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Removal of Endocrine Disrupting Compounds Using Membrane Bioreactor

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemical Engineering

by

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University of Basrah
Bachelor of Science in Chemical Engineering, 2011

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ABSTRACT

The presence of endocrine disrupting compounds (EDCs) and pharmaceutically active compounds (PhAC) such as pesticides, personal care products, antibiotics and pharmaceutical compounds, in sewage, industrial, and domestic waters has extensively become the major concern for health and environmental organizations. These compounds have the ability to interact with mammalian endocrine system and disrupting their functions. The traditional activated sludge processes are designed to degrade solids, organic carbon and nitrogen loading. Although several treatment steps in a wastewater treatment plant can contribute to partial removal of EDCs, effective removal has been a challenge due to their resistant chemical and biological degradation and extreme low concentrations. Membrane bioreactor (MBR) used in this study is novella better water reclamation technology that shows several advantages including stable operation conditions due to long solid retention time (SRT); concentrated mixed liquor suspended solids (MLSS); and low F/M ration in comparison with conventional wastewater treatment. This research will utilize these advanced membrane technologies to develop wastewater treatment processes for removal of EDCs in order to recover and reuse wastewater to augment drinking water supplies. A set of model EDCs including acetaminophen, amoxicillin, atrazine, estrone, and triclosan were selected to study the removal by membrane bioreactor. Those compounds were chosen based on their concentrations present in Oklahoma and Arkansas wastewater and to represent each group of compounds. Optimized HPLC method was used for detection of these model compounds. A Lab-scale MBR operated with real wastewater was tested under different operating conditions, such as retention time and volatile suspended solids concentrations to remove the spiked EDCs. The module MBR can reach desired chemical oxygen demand COD (< 30 ppm), Total nitrogen <10 ppm, and Nitrate nitrogen < 5 ppm in

different retention times. MBR have shown removal of amoxicillin, acetaminophen, triclosan with the efficiency can reach 100% while (50-55) % removal of atrazine can be achieved. Estrone disappearance was also more than 90%.

DEDICATION

To my beloved parents, ***Mrs. Azhar Almosowy*** and ***Mr. Ali Kamaz***.

ACKNOWLEDGEMENTS

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CHAPTER 1 INTRODUCTION

This chapter provides a general background about the extesitance, classifications and source of contamination of EDCs with their impact on both human health and aquatic systems. It also covers the role of membrane bioreactors on the removal of trace contituents and their benefits compared to the conventional activated sludge. The objectives of this research and the thesis organization are covered in this chapter.

1.1 BACKGROUND

Water consumption, potable water in particular, increases every year due to population growth, urbanization, industrial development as well as changes in agricultural and land use practices (Falconer, Chapman, Moore, & Ranmuthugala, 2006). The demand for water reuse requires the wastewater industry to comply with more restricted effluent regulations, aimed at reducing or eliminating adverse effect of wastewater discharge on human health. The presence of endocrine disrupting compounds (EDCs) in industrial and domestic sewage has become a major concern for health and environmental organizations (Yoon, Westerhoff, Snyder, & Wert, 2007). More than 70,000 chemicals are found to have endocrine-disruptive potential (Gillesby & Zacharewski, 1998). They consist of organic compounds from a variety of sources including pesticides, personal care products, antibiotics and pharmaceutical compounds (PhACs), other manmade chemicals or natural hormones as well as inorganic materials such as aluminum, arsenic and other metallic or organometallic compounds.

These compounds have the ability to interact with the mammal's endocrine system and cause disruption for that system's functions. The traditional wastewater treatment process such as activated sludge is designed to remove solids, organic loading, and pathogens. Although several treatment steps in a wastewater treatment plant can contribute to the partial removal of EDCs, complete removal has been proven to be a challenge due to high variety, extreme low concentration and unique characteristics of EDCs. Biological degradation and transformation in the activated sludge process, adsorption to the activated carbon in the filtration process, and oxidation by various disinfectants (such as UV, ozone and chlorine) may decrease the amount of EDCs, though there is still a considerable uncertainty regarding the level of EDC removal (Snyder, Westerhoff, Yoon, & Sedlak, 2003). As conventional wastewater treatment fails to sufficiently eliminate those contaminants, novel sophisticated technologies should be considered as alternatives (Spring, Bagley, Andrews, Lemanik, & Yang, 2007).

Membrane bioreactor (MBR) is considered to be one of the potential alternatives shows several advantages like: stable operation conditions due to long solid retention time (SRT); concentrated mixed liquor suspended solids (MLSS); and low food to microorganisms F/M ratio in comparison with conventional wastewater treatment (Meng, Chae, Shin, Yang, & Zhou, 2012). Previous studies have indicated that several membrane based technologies show potential as cost effective methods for clearance of EDCs from wastewaters, ranging from complete removal of certain compounds to very low removal efficiency for many others (Tadkaew, Hai, McDonald, Khan, & Nghiem, 2011). The reason behind this has not been understood clearly. Physicochemical properties of EDCs, treatment techniques and operating conditions can be the key contributors to the fate and removal of these emerging contaminants from the wastewater streams. The mechanisms controlling the removal efficacy during wastewater treatment have been widely investigated during disappearance of such micropollutants. Biological and chemical conversion and adsorption were confirmed as the main removal mechanisms in wastewater treatment (H. S. Chang, Choo, Lee, & Choi, 2009). Among the membrane-based technologies tested, microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) showed EDC removal to various degrees, but each has its own advantages and disadvantages (Alturki et al., 2010a; Cases, Alonso, Argandoña, Rodriguez, & Prats, 2011; Kimura et al., 2003; Le-Minh et al., 2010; Schäfer, Nghiem, & Waite, 2003). This study evaluated the removal of the five selected EDCs using a lab-scale MBR fed with real wastewater. The focus of this study is to identify and differentiate the removal mechanisms of the selected EDCs with operation conditions of the MBR.

1.2 OBJECTIVE OF THIS RESEARCH

The main objectives of the present investigation are focused on the following aspects;

1. Development of an appropriate model EDC containing feed streams and analytical methods to detect EDCs at concentrations of relevance to wastewater treatment facilities.

   Five model EDC compounds representative of wastewater with domestic, agricultural and industrial input were chosen. These five compounds are acetaminophen, amoxicillin, atrazine, estrone and triclosan. We have developed the analytical methods to detect these EDCs at concentrations relevant to wastewater treatment.

2. Optimization of the detection of EDC model compounds using HPLC and improvement of the analytical performance like sensitivity and detection limits.

   The HPLC analysis of EDC compounds was optimized to improve the sensitivity and detection limit. The detection limit is reduced to below 12.5 ppb for these five compounds by optimizing the HPLC elution buffer as well as the solvent media.

3. Determination of the removal of EDCs by membrane bioreactors (MBR) under various operating conditions.

   A laboratory scale MBR system mimicking industrial wastewater treatment facilities was custom designed and constructed by Lantian Inc. Investigation the EDC removal with MBR under different operating conditions for the spiked EDC model compounds in combinations has conducted.

1.3 THESIS ORGANIZATION

This thesis consists of five chapters. Chapter 1 presents a brief introduction to this study followed by chapter 2 which presents a literature review of EDCs, occurrence of EDCs in wastewater streams, their removal by membrane bioreactors compared to conventional wastewater treatment, and the removal mechanism during the treatment. Chapter 3 discusses the removal of endocrine disrupting compounds from wastewater streams by using lab-scale membrane bioreactor and stand alone filtration. Chapter 4 provides the summary of the work and conclusions drawn out of this study and recommendations for future research.

CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

Due to the increased number of trace contaminants detected in wastewater streams and the fact that endocrine disrupting compounds have adverse effects on the human endocrine system, investigation of the removal of these macro contaminants grabs considerable attention of many researcher worldwide (Snyder et al., 2007; Xue et al., 2010). They can naturally be generated in the aquatic environment due to human and mammal activities or industrially synthesized and released into a water body. In addition, they can be classified into three major categories industrial, domestic, and agricultural compounds such as pharmaceutically active compounds, personal care product and herbicides/ pesticides (Zhang & Zhou, 2008).

The concept of involving membranes in wastewater treatment was first introduced by Dorr-Oliver Inc. right after commercialized polymeric microfiltration and ultrafiltration membranes (Radjenovi, 2008). The flat sheet membranes were utilized to separated activated sludge with cross flow filtration. MBRs are designed with the separation membrane filters located outside the reactor, which is later called side stream MBR, and relied on high transmembrane pressure (TMP) to push water through the membrane. Even though the idea of combining membrane technology with conventional wastewater treatment was attractive to various application, it did not find its way to be widely deployed due to the high cost of membranes and extreme operating conditions while the value of the product is not economical. Another reason behind the lack of interest in using membrane in wastewater treatment was the potential sever fouling of the employed membrane and regular regeneration protocol.

Most of the applications before 1990 were in treating industrial wastewater to meet the required regulatory limits. However, the MBR breakthrough in 1989 when Yamamoto and coworkers demonstrated the idea of submerging the membranes in the bioreactor and suppling cross bubble to generate continuous turbulence that aims to prevent or mitigate the fouling of the membranes. They successfully showed by submerging a membrane in the aerated tank not only the transmembrane pressure would be lower by two order of magnitude but also the no fouling was observed for long term operation (Chiemchaisri & Yamamoto, 1994). With the membrane directly submerged in the aerated bioreactor, submerged MBRs are usually preferred to side stream configuration, particularly for domestic wastewater treatment purposes. Because the membrane is submerged in the aerobic tank, no additional cost is required to supply an aerator specified for the membrane. Since then, the number of MBRs treating municipal wastewater was found to increase while the MBR market is currently experiencing accelerated growth (Scott, n.d.).

This chapter covers the existence of endocrine disrupting compounds in water environments, their impact on human health and aquatic species, and their removal mechanisms and fate in water. This presents the role of membrane bioreactors in removing these constituents the potential removal mechanisms.

2.2 ENDORCIRNE DISRUTPING COMPOUNDS

Endocrine disruptors are chemicals can interfere with the endocrine system of human and wildlife animals to produce adverse developmental, reproductive, and immune effects. These macro-contaminants can be categorized into three major groups; agricultural, industrial, and domestic chemicals, figure 2.1 illustrates the subgroups of these constituents. There is a massive range of substances are thought to cause endocrine disruption, including pharmaceuticals, pesticides plasticizers, and natural hormones (Ballschmiter, 2001). Endocrine disruptors can be found in many everyday products including plastic bottles, metal food cans, detergents, flameretardants, food, toys, cosmetics, and pesticides. There has been an ongoing argument over endocrine disruptors, to ban them from markets by regulators while regulators and some scientists are calling for further investigations and studies. Many of these EDCs have not been regulated yet; however, some endocrine disruptors have been identified and banned from the market such as triclosan by Food Drug Association (FDA) for its contribution of increasing antibacterial and bacterial resistance (U.S. Food & Drug Administration, 2016). Furthermore, it is unclear whether some EDCs on the market are actually harmful to humans and wildlife at the trace concentrations. Several investigators (Kumar & Xagoraraki, 2010; Owens, 2015; Schwab et al., 2005) have evaluated the potential effects from exposure to pharmaceuticals in water by comparing exposures to therapeutic doses divided by uncertainty factors to extrapolate safe levels for populations including sensitive individuals. On the other hand, researchers have tested and demonstrated the health risks of pharmaceuticals in drinking water based on no effect levels from animal toxicity studies or human exposures (Christensen, 1998; Schulman, Sargent, Naumann, Faria, & Dolan, 2009).

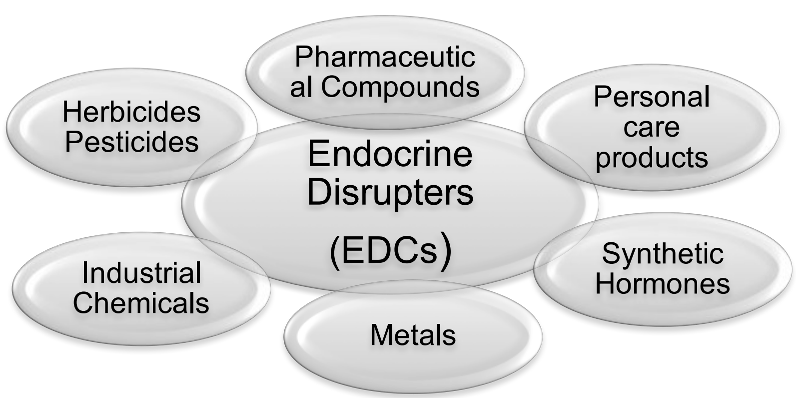

Figure 2.1 Representative diagram of EDCs in the environment

2.2.1 Naturally occurring EDCs

Natural steroid hormones such as estrone (E1), estradiol (E2) and estriol (E3) are widely found in wastewater streams mainly resulting from human urines. The most impact of these compounds is on the aquatic species in down streams waters that poses elevated dosages of estrogenic compounds. Human excretes estrogens from the body, even without taking hormonal drugs. As a result, natural hormones are believed to present at a wide range of concentrations in wastewater from households, which are conveyed to the wastewater treatment plant through the sewer system. These compounds can cause feminism at certain dosage to the exposed fish (Alan et al., 2008). The existence of estrogenic chemicals in surface waters and wastewater is of concern not only because of penetration of these compounds into groundwater, but also as to their accumulation in bottom sediments resulting in risking aquatic species life (Belfroid et al., 1999). For instance, as low concentration as 4 ng/L of ethinylestradiol can block the development of secondary sexual characteristics for fathead minnows males (Sohoni et al., 2001).

2.2.2 Domestically produced EDCs

These EDCs are extensively used as household products and thus posing potential health risks for humans exposed to some of them at certain concentration. It is essential to restrict the releases of these chemicals into water bodies. Pharmaceutically active compounds include prescription drugs, over-the-counter therapeutic drugs, and veterinary drugs and personal care products such as microbial disinfectants represent the major portion of the domestically produced EDCs (Maeng, Sharma, Lekkerkerker-Teunissen, & Amy, 2011). The health risks of these contaminants are a real concern for preserving a healthy ecosystem and aquatic life creatures and for water reuse purposes (Kashiwada, Ishikawa, Miyamoto, Ohnishi, & Magara, 2002).

Even though concentrations of pharmaceuticals in the aquatic environment are generally reported to be low, these compounds possess a high biological activity, often associated with a high stability, and their potential impact on aquatic wildlife even at trace levels (Collier, 2007). Bisphenol-A (BPA) is one of the most common endocrine disrupting chemicals found in every house, a monomer for the production of polycarbonate and epoxy resins found in some plastics that has been linked to heart disease, infertility and behavioral and developmental problems in children exposed in utero (vom Saal & Hughes, 2005).

2.2.3 Agricultural EDCs

Pesticides are one class of compounds that may produce a wide range of toxic side effects that are potentially hazardous to the environment despite their benefits. Pesticide usage has dramatically increased over the last decades to reach an average estimation of 5.53×10^{8} kg in the United State as active ingredient (AI) and 2.593×10^{9} kg used worldwide during 1995. These chemicals are used as a form of herbicides to kill competing vegetation and promote healthy growth (D. W. Kolpin, Thurman, & Linhart, 1998). The two of types of agricultural herbicides that widely used in the U.S. are the chloroacetamides and the triazines. For example, in 1997, approximately a range of $51.2\text{-}58.9\times10^{6}$ kg active ingredient of the chloroacetamide herbicides such as alachlor, metolachlor, acetochlor, and dimethenamid and $44.45\text{-}50.34\times10^{6}$ kg of the triazines such as atrazine, and simazine were applied to crops (Hladik, Hsiao, & Roberts, 2005). Their extensive past or present use contributes to their prevalence as environmental contaminants in groundwater and surface water (Hayes et al., 2002).

Koplin et al. studied the occurrence of selected pesticides and their metabolites in near surface aquifers across the Midwest in the U.S. The results revealed that five of the six most frequently detected compounds were pesticide metabolites. Hence, they concluded that metabolites could be found more frequently in groundwater than their parent compounds (Dana W. Kolpin, Michael Thurman, & Goolsby, 1996). Due to the rising concern of the possible adverse effects of pesticides on human health and environment, many countries trying to minimize the usage of these chemicals by optimizing the herbicides dosage, especially in Europe (Kudsk, 2008). Besides the use of these compounds as herbicides or pesticides, some synthetic steroid hormones are used as growth promoters in beef cattle. It has shown that the soil and run-off from large feedlots contain large amounts of bioactive steroids that may affect wildlife and the environment around these cattle feeding operations (Bartelt-Hunt et al., 2012).

2.3 SOURCE OF EDCS IN WATERS

Endocrine disrupting compounds enter the environment in various ways. For example, pesticides/herbicides are released at their usage point such as farms; industrial chemicals are released by different ways, leaking or leaching either during a product's lifetime or after ultimate disposal (Campbell et al., 2006). Natural hormones are released by different kinds organisms and enter into the environment directly or through some of the biological persistent compounds as they have passed through wastewater treatment plants (Ingerslev, Vaclavik, & Halling-Sørensen, 2003). Once a substance has passed through the environment, it can undergo different fates, such as dissolved in a surface water body, penetrate to a near aquifer reservoir, or settle down and embedded inside the sediments. Whereas wastewater treatment facilities have been utilized to serve as the major sources for biologically persistence EDCs, the actual source of them comes from upstream discharges to the treatment facilities. A few of these upstream sources include natural hormones and pharmaceutical estrogens flushed down home toilets, household cleaners containing NP, industrial processes that use cleaners and plastics (Snyder et al., 2003). Figure 2.2 demonstrates the occurrence of EDCs in different water sources.

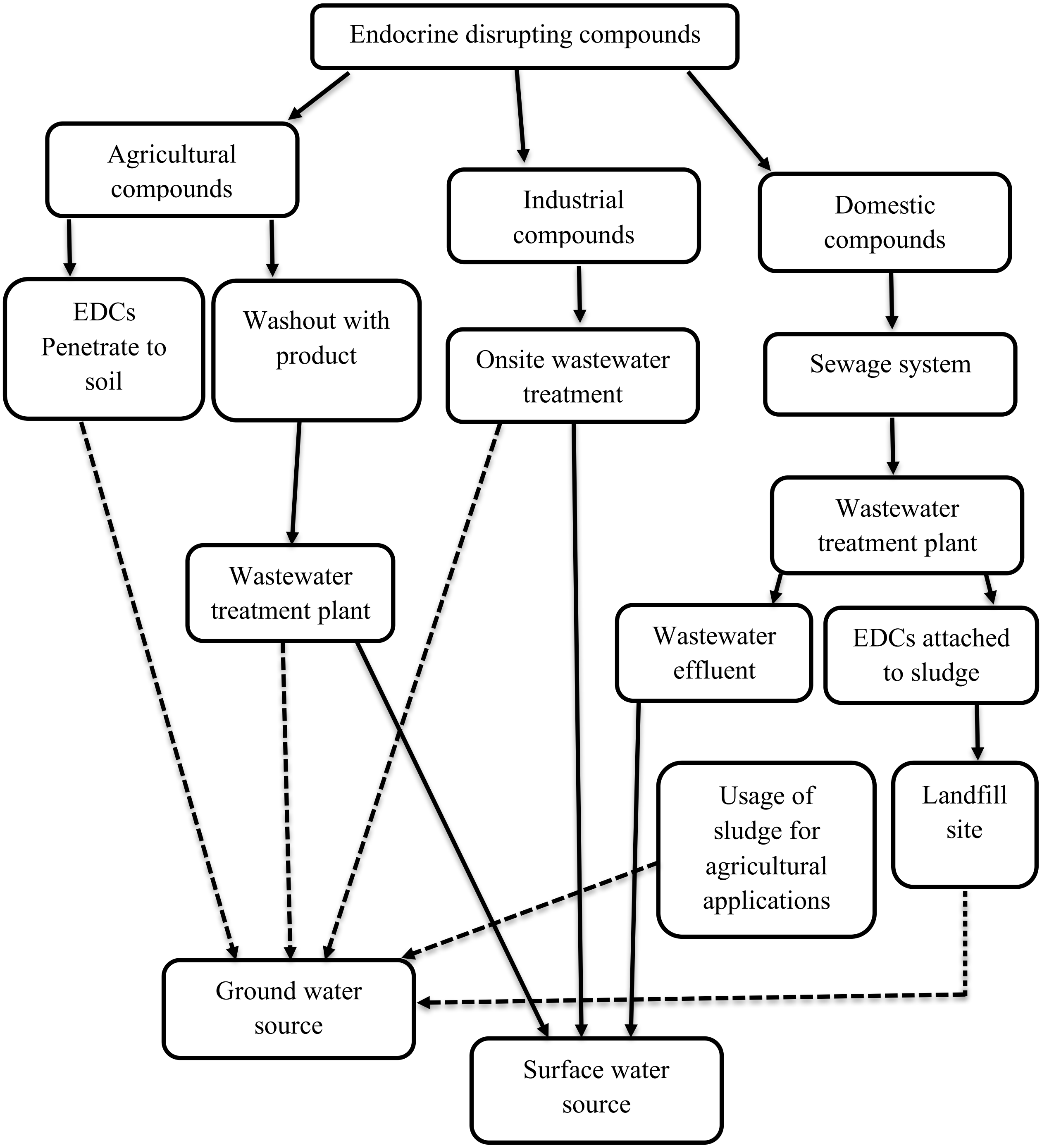

Figure 2.2 Schematic diagram for the occurrence of EDCs in water sources

2.4 MEMBRANE BIOREACTORS

Membrane bioreactor (MBR) technology, which is a combination of biological activated sludge process and membrane filtration, has became more favorable and abundant in last couple years for the treatment of many types of wastewaters. On the other hand, the conventional activated sludge process can not acclimatize with variuos wastewater composition or fluctuations of wastewater flow rate (Visvanathan, Aim, & Parameshwaran, 2000). MBR technology is also utilized in cases, where more stringent rquirements placed on the quality of effluent that can not be fulfilled with CAS. The upgrade of conventional process is continued to be more abundant even though the capital and operational costs of the MBRs exceed the costs of conventional process, (Le-Minh et al., 2010). More restricted wastewater quality requirements, growing demand for water reuse, and increasing of water price could be the reasons for moving towards MBRs (Howell, 2004). With a better understanding of endocrine disrupting contaminants occurrence and fate in wastewater, and their biodegradability, MBR might become a necessary upgrade of conventional activated sludge technologies to attain the regulatory requirements for wastewater discharge (WWTPs) (Bolong, Ismail, Salim, & Matsuura, 2009).

This can be fulfilled by the sludge retention on the membrane surface, which can promote microbial degradation, and physical retention of all molecules larger than the molecular weight cutoff of the membrane (Liu, Kanjo, & Mizutani, 2009). However, the removal of EDCs in MBR system can be affected by sludge age, concentration, and existence of anoxic and anaerobic compartments, composition of wastewater, operating temperature, pH and conductivity (Radjenovi, 2008). According to Melin (2006), MBRs can be operated at independent selection of hydraulic retention time (HRT) and sludge retention time (SRT), which means a more flexible control of operational parameters. More efficient treatment of high strength wastewater could be achieved by MBRs due to high sludge concentrations in the bioreactor. With long sludge retention time, this allows the development of specialized, slow-growing microorganisms able to remove low-biodegradable pollutants contained in wastewater, resulting in improved removal of recalcitrant compounds (Melin et al., 2006).

MBR exist in wastewater treatment in different configurations depending on the position and the driving force of the membrane. There are two main MBR configurations; submerged membranes and external filtration mode (side-stream configuration), figure 2.3 **a** and **b** shows a schematic diagram of both configurations. The MBR market is currently facing an accelerated growth due to the increase number of MBRs treating municipal wastewater worldwide. Over a period of 5 years, from 2003 to 2008, the global MBR market growth has doubled and reached a market value of $217 million in 2005 (Radjenovi, 2008).

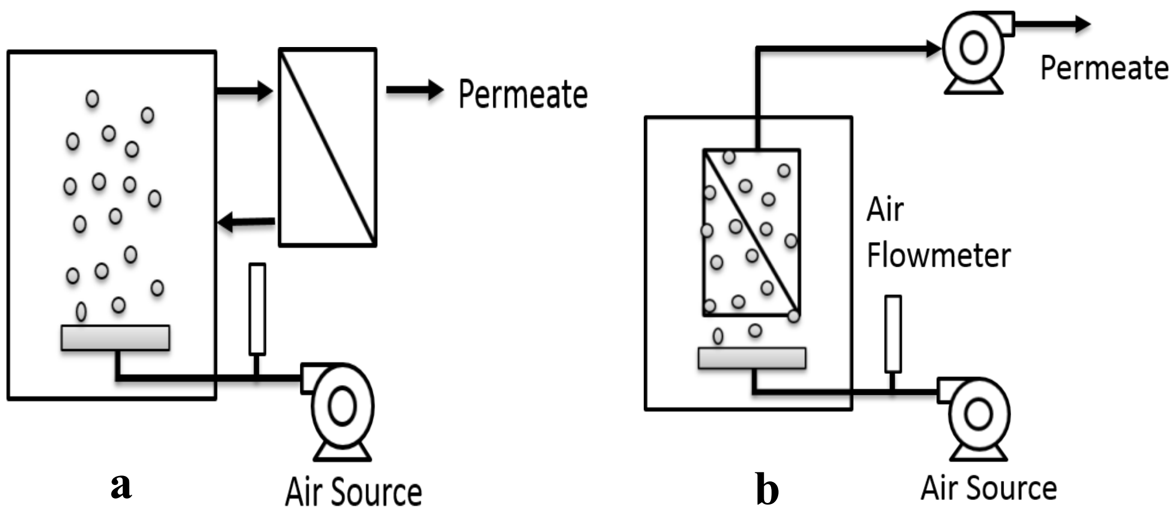

Figure 2.3 **a**) external filtration mode **b**) submerged membrane bioreactor

2.4.1 Removal and fate of EDCs in membrane bioreactor

Micro-constituents are commonly present in waters at low concentrations, ranging from a few ng/L to several µg/L. Because of their low concentration and diversity in waters, they not only complicate the associated detection and analysis procedures but also generate challenges for water and wastewater treatment processes. As a consequence, existing conventional activated sludge (CAS) wastewater treatment plants are not particularly designed to remove these micropollutants (Bolong et al., 2009). Therefore, many of these micropollutants are able to pass through wastewater treatment processes and become threats to wildlife and make difficulties for drinking water industry. Additionally, regulations and monitoring actions for micropollutants have not been well established in most of the wastewater treatment plants. The removal and fate of endocrine disrupting compounds has been investigated worldwide (Bolong et al., 2009).

MBR is able to effectively remove a wide range of EDCs including compounds that are resistant to activate sludge process and constructed wetland (Ahmed et al., 2017; Radjenović, Petrović, & Barceló, 2009). According to a study done by Arriaga et al. (2016), effluent from a full scale wastewater treatment plants using a submerged MBR system have exibited more efficient way to improve the removal of organic matter and trace contaminants such as EDCs with longterm removal effeciency and microbial stability (Arriaga et al., 2016). Table 2.1 reveals the removal efficieny for selected EDCs reported in the literature.

Table 2.1 The removal of selected endocrine disruptors by using membrane bioreactor

Endocrine disrupting compounds	**Removal efficiency (%)**	**Reference**
Acetaminophen/ analgesic	87.1	(Nguyen, Hai, Kang, Price, & Nghiem, 2013)
Atrazine/ herbicide	6.8	(Song et al., 2016)
Estrone (E1)/ hormone	96.5	(Song et al., 2016) (Nguyen et al., 2013)
Triclosan/ antibacterial agent	99.1	(Luong et al., 2014)

2.4.2 Comparison between CAS and MBR

Similar to Conventional Activated Sludge (CAS), MBR consists of an aerated tank for biological oxygen demand (BOD) and chemical oxygen demand (COD) removal, which relies on facultative heterotrophic bacteria. The aeration rate in MBRs is governed by the amount of air required to clean the membrane and prevent the formation of biological cake on the membrane surface that leads to sever reduction in the flux. As a result, the oxygen level is slightly higher in MBR than CAS (Brindle & Stephenson, 1996). Furthermore, nitrification takes place in the aerobic tank. Thus, the conversion of ammonium into nitrite and subsequent oxidization to nitrate is highly sufficient in MBRs rather than CAS due to the higher concentration of dissolved oxygen. In addition, anoxic tank is linked to the process to achieve denitrification with a particular attention is paid to the recycled mixed liquor suspended solids to control low level of dissolved oxygen and promote denitrification.

The solid retention time for MBR is generally longer that for CAS where it is ranging from 5 to 30 days with significantly lower food to microorganisms ratio which allows the complete degradation to happen. Consequently, the wastage of the sludge for MBRs is seemingly less than in the conventional activated sludge. Additionally, the settled sludge in the bottom of the aeration tanks contains 60% inorganic compounds (Witzig, Manz, Szewzyk, & Kraume, 2002).

Nonetheless, MBR offer advantages compared to conventional systems. The membrane allows the detention of particulate matter leading to an effluent free of suspended solids. MBRs achieve high SRTs associated with small reactor volume and as degradation is a function of the operated SRT, this fact represents another advantage of MBRs in comparison to conventional systems (Clara et al., 2005). Especially in regions with no suitable receiving waters or where a reuse of the treated wastewater is planned, MBRs represent an attractive solution due to the mentioned advantages. Several studies have been reported based on the removal of micropollutants by MBR treatment. In the case of macro-contaminants with an intermediate removal between 15 to 80% with activated sludge treatments, MBR treatments can generally further reduce micropollutant concentrations by 20 to 50% (Grandclément et al., 2017).

MBRs are preferably over CAS for several features;

1- Small reactor volume: since the separation of water from sludge is taking place by the membrane, the MBR can be operated at high level of total suspended solids which mostly microorganisms. Consequently, similar quantity of permeate can be gained with a small reactor.

2- Permeate quality: The MBR permeate has zero total suspended solids unlike the CAS permeate which is normally below 30 ppm. Moreover, turbidity is more stable for the MBR's permeate compared to effluent from CAS.

3- Shorter hydraulic retention time: that is due to the high concentration of microorganisms in the reactors.

4- Effluent stability in terms of wastewater quality parameters.

CHAPTER 3 REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS USING MEMBRANE BIOREACTOR

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3.1 ABSTRACT

The presence of endocrine disrupting compounds (EDCs) and pharmaceutical active compounds (PHACs) in sewage, industrial, and domestic waters has become a major health and environmental concern. The traditional activated sludge process is designed to eliminate solids, carbon and nitrogen species from wastewaters, but not trace contaminants such as EDCs. Membrane bioreactor (MBR) has become a preferred method for treating municipal and other industrial wastewaters. Here efficacy and mechanisms for the removal of selected EDC compounds from municipal wastewaters using a lab-scale MBR consisting of an anoxic and an aerobic digestion tank were investigated for the purpose of recovering and reusing wastewater effluent to augment drinking water supplies. Five EDCs/PHACs including acetaminophen, amoxicillin, atrazine, estrone, and triclosan were chosen based on their abundance in the local wastewater and to represent classes of EDC compounds. Analytical method based on performance liquid chromatography (HPLC) were established to determine these EDC compounds at concentration as low as 5 parts per billion (ppb). The EDC compounds were spiked in the municipal wastewater at 1 and 5 ppm levels and the degradation/adsorption of the EDCs were measured as a function of hydrolytic retention time (HRT) at a range of volatile suspended solid levels respectively. Except atrazine, four selected EDC compounds can be completely eliminated from the wastewater effluent after 8 ~ 20 hours of operation. Significant mechanistic insights into the degradation of EDCs were obtained.

Keywords: Wastewater Treatment; Endocrine Disrupting Compounds; Membrane Bioreactor; Microfiltration

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3.2 INTRODUCTION

Water consumption, potable water in particular, increases every year due to population growth, urbanization, industrial development as well as changes in agricultural and land use practices (Falconer, Chapman, Moore, & Ranmuthugala, 2006). The demand for water reuse requires the wastewater industry to comply with more restricted effluent regulations, aimed at reducing or eliminating adverse effect of wastewater discharge on human health. The presence of endocrine disrupting compounds (EDCs) in industrial, and domestic sewage has become a major concern for health and environmental organizations (Yoon, Westerhoff, Snyder, & Wert, 2007). More than 70,000 chemicals are found to have endocrine-disruptive potential (Gillesby & Zacharewski, 1998). They consist of organic compounds from a variety of sources including pesticides, personal care products, antibiotics and pharmaceutical compounds (PHACs), other man-made chemical compounds or natural hormones as well as inorganic materials such as aluminum, arsenic and other metallic or organometallic compounds.

The traditional wastewater treatment processes are designed to remove solids, organic compounds, and pathogens. Biological degradation in the activated sludge, adsorption to the activated carbon during filtration, and oxidation by disinfectants such as UV, ozone and chlorine may decrease the amount of EDCs present, considerable uncertainty remains regarding the level of EDC removal (Snyder, Westerhoff, Yoon, & Sedlak, 2003). Biological degradation, adsorption and oxidation of EDC compounds are complicated due to the large variety of EDC compounds present, generally sub-ppm level of the individual EDC compound, and the recalcitrant nature of many artificial compounds. Since conventional wastewater treatment processes fail to sufficiently eliminate those contaminants, emergent technologies should be considered as alternatives (Spring, Bagley, Andrews, Lemanik, & Yang, 2007). Membrane bioreactor (MBR) is one of the technologies that demonstrate several advantages: stable operation conditions due to long solid retention time (SRT); concentrated mixed liquor suspended solids (MLSS); and low food to microorganisms (F/M) ratio in comparison with conventional wastewater treatment method (Meng, Chae, Shin, Yang, & Zhou, 2012).

Previous studies have indicated that membrane-based technologies show great potential as cost effective methods for clearance of EDCs from wastewaters. For certain EDC compounds, complete removal had been observed whereas for many others, only partial degradation was detected (Tadkaew, Hai, McDonald, Khan, & Nghiem, 2011). Many factors may contribute to the efficacy of the specific method adopted for EDC removal including the physicochemical properties of compounds, processes and conditions used for the treatment. The mechanistic studies on EDC removal during wastewater treatment have been widely conducted. Biological and chemical conversion and physical adsorption were found to be the main removal mechanisms in wastewater treatment processes (H. S. Chang, Choo, Lee, & Choi, 2009).

However, for each specific EDC compound, its degradation mechanism(s) may be dominated by one or multiple pathways depending on the specific properties of the compound and the approach used.

Removal EDC via direct membrane filtration process, earlier studies show that low pressure microfiltration (MF), ultrafiltration (UF), and high pressure nanofiltration (NF) and reverse osmosis (RO) all demonstrate EDC removal capability to different degrees, but each has its own advantages and disadvantages (Alturki et al., 2010a; Cases, Alonso, Argandoña, Rodriguez, & Prats, 2011; Kimura et al., 2003; Le-Minh et al., 2010; Schäfer, Nghiem, & Waite, 2003). Membrane bioreactor (MBR) combining the activated sludge process with membrane filtration demonstrates real potential for complete EDC removal from wastewaters. MBR process involves biological degradation, physical adsorption, membrane rejection and potential chemical degradation leading to EDC's possible multi-degradation pathways. Here the five selected EDC compounds were evaluated for their degradation processes using a lab-scale MBR fed with real local municipal wastewaters. Each of the five EDC compounds is selected based on its abundance in wastewaters and its functionality. Our MBR system consists of one anoxic (AN) tank, one aerobic (AE) tank and a membrane filtration unit. Under continuous recirculating operation mode, wastewater and part of the sludge were circulating between the two tanks. Both batch mode and semi-continuous operation were tested with EDC degradation. The main focus of this study is to evaluate the efficacy of the selected EDC compounds under different conditions and elucidate the removal mechanisms.

3.3 MATERIALS

Amoxicillin trihydrate (Alfa Aesar), acetaminophen (Tokyo Chemical Industry (TCI), ≥ 98%), estrone (Acros Organics, 99+%), atrazine (Tokyo Chemical Industry (TCI), ≥ 97%), and triclosan (Alfa Aesar, 99%), liquid phenol (Sigma-Aldrich, ≥ 89%), sodium nitroprusside dihydrate (Fluka, ≥98%), sodium hydroxide (Amresco), sodium hypochlorite (VWR, 4–6%), were all used as received with no further purification. Acetonitrile (EMD Millipore, HPLC grade), methanol (EMD Millipore, HPLC grade), and de-ionized (DI) water (Milli-Q, 18.2 MΩ cm) were employed as the mobile phase for HPLC analysis.

3.4 METHODS

3.4.1 Selection of EDC model compounds

Five EDC compounds were selected based on their abundancy and functional classes in the wastewater streams of Arkansas and Oklahoma regions. These five EDCs are acetaminophen, amoxicillin, atrazine, estrone, and triclosan. Table 3.1 lists the formula, usage/class, functional group(s), molecular weight (MW) and hydrophobicity. Their molecular structures are shown in figure 3.1. These compounds represent four different classes of artificial chemicals including pharmaceutically active compounds (acetaminophen, amoxicillin), pesticides (atrazine), steroid hormones (estrone), and personal care products (triclosan). All compounds were stored at the room temperature except amoxicillin, which was kept in a refrigerator at 4 $^{\circ}$C. In accordance with their water solubility, acetaminophen and amoxicillin were dissolved in water before spiking; while atrazine, estrone, and triclosan were dissolved in ethanol/water mixture in 15 ml centrifugal tubes and sonicated for 15 minutes to create a homogenous mixed solution.

Table 3.1 The physicochemical properties of the selected compounds

Compound	Formula	Usage/Class	Functional Group	MW g/mol
Amoxicillin	$C_{16}H_{19}N_3O_5S$	Pharmaceutical/ Antibiotic	Ketone, Carboxyllic Acid, Amine, Phenol, Amide	365.40
Acetaminophen	$C_8H_9NO_2$	Pharmaceutical/ Analgesic	Benzene, Alcohol, Amide	151.16
Atrazine	$C_8H_{14}ClN_5$	Herbicide/ Pesticide	Alkyl Halide, Amide	215.69
Estrone	$C_{18}H_{22}O_2$	Hormone/ Steriod	Alcohol, Benzene	270.37
Triclosan	$C_{12}H_7Cl_3O_2$	PCP/ Antibacterial	Alkyl Halide, Alcohol, Ether, Benzene	289.54

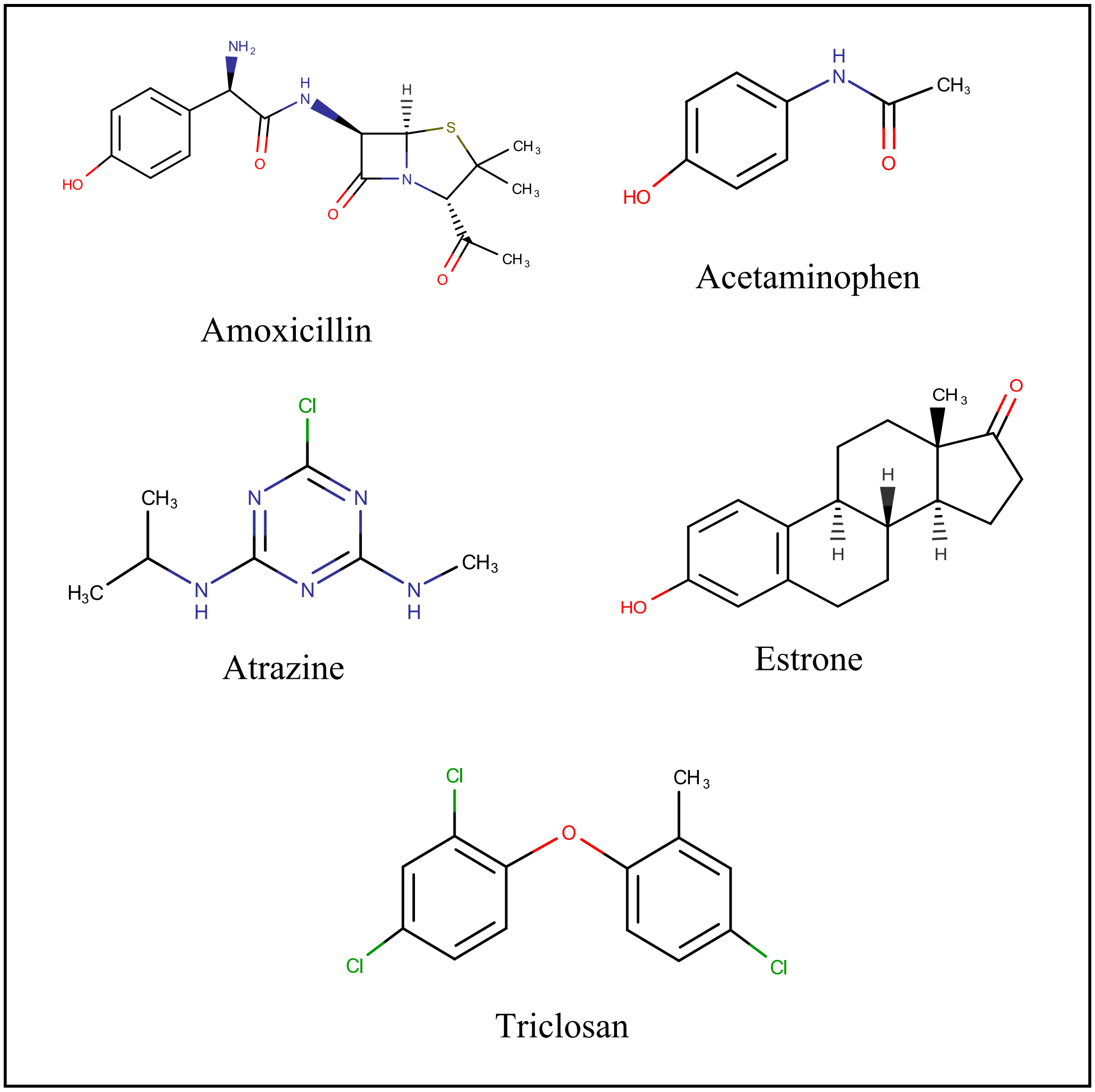


Figure 3.1 The molecular structures of the five selected EDC compounds

3.4.2 Detection of EDC compounds

High Performance Liquid Chromatography (HPLC) was used to detect EDC compounds during various stages of MBR treatment. The HPLC instrument was equipped with a Luna C18 column (5 µm, size 250*4.6 mm, from Phoenix, USA). The mobile phase was a mixture of acetonitrile and DI water at a flow rate of 0.75 mL min^{-1}, with a linear gradient varying from 10 to 100% of acetonitrile during the 35min run followed by 5 min of DI water. The column temperature was kept at 29 °C. The injection sample volume was 100 microliter (μL). A diode array detector (DAD) was used to detect the selected EDCs. An initial scan ranging from 194 to 270 nm was performed for each compound and the wavelength exhibiting highest sensitivity was chosen for the detection of each compound. Prior to HPLC run, in order to remove any sludge from water, samples were centrifuged for 5 min at 1000 rpm followed by filtration through a 0.05 μm syringe filter. The detection limit for triclosan was 12.5 ppb and 5 ppb for the other four EDCs. Table 3.2 shows the HPLC detection limit and the wavelength for the measurement. Figure S1 in supplemental document shows the standard curve of each compound and minimum detection limit. Total ammonium nitrogen (TAN), nitrate nitrogen (NO_3-O), chemical oxygen demand (COD), dissolved oxygen (DO), and total suspended solids (TSS) were monitored during the experiments following the previous protocols (APHA/AWWA/WEF 1998). For Do measurements, SympHony ™ dissolved oxygen probe is used from VWR International. High range up to 1500 ppm COD kits were purchased from (CHEMetrics) and based on the UV absorbance using spectrophotometer the COD concentration was measured in the tested samples. Nitrate nitrogen reagent powder for 5 mL sample was purchased from (HACH) with photometric analysis to quantify nitrate concentration. Total ammonium nitrogen reagent was prepared in the lab using titration method and spectrophotometer at optimized light absorbance was used to gauge its concentration.

Table 3.2 The detection limits of the studied compounds

Compound	**HPLC detection limit (ppb)**	**Wavelength Detected (nm)**
Amoxicillin	5	198
Acetaminophen	5	198
Atrazine	5	222
Estrone	5	194
Triclosan	12.5	198

3.4.3 Membrane bioreactor

The lab-scale MBR system consists of an anoxic (AN), an aerobic (AE) and a membrane filtration tank as shown in Figure 3.2. Each of the AN and AE tank is approximately 35 L and the filtration tank is about 20 L. The microfiltration membrane used in the filtration tank was provided by Lantian corporation (Lantian Inc., China) with a pore size of 0.08 μm and an effective surface area of 0.1102 m^2. While the aerobic tank is continuously aerated with a sparger, the anoxic tank has a mechanical mixer to provide homogenous mixing. In order to reduce membrane fouling, the submerged membrane tank has a separate sparger that supplies coarse bubbles. Wastewater after primary treatment was collected from the Westside wastewater treatment plant at Fayetteville, Arkansas. The wastewater at this stage contains mainly dissolved organic matter and nutrients (carbon, nitrogen and phosphorus), and is roughly free of most of the suspended solids. Activated sludge was collected from both anoxic and aerobic treatment units of the plant and immediately seeded into anoxic and aerobic tanks of the lab-scale MBR, respectively. Fresh wastewater collected was injected into the MBR as feed. At the beginning, both compartments were fed with 10 L of spiked actual fresh wastewater with continuous mixed liquor suspended solids circulation between AE and AN tanks. After certain HRT, MLSS from aerobic tank was moved to the filtration tank containing submerged membrane. Samples were collected at various stages of treatment and different retention time to investigate the removal of model EDCs. Total ammonium nitrogen (TAN), nitrate nitrogen, chemical oxygen demand (COD), dissolved oxygen (DO), and total suspended solids were monitored during the experiment. Once the stable state was reached, EDCs compounds at 1 ppm were spiked to anoxic tank. The concentrations of EDCs during the anoxic, aerobic treatment and in the effluent were monitored with HPLC.

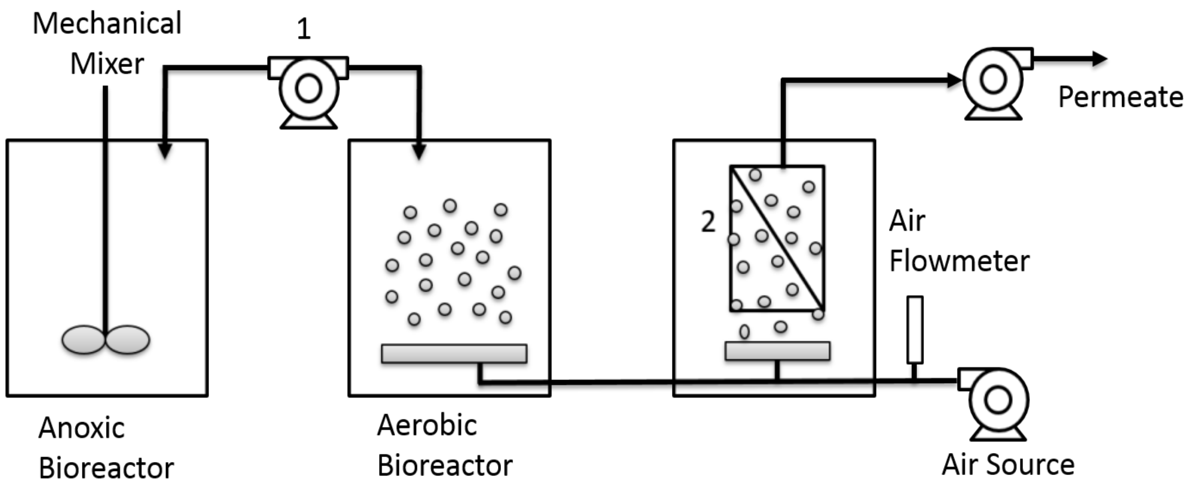


Figure 3.2 Schematic diagram of membrane bioreactor with circulation: 1- Peristaltic circulating pump; 2- Submerged membrane

3.5 RESULTS AND DISCUSSION

3.5.1 Overall performance of MBR

Wastewater quality parameters were monitored daily to evaluate the overall performance of the MBR. COD in the influent wastewater ranges from 155-754 ppm and decreased to 10-22 ppm in the MBR effluent with a mean removal efficiency of over 95%. TAN in the wastewater decreased from 31-41 ppm to 0.02-0.06 ppm in the effluent with a mean removal efficiency of 98%. Nitrate nitrogen (NO_3-N) increased up to 20 ppm in the aerobic reactor due to nitrification, and eventually decreased to 0.3 ppm in the effluent due to denitrification after recycling MLSS from aerobic to anoxic tank and vice versa. Figures 3.3-3.5 demonstrate the variations of COD, TAN and NO_3-N during one of the continuous recirculating operations before EDC spiking studies. The COD in the initial wastewater was just below 500 ppm, however, after spiking with EDC, the level increases to over 1000 ppm. This is due to the addition of EDC compounds and ethanol as a solvent for dissolving some of the otherwise unsolvable compounds. More details on the degradation of the COD, TAN and NO_3-N will be discussed in more detail later.

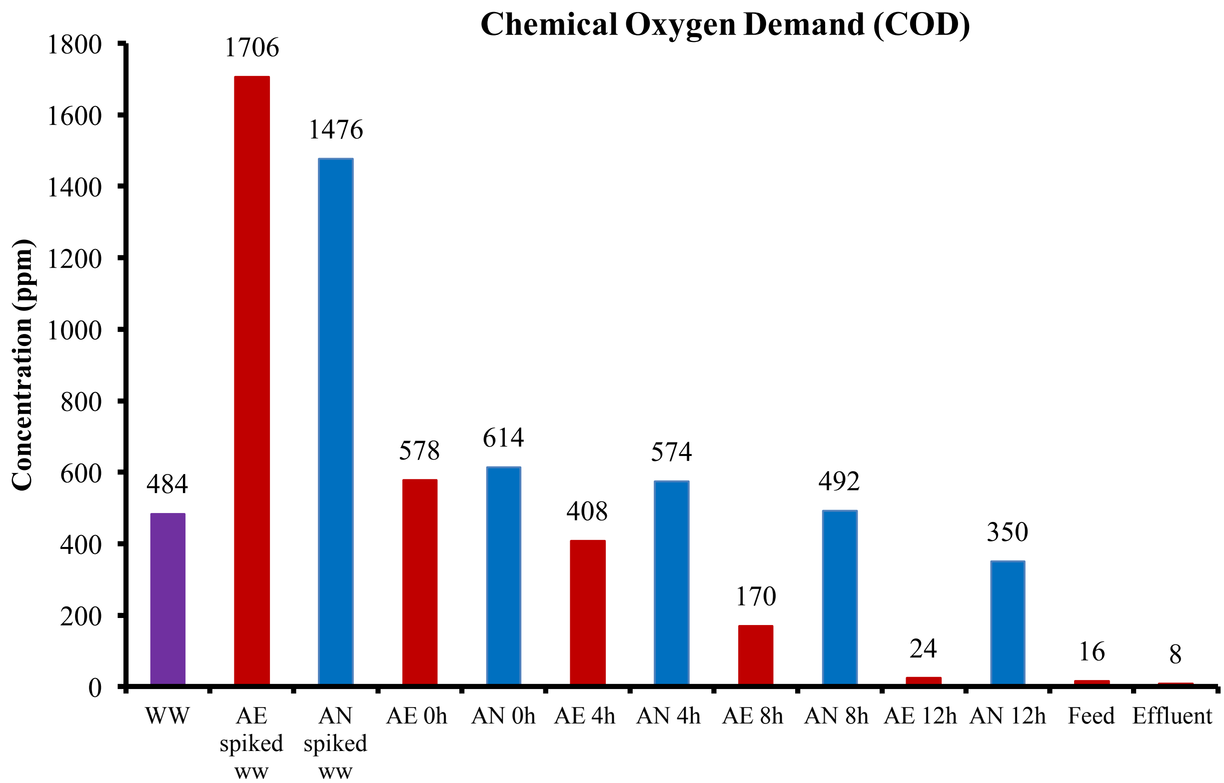


Figure 3.3 The variation of COD during 12 hours of semi-continuous MBR operation with Fayetteville wastewater and sludge as well as COD values before and after membrane filtration. The TSS in the AN and AE tanks are about 5100 and 6500 mg/L respectively.

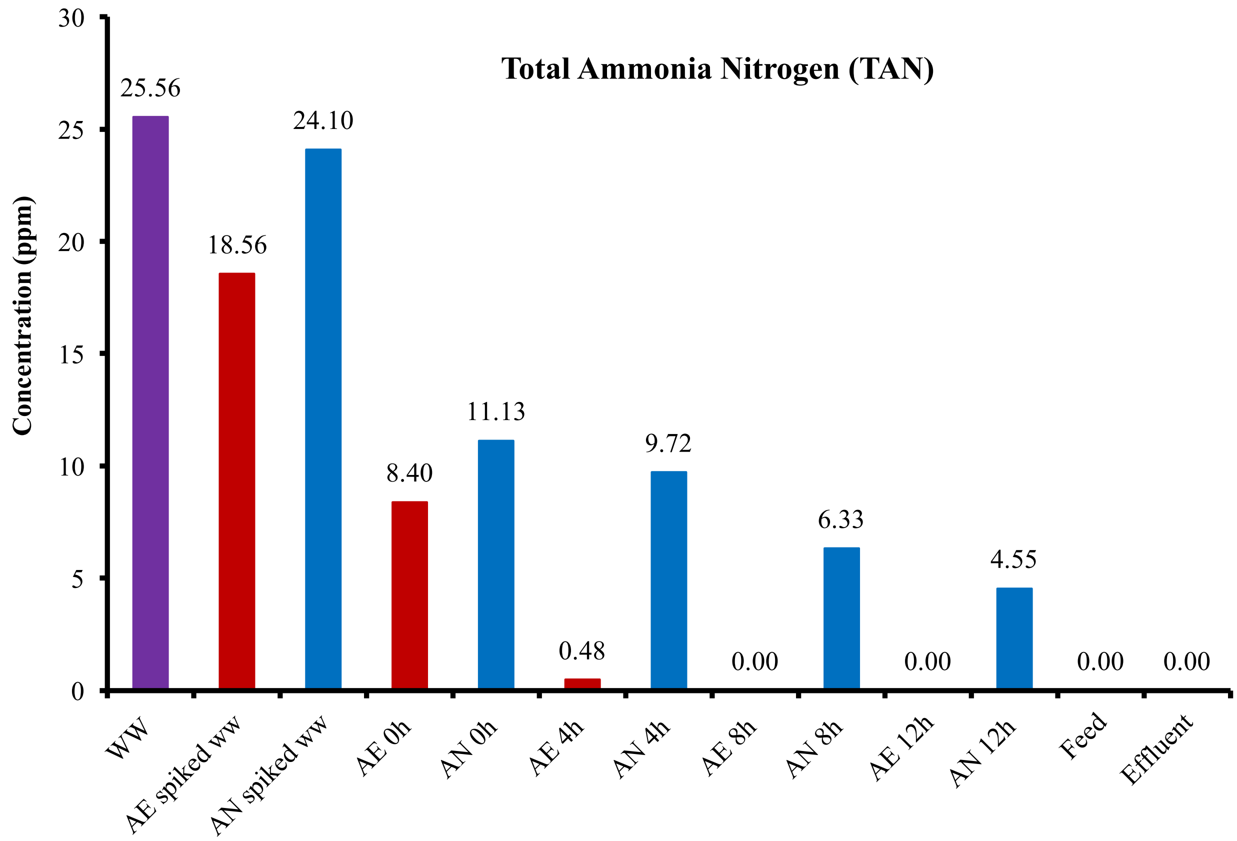


Figure 3.4 The variation of TAN during 12 hours of semi-continuous MBR operation with Fayetteville wastewater and sludge as well as TAN values before and after membrane filtration. The TSS in the AN and AE tanks are about 5100 and 6500 mg/L respectively.

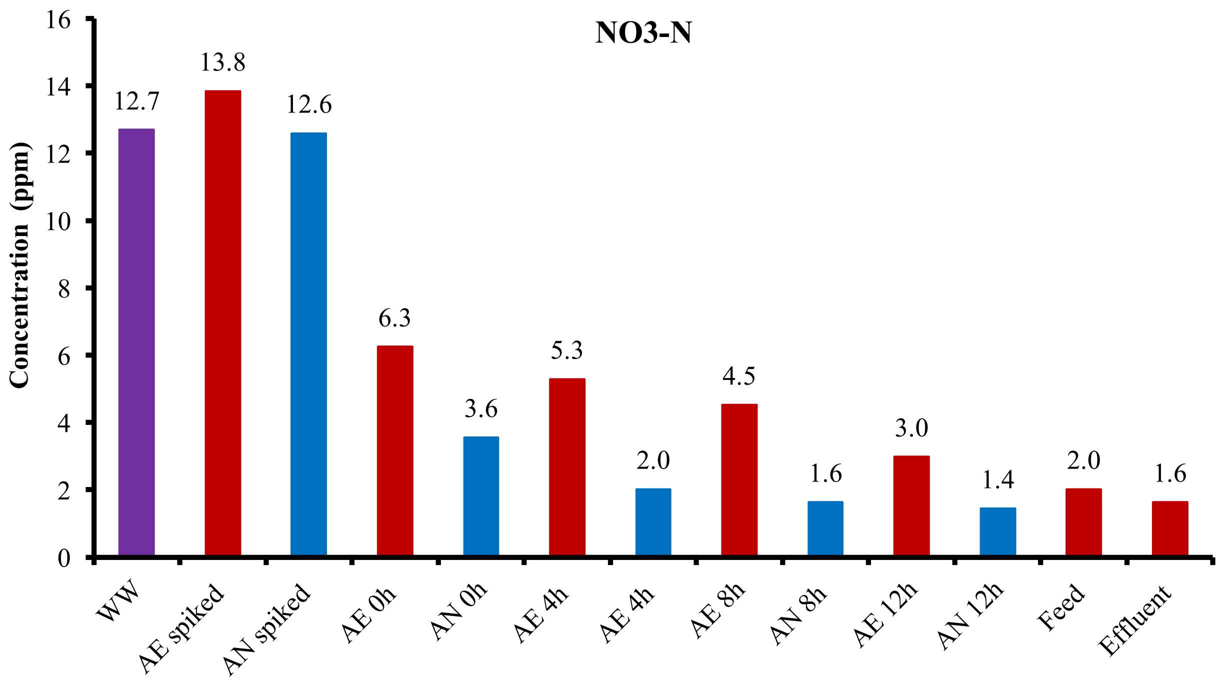


Figure 3.5 The variation of NO3-N during 12 hours of semi-continuous MBR operation with Fayetteville wastewater and sludge as well as NO3-N values before and after membrane filtration. The TSS in the AN and AE tanks are about 5100 and 6500 mg/L respectively.

3.5.2 EDC Removal with Lab-scale MBR

Few studies have reported the removal of multiple EDCs and PhACs from real wastewater by MBR. It is well reported that the removal efficiency of organic matter increases over longer hydraulic retention time (HRT) and solid retention time (SRT) in both aerobic and anoxic tanks (Cirja, Ivashechkin, Schäffer, & Corvini, 2008; Tobergte & Curtis, 2013). This indicates that HRT is an important parameter that affects EDC removal. The longer is the HRT, the greater the time is available for biodegradation of selected EDC compounds. Therefore, MBR was operated under various retention times and then 12 hours selected as the optimized time for the degradation of EDCs and achieving wastewater quality parameters. Furthermore, since the submerged membrane has a nominal pore size of 0.04 µm much larger than the sizes of these selected EDC compounds, the rejection of these compounds by the membrane due to size exclusion can be negligible. However, EDC compounds could potentially adsorb onto the membrane surface or inner pores.

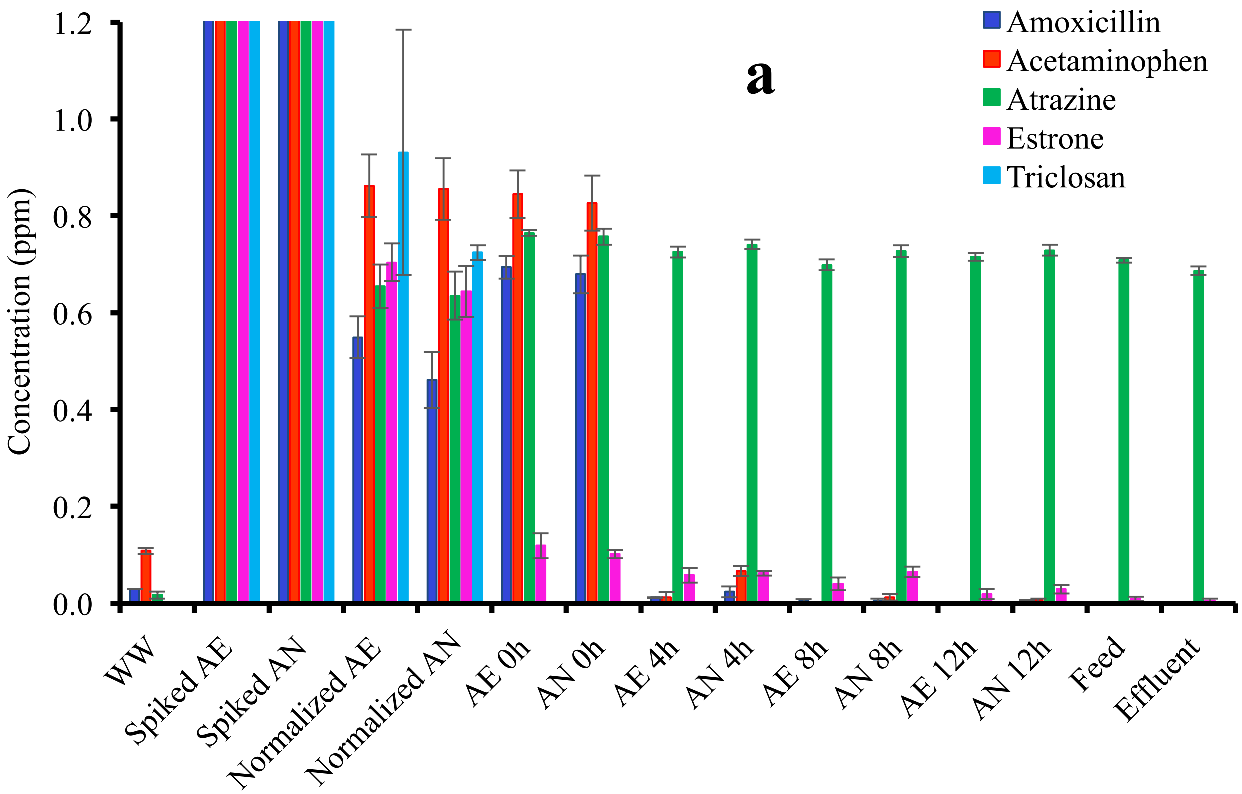
3.5.3 MBR operated with continuous recirculating mode

Wastewater with spiked EDCs was injected into both aerobic and anoxic tanks with 12 hours of hydraulic retention time (HRT) and continuous circulation of mixed liquor suspended solids (MLSS) containing wastewater and suspended solids between the two tanks. The flow rate for the recirculation was adjusted so that only a small percentage of the AN and AE sludge was recirculated and that the dissolved oxygen (DO) levels in the two tanks were within the aerobic and anoxic desired ranges. The flow rate also depends on the total suspended solids (TSS) in the tanks. Several different MBR runs were conducted with different TSS levels. Figures 3.3-3.5 show the COD, TAN, and NO_3-N for one of the MBR runs with TSS levels in the AN and AE tanks kept at 5100 and 6500 mg/L respectively. The flow rate for this run was kept at 31 mL/min. The DO level in the AE tank was maintained above 2-4 mg/L whereas it was below 0.5 mg/L in the AN tank. Samples were taken from both tanks every four hours, and after 12 hours of HRT from the feed (AE tank) and permeate of the membrane as well.

As shown in Figure 3.3, COD of the wastewater was just below 500 ppm. However, COD increased to about 1700 and 1500 ppm in the AE and AN tanks respectively after EDC spiking. This increase was due largely to the added solvent ethanol used to dissolve the selected EDCs. Similar results were reported in earlier studies (Trussell, Merlo, Hermanowicz, & Jenkins, 2006). After the spiked wastewater was added to AN and AE tanks, the COD level dropped to around 600 ppm in both tanks. After 4, 8 and 12 hours of digestion, the COD level in the AE tank reduced to around 400, 170 and 24 ppm respectively whereas the COD level in the AN tank reduced to about 570, 500 and 350 ppm respectively. It is clear from aerobic process is much more efficient in reducing the COD. The feed was taken from the AE tank and permeate was drawn after the filtration with the MF membrane. The COD levels in the feed and effluent were 16 and 8 ppm respectively.

The TAN levels in the AE and AN tanks follow somewhat different patterns as shown in Figure 3.4. The initial TAN in the wastewater was around 25 ppm. After spiking with EDC, it reduces slightly. After EDC spiked wastewater was added to the AN and AE tanks, the TAN levels in the AE and AN were around 8 and 11 ppm respectively. After 4 hours of HRT, the TAN level in the AE tank reduced rapidly to below 1 ppm due to the conversion of TAN to nitride by the microorganisms. During the subsequent HRT, no TAN was detected. As ammonium (NH_4^+) conversion to nitride (NO_3^-) or nitrite (NO_2^-, unstable) requires the presence of oxygen, the oxidation of TAN occurs largely in the AE tank. However, due to the recirculation of the MLSS between the AE and AN tanks, the TAN level in the AN tank reduced somewhat slowly to about 9.7, 6.3 and 4.6 ppm after 4, 8 and 12 hours of HRT. As the feed was taken from the AE tank, no TAN was detected in the feed and also in the filtrate.

Figure 3.5 shows the change of NO_3-N in the AE and AN tanks respectively. As the oxidation or nitrification of TAN to NO_3^- occurs in the AE tank and the reduction or denitrification of the NO_3^- to N_2 occurs in the AN tank, the amount of NO_3^- in the two tanks reduces slowly during the recirculation operation. The amount of NO_3-N reduced to 5.3, 4.5 and 3.0 ppm from the initial 6.3 ppm in the AE tank after 4, 8 and 12 hours of HRT. The concentration of NO_3-N in the AN tank reduced to 2.0, 1.6 and 1.4 ppm after 4, 8 and 12 hours of HRT with the initial concentration of 3.6 ppm. As NO_3^- is a small anion, low concentrations of NO_3-N remained in the feed and filtrate. The degradation of three indicators (COD, TAN, and NO_3-N) over 12 hours of HRT with specified operation parameters demonstrate that our MBR system consisting of the AE and AN tanks are working properly to reach desired wastewater treatment standards and that the microorganisms are healthy. Therefore, it is possible to investigate the degradation of selected EDCs with our current MBR system at these operation conditions.



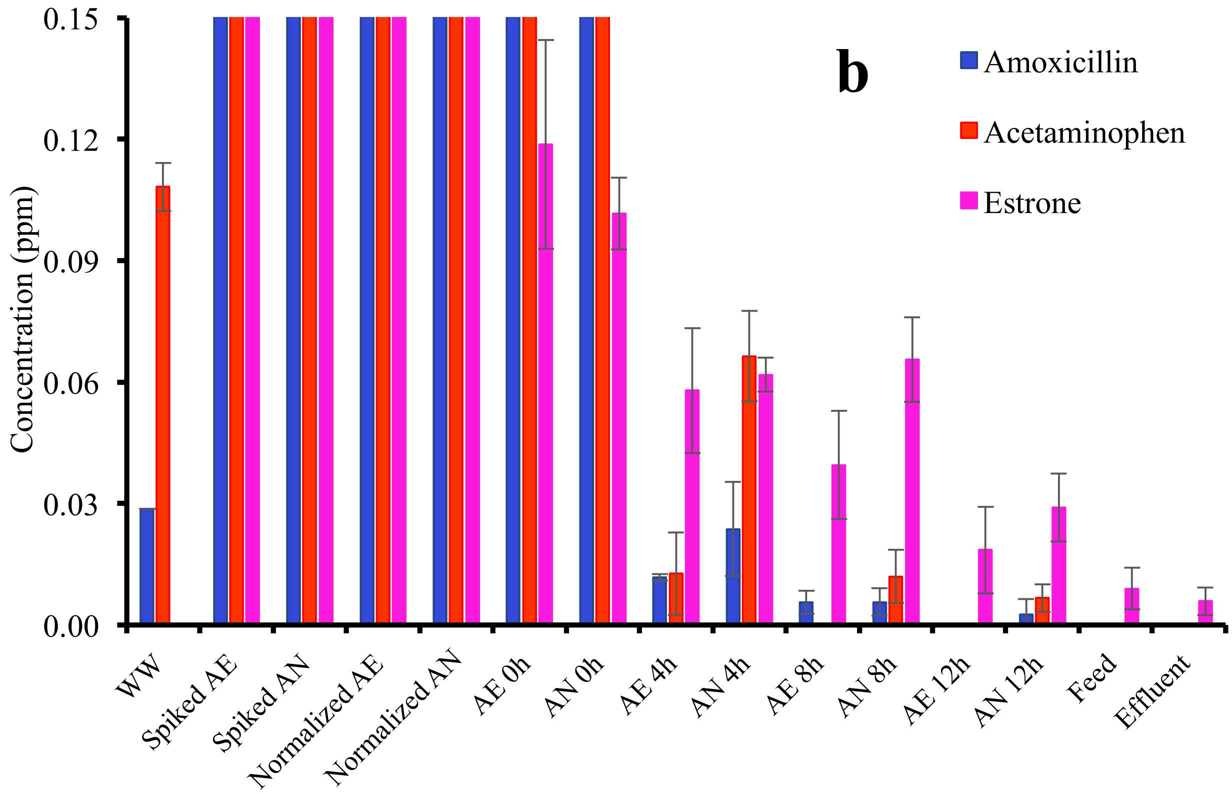


Figure 3.6 Concentrations of all 5 EDC compounds (**a**) and amoxicillin, acetaminophen, estrone only (**b**) at different stages of treatment with wastewaters spiked with 5 EDC compounds at 1 ppm level in both aerobic (AE) and anoxic (AN) tanks. The concentrations of total suspended solids in AN and AE tanks were about 5100 and 6500 mg/L respectively**.** Different scales for **a** and **b** are used to illustrate the data more clearly. The error bars represent the standard deviation of six replicates. Experiments were conducted at room temperature 25^{o}C.

Figures 3.6**a** and 3.6**b** show the concentrations of the model EDCs during different stages of MBR treatment and in the feed as well as in the effluent. The EDC compounds were spiked in the fresh wastewater collected from Fayetteville facility. The wastewater spiked with EDCs were then fed into the AN and AE tanks containing sludge. The targeted concentration for each compound is 1 ppm. Three sets of experiments were conducted at the same conditions except that there was some slight variation in TSS. The TSS for these three experiments are 6040, 6500, 6610 mg/L for the AE sludge and 5370, 5090 and 4610 for the corresponding AN sludge. There were two measurements for each sample. The results and error bars shown in Figures 3.6**a** and

3.6**b** are averages of a total of 6 measurements. As can be seen from Figure 5a, the wastewater collected from Fayetteville facility contains small amount of amoxicillin, acetaminophen and atrazine at ~100 ppb or below. The initial concentrations of EDCs after normalizing to the volume of the AE and AN tank volume were between 0.5 and 1 ppm depending on the specific EDC compound and sludge type. The reason for such variation is likely due to the presence of particulates and solids in wastewater that could adsorb these EDC compounds, apparently at different efficiencies. After equilibrate the spiked wastewater with AE/AN sludge to start the MBR process, the concentrations of the EDC compounds demonstrated significant reduction for some of the compounds. It can be seen that triclosan was completely adsorbed by the sludge at the very beginning. As triclosan is an antimicrobial agent, it has a strong interaction with the microorganisms in the sludge and was completely adsorbed. The concentration of estrone is also reduced by more than half at the beginning with less than 200 ppb remaining in the AN and AE tanks, which can be seen more clearly in Figure 5b with an enlarged scale. Since estrone is a hormone, it should be easy for organisms to intake via the cell membranes. However, the degradation or reduction of estrone is rather slow. Even after 12 hours of HRT, estrone remains in both the AE and AN tanks at a concentration of about 20 ppb or higher. It also appears that aerobic sludge is more efficient in digesting estrone with a higher rate of reduction. Hu et al. (2007) and her colleagues studied the removal of hormones and their conjugates using three pilot-scale and lab-scale MBRs run with raw wastewater. They found that the removal efficiency ranged between 91.4%-80.2% in MBR systems (Hu, Chen, Tao, & Kekred, 2007). Our results are in agreement with their findings. It seems that amoxicillin can be reduced to concentrations less than 30 ppb in both AE and AN tanks only after 4 h. Amoxicillin is an antibiotic agent and should be easily adsorbed by or interacting strongly with bacteria in the sludge. Acetaminophen is a hydrophilic drug compound and is seen to be removed completely in the aerobic tank whereas its concentration in anoxic tank remains at 10 ppb level even after 12 hours of retention time. Since the feed was taken from the AE tank, both amoxicillin and acetaminophen were not detected in the feed and effluent from the filtrate. Unlike other EDCs, atrazine was neither removed by the reactors nor by the membrane. The concentration of atrazine remained constant over the period of 12 h MBR operation. The persistence of atrazine in the MBR can be attributed to its unique structure and that the low concentration (< 20 ppb) of atrazine in wastewater suggesting that the microorganisms have not yet adapted to the digestion of atrazine. The removal efficiency of atrazine in this study is in agreement with previously reported value (Tadkaew et al., 2011).

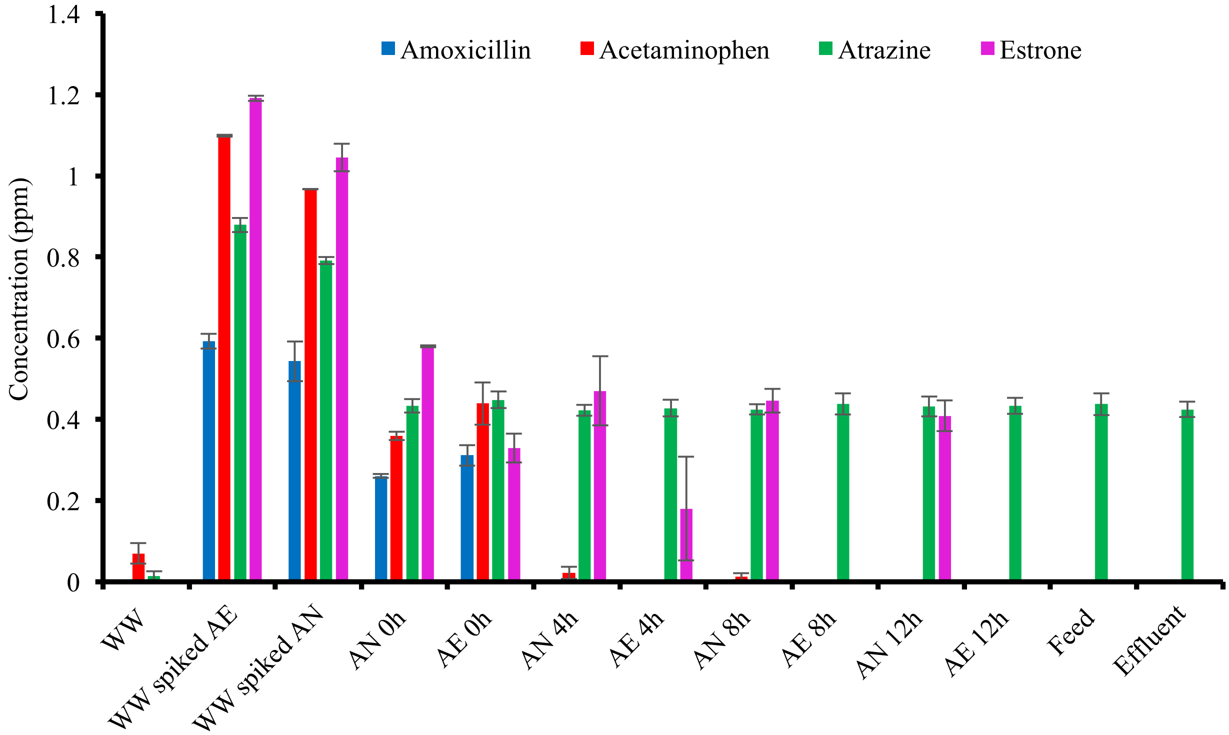


Figure 3.7 Concentrations of the selected 5 EDC compounds in Fayetteville wastewater, at different stages of treatment, feed, and effluent with continuous recirculating between AE and AN tank. The concentrations of total suspended solids in AN and AE tanks were about 4000 and 4200 mg/L respectively. The error bars represent the standard deviation of two replicates.

In order to understand the effects of TSS level on the removal efficiency of 4 EDC compounds without triclosan, EDC concentrations at different stages of treatment were shown in figure 3.7 with TSS of 4000 and 4200 mg/L respectively for the AN and AE tanks. The initial concentrations of EDC compounds in the AE and AN were close to 0.5 ppm. It can be seen that amoxicillin and acetaminophen were completely removed after 12 hours of retention time. Estrone was completely removed in the aerobic tank, but its concentration in the AN tank only reduces from the initial 0.5 ppm to about 0.35 ppm after 12 hours of operation. Clearly, anoxic sludge was not able to degrade estrone. The degradation of estrone occurs largely in the aerobic tank suggesting an oxidative process. Similar to the previous studies (Campo, Masiá, Blasco, &

Picó, 2013; Loos et al., 2013; Terzic et al., 2008), atrazine was found to be recalcitrant in both AE and AN tanks. Fayetteville sludge was not able to removal atrazine.

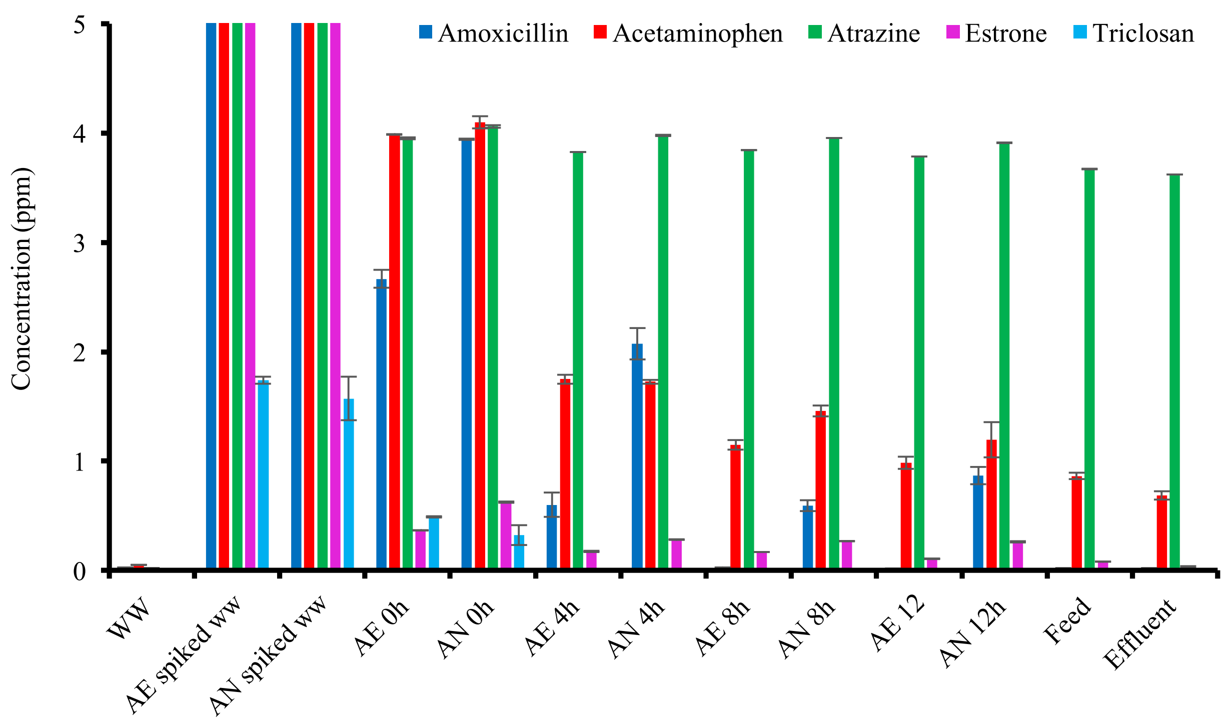


Figure 3.8 Concentrations of 5 EDC compounds different stages of treatment spiked at 5 ppm level in both aerobic (AE) and anoxic (AN) tanks for a total of 12-hour HRT. The concentrations of total suspended solids in AN and AE tanks were about 5370 and 5850 mg/L respectively. The error bars represent the standard deviation of two replicates. Experiments were conducted at room temperature 25^{o}C.

In order to investigate the capacity of the sludge for EDC removal, a higher concentration of EDC compounds at 5 ppm level were spiked under the similar operation conditions when EDC compounds were spiked at 1 ppm level. The TSS in the AE and AN tanks were measured at 4370 and 5850 mg/L respectively. Figure 3.8 shows the concentrations of EDC compounds during the 12 hours of MBR operation as well as their concentrations in the feed and filtrate. After spiking the wastewater with the EDC compounds to target 5 ppm in the AE and AN tanks, triclosan can be seen was largely adsorbed by any particulate matter in the wastewater immediately with less than 2 ppm detected by HPLC. After the spiked wastewaters were equilibrated with the AE and AN sludge, only about 0.5 and 0.3 ppm of triclosan were detected in the aerobic and anoxic tanks respectively similar to earlier observation that triclosan is rapidly adsorbed by the sludge. Similar to triclosan, estrone was also seen being rapidly adsorbed by the sludge with about 0.4 ppm and 0.6 ppm detected in the aerobic and anoxic tanks respectively. There is some adsorption of amoxicillin by the aerobic sludge, but the concentrations of amoxicillin, acetaminophen and atrazine remained closely to 4 ppm levels after equilibrating the spiked wastewater with the sludge.

The degradation of EDCs over time demonstrates interesting trends. Amoxicillin was rapidly degraded by the aerobic sludge and was completely removed in the AE tank after 8 hours of HRT. However, its degradation by the anoxic sludge is relatively slow with over 0.6 ppm detected even after 12 hours of HRT. Our earlier studies show that amoxicillin can be rapidly digested by both the aerobic and anoxic microorganisms. However, at higher concentration of spiking, the anoxic sludge of digesting amoxicillin is no longer complete suggesting that amoxicillin to sludge ratio has reached its optimal level and there is an over-saturation of the substrate. The degradation of acetaminophen shows similar trend except that over-saturation is observed for both the aerobic and anoxic sludge. Even after 12 hours of HRT, over 1 ppm of acetaminophen was detected in the AE and AN tanks. As a result, the feed and filtrate also showed high concentrations of acetaminophen. No much adsorption of acetaminophen was found on the PVDF MF membranes used. Triclosan was completely removed after 4 hours of HRT suggesting the high capacity of the sludge for its adsorption. Estrone, even though significantly adsorbed by the sludge, over 150 ppb estrone was observed even after 12 hours of operation suggesting a slow biodegradation process as observed before. Atrazine was found to be recalcitrant with very little adsorption or biodegradation by the sludge.

In order to test the HRT on EDC degradation, 20 hours of HRT for the degradation of EDCs spiked at 5 ppm level were conducted as shown in figure 3.9. The experiments were carried out immediately after the previous experiments thereby the same levels of TSS in sludge were measured. Similar patterns are observed for amoxicillin, estrone, triclosan and atrazine. However, after 20 hours of HRT, all five EDC compounds except atrazine were removed. Surprisingly, the concentration of acetaminophen in the AE and AN sludge reduced significantly compared to the previous studies. One possible explanation is that the microorganisms have adapted to digest acetaminophen more efficiently. However, the exact reason remains elusive.

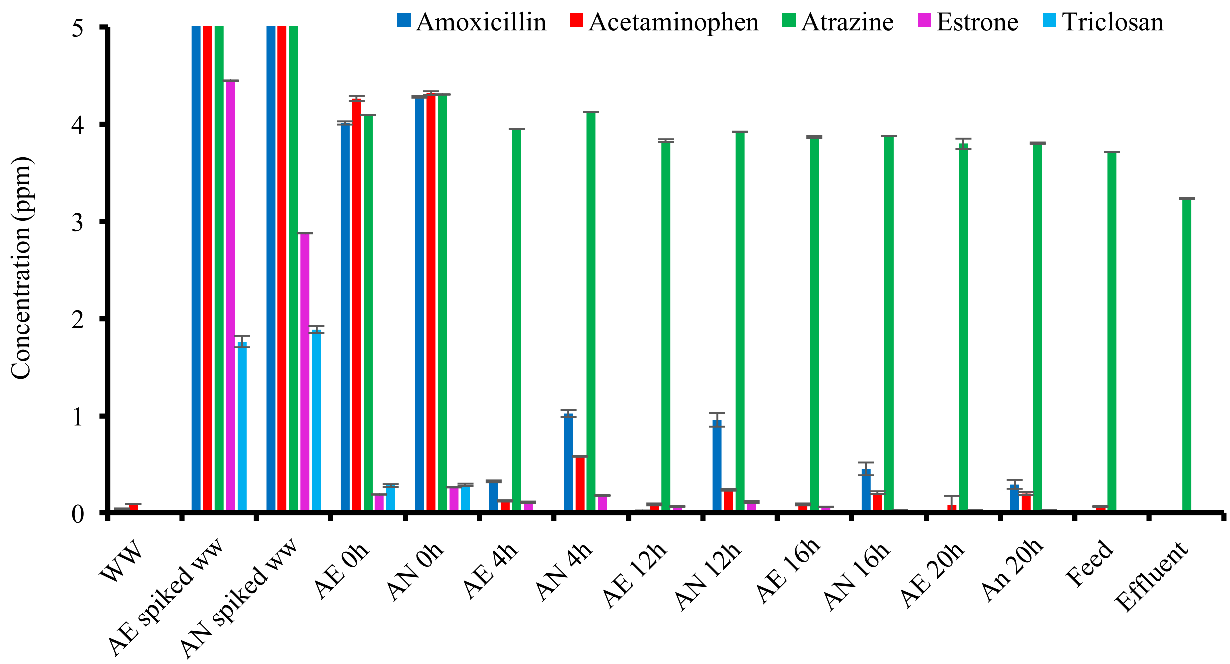


Figure 3.9 Concentrations of 5 EDC compounds different stages of treatment spiked at 5 ppm level in both aerobic (AE) and anoxic (AN) tanks for a total of 20 hours of HRT. The concentrations of total suspended solids in AN and AE tanks were about 5370 and 5850 mg/L respectively. The error bars represent the standard deviation of two replicates. Experiments were conducted at room temperature 25^{o}C.

3.5.4 The effect of the Physicochemical properties on the removal of the selected EDCs

Table 3.3 shows selected physicochemical properties of the investigated compounds. Some correlation between the properties and the corresponding removal efficiencies in the MBR can be found. Triclosan is the most hydrophobic and an antimicrobial agent, it is expected that it should strongly interact with the bacteria in the sludge as was observed in this study. Hydrophobic adsorption of this compound to the membranes of the bacteria is expected. On the other hand, amoxicillin is an antibiotic drug and is highly solvable in water, it is expected that it should also interact strongly with the bacteria, but the mechanism(s) for its removal is probably via biodegradation rather than via hydrophobic adsorption due to the relative slow removal process observed. Estrone is an estrogen and relatively hydrophobic. It should be able to be adsorbed by the tissue or microorganisms as was seen in the studies. The mechanism for its removal appears to be adsorption followed by biodegradation. The ring structure of atrazine is not naturally occurring thereby it appears not be biodegradable by the microorganisms in Fayetteville wastewater treatment facilities. It is somewhat hydrophilic due to the N replacement of the C atom on the ring structure. Therefore, it seems that it is neither adsorbed by the sludge nor biodegradable. Acetaminophen is a hydrophilic drug molecule. Its amide bond and hydroxyl group are occurring abundantly in nature and probably serve as effective substrate for bacteria digestion.

Table 3.3 Selected physicochemical properties of the investigated trace organic compounds.

EDCs	Water solubility (mg/L) [a]	Hydrophobicity Log D at (pH 8) [a]
Amoxicillin	3430	-2.56
Acetaminophen	14000	0.33
Atrazine	34.7	2.63
Estrone	30	3.68
Triclosan	10	4.76

[a] Source: PubChem open chemistry Database. https://pubchem.ncbi.nlm.nih.gov/compound/atrazine#section=Top
Log *D* is logarithm of the distribution coefficient which is the ratio of the sum of concentrations of all forms of the compound (ionised and unionised) in octanol and water at a given pH.

3.6 CONCLUSIONS

Studies were conducted to investigate five selected EDC compounds for removal from wastewater using a continuous recirculating custom-made MBR system. It was found that MBR is efficient and effective to remove amoxicillin, acetaminophen, estrone, and triclosan. Atrazine is recalcitrant with only about 5% removal. The probable mechanisms for the removal of the selected EDC compound in MBR were discussed and correlated to some of their properties. The effects of sludge concentration and HRT on EDC removal was also discussed.

3.7 ACKNOWLEDGMENT

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CHAPTER 4 WORK SUMMARY AND RECOMMENDATIONS

4.1 WORK SUMMARY AND CONCLUSIONS

A lab scale membrane bioreactor was used to investigate the removal of endocrine disrupting compounds from actual spiked wastewater. These macro-contaminants are responsible for disrupting the endocrine system by mimicking or blocking the natural hormones and many of them posing divers hazards to the aquatic species. The MBR was equipped with ultrafiltration membrane and seeded with sludge from the city of Fayetteville wastewater treatment plant. An analytical method using HPLC was developed and optimized to detect the tested compounds at trace concentration to simulate their occurrence in actual water streams. The treatment system was run at various operating conditions such as batch and semi-continuous mode, different total suspended solids concentration, and vary hydraulic retention time to have insightful understanding of their effects on the removal of EDCs. Furthermore, the influence of the physicochemical properties upon the interaction with sludge and which ultimately leads to their removal.

MBR shows different removal efficiencies varying from low removal of atrazine <10% to complete removal up to level below the detection limits for amoxicillin, acetaminophen, and triclosan. Operating the MBR at different TSS concentrations have demonstrated serious effects on the uptake of the biodegradable compounds by the sludge where low TSS MBR takes longer retention time to removed amoxicillin, estrone and acetaminophen than high TSS MBR. However, for the specified retention time, both systems were able to eliminate amoxicillin, acetaminophen, and triclosan while atrazine concentration was stable over the treatment time with overall removal below 10%. Estrone showed significant difference when the MBR was operated at low and high TSS. Estrone is completely removed in the aerobic tank whereas it is largely retained in the anoxic tank. It is noteworthy that the chemical properties of the selected compounds play a significant role in predicting and removal pathway. A clear correlation between the water solubility and the removal efficiency shows that compounds with high solubility have high removal efficiency while compounds with high molecular weight are more biodegradable than those with low molecular weight. The reason behind this hypothesis is that compounds with high molecular weight usually have more active branches that are ready targets for the microorganisms to ignite biodegradation. In conclusion, the removal of trace organic compounds is significantly governed by biodegradation and adsorption simultaneously. Nevertheless, chemical properties and molecule active groups can play a major role in the removal of trace organic compounds with low hydrophobicity because the adsorption for such compounds can be negligible.

4.2 RECOMMENDATIONS

Since there are many compounds are thought to possess endocrine effects, it is recommended to extend the number of the investigated compounds which might give a better removal comparison with respect to their removal. Even though HPLC is an accurate method to detect small group of EDCs, more advance detection tools such as GC-MS and LC-MS are more precise and could be used to detect a wide range of contaminants.

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APPENDICES

APPENDIX A1

Standard operation procedure (SOP) and experimental setup

These experiments were conducted to evaluate the removal of endocrine disrupting chemical using a lab-scale membrane bioreactor. All the equipment was placed inside a hood in Lab No. 140 at the Cato Springs Research Center (CSRC). The personal protection equipment required to perform this experiment are goggles, lab coat, and appropriate gloves. In addition, each AE and AN tank were placed in plastic containers that can handle the excessive MLSS in case of emergency overflow or peristaltic pump flaw.

MBR operation

The lab scale MBR system comprise of anoxic, aerobic and membrane filtration tanks and it was constructed by Lantian, Inc in China, as shown in figure A.1 and figure A.2. The volume for each compartment is about 16 L in each tank. Lantian also provided the initial MBR filtration membrane with 0.08 µm pore size. The aerobic tank has air diffuser to supply bubbles of air to the system. The submerged membrane tank has a separate sparger to supply coarse bubbles that aim to generate turbulence that reduces the potential membrane fouling. Wastewater after primary treatment was collected from Fayetteville West Side Wastewater Treatment Plant (15 South Broyles Avenue, Fayetteville, Arkansas 72704). Activated sludge was taken from both the anoxic and aerobic compartments and was used in the anoxic and aerobic tanks of our MBR system, respectively. Depending on the hydraulic retention time (HRT), the circulation flow rate was adjusted. At the beginning, both compartments were fed with spiked actual fresh wastewater with continuous mixed liquor suspended solids circulation. After 12 hours of HRT, MLSS from aerobic tank was moved to the filtration tank containing submerged membrane. Samples were collected at various stages of treatment and different retention time to investigate the removal of model EDCs. Total ammonium nitrogen (TAN), nitrate nitrogen, chemical oxygen demand (COD), dissolved oxygen (DO), and total suspended solids were monitored during the experiment. Once the stable state was reached, EDCs compounds at 1 ppm were spiked to anoxic tank. The concentrations of EDCs during the anoxic, aerobic treatment and in the effluent were monitored with HPLC.

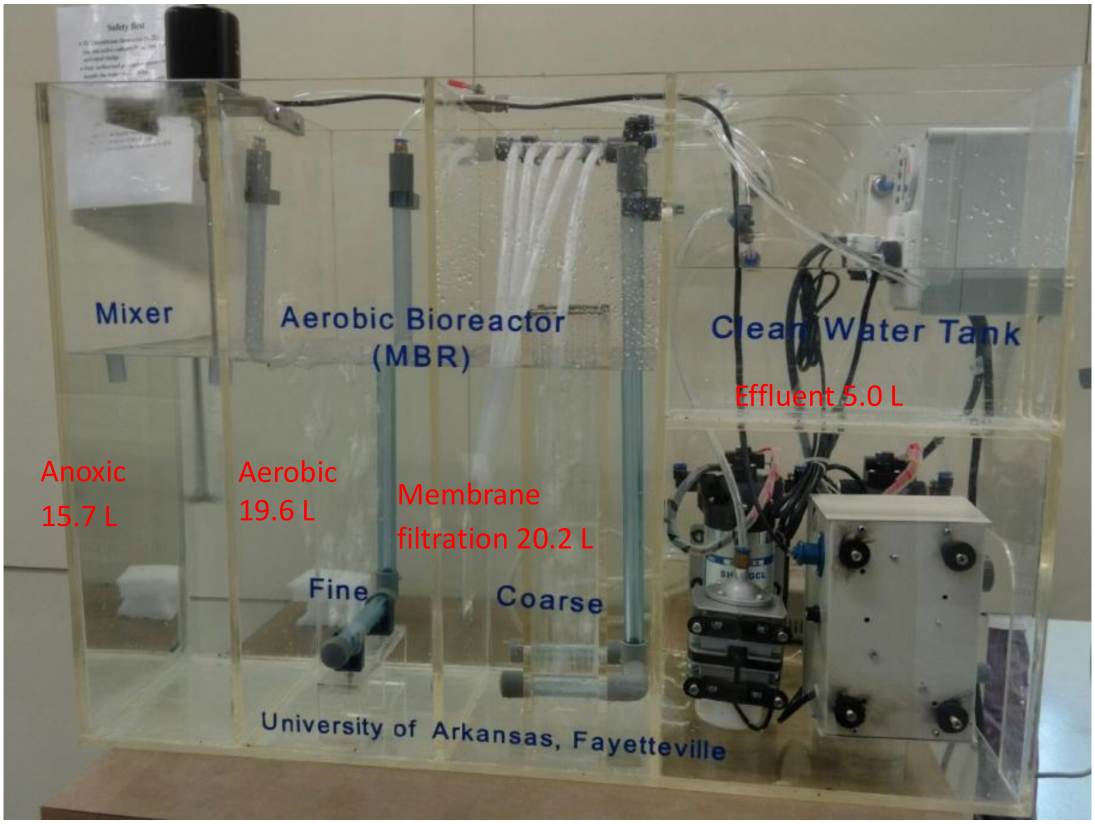

Figure A.1 Laboratory scale MBR from Lantian Inc.

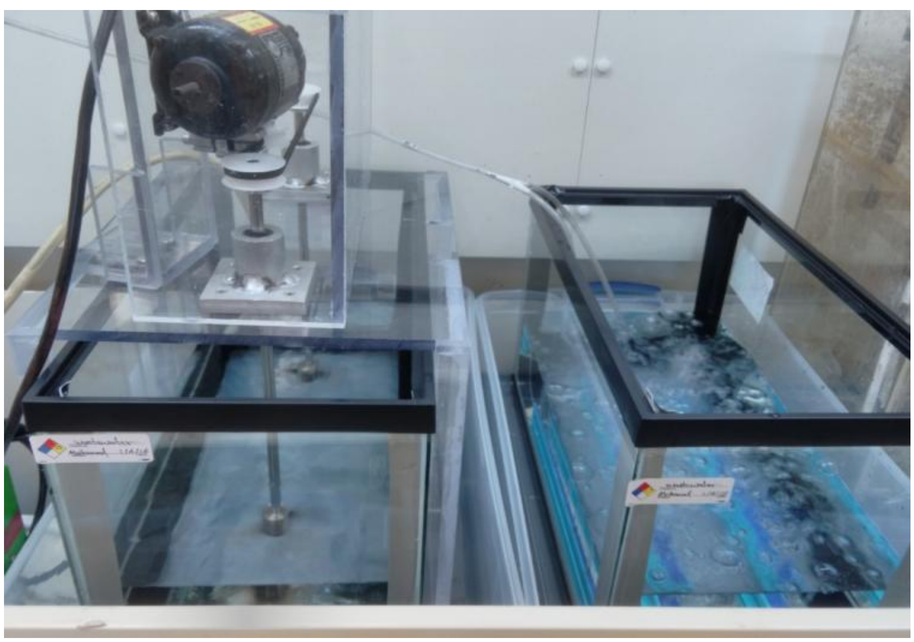

Figure A.2 Custom-designed anoxic and aerobic laboratory scale MBR with glass tanks and continuous circulation

APPENDIX A2

Chemical and materials used

Table A.1 Chemicals used. Adapted from SDS

Material Name	Hazards	Purity	Provider
Methanol	Highly flammable material. Toxic	HPLC grade	EMD Chemicals
Acetonitrile	Highly flammable material. Toxic	HPLC grade	Macron Fine Chemicals™
Amoxicillin	May cause allergy or asthma if inhaled	>99%	Alfa Aesar
Acetaminophen	Harmful if swallowed and may cause genetic effects	>98%	TCI
Atrazine	Cause eyes and skin irritation	>97%	TCI
Estrone	Harmful if inhaled	>99%	Acros Organics
Triclosan	Very toxic to aquatic life	>99%	Alfa Aesar
Liquid phenol	Toxic, serious eye damage, chronic hazards to aquatic life	≥ 89%	Sigma-Aldrich
Sodium nitroprusside dihydrate	Skin irritation, severe over exposure might cause death	≥98%	Fluka
Sodium hydroxide	Very hazardous in case of skin contact	NA	Amresco
Sodium hypochlorite	May cause severe irritation and burns for eyes and skin	4–6%	VWR International

*All chemicals were all used as received with no further purification

APPENDIX A3
Standard curves of the investigated contaminants using HPLC and water quality parameters by spectrophotometer

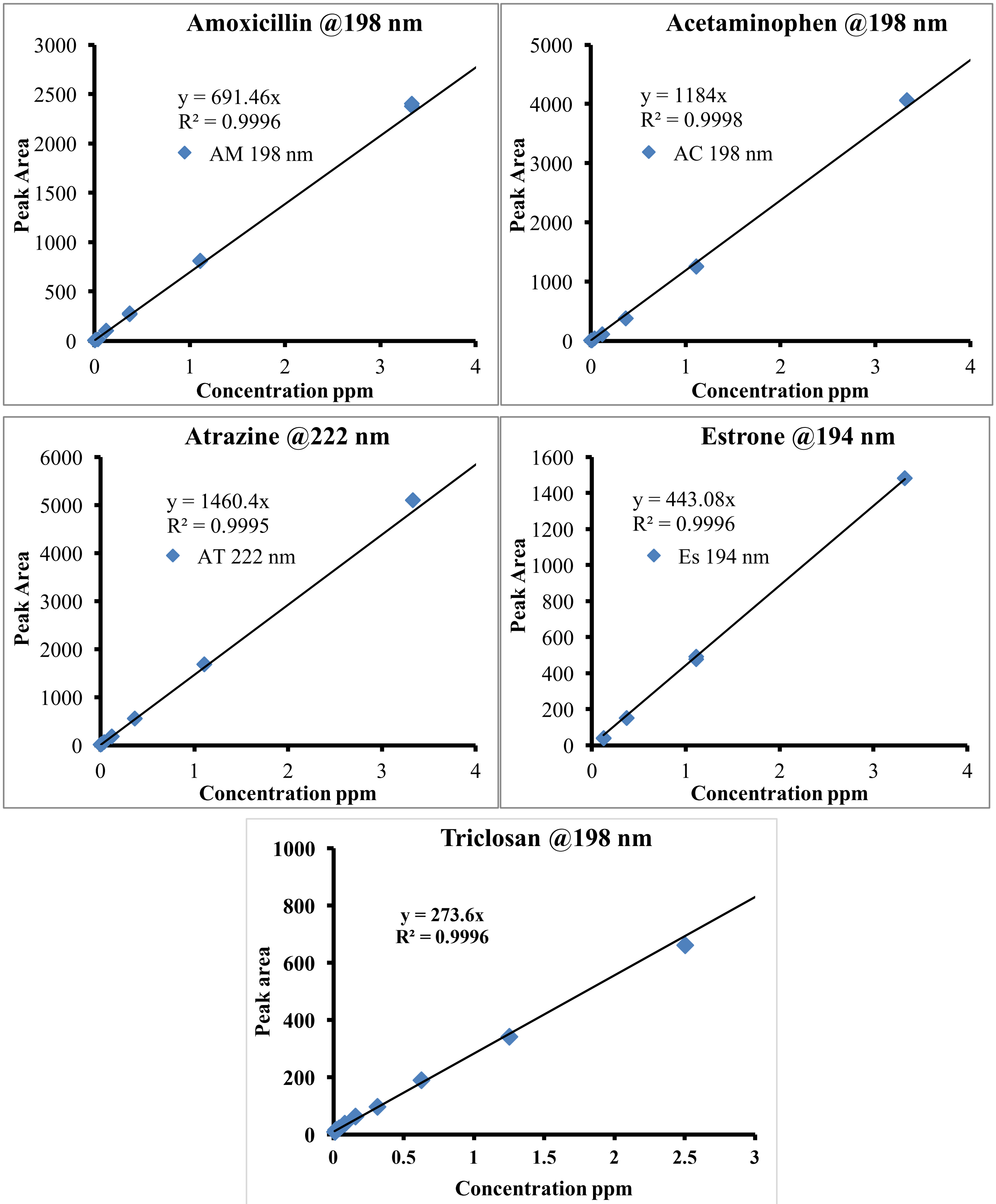

Figure A.3 Standard curves of the studied EDCs using HPLC

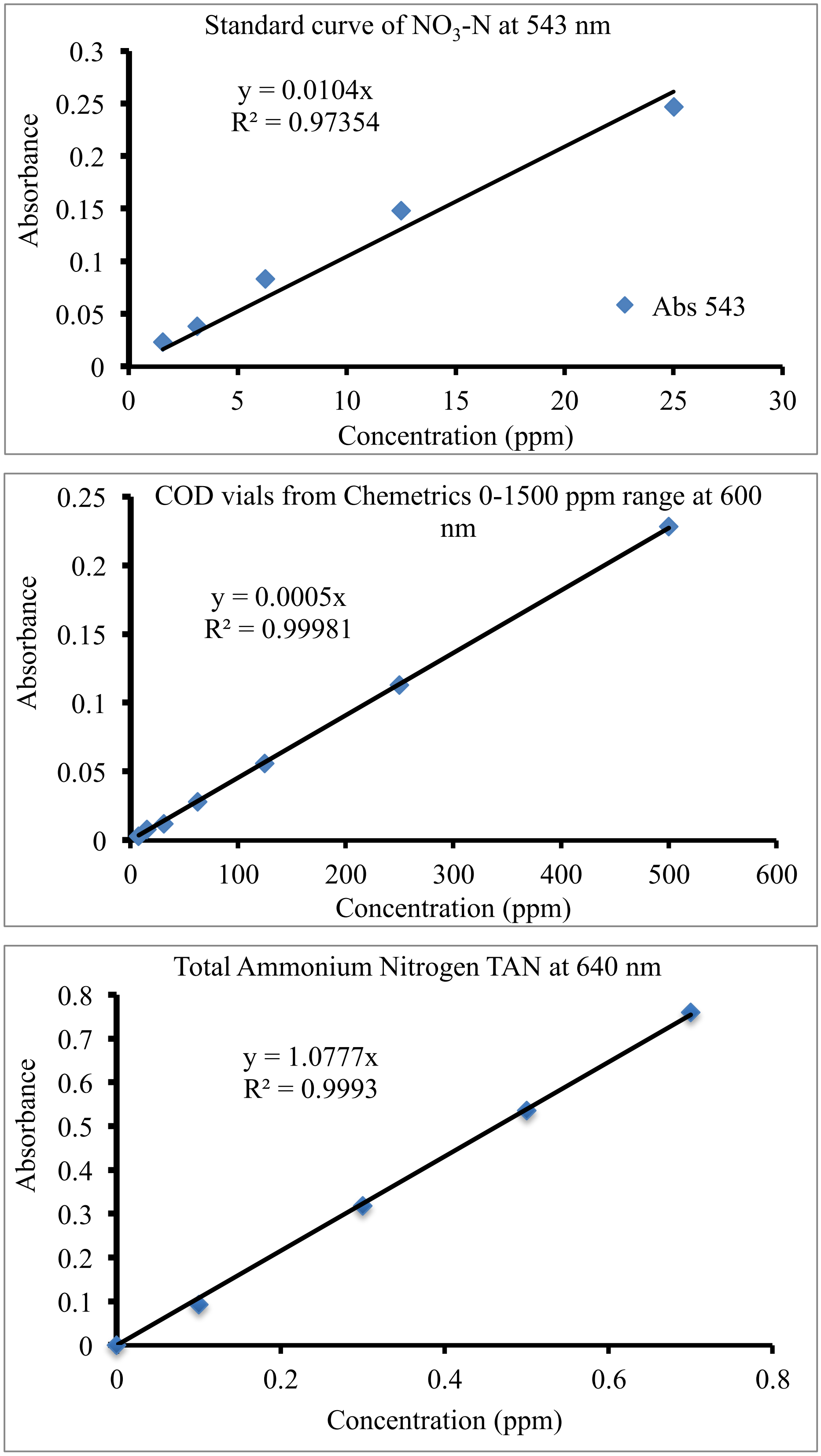

Figure A.4 Standard curves of COD, TAN, and NO_3-N using spectrophotometer