and reduce the biodegradation rate of E2 compared to the biodegradation under methanogenesis condition. This finding suggests that true anaerobic conditions (methanogenic) are needed for E2 biotransformation.

# CHAPTER 8: ANAEROBIC MEMBRANE BIOREACTOR FOR 17β-ESTRADIOL REMOVAL

## 8.1. Abstract

Around 600 µg/L of E2 was spiked in to the leachate fed to the An-MBR when the An-MBR was operated at steady state at 90-92% of COD removal with HRT =2.3 days. The E2 removal efficiency of the An-MBR can be achieved consistently above 90% (maximum of 98% or 1.7 log removal). With the addition of PAC to the reactor, E2 was not detected (detection limit of 4 ng/L) in the effluent of both MF and UF membrane, corresponding to a removal efficiency of 99.993% (5.17 log).

## 8.2. Introduction

The presence of contaminants in landfill leachate is because municipal landfills are essentially the resting ground of society's wastes. Landfills contain a variety of high potential sources of micropollutants such as pesticides, pharmaceuticals, personal care products, and cosmetics. When these wastes are buried in the landfill, hydrolytic processes occur and release the micropollutants into leachate. Many studies have demonstrated the presence of EDCs and PPCPs in landfills. For example, Bisphenol-A concentration in landfills have been reported between 0.3-17,200 µg/L (median: 269 µg/L) by Yamamoto et al. (2001) and between 0.15-2980 µg/L by Yasuhara et al. (1997).

The concentration of nonylphenol in leachate was detected at 2.8μg/L by Behnisch et al. (2001). Landfill leachate can reach to the environment through groundwater or wastewater if the leachate is not collected. Once in the groundwater, controlling effects of micro pollutants to the environment will be very difficult. Therefore, it is necessary that landfill leachate has to be collected and treated in a proper way.

The 21$^{st}$ century also remarked with the rapidly increasing application of MBRs in wastewater treatment. MBRs show more advantages compared to conventional method and being considered as state of the art in wastewater treatment. Recently, the potential of removal of micropollutants in the wastewater by MBR technology has attracted a lot of attention from scientists. Many studies were implemented to answer the question if MBRs can enhance the removal of micropollutants. The higher removal efficiencies of MBR to micropollutants compared to CAS were reported in many reports (Clara et al., 2004; Lyko et al., 2005; Radjenovic et al., 2007). Most of the research for removal of the micropollutants by MBR was conducted with wastewater treatment. In this chapter, the potential of the MBRs for removal of micropollutants will be tested in landfill leachate which has higher organic strength than wastewater to see if the AnMBR can both reduce the COD in the leachate and also remove the micropollutants (e.g. E2).

## 8.3. Materials and Methods

Description of the reactor configuration, anaerobic sludge used for the reactor and analytical methods can be found in Chapter 3, Materials and Methods. The reactor was fed OR leachate as previously described in Chapter 5. In this chapter, the same HRT and

organic loading were continued but E2 was added to the feed (Stages IIc and IId). PAC was added in the final stage (IId). The stages are indicated in Table 8.1.

E2 was added into the landfill leachate at the concentration around 600µg/L when the AnMBR was at the steady state and operated for 288 days. E2 was not detected in the raw landfill leachate from Polk County. The reactor of the AnMBR system was operated at HRT =2.3 days, temperature of 35$^{o}$C. The MLVSS of the sludge blanket in the reactor was from 10,000 mg/L-13,000mg/L.

## 8.4. Results and Discussion

### 8.4.1. COD Removal and Performance of Membranes in the AnMBR System

The performance of the AnMBR for removal of COD is shown in Figure 8.1a. The COD removal efficiency was consistently above 89%. The COD concentration in the effluent of MF and UF was around 1800-2200 mg/L. The COD in UF effluent was only slightly lower than that in MF effluent which is around 40 mg/L.

The flux of MF and UF was operated around 25-40 LMH/bar and 5-15 LMH/bar, respectively. During some period of operation, the fluxes were less due to membrane fouling. However, after cleaning-in-place (by applying a zero TMP cross flow through the membrane for 3-5 hours) the membrane fluxes were restored to the original values. This phenomenon can be observed on day 390.

### 8.4.2. AnMBR Performance for E2 Removal

The initial concentration of E2 added into the landfill leachate averaged around 600µg/L. E2 was still detected in UF and MF effluents at an average value of 35 µg/L

**Table 8.1:** Stages of operation of the AnMBR reactor.

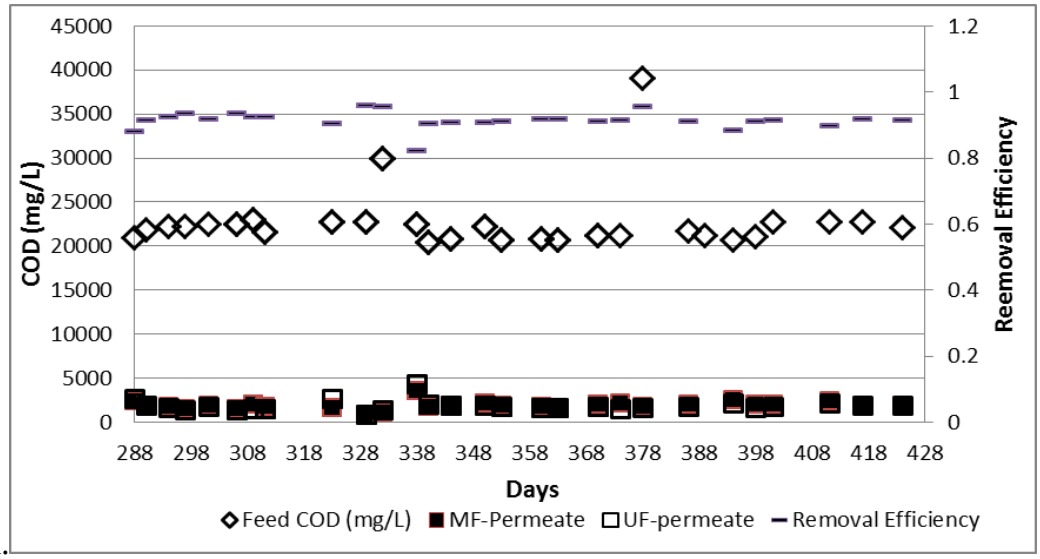
| Chapter | Day | Stages | Feed | OLR ($kg/m^3day$) | HRT (d) | membrane | E2 | PAC | Note |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 36-53 | Ia | Dextrose | 3.9 | 4.16 | MTR UF | -- | -- | |
| 5 | 55-80 | Ib | Synthetic Leachate | 3.9 -5 | 4.16 | MTR UF | -- | -- | |
| 5 | 0-207 | IIa | OR Leachate | 3.6-4.8 | 4.4 | Orelis UF/MF | -- | -- | After Reactor modification |
| 5 | 207-288 | IIb | OR Leachate | 7.3-9.3 | 2.3 | Orelis UF/MF | -- | -- | HRT change |
| 8 | 289-385 | IIc | OR Leachate | 8.9-13 | 2.3 | Orelis UF/MF | 600 mg/L | -- | E2 added to leachate |
| 8 | 386-428 | IId | OR Leachate | 9-9.7 | 2.3 | Orelis UF/MF | 600 mg/L | 1g/L | PAC added to reactor |

Note: Phase I was operated in 80 days and the initial day of phase I was marked at 0
Phase II was operated in 425 days and started after phase I. The initial day of phase II was marked at 0

and 33µg/L, respectively. The highest removals of E2 by UF-AnMBR and MF-AnMBR were 98.3%and 97.6%, respectively (about 1.7 log removal). Similar to COD removal performance, the concentration of E2 in UF membrane was just slightly lower than in MF membrane. The removal of E2 can be explained by the adsorption of E2 onto the sludge, which was showed in the previous chapter. E2 adsorption to the sludge (or possibly colloidal organic material) was retained in the reactor the MF and UF membranes. The reason that E2 was still detected in the effluent of UF and MF membranes is because an equilibrium concentration of E2 exists between the liquid and solid (sorbent) phases. The exact concentration in the liquid phase depends on the strength of the sorbent. Insufficient contact time may be another reason. However, this is less likely to be the case because the HRT of the system (2.3 days) is longer than the time required for the E2/biomass system to mostly reach equilibrium (1 day), as shown in the previous chapter (Figure 6.3). Therefore, limitation in adsorption capacity of sludge and colloids in the anaerobic reactor is likely the main contribute to the presence of E2 in the effluent.

If the limitation in adsorption capacity of sludge is considered as the sole reason for the presence of the E2 in the effluent, it can be expected that the concentration of E2 will sharply increase after the sludge in the reactor is saturated. However, figure 8.2.a. and 8.2.b. showed that the concentration of E2 in both UF and MF effluent did not increase or significantly change over time. Thus, there should be another mechanism existing along with the adsorption mechanism. That mechanism can be biodegradation, biotransformation or both. This hypothesis was supported by the results from chapter 7. E2 was found to transform to E1 and then both E2 and E1 disappeared from the liquid phase. However, E1 was not detected in both UF and MF effluent. It might be

a.

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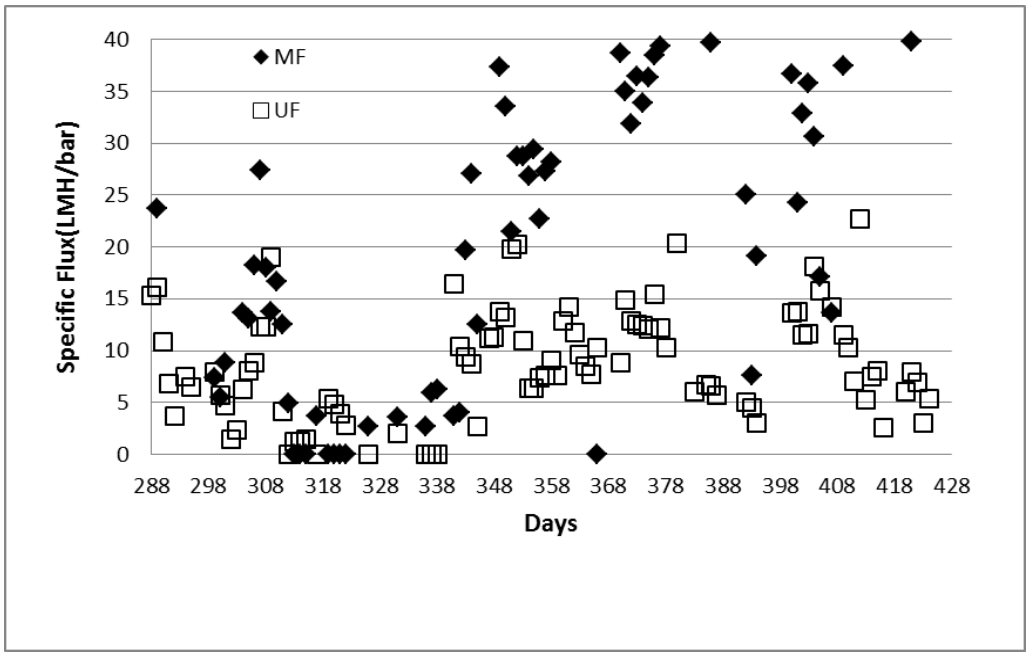


**Figure 8.1:** AnMBR performance during Stages IIc and IId. a.COD removal performance of the AnMBR, b. Membrane specific fluxes of the AnMBR.

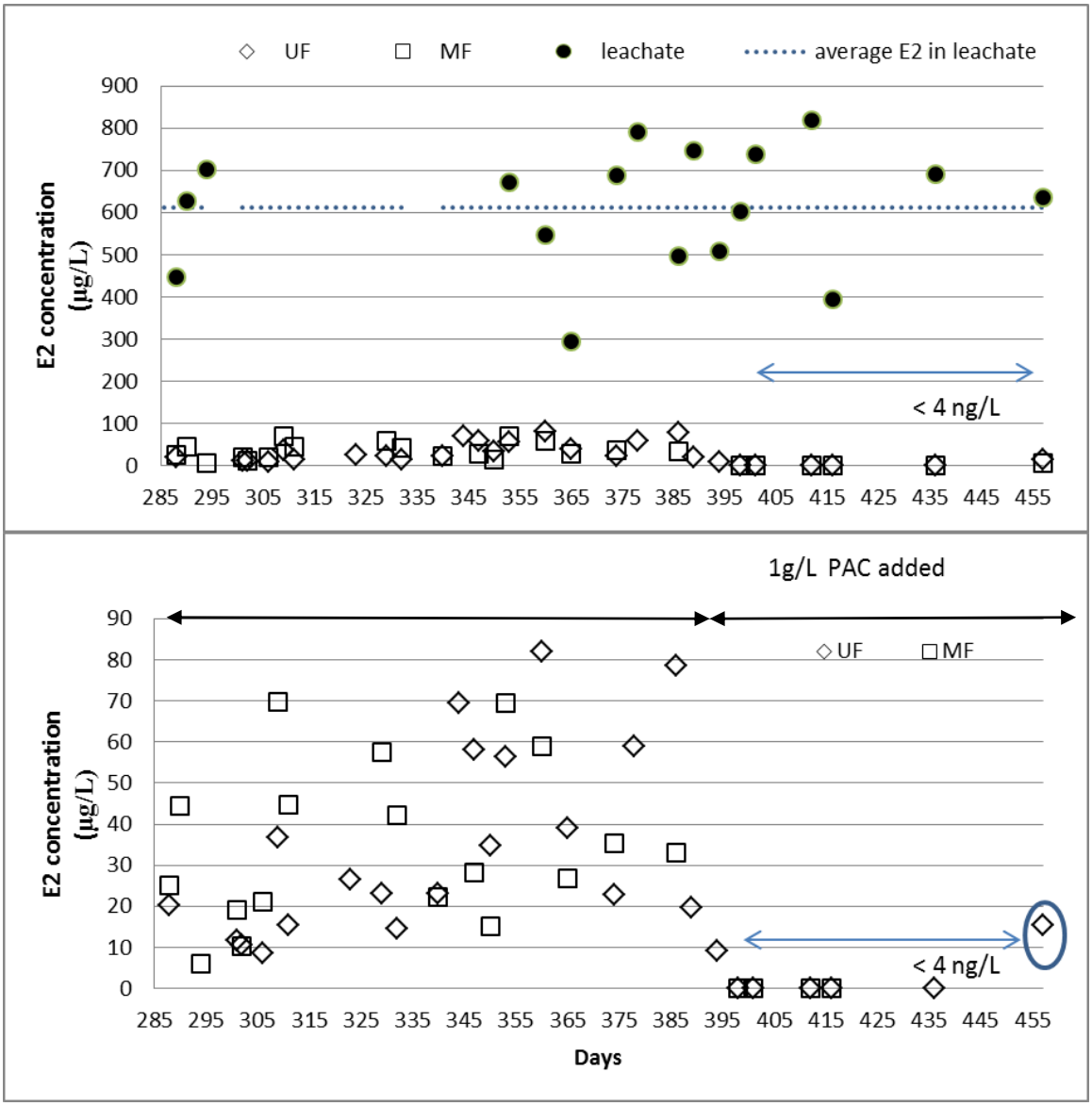


**Figure 8.2:** Influent and effluent E2 profile in the AnMBR system (the second figure is an expanded plot on the Y-axis to better show effluent concentration data).

hypothesized that E1 was just an intermediate product and further biodegradation beyond E1 occurred.

### 8.4.3. Potential for Removal of E2 in the AnMBR with PAC Addition

Although significantly reduced (up to 98%), E2 was still detected in both MF and UF effluents. There are two potential strategy options which might help to improve the removal of E2 in the AnMBR. Option 1 is to increase HRT (to allow more contact time). In chapter 7, biodegradation was found to contribute an important role in removal of E2. The intention of Option 1 is to provide the anaerobic microorganisms more contact time with E2 to be able to degrade E2 completely from the liquid phase. However, this option may not be realistic for a full-scale system. To keep the same loading rate of leachate and to be able to increase the HRT, the size of the anaerobic reactor has to be increased. This would bring issues in cost and available space. Option 2 is to attempt a decoupling of CRT and HRT, so as to keep the chemical (E2) in the system longer than the liquid. In order to accomplish this, a strong adsorbent would need to be added to the system. As shown in Chapter 6, Norit 20B PAC has a high potential of retaining E2. Although Norit 20B PAC cannot enhance the biodegradation of E2 but it does not inhibit either as found in Chapter 7. Therefore, Norit 20B PAC was selected for addition into the anaerobic reactor in order to enhance the removal of E2. Norit 20B PAC was added on the 386$^{th}$ day of the AnMBR operation. After the PAC added, E2 in both MF and UF effluent decreased very quickly from 40-60 µg/L to less than 10µg/L in the first 8 days. Then after 10 days, E2 disappeared entirely (to below detection limit of 4 ng/L) from both effluents, corresponding to a removal efficiency of 99.993% (or 5.2 log). This result

demonstrates that PAC has the ability to enhance the removal of E2 in AnMBR system. Likely, the addition of PAC also benefited the anaerobic process by aiding the anaerobic microorganism to form granules, which help maintain interspecies hydrogen transfer between methanogens and fermenters, a necessary syntrophic relationship in healthy anaerobic digestion.

## 8.5. Summary and Conclusion

The AnMBR has tremendous potential from removing micropollutants such as E2 from landfill leachate. The highest removal efficiency that the AnMBR can reach was around 98% for both UF and MF membrane (1.7 log). For a hybrid AnMBR, in which PAC (Norit 20B) was added, the concentration of E2 decreased to below detection limit (4 ng/L) in both UF and MF effluents (corresponding to 99.993% or 5.2 log removal). Enhanced retention of E2 by PAC increases the likelihood of E2 biodegradation in the AnMBR, as was shown to be possible in Chapter 7.

# CHAPTER 9: CONCLUSION AND RECOMMENDATIONS

## 9.1. Summary of Results and Significant Findings

The AnMBR was designed, fabricated and tested for 80 days during this study. The reactor required about 70 days of acclimation and startup to reach the stable condition in term of reduction of COD in landfill leachate. The reactor operated with greater efficiency for COD removal (average of 92%) when operated at a HRT of 2.3 days (corresponding to an organic loading rate of 3.9-4.8 kg COD/$m^3$day) compared to HRT of 4.4 days (OLR of 7.9-9.3 kg/$m^3$day). The COD removed was readily converted to methane gas at a ratio of 0.40L $CH_4$/g COD removed (at 37°C), demonstrating one of the important advantages of anaerobic process which is the ability to produce energy from waste. At a COD removal efficiency of 92%, the influent COD of 20,000 mg/L was reduced to an effluent level of 1500-2000mg/L. Because this amount is still much higher than permitted levels for surface water discharge, the AnMBR should be considered an efficient pretreatment method for either discharge to a municipal WWTP (where it undergoes further treatment along with domestic wastewater) or further onsite treatment (e.g., a second aerobic MBR or NF/RO process), possibly for reuse. This research also showed that fouling of the membranes in the AnMBR can be a constraint (for the specific lab-scale membrane modules utilized, feed side channel blocking was more problematic than actual fouling). However, for lab-scale AnMBR used in this study, the fouling could

be managed if cleaning was applied on a weekly cycle. For a full-scale AnMBR, where a number of membrane configurations are available from different vendors, vendor-specific fouling management and cleaning protocols would be considered.

The series of batch experiments to test the adsorption potential of the EDCs (E2, E1, and EE2) showed that all three compounds were able to adsorb to both sludge and PAC. PAC shower higher potential on adsorption of E2, E1 and EE2 than the anaerobic sludge (10 times higher). The sorption affinity to anaerobic sludge for the three compounds followed the order E2>EE2>E1, which correlated to the order of $K_{ow}$ of three compounds. These results can be used to predict the behavior of other EDCs compounds which have similar characteristic as E2, E1 and EE2 such as E3, BPA etc. The EDCs would be expected to equally sorb to Norit 20B PAC and follow similar $K_{ow}$ order for adsorption on anaerobic sludge.

From the batch bioassays, the finding that biotransformation of E2 occurred (beyond E1) under anaerobic conditions in leachate was a major finding of this research. The biodegradation contributed around 68±13% overall of total removal mechanisms of E2 (exclude the loss of E2 to tubing or bottles). The batch bioassay results showed strong evidence for the role of biodegradation on the removal of E2 in the AnMBR treatment system. It confirmed that the AnMBR has the potential to biologically remove the EDCs in landfill leachate beyond mere sorption. In batch studies, it was found that the E2 was mostly undetected from the liquid phase after 25 days of incubation under methanogenic conditions. For the AnMBR which operated with a HRT of 2.3 day, E2 was reduced by about 98% but still remained in the MF and UF effluents at average concentrations of 35 and 33μg/L, respectively. While it is not feasible for a biological treatment system to

operate at a HRT of 25 days (if so, either the reactor volume would be enormous or the flow rate would be unacceptably low), there are strategies to increase the chemical incubation time in the system by decoupling the CRT from the HRT. To this end, PAC was added to increase the retention of E2 within the AnMBR, with the expectation of enhanced removal. The result was that E2 was reduced from 600 µg/L to non-detect levels (less than 4 ng/L) in the effluent, or more than 99.9993% (5.2 log) removal. From earlier bioassays, it was shown that the addition of PAC neither enhanced nor inhibited E2 degradation in a batch system. Hence, there is reason to conclude that the addition of PAC to the AnMBR immediately affected a 5.2 log removal of E2, and the resulting enhanced retention of E2 in the system would facilitate further biotransformation or biodegradation of E2. The observed biodegradation of E2 in batch bioassays suggest that E2 is most likely not simply accumulating in the AnMBR system but is being removed. Any recalcitrant or accumulated portion of E2 would eventually be removed from the bioreactor through sludge wasting (a regular maintenance protocol) and can be concentrated and ultimately destroyed (for example in a hazardous waste incinerator).

The batch bioassay experiments involving additional electron acceptors (sulfate and nitrate) showed that the additional electron acceptors delayed methanogenesis and reduced the biodegradation rates of E2 (compared to methanogenic series without additional electron acceptors). While further investigation may be needed to delineate the exact nature of interference on E2 removal, this finding suggests that wastewater or leachate which has high concentration of nitrate and sulfate may experience less anaerobic removal of E2. Therefore, for these wastewaters, it may be necessary to employ another strategy (e.g., pretreatment, or a denitrification or sulfate reduction stage)

to reduce these competing electron acceptors from the AnMBR influent in order to facilitate higher removal of EDCs.

One of the significant findings in this research was that the hybrid AnMBR has a very high removal efficiency for E2 from the landfill leachate (99.993%), corresponding to an increase in removal efficiency from 1.7 log to 5.2 log. The success of decoupling CRT and HRT by adding PAC can be very important for developing efficient strategies to significantly remove micropollutants from landfill leachate. While studies have been reported on the removal of E2 from domestic wastewater, according to our literature review, this is the first application of AnMBR for removal of EDCs from landfill leachate. Results of this study confirmed the ability of high efficient reduction of organic strength from leachate by AnMBR. More important, the AnMBR showed the near complete (5.2 log) removal of E2 from the leachate with the assistance from PAC. Therefore, this study has demonstrated the potential of applying AnMBR for the *simultaneous* reduction of high strength organic matter (COD) and also emerging contaminants of concern which are present at trace concentrations (micropollutants) from high strength wastewater such as landfill leachate.

The intellectual merit and broader impacts of this dissertation are as follows:

Intellectual Merit:

- Knowledge about behavior and removal mechanisms (sorption and biodegradation) of E2 and other estrogenic compounds in anaerobic systems.
- Proof-of-concept on a new approach to treat leachate with the potential of removing organics, nutrients, and a variety of xenobiotic compounds.

- A reference for improvement of AnMBR to remove E2 and other estrogenic compounds in landfill leachate.

Broader Impacts:

- Findings can be applied to other types of wastewater systems (e.g. municipal and industrial).
- Providing information which can educate the general public on the effects of micropollutants on human. The research can help encourage the public to avoid disposing medicine or pharmaceutical products into house hold trash.

## 9.2. Recommendations for Future Research

A number of questions have been generated from this study, which merit further future research.

- Due to limitations in permeate flux from the membrane modules utilized, the AnMBR was only tested under two HRTs which are 2.3 days and 4.4 days. It may be interesting to push the AnMBR to lower HRTs (hence higher volumetric loading) in order to determine its limits for reduction COD as well as E2. It can be expected that lower HRT might overwhelm the sorptive capacity of the system and result in less removal of E2.
- Adsorption study in this research included three compounds E2, E1, EE2. The isotherm adsorption curves of these three compounds were not exactly as expected. The finding might be due to sorptive competition of three compounds to the adsorption sites of sludge as well as PAC. Additional studies on multi-component sorption can lead to better understanding on this issue.

- Organic material in the leachate, as well as soluble microbial products (produced by anaerobic microbes) may potentially both interfere with (through sorption competition) or enhance (by increasing the organic content of the PAC) the sorptive capacity of the PAC for the target micropollutants.
- The interaction between attached microbial growth (biofilm) and the PAC is mostly unknown. It is assumed that microbes would be able to access sorbed micropollutants (as shown by bioassay results which showed that PAC did not inhibit E2 removal). Similar conclusions have been reached in GAC biofiltration systems used for removing trace organic compounds from drinking or wastewater. However, it is unknown how the microbes would interact with leachate organic matter or soluble microbial products which have been sorbed to PACs, or how long-term accumulation of these macro-organic matters on PAC would affect the bioavailability of sorbed micropollutants.
- In this study, E2 was the main EDCs selected for study. However, in different actual landfill systems, there will likely be a variety of EDCs such as BPA, Estriol, nonylphenol, halogenated organics, etc. Hence, there may be competition from all the EDCs on adsorption as well as biodegradation. Therefore, the behavior of each compound might change compared to the behavior in single compound system. Further investigation on this and the previous points would yield better understanding on designing a robust hybrid AnMBR system which could target a suite of micropollutants with a range of properties.

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# APPENDICES

# Appendix A: Pretreatment of Landfill Leachate by Filtration[1]

## A.1. Abstract

Landfilling has become the most common and socially accepted way to dispose municipal wastes. Generation of leachate due to rainwater percolation through the wastes has become a major environmental concern. Landfill leachate contains high concentration of organic and inorganic contaminants which can harm the environment and species health. This research was undertaken to study landfill leachate treatment by anaerobic processes and investigate some filtration methods as pretreatment in order to enhance the biodegradation of leachate in anaerobic processes. Different sizes of filters range from Coffee filter size to Microfiltration membrane (MF) size (0.1µm) and Ultra filtration (UF) membrane size (0.01µm) were tested. The MF and UF experiment were conducted by a cross flow membrane system with dual membrane units. Parameters such as pressure, flow rate were collected by an acquisition box (HOBO) which was connected to a computer. Then the pretreated (filtered) leachate was transferred to series of serum bottles to test the biodegradability and COD, $NH_4^+$, TS, TSS, TOC, TN removal efficiency of anaerobic processes to each type of pretreated leachate. The ultrafiltration was effective at removing suspended solids with a removal rate of about 80% and decreasing the turbidity of the leachate from 26.6 NTU to 2.5 NTU. It is suspected that other contaminates did not have very high removal rates because they were present in a

[1] Summer work with two REU students Gerlindie Wolf and Hildamarie Cáceres in summer 2008 and 2009

dissolved form. Further reduction of the dissolved contaminates will take place in the bioreactor using biological methods.

## A.2. Introduction

Landfill leachate is a type of wastewater that is usually very toxic and has a complex chemical composition. All landfills generate leachate, some more than others, depending on the geographic location and the surrounding weather. A general problem associated with leachate is that each landfill generates a different type depending on what is in the landfill and the weather in the surrounding area (Ziyang et al., 2007; Pavelka et al., 1993). Because of this problem it has been difficult to come up with a method of treatment for landfill leachate. It is even recommended that different treatment methods be used for leachate samples of the same landfill if they are in different periods of degradation (Ziyang et al., 2009).

Many people recognize the proper treatment and disposal of landfill leachate as the most significant problem associated with solid waste landfill operations (Pavelka et al., 1993; Pi et al. 2009; Bohdziewicz et al., 2008). Solid waste disposal through landfills is one of the most common methods for disposal used globally today. This is due to the economic advantage of its use (Renou et al., 2008). Currently more than 80% of waste entering landfills does not undergo pretreatment of any kind (Ziyang et al., 2009). As a result of this there is a lot of leachate that is generated and must be disposed of. Currently, many landfills that generate a large amount of leachate send the leachate to a wastewater treatment plant or pay large amounts of money to have it shipped to a facility

that is able to treat it. Since the leachate is so toxic, and has such a complex chemical composition, most wastewater treatment plants are unable to fully treat it correctly. Not all of the chemicals, pharmaceuticals and other water pollutants are fully removed, causing problems in surroundings areas and wildlife. Needless to say, leachate becomes a burden on the area where is it shipped. The ideal treatment method of landfill leachate would be one that could be carried out on site. Treating leachate effectively onsite would save the landfill company a lot of money because they would not have to pay someone to ship it to a treatment facility, also they could ensure the quality of the effluent product themselves. There is also the possibility to recycle the treated leachate through the landfill.

Treating leachate onsite would allow a landfill facility to become more sustainable, an ongoing revolution in our world today. To be sustainable one must integrate the economic, social and environmental spheres of life in order to meet the needs of the present without compromising the ability of future generations to meet their own needs (UN). By developing an onsite treatment method a landfill could meet the criteria of this definition in the following ways. By treating leachate on its own a landfill facility could ensure the quality of its effluent leachate therefore making sure that the surrounding environment is not harmed.

On site leachate treatment would save the landfill money and therefore become economically more efficient and spend money of other areas of the landfill. Finally, onsite treatment would be socially beneficial and sustainable because residents in that

area might not have to pay as much in taxes for leachate treatment since the landfill is spending less.

Generally leachate is composed of many constituents that pose a threat to human and environmental health. Average landfill leachate has a chemical oxygen demand (COD) that is much higher than drinking water, which indicates severe organic contamination. The COD concentration of typical landfill leachate is about 10,000-20,000 mg/L. It has been proven that COD in leachate can be reduced to about 500-800 mg/L with biological treatment (Ziyang et al., 2007). The amount of COD can depend on the age of the landfill, older landfills usually have a lower COD value because the organic compounds present degrade over time (Pavelka et al., 1993; Pi et al., 2009). Other parameters of concern are high amounts of total suspended solids, total solids, total phosphorous, total nitrogen and presence of heavy metals. Leachate normally has a very high concentration of suspended solids leading to a high turbidity and discoloration of water. On average landfill leachate contains anywhere from about 800-2000mg/L of suspended solids. Heavy metals including Zinc, Lead, Nickel, Iron, Magnesium, Arsenic, and many others, can pose a threat to increase the toxicity of the leachate. Several treatment methods are available, however some are better understood and more widely practiced than others. A treatment method that has recently gained a lot of attention is the use of a membrane bioreactor (MBR). Often a membrane is used in a multi-step system, including other treatment methods such as air stripping and coagulation. Ultrafiltration is a sieving process and is used to facilitate separation, concentration and fractionation (Pi et al., 2009). The membrane traps particles that are larger than the pore size, letting only

the clean permeate pass through. Ultrafiltration is a very effective method of removing macromolecules, however it depends on the type of material that the membrane is composed of (Renou et al., 2008).

Based on the literature research and the knowledge of my mentor and PI, the treatment method of leachate chosen was a membrane filtration system, using an ultrafiltration membrane. It was found that a membrane filtration system when used alone or with another treatment method often had a removal rate of COD at least 84%. This method of preliminary treatment of leachate is relatively simple, reliable and does not require high-energy costs. It is a relatively cheap method, with the only variable factor of cost being the replacement of the membrane depending on the level of leachate contaminant. The membrane would serve as a pretreatment of the leachate, which would then undergo further treatment in an anaerobic bioreactor. This system is simple in design, yet effective and compact.

## A.3. Material and Methods

Once it was decided that an ultrafiltration membrane would be used for the pretreatment of leachate construction of the filtration system begun. It was decided that a Rayflow module, a glass case-like house for the membrane, would be used and attached with fittings and tubing's to the rest of the system. Leachate is pumped to the Rayflow module and through the membrane using a pressure force. As the pressure is increased by turning a dial located on the Rayflow module the force increases and the

flow of permeate would also increases. The Rayflow module actually housed two membranes that were set up in series, so that each time the leachate passed through the system, it passed through two membranes. The area of the membranes used was 0.02 m^2. Before the membrane was used, it was activated in ethanol. The filtered leachate, also known as the permeate, would be collected in a basin below the system. A rain gauge and flow meter were attached to the system so that the flow of permeate could be measured. Two pumps were included in the system, one to pump the leachate through the Rayflow module and membrane, and one to return the permeate back to the leachate tank for recirculation through the system for further treatment.

The data from the system was collected by a HOBO weather station and could be logged at intervals chosen by the user. Two pressure transducers were calibrated using carbon dioxide gas, and calibration curves were generated. This is extremely useful so that the voltage could be automatically measured while the system was running. After the system was successfully built it was tested several times with clean water to ensure the reliability of the system and HOBO logger. During this trial period the pressure was adjusted, increasing slightly to make sure that the system could handle higher pressures. The system was tested with water at the following pressures: 0psi, 2psi, 5psi, 10psi, and 15psi. It was expected that once the filtration of leachate began then more force would be needed since the leachate contains much more suspended solids than water.

Samples of leachate were collected directly from the Polk County Landfill site in Winter Haven Florida. The Polk county landfill has three stages, each of varying age. Stages 1 and 2 are about 10 years and stage 3 has recently opened, only about 3 years old.

The leachate from phases 1 and 2 was collected together as a mixed sample due to the nature of the landfill leachate system.

Once the samples were brought back to the lab, they were analyzed and tested for many compounds. First the leachate was tested for solids content. Leachate from phases 1 and 2 and phase 3 was tested for total suspended solids and total solids, and then the value of the total dissolved solids could be obtained. Solids are an important thing to test for because they are an indicator or water turbidity, and water quality. The leachate was then tested for different elements and compounds such as total phosphorous, total nitrogen and ammonium content. Nitrogen and phosphorous are necessary elements for human and plant growth, however too much of these elements leads to unfavorable environmental conditions in water bodies. It is important that nitrogen and phosphorous be removed from the leachate during treatment. To test for total nitrogen and total phosphorous the procedure from the Hatch book was followed, using Hatch sample vials. Each sample had to be treated with reagents and digested in order to produce a correct reading from the Hatch ultraviolet machine. Another important measurement that was taken was the chemical oxygen demand of each phase of leachate. COD measures the amount of organic compounds in water, and therefore is an indicator of the level of organic pollutants. The testing for COD also followed a Hatch procedure and testing in the Hatch ultraviolet machine. The leachate samples were also tested for ammonium content using a Vernier ammonium probe and a logger pro data acquisition device.

During the testing process for total phosphorous, total nitrogen and chemical oxygen demand if was found that the concentrations of these contaminates were too high

for accurate readings. Therefore, the leachate samples needed to be diluted accordingly to fit the appropriate range.

After all of the initial concentrations of contaminates were obtained, and the initial conditions of the leachate were know, filtration of the leachate could begin. It was decided that the combination phase 1 and 2 leachate would be used for filtration. This was because it had a composition that was more similar to the types of leachate that were tested in several of the papers found during literature research. The Phase 1 and 2 leachate was filtered through the ultrafiltration membrane system for a total of 6 and a half hours. A two-liter sample size was used to ensure that enough of the leachate would be recalculated through the system and filtered more than once. Data regarding was collected at one-minute intervals using the HOBO weather station. This data was then analyzed in using an excel spreadsheet to see the results of the filtration.

The permeate was then tested for the same parameters listed above using the same methods. In some cases dilution was not necessary because some of the contaminate was removed during the filtration process. After all of the post filtration leachate was tested the results could be analyzed to see the effectiveness of the filtration system.

## A.4. Results and Discussion

Pretreatment of landfill leachate by MF membrane: the removal rate of suspended solids was about 80%, a relatively good removal rate. However, the removal rate of total solids was about 8%. This means that many of the solids that are present in the Polk County landfill leachate are dissolved solids. These dissolved solids were not filtered out

because they were too small to be trapped in the ultrafiltration membrane and as a result showed a moderately high concentration in the post treatment testing of the leachate. One constituent that shows vast improvement was the measured turbidity of leachate. Turbidity is the measure of cloudiness of water visible to the naked eye, and is often associated with testing for water quality. Prior to filtering the turbidity was 26.6 NTU, however after filtering the turbidity was 2.5 NTU, and much clearer. This is mainly due to the effective removal of suspended solids, which allowed for less turbid water.

The removal rate for the measured chemical oxygen demand was about 31.6 %. For a pretreatment method this is a relatively good value. The COD/TSS ratio before filtering was 1.22 and after filtering was 4.16. After filtering the leachate the ratio was much higher, meaning the remaining COD present was in the dissolved form. These dissolved particles were not trapped in the membrane, and it is necessary to use a biological method such as a MBR for further reduction.

It is possible that the removal rate of total nitrogen, total phosphorous and ammonium was relatively low due to the fact that these contaminates were present in leachate is the form of a dissolved solids. Since dissolved solids were too small to be filtered out of the leachate they were still present after the ultrafiltration treatment. Phosphorous does have an insoluble form, meaning that some of it was particulate, allowing for about 50% removal.

Appendix A (Continued)

**Table A.1:** Leachate characterization results.

| Contaminant | Before Filtering | After Filtering | % Removal |
|---|---|---|---|
| TS (mg/L) | 6494 +_ 103 | 5952 +_ 126.1 | 8.3 |
| TSS (mg/L) | 1331 +_ 2825 | 267.5 +_ 25 | 79.9 |
| TDS (mg/L) | 6147.14 +_ 209 | 5685 +_ 101.5 | 7.5 |
| Total P (mg/L) | 8.1 +_ 3.3 | 4.2 | 48.1 |
| Total N (mg/L) | 640 +_ 73.7 | 520 | 18.7 |
| Ammonium (mg/L) | 454.27 +_ 58.75 | 312 | 31.3 |
| COD (mg/L) | 1630 | 1115 | 31.6 |
| pH | 7.39 | 8.35 | NA |
| Turbidity (NTU) | 26.6 | 2.5 | 90.6 |

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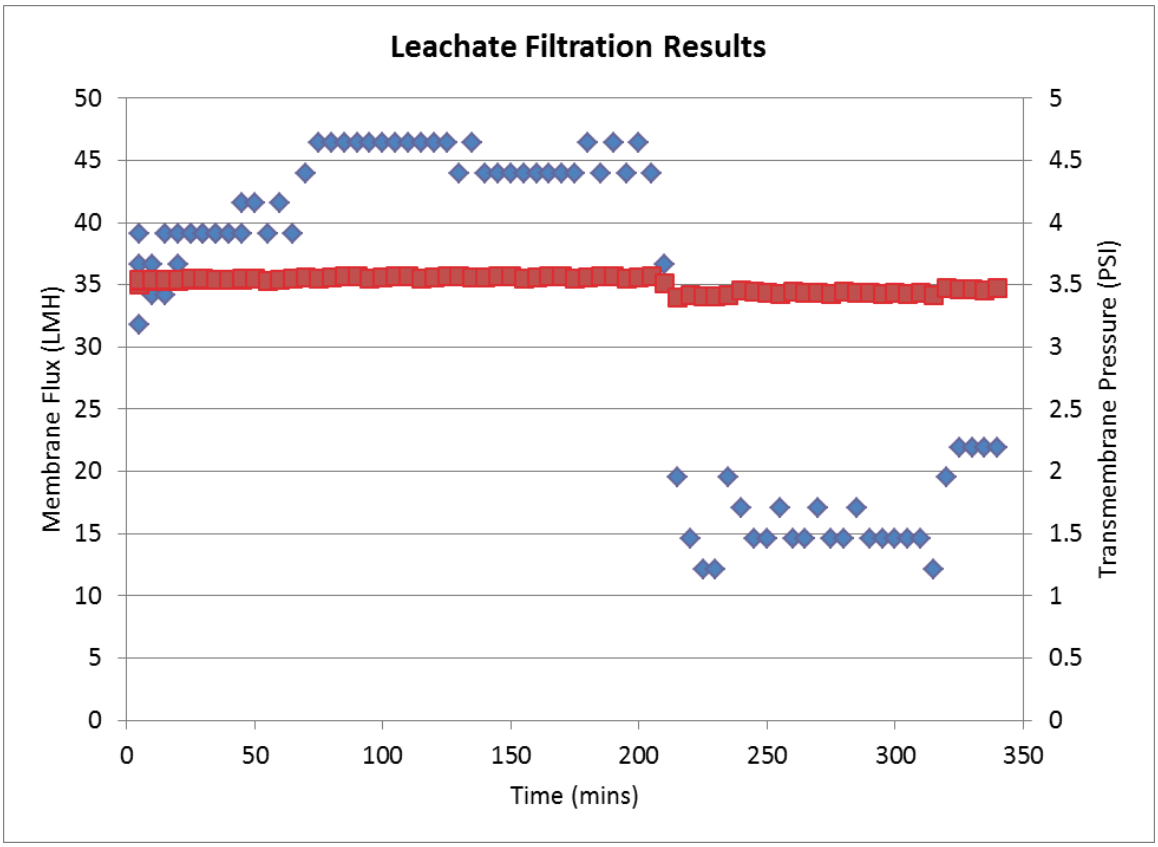


**Figure A.1:** Membrane flux vs. transmembrane pressure for leachate filtration.

Figure A.1 shows that there is a linear relationship between the trans-membrane pressure and membrane flux. Membrane flux represents the amount of permeate that is being produced. This means that as the trans-membrane pressure increases so does the membrane flux. This shows that the membrane flux is highly dependent of the TMP. Even the slightest change in TMP can cause the flux to drop considerably. Using these graphs the optimum operating point can be determined. This point is just before the maximum flux is reached, because any higher will cause a drop in flux due to fouling of the membrane.

Overall this system is effective at removing contaminates from water, however it is evident that further treatment is necessary. It is important to remember that the ultrafiltration membrane system is intended to be used as a pretreatment for leachate to then be further treated in a membrane bioreactor.

Pretreatment of landfill leachate by different filtration sizes and adding PAC for help to remove aromatic organic compounds in landfill leachate: different filtration methods were also tested to determine if there's an effective to remove recalcitrant compounds which are tough to remove from biological processes. PAC also tested to determine if PAC can enhance the filtration process. The filters selected in the study were coffee filter, 934-AH filter (fisher scientific, US) which has pore size of 1.5 µm, 0.7 µm and 0.45 µm glass fiber filter. The experiment was set up into two series. In the first series, the leachate was filtered separately through each of those filters. In the second series, PAC was added into the leachate and well mixed 1 hour before filtered through the

above filters. The PAC selected in the research was Norit 20B. Then the liquids before and after filtration were used to analyze COD, aromatic, $NH_4^+$.

Figure A.2 showed that the filtration by itself did not remove any organic compounds which can be able to adsorb the 254nm, normally the organic compounds having the aromatic ring. However, with the set of PAC added, the UV-absorbance at 254nm are lower than absorbance in the leachate, 0.7-0.8 ABS compared to 0.95 ABS in the leachate. The result implicated that the PAC can remove the aromatic compounds in the leachate. The PAC combined with the smaller size of filtration shows higher removal efficiencies. The smaller size of filtration has more chance to retain the PAC containing aromatic compounds.

## A.5. Conclusion

This laboratory experience was very beneficial to me as an engineer. I learned many new things about developing wastewater treatment technologies I gained useful experience in the lab and got practice at using testing machine such as the Hatch machine, HOBO weather device, Vernier testing probes and many other pieces of equipment.

The use of a Rayflow module system containing an ultrafiltration membrane is a good choice for the pretreatment of leachate. The ultrafiltration was effective at removing suspended solids and decreasing the turbidity of the leachate. As a pretreatment step the UF membrane was very effective. Since leachate is often so contaminated it often requires a multistep treatment process, and it is now evident why this is true. Different

steps contribute differently to the removal of contaminates. It is suspected that other contaminates did not have high removal rates because they were present in a dissolved form. Further reduction of the dissolved contaminates will take place in the bioreactor using biological methods.

Appendix A (Continued)

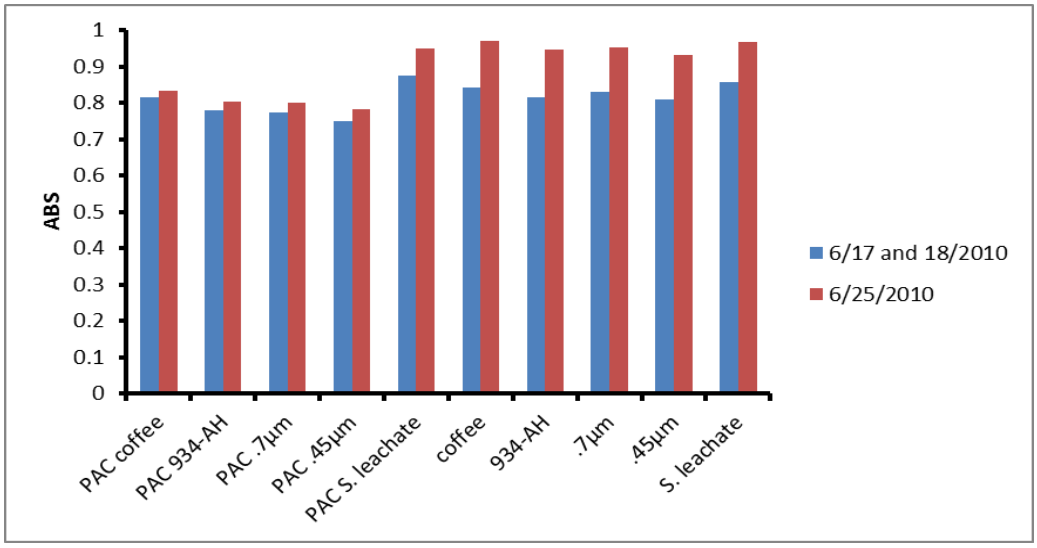


**Figure A.2:** Profile of the leachate after filtration by different filter sizes at UV 254 nm.

A.6. References

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Appendix B: Sequential MBR-UV Treatment for Landfill Leachate[2]

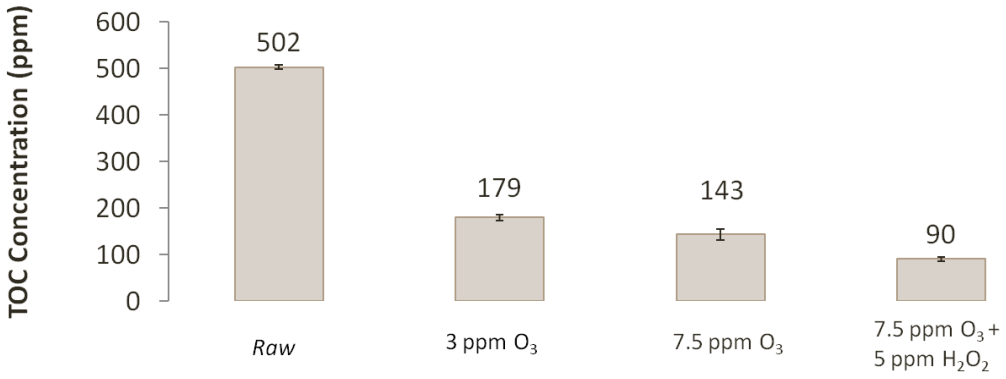


**Figure B.1:** TOC removal of post anaerobic incubation after AOP.

[2] Collaboration with Dr. Michael Watts and his Masters Student, Andres Lastra at Florida State University. The project was funded by Hinkley Center for Solid & Hazardous Wastes Management

Appendix B (Continued)

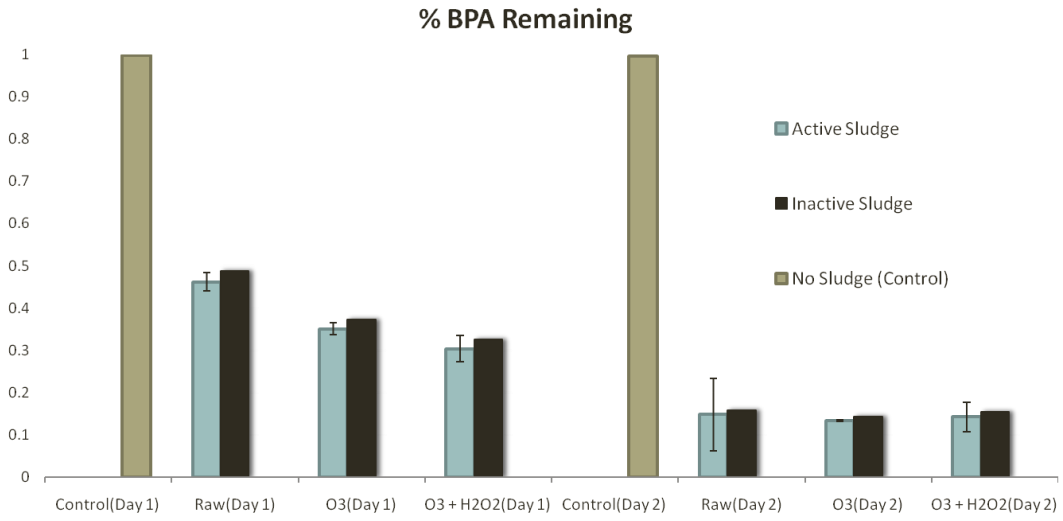


**Figure B.2:** Remaining profile of BPA after 2 days anaerobic incubation of samples which were pretreated by AOP.