I, William E Platten III, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Environmental Engineering.

It is entitled:
Fate of Emerging Contaminants in Biomass Concentrating Reactors (BCR) under Conventional Aerobic and Aerobic/Anoxic Treatment

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Fate of Emerging Contaminants in Biomass Concentrator Reactors (BCR) under Conventional Aerobic and Aerobic/Anoxic Treatment

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Abstract

The Biomass Concentrator Reactor (BCR), an innovative membrane bioreactor (MBR), was investigated for treating municipal wastewater containing a suite of emerging contaminants, also known as Chemicals of Concern (COC). Traditional treatment, known as the conventional activated sludge process, has been shown to be ineffective for treating micropollutants in municipal wastewater. MBRs have shown promise in attenuating these chemicals, but are much more expensive to build and operate. The BCR is an MBR design that attempts to mitigate the more costly aspects of a membrane system.

The BCR utilizes gravity and a pressure head of only 2.5 cm to separate the biomass from the treated effluent using a thick membrane with large, tortuous path pores. Three systems were tested in this research. Two systems treated a synthetic wastewater as well as the COCs under aerobic and hybrid aerobic/anoxic conditions. The third system was operated under aerobic/anoxic conditions for treating a real wastewater stream. All three systems were monitored for traditional municipal wastewater constituents, i.e., chemical oxygen demand, nitrogen, suspended solids, while the two synthetic wastewater reactors were also monitored for removal of the COCs. The chemicals examined were caffeine, carbamazepine (CMP), testosterone, progesterone, ethinylestradiol (EE2), triclosan, and nonylphenol.

The aerobic and hybrid synthetic systems were able to reduce the COD levels by 93% and 98%, respectively, while ammonia nitrogen was reduced below 0.3 mg/L in both systems and total nitrogen was reduced by 24% and 90%, respectively. The real wastewater system achieved 93 % COD reduction, ammonia nitrogen removal below 0.1 mg/L, and total nitrogen removal of 46%. The real wastewater system was unable to match the hybrid synthetic system for nitrogen removal because the wastewater contained limited amounts of COD needed for denitrification. For the duration of all the experiments, the effluent suspended solids were below 1 mg/L, indicating complete retention of the biomass by the membrane. The COCs were removed by over 90% in the synthetic systems, except for CMP and EE2.
CMP was initially removed by 50%, but the removal was determined to be due to adsorption to the membrane and subsequently reduced to zero after the membrane became saturated. Removal of EE2 was over 90% in the aerobic reactor, but variable in the hybrid. Further investigation confirmed a relationship between oxygen levels and an abiotic transformation, likely oxidative coupling, previously reported in the literature. The recycling between the aerobic and anoxic sections of the hybrid reactor produced variable oxygen conditions and resulted in varying rates of transformation, which explains the inconsistent results in that reactor. Overall, the BCR produced results greater than the conventional activated sludge process and comparable to other MBR systems.
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1 Introduction

The goal of this research was to evaluate the effectiveness of the Biomass Concentrator Reactor (BCR) for treating a complex, synthetic wastewater containing a suite of emerging contaminants. Emerging contaminants, also known as chemicals of concern (COCs) include a wide range of chemicals, such as pharmaceuticals, hormones, and pesticides, many of which are classified as endocrine disrupting chemicals (EDCs). The compounds studied were caffeine (CAF), carbamazepine (CMP), testosterone (TES), progesterone (PRO), ethinylestradiol (EE2), triclosan (TCS), and nonylphenol (NP). These contaminants, and many others, have been found widely distributed in water and wastewater (Heberer, 2002a; Heberer et al., 2002). Conventionally operated wastewater treatment plants (WWTP) have proven ineffective for removing them from the waste stream. The chemicals are usually found at very low concentrations, and WWTPs are not designed to remove these micro-pollutants (Xue et al., 2010). Membrane bioreactors have been shown to be more effective in treating them, but their high cost and energy requirements have limited their adoption (De Wever et al., 2007). Thus, the BCR was investigated as an alternative, having previously achieved success in treating low-level gasoline contaminants in drinking water (Zein et al., 2004).

Three different systems were operated during this research under differing conditions. Two systems were operated simultaneously and were fed a synthetic wastewater mixture as well as the COCs. The reactors differed in the method of treatment used; one system was fully aerated for organic carbon and ammonia oxidation while the other system contained an anoxic section as well as an aerated section for nitrate reduction in addition to organic carbon and ammonia oxidation. Operational parameters, the solids retention time and recycle ratio, were changed to assess the impact on traditional performance criteria as well as COC removal. The third system was operated separately from the other two and was fed a real wastewater. No COCs were fed, as the intent of this reactor was to determine the impact a variable wastewater stream would have on the BCR. The system had aerobic and anoxic sections and was monitored for traditional performance criteria.
2 Literature Review
2.1 Chemicals of Concern

Wastewater treatment has reached a level of maturity where removing just the fundamental contaminants, chemical oxygen demand (COD), nitrogen, and phosphorus, is no longer enough to protect the environment and the population. The proliferation of synthetically produced products including pharmaceutical drugs or chemical residues has produced waste streams with hundreds of micropollutants. Many of these chemicals of concern, which includes pharmaceuticals, hormones, pesticides, and chemicals from everyday personal-care products, have known health effects even when present at very low concentrations, such as endocrine disrupting chemicals and hormones; others do not have known dangers at low concentrations, but their increasing ubiquity raises concerns, such as caffeine and pharmaceuticals.

These COCs are beginning to reach waters used for drinking water (Heberer, 2002a; Heberer, 2002b; Heberer et al., 2002; Kimura et al., 2005, Xue et al., 2010). One of the largest sources for this contamination is the municipal wastewater stream. Traditional wastewater treatment, consisting of a conventional activated sludge (CAS) process, has proven unsuitable for the task of removing these chemicals (Heberer, 2002a; Heberer, 2002b; Kimura et al., 2005; Radjenovic et al., 2009). CAS treatment plants are design to remove the major constituents, carbon and nutrient sources, in wastewater that may affect the immediate health of receiving waters, not the low concentration chemicals. Reliance on biomass settling, short solids retention times (SRT), lack of control over SRT and hydraulic retention time (HRT), and potential loss of biomass combine to select for a culture that will not target many of the COCs (Cicek et al., 2001; LeClech, 2010). The effluents from these traditional systems contain very low, yet biologically and chemically significant amounts of COCs. A new process or treatment plant design is needed to be able to control this growing group of chemicals.
2.2 Membrane Bioreactor

A technology that has shown promise for attenuating these chemicals is the membrane bioreactor, or MBR. Developed in the 1960s, the MBR works by using a membrane, made from a variety of materials, to physically separate the biomass/sludge from the treated effluent/permeate. The effluent is forced, usually with pumps, through the membrane while the biomass is held inside the reactor by the small pores of the membrane. Typically, an MBR system is placed after or within the aerobic stage of a CAS process and takes the place of a secondary clarifier/settling tank (De Wever et al., 2007). Membranes can produce excellent effluent quality, even surpassing the CAS process in terms of COD, biochemical oxygen demand (BOD), ammonia, and total and volatile suspended solids (TSS and VSS) (De Wever et al., 2007; Mallevalle et al., 1996; Radjenovic et al., 2009). MBRs can achieve effluent suspended solids of less than 1 mg/L and up to 99% COD removal, much better removal than a CAS system, which is typically limited to a maximum of 95% COD removal and fluctuating effluent suspended solids (Le-Clech, 2010).

Beyond the improved traditional performance, numerous advantages are gained by using an MBR. Two literature reviews, Le-Clech et al. (2006) and Le-Clech (2010), present a thorough explanation of the advantages. The replacement of the secondary clarifier by the smaller membrane system shrinks the space required for the treatment plant. Additionally, the removal of the secondary clarifier leads to an improvement in the effluent from the membranes. The higher quality comes from the complete retention of the solids within the reactor side of the membrane. Clarifiers rely on the biomass settling characteristics to separate it from the water, which is not always reliable. A small amount of the biomass is allowed to go over the weir and exit the system with the effluent, contributing to the lower effluent quality. The CAS-clarifier system must be maintained within a specific set of parameters, primarily HRTs, SRTs, and TSS, to maintain the optimal operation of the clarifier. The MBR system allows for variation in these parameters without significant determent to the quality of the effluent. SRT is a term used to describe the diversity of culture in the biological reactor, and it is equivalent to the doubling time of the slowest growing organism that can survive in the biological reactor. The longer SRT coupled with
the lack of any washout of biomass over a weir can lead to the growth of slow-growing specialty microorganisms and increased diversity that can be essential for treating the previously mentioned COCs. The HRT is also unrelated to the SRT in an MBR, allowing it to be adjusted based on the incoming flow. Higher TSS is generally associated with longer SRTs and provides a lower food to microorganisms (F/M) ratio. The lower F/M ratio provides another method for improved COC removal by forcing the biomass to utilize all possible sources of nutrition. All solids’ wasting is done intentionally in an MBR, unlike in traditional settling tanks where filamentous bacteria and poor flocculation act as a selection process, eliminating certain types of organisms from the reactor. The more diverse community allowed by the MBR in addition to the higher biomass concentration could increase the effectiveness of process for control of the emerging contaminants.
2.3 Preliminary Studies of MBRs and COCs

Applying this membrane technology to the control of these emerging contaminants has been shown to be promising. Research on adsorbable COCs, nonylphenols and estrogenic chemicals, found that these were eliminated more readily in MBRs than in CASs, attributing it to the attachment of the chemicals to the biomass and the MBR being able to retain more of the biomass than the CAS (Lyko et al., 2005). In a side-by-side comparison of an MBR and a conventional activated sludge system (CAS), De Wever et al. (2007) assessed micropollutant removal of both treatment systems. The MBR was shown to achieve superior performance for many of the chemicals, while neither system was able to attenuate certain chemicals. Kimura et al. (2005) found similar results. In their study, they were able to attribute the removal to the structural makeup of the chemical. The MBR was able to remove the chemicals with more complex structures better than the CAS, while both systems were able to remove the chemicals with simpler structures and neither could remove chlorine-containing chemicals. More recent results from Radjenovic et al. (2009) and Xue et al. (2010) further support these findings. A study of CAS and MBR pilot plants found varying degrees of removal for a suite of pharmaceuticals, with the MBR performing better than the CAS system (Radjenovic et al., 2009). Of note was the complete lack of removal of carbamazepine. Additionally, this study compared two different MBRs, one using ultrafiltration (pore size of 0.05 µm) and one using microfiltration (pore size of 0.4 µm). The comparison of the two found slightly better removal in the microfiltration system, attributed primarily to the higher TSS within the microfiltration system. Xue et al. (2010) monitored a full-scale treatment plant for 19 different micropollutants. The plant contained an anaerobic stage, anoxic stage, and an aerobic stage with a membrane tank following the aerobic stage. Their findings also showed a range of removal efficiencies similar to the previously mentioned studies. Interestingly, they followed the chemicals through the plant and recorded the concentration differences between the different sections. They demonstrated the impact that each process, anaerobic/anoxic/aerobic treatment and membrane separation, in a treatment plant has on the removal of the compounds studied.
2.4 Biomass Concentrator Reactor

There are, however, several drawbacks to the MBR that have limited their inclusion in treatment systems. MBRs require a larger capital investment for the membranes and the infrastructure to handle them. They also require large pumps operating continuously to provide the pressure differential to force the water through the membrane. The intense pressure causes clogging of the membranes, requiring frequent backwashing and regeneration cycles to extend their lifetime, which adds additional running costs.

The BCR is a specific type of MBR that tries to mitigate some of the more costly aspects of the standard MBR system. It still works by separating and concentrating biomass on one side of a membrane and allowing the treated water to permeate through and exit the reactor on the other side. The difference is in how the water is passed through the membrane. Usually, these membrane systems require a vacuum on the permeate side or applied pressure on the mixed liquor side to achieve the results. A gravity-flow BCR, developed by the University of Cincinnati in partnership with EPA-NRMRL (Patent No. 6821425 issued Nov. 23, 2004), uses a porous barrier through which treated water permeates solely under the pressure of gravity, and the suspended solids are retained or concentrated effectively. This technology uses thick membranes (~3 mm) comprised of fused polyethylene beads resulting in a separation mechanism involving deep filtration through tortuous paths having an effective pore size range from 18 to 28 µm. Solids/liquid separation across this membrane is achieved using a head differential of less than 2.5 cm. The typical membrane used in commercial MBR systems has a pore size range from 0.02 to 0.5 µm, much smaller than the BCR membrane. The advantages of the BCR come from allowing gravity instead of pumps to force the water through pores that are much larger, but are still able to retain the biomass because of the significantly lower pressure and tortuous pore structure.

The BCR system was initially developed for treating low concentration gasoline additive contamination in drinking water aquifers. In a study conducted in the field, Zein et al. (2004) were able to biologically treat groundwater contaminated with benzene, toluene, ethylbenzene, and xylenes (BTEX) and a variety
of fuel oxygenates to effluent concentrations lower than 5 µg/L (< 1 µg/L for the BTEX). Of special significance was the fact that this effluent quality was achieved with feed groundwater containing very low concentrations of the contaminants (1-5 mg/L). This study demonstrated the significant feature of biomass retention in treating water containing low concentrations of contaminants. Several other studies were conducted, confirming the designs effectiveness for completely retaining the biomass while reducing the hydrocarbon and oxygenate contamination (Zein et al., 2006; Capodaglio et al., 2010; Medella et al., 2011). The BCR was successfully adapted for treating municipal wastewater by Scott et al. (2013). Municipal wastewater is much more complex and utilizes a much more diverse, faster growing culture than the previous studies. Side-by-side evaluation of a nitrifying system and a nitrifying-denitrifying hybrid system using a synthetic wastewater resulted in a chemical oxygen demand (COD) reduction of over 90% and almost complete nitrification in both systems. In addition, the BCR was able to reduce the total nitrogen in the hybrid system by 67%. Their work served as a preliminary study for this research.
2.5 EE2 Removal Mechanism

EE2 was monitored for removal by the BCR along with several other chemicals. Its removal in wastewater treatment has proven erratic and unpredictable. Removal efficiencies range from 30% - 90% (Esperanza et al., 2004; Lyko et al., 2005; Esperanza et al., 2007; Abegglen et al., 2009; Xue et al., 2010). Esperanza et al. (2007) noted a loss of estrogenic compounds in the presence of biomass that could not be attributed to biological degradation. They postulated that either irreversible adsorption or an abiotic transformation were responsible for the loss. Building on this work, members of the same research group investigated the issue further. Through a series of experiments, they discovered that estrogens undergo an abiotic transformation when in the presence of oxygen and rabbit food, a surrogate for the organic material in municipal wastewater (Marfil-Vega et al., 2010; Marfil-Vega et al., 2011; Marfil-Vega et al., 2012). They proposed oxidative coupling as the mechanism. The dependence on oxygen explains why removal efficiencies for EE2 are unpredictable as aeration methods and biomass composition vary greatly from facility to facility. In this research, an experiment similar to those conducted by Marfil-Vega et al. (2012) was performed to verify their findings using biomass from the reactors instead of rabbit food.
3 Materials and Methods
3.1 Reactor Design
3.1.1 Construction

The three reactors used in this study were, for the most part, the same. The largest difference was the inclusion of a separator between the aerobic and anoxic sections in the two hybrid reactors. Schematics of the reactors can be found in the Appendix, Figures 6.2-1 and 6.2-2. Figure 6.2-1 shows the synthetic reactors, hybrid and aerobic (conventional) while Figure 6.2-2 shows the real wastewater reactor.

The reactors were constructed from a Plexiglas cylinder divided into two sections. The upper section contained a platform to hold the membrane and provide aeration to the upper section. In the hybrid design, the lower section was separated from the upper section using a plastic cone with a ~7 cm hole in the middle. The cone facilitated the downward flow of the mixed liquor and prevented mixing between the aerobic and anoxic sections. In the aerobic design, a second aerator was placed towards the bottom of the lower section for additional aeration and mixing. Ports were mounted in the lower section for the introduction of the wastewater. This was important for the hybrid design because the COD in the wastewater was required for denitrification to occur in the lower section. In both designs, the lower section was shaped as a cone at the very bottom and a port allowed for the pumping of the mixed liquor back up to the top section. In the aerobic system, the pumping was done purely for mixing and was not controlled, but, in the hybrid system, it was controlled and varied as a testing parameter, the recycle ratio.

The anoxic section in the hybrid design was constantly recirculated using an inline pump to ensure complete mixing. The effluent was collected from the permeate side of the membrane and exit the reactor through a port in the sidewall. A vertical tube was connected to this port and the effluent flowed out of the top of this tube, allowing for control of the effluent water level. Because the water flowed only by gravity, the effluent water level also controlled the water level inside the reactor.

A single membrane unit was placed into the top section of each reactor. The membrane unit was a pleated, cylindrical filter available commercially from Porex Corp. (Atlanta, GA). The membrane was
25.1 cm long with a diameter of 15.5 cm and an approximate area of 0.62 m². The membrane was 3-4 mm thick with a pore size between 18-28 µm constructed from packed polyethylene beads. The thickness and construction method produced tortuous-path pores, which were essential for the BCR design. The unit was capped on top with an air vent connected to the side of the reactor to allow any buildup of pressure to be released. The bottom of the unit was secured to the platform, which contained a hole in the middle for the effluent to flow out the side of the reactor to the tube that controlled the water level. The membrane units were cleaned chemically whenever they clogged, first soaking for 24 hours in 10% nitric acid solution followed by a 10% Clorox solution.

3.1.2 Operating Parameters
The reactor volume for each system varied slightly due to minor differences in construction. The aerobic system was 28 L; the synthetic wastewater hybrid system was 27.5 L, 12.5 L for the aerobic section and 15 L for the anoxic section; and the real wastewater hybrid system was 28 L, 14 L for the aerobic section and 14 L for the anoxic section. The aerobic section for the synthetic wastewater hybrid was slightly lower than the anoxic section because the membrane unit occupied approximately 2 L, and this was not accounted for during the construction of the system.

The two synthetic wastewater systems were operated at 6, 15, and 30 day SRTs, controlled by wasting 1/SRT each day (i.e., 1/6th of the volume of the reactor was wasted each day). For the hybrid, the waste volume was divided proportionally between the two sections. The flow rate in for these systems was ~71 L per day, resulting in an HRT of ~9 hours. The recycle ratio of the hybrid system was tested at 3Q, 4Q, and 5Q, Q referring to the influent flow rate.

The real wastewater system was operated at an SRT of 20 days. The waste volume was taken equally from both sections, 0.7 L per day. The influent flow rate was ~72 L per day, resulting in an HRT of ~9 hours. The recycle ratio was tested at 3Q and 5Q.
3.2 Wastewater
3.2.1 Synthetic Wastewater

The wastewater feed was selected to simulate a medium strength wastewater of 300 mg/L of COD and total Kjeldahl nitrogen (TKN) of 40 mg/L. This synthetic wastewater contained a mixture of proteinaceous matter, carbohydrates, starches, fatty acids, ammonium, phosphates, and a myriad of trace macronutrients and micronutrients needed to support microbial growth. To introduce them into the reactors, the components were split into three solutions: organic feed, buffer, and nutrients. The organic feed contained the majority of the COD and nitrogen, the buffer was used to regulate the pH, and the nutrients contained the micronutrients required by the biomass. All three solutions were prepared in concentrated form and then pumped into the reactors alongside a stream of dechlorinated, deionized tap water. The full list of constituents in each solution can be seen in Appendix A, Table 6.0-1.

3.2.2 Chemicals of Concern

Seven COCs were monitored for removal in the BCR. They were caffeine (CAF), carbamazepine (CMP), testosterone (TES), progesterone (PRO), ethinylestradiol (EE2), triclosan (TCS), and nonylphenol (NP). The chemicals and their structures are shown in Table 6-1 in the Appendix. All the chemicals were obtained from Sigma with the exception of NP, which was obtained from Fluka, with greater 97% purity. A stock solution of the COCs was prepared in acetone, which was then added to the buffer solution such that the final influent concentration was ~10 µg/L. Surrogates were used to verify the recovery of the COCs during extraction and processing. Surrogates for CAF (d3-CAF), TES (d3-TES), EE2 (d4-EE2), and NP (13C6-N-P-NP) were used.

In addition to these seven COCs, three additional COCs were included in the influent but were excluded from the results: atrazine (ATR), NP-monoethoxylate (NP1EO), and NP-diethoxylate (NP2EO). Surrogates were used for ATR (d5-ATR) and NP2EO (d2-NP2EO). Problems with quantifying these chemicals using the liquid chromatography method made the results unusable. Atrazine recovery was inconsistent and did not correlate with the atrazine surrogate in the effluent and influent samples. The
calibration was consistent and reproducible, leading to the assumption that some part of the matrix was causing the issue. The NP-ethoxylates were detectable using a "clean" instrument in a matrix of only water and solvent, but once effluent samples were analyzed, the signal to noise for the two compounds was very high, making quantification impossible.

3.2.3 Real Wastewater

The wastewater was taken from a 48” diameter sewer main running under the University of Cincinnati west campus in Cincinnati, Ohio, USA. Upstream of the connection were several restaurants, dorms, classroom buildings, and office buildings as well as runoff collection drains. All of these sources were assumed to have contributed to the sewage stream, though the exact contributions of each are unknown and most likely varied widely over time.

The pumping system consisted of a large storage tank, a primary settling tank, and several pumps. Wastewater was pumped from the sewer main into the storage tank and, subsequently, up to the lab and into the settling tank. Within the settling tank, the sewage was allowed to separate, with the solids and grit settling to the bottom and the fats and oils floating to the surface. A port located just below the middle of the tank was used to supply the sewage to the reactor.

The system was initially run without a buffer; however, the pH in the system varied significantly and was often below 7, which is not optimum for nitrification. A Na₂CO₃ solution (16 mg/L) was added to control the pH by pumping it into the aerobic section at a flow of ∼1 L per day, maintaining a pH between 7.4 and 8. The flow rate was adjusted, as needed, dependent on the wastewater.
3.3 Sampling Procedures

3.3.1 Synthetic BCR Sampling

The synthetic BCRs were sampled as grab samples on the day of analysis, conducted on a weekly basis. Since the influent was broken into 4 components (organic feed, buffer, nutrients, and DI water), the influent samples were collected from the reservoirs and analyzed separately. Additionally, the reservoirs served both systems, so the influent was analyzed once and the data was included in the results for both systems. The effluent samples were collected directly from the effluent line. The mixed liquor was wasted daily and samples were collected from the volume wasted.

3.3.2 COC Sampling

After stable conditions were reached at each SRT and recycle ratio for the synthetic BCRs, samplings were conducted for the COCs. Stable conditions were assumed to occur after operating for at least three times the length of the SRT for changes to the SRT and after three weeks for changes to the recycle ratio. Samples were collected as grab samples from the effluent, the buffer reservoir, and the mixed liquor.

3.3.3 Real Wastewater BCR Sampling

During the start-up period for the reactor, grab samples were collected twice per week from the influent, effluent, and mixed liquors. On day 102, a composite sampler was put in place so that influent and effluent were monitored over 24-hour periods by collecting 20 mL of each stream every 15 minutes. The apparatus was placed in a refrigerator at 4°C to limit further biological activity. Every 24 hours, the sampling bottles were replaced and kept at 4°C until analysis could be performed. Mixed liquor samples were collected as grab samples and analyzed along with the stored composite samples 3 times per week.
3.4 Analytical Methods
3.4.1 Traditional Wastewater Constituent Methods

The reactors were monitored for traditional wastewater constituents. Influent and effluent samples were analyzed for COD, ammonia, total Kjeldahl nitrogen (TKN), and nitrite and nitrate. The reactor mixed liquors was analyzed for TSS and volatile suspended solids (VSS). Because the real wastewater system could potentially contain inorganic or non-biodegradable components, TSS and VSS were also conducted on the influent collected for this system. The methods used for these different analyses were: COD method 8000 by Hach, ammonia method 4500 (Orion 9512HPBNWP Ammonia Electrode), TKN method 4075 by Hach, nitrite and nitrate method 4110B (Dionex DX500 Ion Chromatograph), and TSS/VSS method 2540D (Hach, 1992; APHA, 1998). The mixed liquors were monitored for pH daily using an Oakton WD-35801-00 pH Electrode.

3.4.2 COC Sample Preparation

The samples were prepared for extraction after sampling. The preparation and extraction methods were based on those used previously by the research group (Esperanza et al., 2004; Esperanza et al., 2007; Marfil-Vega et al., 2010; Marfil-Vega et al., 2011; Marfil-Vega et al., 2012). The effluent and buffer samples were concentrated with C-18 SPE and the mixed liquor samples were first extracted with Accelerated Solvent Extractor (ASE) extraction and then cleaned up with alumina.

For C-18 SPE, the effluent samples were filtered through 1.2 µm filter and then 100 mL was poured into serum bottles, in triplicate. The influent samples as well as the calibration curve were prepared by diluting the buffer or COC stock solution with 100 mL of the dechlorinated, deionized tap water fed into the synthetic wastewater systems. The buffer and tap water were first adjusted to a pH of ~7.1. Then, serum bottles were filled with 100 mL of the tap water and 250 µL of the buffer and appropriate volumes of the COC stock solution were added to individual serum bottles. The buffer was extracted in triplicate. For all the samples, 250 µL of surrogate stock solution was added while, for the calibration curve, an appropriate volume was added. A second set of samples, influent and effluent, were prepared because the
C-18 method was slightly different for CAF and needed to be extracted separately from the other COCs. SPE was not done on the CAF calibration curve. An appropriate amount of the COC standard solution was spiked directly into reaction vials for these standards.

For the ASE extraction and alumina cleanup, a volume of mixed liquor was filtered and then the filter and suspended solids were placed into an 11 mL ASE extraction cell. The volume of mixed liquor was 50 mL for the 6-day and 15-day SRT periods and 25 mL for the 30-day and second 15-day SRT periods. Diatomaceous earth and 250 µL of surrogate stock solution were added to the cell and then it was extracted via ASE. After the ASE extraction, alumina cleanup was performed on the extracts.

### 3.4.3 COC Solid Phase Extraction

Solid phase extraction was performed on the COC samples. After sampling and preparing the samples, they were loaded on C-18 SPE cartridges (Supelclean ENV1-18 SPE, Supelco). The cartridges were first rinsed with 10 mL of acetone, 10 mL of methanol, and 10 mL of Super Q water, in order. The samples were loaded by pulling a vacuum on the cartridge manifold and pulling the samples through Teflon tubing at ~ 5 mL per minute. After loading, the serum bottles were rinsed with 30 mL of Super Q water, which was loaded onto the cartridges, and the cartridges were rinsed. The rinse step differed slightly for the CAF and the other COCs. The set of cartridges for CAF were rinsed with 6 mL of Super Q water, while the cartridges for the other COCs were rinsed with 6 mL of freshly prepared 35% (v/v) methanol in Super Q water. After rinsing, the cartridges were dried by pulling a high vacuum on the manifold, allowing air to pass through the cartridges, for 15 minutes. After drying, the COCs were eluted with 6 mL of 50% (v/v) methanol and acetone followed by 6 mL of acetone.

### 3.4.4 Suspended Solids ASE Extraction and Cleanup

After the suspended solids were prepared in the extraction cells, the cells were placed in the ASE and the extraction method was performed. The samples were extracted with 50% (v/v) methanol and acetone under the following conditions: 2 extraction cycles, 100 °C, and 1500 psi. The extracts were evaporated,
redissolved, and then loaded onto alumina cartridges. The alumina cartridges were conditioned with 9 mL of 30% (v/v) methanol in acetone and then 20% (v/v) dichloromethane (DCM) in isooctane. The extracts were redissolved with 0.4 mL of DCM, which was loaded onto the alumina, followed by 1.6 mL of isooctane, which was also loaded onto the alumina. Three rinses of the extract vial were conducted with 0.5 mL of 20% (v/v) DCM in isooctane, each loaded onto the alumina. After all the extracts were loaded, the cartridges were rinsed with 9 mL of hexanes. The COCs were then eluted with 9 mL of 30% (v/v) methanol in acetone.

### 3.4.5 Derivatization

The C-18 SPE extracts and alumina cleanup extracts were evaporated to ~1 mL in a warm water bath with nitrogen gas and transferred to 2 mL reaction vials. Then, along with the CAF calibration curve that was created directly in the reaction vials, they were evaporated completely. The samples were then derivatized by first adding 100 µL of 0.1 M NaHCO3 buffer and 100 µL of 1 mg/mL dansyl chloride in acetone. The vials were mixed and then heated at 60 °C for 15 minutes. Water, 700 µL, was added to each sample and, after mixing, the samples were transferred to autosampler vials for analysis via liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS).

### 3.4.6 LC/MS/MS Method

The COCs were quantified with a Model 1200 series liquid chromatograph connected to a Model 6410 tandem Triple Quadrupole Mass Spectrometer by Agilent (Palo Alto, CA). An analytical column Zorbax Eclipse EBD C18 (2.1 x 50 mm with a particle size of 3.5 µm) was used for analyte separation. The binary pump was operated at 0.4 mL/min with two eluents: (A) water with 5 mM ammonium formate and (B) methanol with 5 mM ammonium formate. The run was 25 minutes long with a 3-minute post run, utilizing a gradient for separation. At the beginning of the run the mobile-phase composition was 90% A and 10% B. The gradients strength was increased to 100% B at 20 minutes and then back to the starting composition, 90% A and 10% B, at 20.1 minutes until the end of the run. The COCs and their surrogates
were detected by tandem mass spectrometry. With the electrospray in positive mode, the compounds produced precursor and product ions for quantitation as well as for confirmation, listed in Table 3.4-1. Electrospray parameters were gas temperature, 350 °C; gas flow, 10 L/min; and nebulizer, 60 psi. The capillary voltage was different for some of the chemicals, so the run divided into 5 segments, shown in Table 3.4-2.

**Table 3.4-1: COC and Surrogate Precursor and Product Ions**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Precursor Ion m/z</th>
<th>Product Ion m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAF</td>
<td>198.2</td>
<td>138.0</td>
</tr>
<tr>
<td>CAF Confirmation</td>
<td>198.2</td>
<td>110.0</td>
</tr>
<tr>
<td>d3-CAF</td>
<td>195.0</td>
<td>138.0</td>
</tr>
<tr>
<td>d3-CAF Confirmation</td>
<td>195.0</td>
<td>110.0</td>
</tr>
<tr>
<td>CMP</td>
<td>237.3</td>
<td>194.3</td>
</tr>
<tr>
<td>CMP Confirmation</td>
<td>237.3</td>
<td>179.2</td>
</tr>
<tr>
<td>TES</td>
<td>289.3</td>
<td>97.1</td>
</tr>
<tr>
<td>TES Confirmation</td>
<td>289.3</td>
<td>109.1</td>
</tr>
<tr>
<td>d3-TES</td>
<td>292.3</td>
<td>97.1</td>
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</tr>
<tr>
<td>PRO</td>
<td>315.3</td>
<td>97.2</td>
</tr>
<tr>
<td>PRO Confirmation</td>
<td>315.3</td>
<td>109.0</td>
</tr>
<tr>
<td>EE2</td>
<td>530.3</td>
<td>171.2</td>
</tr>
<tr>
<td>EE2 Confirmation</td>
<td>530.3</td>
<td>156.2</td>
</tr>
<tr>
<td>d4-EE2</td>
<td>534.3</td>
<td>171.2</td>
</tr>
<tr>
<td>d4-EE2 Confirmation</td>
<td>534.3</td>
<td>156.2</td>
</tr>
<tr>
<td>TCS</td>
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<tr>
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</tr>
<tr>
<td>NP</td>
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<td>NP Confirmation</td>
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</tr>
<tr>
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<tr>
<td>13C6-NP Confirmation</td>
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<tr>
<td>ATR*</td>
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</tr>
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<tr>
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<td>127.2</td>
</tr>
<tr>
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<tr>
<td>NP2EO*</td>
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<td>183.2</td>
</tr>
<tr>
<td>NP2EO Confirmation*</td>
<td>326.3</td>
<td>121.2</td>
</tr>
<tr>
<td>d2-NP2EO*</td>
<td>328.3</td>
<td>185.1</td>
</tr>
</tbody>
</table>

*Difficulty with quantification/reproducibility was experienced for these chemicals
Table 3.4-2: LC/MS/MS Run Segments and Capillary Voltage

<table>
<thead>
<tr>
<th>Segment</th>
<th>Start Time (minutes)</th>
<th>COCs Analyzed</th>
<th>Capillary Voltage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>CAF</td>
<td>3500</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>CMP ATR</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>10.5</td>
<td>TES PRO</td>
<td>2000</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>NP1EO NP2EO EE2</td>
<td>3000</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>TCS NP</td>
<td>4000</td>
</tr>
<tr>
<td>Post Run</td>
<td>20.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3.5 EE2 Removal Mechanism Experiment

An investigation into the removal mechanism for estrogens, specifically EE2, was conducted. An experiment similar to those performed by Marfil-Vega et al. (2012) was done, involving EE2 and biomass from the hybrid reactor. A series of serum bottles were filled with effluent from the reactor, which had been spiked with EE2, and then biomass from the reactor was added. The experiment was conducted under aerobic and anaerobic conditions to determine the role of oxygen in the removal. An identical set of serum bottles was spiked with sodium azide to inhibit biological activity to determine if any non-biological removal was occurring.

The effluent was collected from the reactor and split into two portions. One portion was left mixing open to the atmosphere while the second was bubbled with nitrogen gas to remove any oxygen and then placed in an anaerobic chamber. The wasted biomass from the reactor was collected and combined, then split into two portions. Again, one portion was left mixing open to the atmosphere while the second was bubbled with nitrogen gas and placed in an anaerobic chamber. A solution of EE2 was prepared and spiked into the effluents and allowed to mix. The effluents and biomass were then placed into the serum vials, 90 mL of effluent and 10 mL of biomass. They were capped and tumbled end-over-end for 30 minutes before the initial samples were sacrificed. Background controls were created from the effluent and biomass before the addition of the EE2 into the effluent to determine how much EE2 was present without the addition. Since the reactor from which the effluent and biomass were collected was actively being fed EE2, it was necessary to determine the background levels. In total, four combinations were tested in triplicate in addition to the background control: aerobic, aerobic killed control, anoxic, and anoxic killed control. Bottles were sacrificed at 0, 2, 4, 8, and 12 days.
4 Results and Discussion
4.1 Evaluation of an aerobic, gravity-flow membrane bioreactor for removing chemicals of concern from a synthetic wastewater

4.1.1 Introduction

Micro-pollutant chemicals are increasingly becoming a concern in the environment. These chemicals of concern (COCs) are being found widely distributed in water and wastewater systems (Heberer, 2002a; Heberer, 2002b; Heberer et al., 2002; Kimura et al., 2005, Xue et al., 2010). COCs include pharmaceuticals, hormones, pesticides, and chemicals from everyday personal-care products. Many of them have known health effects even when present at very low concentrations, such as hormones; others do not have known dangers at low concentrations, but their increasing ubiquity raises concerns, such as caffeine and pharmaceuticals. Once in the environment, they pose a threat to plants and wildlife as well as the possibility of being introduced into the drinking water supply. In addition, many of these chemicals are persistent and can accumulate over time in soils and sediments, posing an even greater combined risk if released at a future time.

Wastewater treatment plants have been shown to be a major source of these chemicals. Conventional activated sludge (CAS) treatment has not proven to be an effective barrier to these types of chemicals (Heberer, 2002a; Heberer, 2002b; Kimura et al., 2005; Radjenovic et al., 2009). CAS treatment plants are designed to remove major constituents, carbon and nutrient sources, in wastewater that may affect the immediate health of receiving waters, not the low concentration chemicals. Reliance on biomass settling, short solids retention times (SRT), lack of control over solids and hydraulic retention times (HRT), and potential loss of biomass combine to select for a culture that will not target many COCs (Cicek et al., 2001; LeClech, 2010).

The membrane bioreactor (MBR) is an alternative treatment process that overcomes some of these limitations and has shown promising results for micro-pollutants. MBRs offer increased control over the operating parameters of a treatment plant, in particular the total control of the solids and biomass. They
eliminate the need for a settling tank, allow for longer SRTs, complete biomass retention, and flexibility in operation. This control increases the diversity of the biomass, allowing specialized cultures to develop and target the low concentration, more recalcitrant COCs. Several studies of MBRs have shown increased removal of COCs compared to CAS (de Wever et al., 2007; Lyko et al., 2005; Radjenovic et al., 2009; Xue et al., 2010). MBRs also have much better traditional effluent quality when compared to CAS (Rosenberger et al., 2002). The advantages of the MBR come at a cost, however, as they are typically much more expensive to both construct and operate.

The gravity flow biomass concentrator reactor (BCR), developed and patented by the University of Cincinnati and the U. S. Environmental Protection Agency (Patent No. 6821425 issued November 23, 2004), is a technology designed to reduce the cost of using an MBR system. Operating at less than 2.5 cm of head, the BCR uses a commercially available membrane to separate the biomass from the treated effluent without the need for effluent side vacuum pumps. The membrane unit is 3-4 mm thick with a pore size between 18-28 µm constructed from packed polyethylene beads. The tortuous path pores allow effluent to pass easily through while retaining the solids inside the reactor. The BCR has been proven effective in treating groundwater contaminated with low concentration gasoline hydrocarbons and additives, such as methyl-t-butyl ether (Zein et al., 2004; Zein et al., 2006; Capodaglio et al., 2010; Medella et al., 2011). Scott et al. (2013) conducted a preliminary study on the ability of the BCR to treat a complex synthetic wastewater. They were able to reduce the influent chemical oxygen demand (COD) by more than 90% and almost completely oxidize the influent ammonia. This study expands upon their work on the aerobic BCR system and, in addition, evaluates its ability to remove a suite of COCs at several SRTs.

4.1.2 Materials and Methods
4.1.2.1 Reactor Design

A detailed description of the BCR system can be found in Scott et al. (2013). The aerobic system from their work was used in this research, a schematic of which can be found in the Appendix, Figure 6.2-1.
The membrane unit was a pleated, cylindrical filter available commercially from Porex Corp. (Atlanta, GA). The membrane was 25.1 cm long with a diameter of 15.5 cm and an approximate area of 0.62 m$^2$. The reactor volume was 28 L. The system was fed a synthetic wastewater, shown in the Appendix, Table 6-2, at 71 L/day, resulting in a hydraulic retention time of ~9 hours. Concentrated mixtures of the wastewater components (substrate, nutrients, and buffer) were pumped into the reactor alongside a stream of dechlorinated and deionized tap water.

The system was operated for a total of 956 days, the first 350 of which are discussed in Scott et al. (2013). Their work covers the startup and operation of the system at an SRT of 6 and 15 days. The system was operated at an SRT of 30 days from day 350 until operation ended on day 956. The SRT was controlled through daily wasting of a portion of the mixed liquor equal to 1/SRT, i.e., 1/30th for the 30-day SRT. For the 30-day SRT period, the target COD was increased to ~300 mg/L by increasing the flow of the substrate, up from ~200 mg/L in the previous work.

4.1.2.2 Chemicals of Concern

The COCs investigated were caffeine (CAF), carbamazepine (CMP), ethinylestradiol (EE2), testosterone (TES), progesterone (PRO), triclosan (TCS), and nonylphenol (NP). All chemicals had a purity of 97% or greater and were obtained from Sigma with the exception of NP, which was purchased from Fluka. The COCs were dissolved in acetone to create a stock solution and then added to the buffer solution such that the final influent concentration of each compound was targeted at 10 µg/L.

4.1.2.3 Sample Collection and Analytical Methods

During the 30-day SRT, the reactor was sampled weekly for COD, ammonia, and total Kjeldahl nitrogen (TKN) in the influent and effluent, nitrite and nitrate in the effluent, and total and volatile suspended solids (TSS and VSS) of the mixed liquor. The samples were collected as grab samples on the day of analysis. The analysis was conducted in triplicate using the following methods: COD method 8000 by Hach, ammonia method 4500 (Orion 9512HPBNWP Ammonia Electrode), nitrite and nitrate method
4110B (Dionex DX 500 Ion Chromatograph), TKN method 4075 by Hach, and TSS/VSS method 2540D (Hach, 1992; APHA, 1998). The mixed liquor was also analyzed daily for pH using an Oakton WD-35801-00 pH Electrode.

COC samples were collected during the 6, 15, and 30-day SRT periods. Three different samplings were conducted during the 30-day SRT to determine if there were any changes over a long period of operation. Samplings were conducted once per week over several weeks after the reactor stabilized at a given SRT. Stability was defined as occurring after at least three times the length of the SRT, i.e., after 90 days for the 30-day SRT. Samples were collected from the effluent and from the influent buffer, which contained the concentrated COCs. The COCs were prepared by solid phase extraction (SPE) with C-18 and analyzed by LC/MS/MS, Agilent 1200 series LC coupled to a 6410 triple quad MS. Biomass samples were also collected, extracted using a Dionex ASE 200 Accelerated Solvent Extraction (ASE), and prepared by SPE with alumina to assess any adsorption to the solids. Detailed information on the sample preparation, SPE, solids ASE extraction, and LC/MS/MS methods can be found in the supplementary information.

4.1.3 Results and Discussion

The reactor performance results from the 6-day and 15-day SRT periods relative to removal of conventional parameters can be found elsewhere (Scott et al., 2013). The reactor performance during the 30-day SRT period and the removal of COCs during all three periods is discussed below.

4.1.3.1 Suspended Solids

Figure 4.1-1 shows the TSS in the reactor mixed liquor and the total solids retained in the reactor. After an initial period of instability with large increases and decreases in the biomass concentration prior to day 500, the TSS increased gradually from 3000 mg/L to around 3600 mg/L. This increase is only marginal and quite unexpected. After increasing the SRT from 6 to 15 days, the TSS concentration more than doubled from less than 1500 mg/L to around 3000 mg/L (Scott et al., 2013). It was expected that this trend would continue as the SRT was increased to 30 days. Although it is not clear what caused this
limited increase in reactor solids, a possible explanation is a shift in the culture toward higher order organisms that graze on the bacteria and limit increased growth.

The VSS was roughly 93% of the TSS, which is higher than normal. The higher VSS may be a result of the synthetic influent, which contained less inorganic material than is typical in a municipal wastewater. For the duration of the testing period, the TSS of the effluent was consistently below 1 mg/L, indicating complete retention of the biomass by the membrane.

4.1.3.2 COD and Nitrogen Removal

The influent and effluent COD over the 30-day SRT period is shown in Figure 4.1-2. The influent was ~300 mg/L while the effluent was consistently less than 30 mg/L with an average of 8 mg/L, resulting in an average removal rate of 97.5%. The effluent quality was comparable to the results obtained during the 6 and 15-day SRT, but, because the influent concentration was significantly higher, the removal rate increased (Scott et al., 2013). The consistency of the effluent despite the increase in COD indicates that influent concentration does not greatly influence the effluent quality, at least within the range of a medium strength municipal wastewater. The high removal rate is not unexpected as typical values for an
MBR range from 85-99% (Kimura et al., 2008). Furthermore, the wastewater was a synthetic mixture with very little non-biodegradable COD.

**Figure 4.1-2: Influent and effluent COD for the 30-day SRT.**

The observed biomass yield was determined from a plot of cumulative VSS wasted from the reactor versus the cumulative COD removed, as shown in Figure 4.1-3. The slope of the resulting line is equivalent to the observed yield. The observed yield was 0.15 g VSS/g COD. This value is low compared to a typical nitrification only treatment system, which is around 0.4 g VSS/g COD, and is likely due in part to the long SRT (Metcalf and Eddy, 2003). Cicke et al. (2001) showed a decrease in yield as the SRT increased; although they still reported a yield of 0.269 g VSS/g COD, for an SRT of 30 days.

**Figure 4.1-3: Biomass yield for the 30-day SRT.**
The low yield agrees with the low TSS data previously discussed and further supports some limitation to the biomass growth.

Nitrogen was monitored in several forms: ammonia, TKN, nitrite, and nitrate. Figure 4.1-4 presents the ammonia and TKN experimental results while Figure 4.1-5 presents the nitrate results. The influent NH$_3$-N was 26 mg/L while the influent TKN was 35 mg/L. The influent did not contain nitrite or nitrate and they were only monitored in the effluent, as products of nitrification. The BCR was able to remove the NH$_3$-N to below 0.2 mg/L, except for one instance around day 550 due to an upset of the reactor because the membrane was clogged and subsequently replaced. The effluent TKN was between 1-3 mg/L. These results suggest that complete or nearly complete nitrification was achieved, as was the case during the two previous SRT periods (Scott et al., 2013). The concentration of nitrite in the effluent was always below 0.1 mg/L, so only the effluent nitrate results are reported in Figure 4.1-5. The nitrate results closely match the theoretical production of nitrate in a nitrifying system based on the

![Figure 4.1-4: Influent and effluent ammonia and TKN for the 30-day SRT.](image-url)
influent TKN. The theoretical production was calculated using Eqn. 4.1-1, where all the forms of nitrogen were expressed as N; $P_x$ and $Q$ represent the daily wasted biomass and daily flow rate, respectively; and 0.12 g N/g VSS is based on TKN digestion of the biomass (Metcalf and Eddy, 2003):

$$NO_x = TKN_{inf} - NH_{3,eff} - 0.12 \times \frac{P_x}{Q}$$

(4.1-1)

From these results, the total nitrogen (TN) removal was calculated, using Eqn. 4.1-2, where all the forms of nitrogen were expressed as N:

$$\%N_{removed} = \frac{(TKN_{inf} - NH_{3,eff} - NO_{3,eff} - NO_{2,eff})}{TKN_{inf}} \times 100$$

(4.1-2)

The average TN removal was 24%, which is expected from conventional treatment systems. The only vector for removal in conventional treatment is biomass production and subsequent removal through wasting. With increasing sludge age, the utilization of nitrogen for biomass production decreases, resulting in low TN removal at high sludge ages. It is also in line with the results of Scott et al. (2013). They found a decrease in TN removal with an increase in SRT from 6 to 15 days. Increasing the SRT to 30 days would be expected to lower TN even further.
Table 4.1 summarizes the experimental results and offers a comparison to other membrane filtration systems in the literature. The COD and TN results reported here compare favorably with MBRs from the literature and further support the claims of Scott et al. (2013) on the capabilities of the BCR.

### Table 4.1-1: Comparison of the Aerobic BCR with Other MBR Results.

<table>
<thead>
<tr>
<th></th>
<th>Feed</th>
<th>HRT (h)</th>
<th>SRT (d)</th>
<th>COD Removal (%)</th>
<th>TN Removal (%)</th>
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</thead>
<tbody>
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<td>Aerobic BCR</td>
<td>Synthetic</td>
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<td>Scott et al., (2013) - Aerobic BCR</td>
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<td></td>
<td></td>
<td></td>
<td>15</td>
<td>93</td>
<td>43</td>
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<tr>
<td>Ueda and Hata, (1999)</td>
<td>Municipal wastewater</td>
<td>13.4</td>
<td>72</td>
<td>93 (TOC)</td>
<td>79</td>
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<td>Rosenberger et al., (2002)</td>
<td>Municipal wastewater</td>
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<td>N/A</td>
<td>90-95</td>
<td>80-83</td>
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<td>Patel et al., (2005)</td>
<td>Synthetic</td>
<td>12</td>
<td>20</td>
<td>99</td>
<td>77</td>
</tr>
<tr>
<td>Kimura et al., (2008)</td>
<td>Municipal wastewater</td>
<td>4.7</td>
<td>29</td>
<td>85 (TOC)</td>
<td>77</td>
</tr>
</tbody>
</table>

#### 4.1.3.3 Chemicals of Concern Removal

The removal efficiency for each of the COCs is shown in Figure 4.1-6. The figure shows the removals for the 6 and 15-day SRTs as well as the three different sampling events for the 30-day SRT. Removal efficiencies were consistently above 90% for all the compounds but CMP, which is a notoriously recalcitrant chemical. The biomass was extracted to determine if any of the chemicals were completely or partially removed by sorption onto the solids. Less the 1% of the daily chemical load was found on the wasted biomass (data not shown). Thus, adsorption was not a major factor in removal.
CAF was reduced by 90%, 95%, and 99% at 6, 15, and 30-day SRTs, respectively. The increase in removal appears linked to the SRT and/or TSS concentration, suggesting biological transformation. Others have found similar results, reporting over 99% removal due to biological degradation under various treatment conditions (Miao et al., 2005; Thomas and Foster, 2005; Xue et al. 2010; Sui et al., 2010; Goa et al., 2012). CAF is not particularly difficult to eliminate; however, its ubiquity makes it an attractive marker for human activity; thus, it is commonly studied alongside other COCs.

CMP was removed by 50% for the 6 and 15-day SRTs, but was not removed at all for the 30-day SRTs. The 50% removal was unexpected, given the difficulty others have observed in removing it from wastewater (Heberer et al., 2002; Radjenovic et al., 2009; Xue et al., 2010; Sui et al., 2010; Fernandex-Fontaina et al., 2012). In almost all cases reported in the literature, CMP was not removed at all. While biological removal cannot be definitively confirmed or refuted in this case, it must be conceded that it is unlikely. CMP was not found adsorbed to the biomass, which is supported by other similar findings (Joss et al., 2005; Miao et al., 2005). However, it was found to adsorb to the membrane. A 3 cm square of the membrane was placed in a solution of 10 µg/L CMP. After four days, the concentration was reduced by
30%, demonstrating some capacity for adsorption. The increase in effluent concentration observed during the 30-day SRT likely occurred because the membrane reached a saturation point and the regeneration procedure was unable to removed the CMP from the membrane surface, allowing it to accumulate throughout the experiment.

TES and PRO were removed to greater than 99% under all conditions, which is consistent with other published results (Esperanza et al., 2004; Lee et al, 2004; Esperanza et al., 2007; Liu et al., 2009). Layton et al. (2000) found that TES is mineralized by biomass from wastewater treatment plants, while Chang et al. (2008) found nearly complete removal of a suite of androgens and progestogens and Fan et al. (2011) concluded that androgens and progestogens are removed by biodegradation. Based on the literature and lack of adsorption to the biomass, biological transformation and degradation are the likeliest mechanisms for removal for both chemicals.

EE2 was removed by >94% increasing to >98% as the SRT increased. Many studies have been performed on EE2 with mixed results, removal rates ranging from 30% - 90% (Esperanza et al., 2004; Lyko et al., 2005; Esperanza et al., 2007; Abegglen et al., 2009; Xue et al., 2010). The studies all have slight differences in the treatment stages and conditions, i.e., aerobic, anoxic, anaerobic, and there is not a clear trend explaining the variations. Through a series of experiments, the inconsistent removal was investigated and it was determined that estrogens undergo an abiotic transformation via oxidative coupling (Marfil-Vega et al., 2010; Marfil-Vega et al., 2011; Marfil-Vega et al., 2012). The variation in the treatment conditions and the levels of oxygenation likely explains the inconsistency of the reported removal rates. In addition, the transformation does not exclude removal by other mechanisms, such as biological degradation or solids adsorption. Since the system in this study was completely aerobic, it maximized the potential for removal. The increased removal observed with increasing SRT could be attributed to any of the removal mechanisms. The increase in TSS provides more abiotic transformation
enzymatic sites and more biomass for degradation, though removal through adsorption was not found, as EE2 was not present in the biomass.

TCS was reduced by 98% while NP was reduced by 99%. TCS was found to be removed by >94% in the literature (McAvoy et al., 2002; Singer et al., 2002; Thomas and Foster, 2005). Reported results for NP are variable, ranging from 75 – 94% (Esperanza et al., 2004; Lee et al, 2004; Lyko et al., 2005; Liu et al., 2009). Many of the studies involved sampling from actual treatment plants, where the influents are variable and treatment processes differ. Also, they likely contained large quantities of NP-ethoxylates, which can degrade to NP during anaerobic treatment. For both chemicals, the removal is attributed to a combination of biological removal and some adsorption to the biomass. In this study, no NP and TCS was found in the biomass extract, indicating only biological removal was involved or that adsorption was not reversible by the extraction method used.

In most of the cases cited above, the COC removal performance was not significantly tied to the mechanism of treatment (CAS v. MBR). CAF, TES, and PRO are eliminated under almost all conditions, while the opposite is true for CMP, not eliminated under any condition. The BCR was slightly better than a CAS process for removing TCS and NP and comparable to data reported for other MBR systems. Removal efficiency for EE2 is heavily dependent on aeration conditions, which vary widely and confound any attempts at a comparison. Unlike reports in the literature, the COCs were not found to significantly attach to the solids. A possible explanation for the low attachment is the relatively high concentration of the COCs in the influent. They are usually detected and reported between 1 ng/L and 1 µg/L, while in this study 10 µg/L was used. Aside from slight increases in the removal of CAF and EE2, increased SRT did not play a significant role in the removal effectiveness for COCs. Additionally, no differences were observed among the three 30-day SRT sampling events. The system was not able to adapt to remove CMP over the extended period of time and the remaining COCs were consistently removed by over 99% during all three events.
4.1.4 Conclusion

The BCR was demonstrated to operate successfully at a 30-day SRT. Under aerobic conditions, COD and ammonia were reduced by ~98%, surpassing the CAS process and matching performance in other MBRs. Seven COCs were monitored at 6, 15, and 30-day SRTs. TES, PRO, TCS, and NP were mostly eliminated with removal efficiencies exceeding 98%, while CAF and EE2 had slightly lower removal, between 90%-99%, and CMP was not eliminated at all. Only a marginal increase in CAF and EE2 removal (<10%) was seen by increasing the SRT from 6 to 30 days and could have been caused by many factors: better acclimation of the biomass, increased TSS, and/or diversification of the microbial culture. EE2 in particular was likely removed better because of the increase in TSS coupled with the aerobic only conditions. In most cases, the BCR matched or exceeded reported results from CAS and MBR system.
4.2 Evaluation of an aerobic-anoxic gravity flow membrane bioreactor for removing chemicals of concern from a synthetic wastewater

4.2.1 Introduction

Wastewater treatment has reached a level of maturity where removing just the gross contaminants, chemical oxygen demand (COD), nitrogen, and phosphorus, is no longer enough to protect the environment and the population. The proliferation of synthetically prepared products including pharmaceutical drugs or chemical residues has produced waste streams with hundreds of micropollutants. Many of these chemicals of concern (COCs), pharmaceuticals, hormones, pesticides, and chemicals from everyday products, are reaching waters used for drinking water (Heberer, 2002a; Heberer, 2002b; Heberer et al., 2002; Kimura et al., 2005; Xue et al., 2010). One of the largest sources for this contamination is the municipal wastewater stream. Traditional wastewater treatment, consisting of the conventional activated sludge (CAS) process, has proven unsuitable for the task of removing these chemicals (Heberer, 2002a; Heberer, 2002b; Kimura et al., 2005; Radjenovic et al., 2009). The effluents from these traditional systems contain very low, yet biologically and chemically significant amounts of COCs. A new process or treatment plant design is needed to be able to control this growing group of chemicals.

A technology that has shown promise for attenuating these chemicals is the membrane bioreactor, or MBR. Membranes can produce excellent effluent quality, surpassing the CAS process in terms of COD, biochemical oxygen demand (BOD), ammonia, and total and volatile suspended solids (TSS and VSS) (De Wever et al., 2007; Mallevalle et al., 1996; Rosenberger et al., 2002; Radjenovic et al., 2009). MBRs can achieve effluent suspended solids of less than 1 mg/L and up to 99% COD removal, much better removal than a CAS system, which is typically limited to 95% COD removal and fluctuating effluent suspended solids (Le-Clech, 2010). The additional control over operating parameters provided by incorporating membrane filtration produces higher quality effluent, but has also limited the adoption of MBRs because the capital and operational costs for such systems are much higher. A specific type of MBR, the Biomass Concentrator Reactor (BCR), was designed to mitigate some of the more costly aspects of the standard MBR system.
A gravity-flow BCR, developed by the University of Cincinnati in partnership with EPA-NRMRL (Patent No. 6821425 issued Nov. 23, 2004), uses a porous barrier through which treated water permeates solely under the pressure of gravity and the suspended solids are retained or concentrated effectively. This technology uses thick membranes (~3 mm) comprised of fused polyethylene beads resulting in a separation mechanism involving deep filtration through tortuous paths having an effective pore size range from 18 to 28 µm. Solids/liquid separation across this membrane is achieved using a head differential of less than 2.5 cm. The typical membrane used in commercial MBR systems has a pore size range from 0.02 to 0.5 µm, much smaller than the BCR membrane. The advantages of the BCR come from allowing gravity instead of pumps to force the water through pores that are much larger, but are still able to retain the biomass because of the significantly lower pressure and tortuous pore structure.

Previous work using the BCR involved treating groundwater contaminated with gasoline hydrocarbons and oxygenates, specifically methyl-t-butyl ether (Zein et al., 2004; Zein et al., 2006; Capodaglio et al., 2010; Medella et al., 2011). In a study conducted in the field, Zein et al. (2004) were able to biologically treat groundwater contaminated with benzene, toluene, ethylbenzene, and xylenes (BTEX) and a variety of fuel oxygenates to effluent concentrations lower than 5 µg/L (< 1 µg/L for the BTEX). Of special significance was the fact that this effluent quality was achieved with feed groundwater containing very low concentrations of the contaminants (1-5 mg/L). This study demonstrated the significant feature of biomass retention in treating water containing low concentrations of contaminants. The BCR system was adapted to treat municipal wastewater, and preliminary results showed promise for COD and nitrogen removal as well as solids retention (Scott et al., 2013). This research builds on the results of the hybrid aerobic/anoxic system and, in addition, examines the removal of a suite of COCs over several operational parameters.
4.2.2 Materials and Methods

4.2.2.1 Reactor

A detailed description of the aerobic/anoxic reactor can be found in Scott et al. (2013) and is shown in the Appendix, Figure 6.2-1. In brief, the reactor was constructed from a Plexiglas cylinder with an upper and lower section separated by a cone. The upper section contained the membrane unit and was aerated while the lower section was operated under anoxic conditions with the cone providing separation. The anoxic section was continuously mixed with a recirculating pump to ensure completely mixed conditions. The membrane unit consisted of a pleated, cylindrical filter available from Porex Corp. (Atlanta, GA). The membrane was 25.1 cm long with a diameter of 15.5 cm and an approximate area of 0.62 m².

Scott et al. (2013) operated the system at a solids retention time (SRT) of 6 and 15 days for Runs 1 and 2, respectively, for a total of 350 days. Operation continued at an SRT of 30 days (Run 3) and then at 15 days again (Run 4) in this study, spanning days 350 to 1,230. The reactor was approximately 27.5 L, 12.5 L for the aerated section and 15 L for the anoxic section, and had a daily flow rate of approximately 71 L with a hydraulic retention time of ~9 hours. The SRT was maintained by wasting an appropriate volume (1/SRT) of mixed liquor each day, divided proportionally between the two sections. The influent was a mix of concentrated wastewater components, detailed in the Appendix, Table 6.1-2, consisting of substrate, nutrients, and a buffer, which were fed alongside a stream of dechlorinated and deionized tap water. Adjustments to the flow rates of these components at the start of the 30-day SRT increased the target COD of the influent to 300 mg/L, up from 200 mg/L used in the previous study.

4.2.2.2 Chemicals of Concern

Seven COCs were monitored for removal in the BCR. They were caffeine (CAF), carbamazepine (CMP), testosterone (TES), progesterone (PRO), ethinylestradiol (EE2), triclosan (TCS), and nonylphenol (NP). All the chemicals were obtained from Sigma with the exception of NP, which was obtained from Fluka, and had a purity greater 97%. A stock solution of the COCs was prepared in acetone, which was then added to the buffer solution such that the final influent concentration was ~10 µg/L.
4.2.2.3 Sample Collection and Analytical Methods

Samples were collected from the influent reservoirs, the effluent, and the mixed liquor on a weekly basis and analyzed for COD, ammonia, total Kjeldahl nitrogen (TKN), nitrite and nitrate, and total and volatile suspended solids (TSS and VSS). The methods used were: COD method 8000 by Hach, ammonia method 4500 (Orion 9512HPBNWP Ammonia Electrode), TKN method 4075 by Hach, nitrite and nitrate method 4110B (Dionex DX500 Ion Chromatograph), and TSS/VSS method 2540D (Hach, 1992; APHA, 1998). Samples were collected as grab samples just prior to analysis and analyzed in triplicate. The pH was also measured daily in the mixed liquor of both sections using an Oakton WD-35801-00 pH Electrode.

After stable conditions were reached at each SRT and recycle ratio, samplings were conducted for the COCs. Stable conditions were assumed to occur after operating for at least three times the length of the SRT for changes to the SRT and after three weeks for changes to the recycle ratio. Samples were collected from the effluent, the buffer reservoir, and the mixed liquor. The effluent and buffer samples were concentrated via solid phase extraction (SPE) with C-18 and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), Agilent 1200 series LC and 6410 triple quad MS. The mixed liquor samples were extracted via Accelerated Solvent Extraction (ASE), prepared via SPE with alumina, and analyzed by LC/MS/MS. Detailed information on the sample collection and preparation, SPE, ASE, and LC/MS/MS methods can be found in the supplementary material.

4.2.3 Results and Discussion

The reactor performance results for Runs 1 and 2 are presented elsewhere (Scott et al., 2013). Results for Runs 3 and 4 are presented below as well as the COC removal results for all 4 periods. Runs 3 and 4 were broken into 3 sections with differing recycle ratios. In the figures, these are delineated with vertical dotted lines with the 3Q section at the start of each SRT, followed by 4Q and 5Q.
4.2.3.1 Suspended Solids

The mixed liquor suspended solids are presented in Figure 4.2-1. The TSS increased significantly during Run 3, up from ~3.5 g in the aerobic section and ~2.5 g in the anoxic section during Run 2 (Scott et al., 2013). The large increase was anticipated because of the longer SRT, but is also likely due to the increase in the target influent COD. When the SRT was changed back to 15 days, the TSS decreased, but did not return to the previous concentrations due to the increased COD. The concentration in the anoxic section was always lower than the aerobic section because the influent wastewater first entered the anoxic section. The change in recycle ratios caused a redistribution of the biomass during Run 3, decreasing the concentration of the aerobic section and increasing the concentration of the anoxic section. Run 4 was much more volatile, particularly immediately after the change in SRT, and the recycle ratio did not have a noticeable effect.

![Figure 4.2-1: TSS (a) in the mixed liquor and Total Suspended Solids (b) in the reactor for Run 3 (30-day SRT) and Run 4 (15-day SRT).](image)

The VSS was roughly 85-90% of the TSS concentration. Due to the lack of inorganic and non-biodegradable solids in the synthetic influent, this ratio is higher than expected in an actual wastewater
system. For the duration of testing, the TSS in the effluent was negligible, <1 mg/L, indicating the membrane was able to completely retain the solids inside the reactor.

### 4.2.3.2 Chemical Oxygen Demand and Nitrogen Removal

The influent and effluent COD results for Runs 3 and 4 are shown in Figure 4.2-2. The BCR was able to reduce the influent COD from ~300 mg/L to an average of ~6 mg/L, with the effluent never fluctuating above 20 mg/L. The removal rate was over 98%. While the removal rate increased due to the increased influent concentration, the effluent concentrations are similar to those found during Runs 1 and 2, indicating the increase in COD had little effect on the BCR (Scott et al., 2013). The high removal is not unexpected as the influent lacked any inorganic or non-biodegradable components. Using an almost identical synthetic influent, ~93% removal was achieved in two pilot scale CAS systems (Esperanza et al., 2004; Esperanza et al., 2007). Also, COD removals in MBRs typically range from 85% to 99% (Kimura et al., 2008). Little difference in performance was observed between the two SRTs and the change in the recycle ratios had little effect.

The observed biomass yield was determined from the cumulative VSS wasted and the cumulative COD removed, as shown in Figure 4.2-3. The slope of the resulting line is the observed yield. For Run 3 the yield was 0.23 g VSS/g COD while for Run 4 it was 0.31 g VSS/g COD. Yield for denitrifying systems
falls between 0.3 and 0.4 g VSS/g COD (Metcalf and Eddy, 2003). Due to the high SRTs, Run 3 is well below this range and Run 4 is at the low end. Cicek et al. (2001) examined the effect the SRT had on the performance of an MBR and found that as the SRT increased the yield decreased. Their data were for a nitrifying only system, so their results are higher, but the overall trends were similar.

![Figure 4.2-3: Observed biomass yield for Run 3 (30-Day SRT) and Run 4 (15-Day SRT).](image)

**Figure 4.2-3:** Observed biomass yield for Run 3 (30-Day SRT) and Run 4 (15-Day SRT).

![Figure 4.2-4: Influent and effluent ammonia and TKN for Run 3 (30-day SRT) and Run 4 (15-day SRT).](image)

**Figure 4.2-4:** Influent and effluent ammonia and TKN for Run 3 (30-day SRT) and Run 4 (15-day SRT).
The influent and effluent were monitored for ammonia and TKN while the effluent was also monitored for nitrite and nitrate during Runs 3 and 4. The ammonia and TKN results are shown in Figure 4.2-4. The influent contained, on average, 28 mg/L of NH$_3$-N and 33 mg/L TKN, while the effluent had 0.1 mg/L of NH$_3$-N and 1.8 mg/L of TKN. The effluent never contained more than 0.3 mg/L of NH$_3$-N and 2 mg/L of TKN. The consistency of the ammonia and TKN removal indicate nearly complete nitrification occurred. The nitrate concentration in the effluent is shown in Figure 4.2-5. Nitrite was never detected above 0.1 mg/L; thus, only nitrate data are presented. The nitrate is compared to the theoretical production based on the influent TKN and the nitrification-denitrification process. The theoretical production was calculated using Eqns. 4.2-1 and 4.2-2, where all the forms of nitrogen were expressed as N; P, Q, and IR represent the daily wasted biomass, daily flow rate, and internal recycle ratio, respectively; and 0.12 g N/g VSS is based on TKN digestion of the biomass (Metcalf and Eddy, 2003):

$$NO_x = TKN_{inf} - NH_3_{eff} - 0.12 \times \frac{P_x}{Q}$$  \hspace{1cm} (4.2-1)

$$IR = \frac{NO_x}{NH_3_{eff}} - 1$$  \hspace{1cm} (4.2-2)
The measured values generally agree with the theoretical values except for the measurements from day 650 to day 780. The pump recirculating the anoxic section began to fail resulting in diminished mixing and improper nitrate removal. The pump was not suspected immediately, so other possible causes for the problem were investigated first, resulting in the long disruption period.

From these measurements, the total nitrogen removed was determined, using Eqn. 4.2-3:

$$\% N_{\text{removed}} = \left( \frac{T KN_{\text{inf}} - NH_3_{\text{eff}} - NO_3_{\text{eff}} - NO_2_{\text{eff}}}{TKN_{\text{inf}}} \right) \times 100$$  \hspace{1cm} (4.2-3)

Three removals were calculated for each run, representing the 3 recycle ratios tested. For Run 3, the removals were 78, 82, and 85% for 3Q, 4Q, and 5Q, respectively. For Run 4, they were 84, 89, and 91% for 3Q, 4Q, and 5Q, respectively. In general, the TN removal was excellent and behaved as expected in a denitrifying system. The removal for Run 4 was higher than for Run 3 because of the increased incorporation of nitrogen into the biomass due to a higher growth rate. There was a marginal increase with higher recycle ratio.

The operating conditions and experimental results are summarized in Table 4.2-1. Also included in the table are some reported results of other MBRs shown for comparison. The results compare well with other MBRs and support the claims of Scott et al. (2013) on the capabilities of the BCR technology.
Table 4.2-1: Comparison of the Hybrid BCR with Other MBR Results.

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<tr>
<th>Feed</th>
<th>HRT (h)</th>
<th>SRT (d)</th>
<th>COD Removal (%)</th>
<th>TN Removal (%)</th>
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<td></td>
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<td>72</td>
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<td>Patel et al., (2005) Synthetic</td>
<td>12</td>
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<td>4.7</td>
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<td>85 (TOC)</td>
<td>77</td>
</tr>
</tbody>
</table>

4.2.3.3 Chemicals of Concern Removal

Figure 4.2-6 summarizes the removal of the COCs by the BCR. A total of 8 sampling events were conducted: one during both Runs 1 and 2 and three during both Runs 3 and 4, one for each recycle ratio. The aim was to evaluate the effect of SRT and recycle ratio, two commonly varied operating parameters, on removal. The COCs were removed by over 90% with the exception for CMP, which is well known to be a recalcitrant chemical. The wasted biomass was extracted to determine if the COCs were removed through sorption. Less than 2% of the daily mass loading was found (data not shown), so adsorption was deemed to not contribute significantly to the removal.

CAF was removed by over 90% for the 6-day SRT and over 99% for the remaining events. Others have shown that CAF is removed in both CAS and MBR systems quite well, >99%, through biological removal (Miao et al., 2005; Thomas and Foster, 2005; Xue et al. 2010; Sui et al., 2010; Goa et al., 2012). The slightly lower removal at the 6-day SRT is likely due to some limitation of the biomass, either lack of acclimation to CAF or the lower solids concentration during that period. CAF is not particularly difficult
to remove, but is important because of its ubiquity and usually high concentrations in wastewater. It can be used as a marker for human activity and is commonly studied alongside other COCs of more significance.

Figure 4.2-6: Percent removal of the COCs for Runs 1 - 4. Three separate sampling events were conducted during Runs 3 and 4, one for each recycle ratio (3Q, 4Q, and 5Q).

CMP was removed during Runs 1 and 2 by ~ 50%, but not at all during Runs 3 and 4. According to the literature, CMP is a notoriously recalcitrant compound, and consequently any removal is unexpected (Heberer et al., 2002; Radjenovic et al., 2009; Xue et al., 2010; Sui et al., 2010; Fernandex-Fontaina et al., 2012). Since CMP was not found on the biomass, which is consistent with results from the literature, and biological removal was unlikely, other sources of removal were investigated. (Joss et al., 2005; Miao et al., 2005). A small test was conducted to determine if there was any adsorption onto the membrane. A 3 cm square of the membrane was placed into a 10 µg/L solution of CMP for 4 days. It was determined that the membrane adsorbed about 30% of the CMP. Adsorption to the membrane explains the partial removal during the first two SRTs. Eventually, the membrane became saturated and the effluent concentration increased. The regeneration method appeared to have been unable to remove the CMP from the membrane surface, allowing it to accumulate.
TES and PRO were removed by more than 99% during all the sampling events. This removal agrees well with reported results in the literature (Esperanza et al., 2004; Lee et al, 2004; Esperanza et al., 2007; Liu et al., 2009). TES was found to mineralize in batch experiments with biomass from wastewater treatment plants while both androgens (TES) and progestogens (PRO) in general are completely removed through biodegradation (Layton et al., 2000; Chang et al., 2008; Fan et al., 2011). Since neither of the chemicals was found adsorbed to the biomass, biological removal is likely the mechanism for removal.

The removal of EE2 ranged from 65% up to 94%, but the deviation of each sampling event was such that the results of each event were not significantly different from each other. While not quite as variable, these results are similar to the range of reported values, removal efficiencies ranging from 30% - 90% (Esperanza et al., 2004; Lyko et al., 2005; Esperanza et al., 2007; Abegglen et al., 2009; Xue et al., 2010). Noticing the unpredictable behavior of estrogens during wastewater treatment, Marfil-Vega et al. performed a series of experiments to explain the variations (Marfil-Vega et al., 2010; Marfil-Vega et al., 2011; Marfil-Vega et al., 2012). They discovered that estrogens undergo an abiotic transformation and linked the transformation to oxygen levels, suggesting oxidative coupling. Since the wastewater treatment processes used at a given treatment plant can vary widely, oxygen levels and therefore estrogen removal vary. The abiotic transformation is also not the only mechanism for removal, as adsorption and biological removal are still possible. In this research, adsorption was not found, so biodegradation and abiotic transformation are the likely mechanisms of removal.

Nearly complete, greater than 99%, removal of TCS and NP was achieved. The TCS removal compares well with the literature, most reporting over 90% (McAvoy et al., 2002; Singer et al., 2002; Thomas and Foster, 2005). Reported NP removal varies widely, 33% - 94% (Esperanza et al., 2004; Lee et al, 2004; Lyko et al., 2005; Liu et al., 2009). Because most of the studies were conducted at actual treatment facilities, influent concentrations vary and operating conditions differ, leading to mixed results by different investigators. For instance, NP-ethoxylates can be found in large quantities alongside NP and
are transformed into NP during anaerobic treatment; therefore, a treatment plant with that particular process would produce large amounts of NP (Lee et al., 2004). The reported results for both chemicals attribute part of the removal due to adsorption onto the solids; however, no adsorption was observed in this study. This indicates either adsorption was not a factor in the removal or the adsorption was not reversible by the ASE method used.

In most of the literature cited above, the treatment method (CAS v. MBR v. BCR) was not an important factor in the removal of the COCs. The results for CAF, TES, PRO, and CMP are consistent. The BCR was slightly more effective than a CAS process for NP and TCS while comparable to other MBRs. EE2 removal was too varied and dependent on oxygen levels to offer a comparison. The COCs were not significantly affected by the SRT or the recycle ratio operating conditions either. CAF removal increased slightly due to the increase in SRT while CMP removal decreased after increasing the COD and SRT, though it's unclear which if either of these changes played a role. The recycle ratio was not anticipated to have a large effect, since nitrifying conditions have not been reported as an influential factor. It could have affected EE2, given the relationship EE2 has with oxygen, but the change in oxygen levels associated with the recycle ratio is minor.

4.2.3.4 Estrogen Removal Mechanism

An investigation of the removal mechanism for estrogens was conducted. An experiment similar to those performed by Marfil-Vega et al. (2012) was done, involving EE2 and the biomass from the reactor. The waste mixed liquor from both sections of the reactor was combined and effluent was collected, then they were each split in half. One half was bubble with nitrogen and then placed in an anaerobic hood while the other was left to sit mixing under normal atmospheric conditions. EE2 was spiked into the two effluents and a series of serum bottles was filled, containing 90 mL of the effluent and 10 mL of the mixed liquor. The serum bottles were then mixed for 30 minutes, tumbling end-over-end, before the initial samples were sacrificed. Additional samples were sacrificed at 2, 4, 8, and 12 days. Killed controls were run
using sodium azide for each sampling event and a background control was run with the initial sampling event to determine the amount of EE2 already present in the two solutions. In total, four combinations were tested, in triplicate, in addition to the initial background control: aerobic, aerobic killed controls, anoxic, and anoxic killed controls.

The results of this experiment are shown in Figure 4.2-7. The graph displays the EE2 concentrations for the four conditions over time as well as the background EE2 that was initially present in the effluent and mixed liquor. Under aerobic conditions, EE2 was quickly removed, but in the killed control only about ~40% was removed. Under anoxic conditions, in both the live and killed control samples, the EE2 was not removed. A small decrease is observed, likely due to variation in processing and recovery. There is a difference in the initial concentrations between the two sets because the EE2 was added to the two effluent solutions separately, possibly causing a small variation in the two solutions, and the bottles were mixed for 30 minutes prior to processing, where transformations could occur. The drop on day 2 for the anoxic conditions is likely an error in processing the samples and not because of any removal. From the data, it is clear that under anoxic conditions, there is no biological removal and that any non-biological transformation is minimal. Under aerobic conditions, biological removal occurred, as displayed by the difference between the live and killed control samples, as well as some non-biological transformation,
displayed by the partial removal in the killed control samples. The aerobic killed control samples show very little removal after the first 2 days, which could indicate that the transformation catalyst is exhausted by the process. This finding supports the claims of Marfil-Vega et al. (2012) that the mechanism for removal of estrogens is due in part to oxidative coupling. It also explains the significant disparities, discussed above, in the removal of EE2 in the literature, where the aeration conditions vary widely from study to study. It can even explain the variation in the removal in this study, where dissolved oxygen levels were only measured occasionally, but not often enough to guarantee they were 100% stable.

4.2.4 Conclusion

The BCR was shown to be an effective technology for treating a complex municipal wastewater. The system, which contained both an aerobic and anoxic section, was operated at an SRT of 6, 15, and 30 days with three different recycle ratios. During Runs 3 (30-day SRT) and 4 (15-day SRT), the biomass were completely retained, COD was reduced by 98%, and NH$_3$-N and TKN were reduced to 0.1 mg/L and 1.8 mg/L, respectively. Total nitrogen was reduced by 78-91%, dependent on the SRT and recycle ratio. Several COCs were monitored for removal alongside the traditional constituents of COD and nitrogen. During all 4 Runs, TES, PRO, TCS, and NP were removed by 95% or better, while EE2 was lower, ranging from 65-94%. CMP was initially removed by 50% during Runs 1 and 2, due to adsorption to the membrane, but was not removed at all after the membrane became saturated. CAF removal improved from 95% to 99% when the SRT was increased from 6 to 15 days. The variability of the EE2 removal was further investigated and confirmed to be dependent on oxygen levels. EE2 was shown to undergo an abiotic transformation in the presence of oxygen, confirming previously reported findings in the literature. Overall, the BCR was able to match or surpass the performance of CAS and MBR systems for treating traditional municipal wastewater constituents as well as micropollutants.
4.3 Evaluation of a gravity-flow membrane bioreactor for treating municipal wastewater
4.3.1 Introduction

Traditional wastewater treatment systems are dominated by large, centralized facilities that remove organic matter and nutrients from water. Design engineers size unit processes based on estimated parameters such as flow rates and mass loadings. Once construction is completed, the resulting infrastructure is often too rigid to accommodate changing conditions that may occur in the served area (Tjandraatmadja et al., 2005). Hence, demands for a higher quality effluent and the need for a more flexible, controllable treatment process have led to the rise of membrane bioreactors (MBRs).

MBRs were developed in the 1960s, but have become very popular in recent years (Mallevialle et al., 1996; Le-Clech et al., 2006). These reactors have several advantages over conventional designs that make them desirable: a reduced footprint, improved effluent quality, total control of biomass wasting, flexibility to operate at a wide range of variables, and flexibility in scale (Cicke et al., 2001; Le-Clech 2010). Hence, MBRs have become preferred in areas where water and land are scarce and where pollutant removal and water recovery/reuse are important. General acceptance of MBRs has been hindered by several disadvantages such as higher capital cost as compared with traditional treatment because of the high cost of the membrane units; higher energy consumption owing to the pumping requirements through the low porosity membranes and extra aeration; membrane clogging that requires wastewater pretreatment; and membrane chemical cleaning or replacement because of fouling (Cicke et al., 2001; Metcalf and Eddy, 2003; Le-Clech et al., 2006; Le-Clech, 2010). These reasons have limited the implementation of MBRs to instances where the need to recover the water outweighs the energy and monetary costs.

An innovative MBR design, the biomass concentrator reactor (BCR), was developed to significantly reduce the energy and cost requirements of an MBR system, while still maintaining complete biomass retention. Co-developed and patented by the University of Cincinnati and the U. S. Environmental Protection Agency (Patent No. 6821425 issued November 23, 2004), the BCR has a 3-4 mm thick
membrane with pore size ranging from 18 – 28 µm constructed from packed polyethylene beads. Flow through the membrane is driven entirely by gravity with a head differential of less than 2.5 cm between the retentate and permeate sides of the membrane. The large pore size of the membrane along with the tortuous, depth filtration mechanism effectively retains the biomass and significantly reduces the pressure required for water to percolate.

The BCR was first developed to treat groundwater contaminated with gasoline (Zein et al., 2004). Several studies confirmed its effectiveness in removing hydrocarbons and oxygenates such as methyl-t-butyl ether, while completely retaining biomass under different conditions and loadings (Zein et al., 2004; Zein et al., 2006; Capodaglio et al., 2010; Medella et al., 2011). Scott et al. (2013) used the BCR system to treat municipal wastewater. Side-by-side evaluation of a nitrifying system and a nitrifying-denitrifying hybrid system using a synthetic wastewater resulted in a chemical oxygen demand (COD) reduction of over 90% and almost complete nitrification in both systems. In addition, the BCR was able to reduce the total nitrogen in the hybrid system by 67% (Scott et al., 2013). The goal of the research reported in this paper is to replicate their results with the hybrid system using actual municipal wastewater in order to assess the impact of inert suspended solids and non-biodegradable organic matter on membrane performance.

4.3.2 Materials and Methods
4.3.2.1 Reactor Design

The BCR was set up as shown in the Appendix, Figure 6.2-2, and its detailed description can be found in Scott et al. (2013), as the system is almost identical to the hybrid unit therein reported. The membrane was a commercially available filter (Porex Corp., Atlanta, GA), which consisted of a pleated, cylindrical module, approximately 15.5 cm in diameter and 25.1 cm in length with a surface area of approximately 0.62 m². The reactor volume (30 L) was equally split between the aerobic and anoxic sections with approximately 2 L occupied by the membrane and the permeate collection section within the membrane module.
Wastewater was continuously fed into the anoxic compartment to facilitate denitrification, while a second pump kept a fixed recycle ratio by returning mixed liquor from the anoxic compartment back up to the aerobic section where the membrane separated the solids from the water. The retained biomass was funneled toward the anoxic stage at a rate equal to the recycle ratio minus the feed flow rate of the influent wastewater. Permeate exited the reactor through a side port located in a vertical tube that allowed for water level control inside the reactor.

The BCR was operated at a solids retention time (SRT) of 20 days (i.e., 1/20th of the volume of the reactor was wasted each day). This waste volume was taken equally from both sections. Wastewater was pumped into the reactor at a flow rate of 72 L/d. The recycle ratio was initially set at 3 times the influent flow rate (3Q) and was increased to 5 times the influent flow rate (5Q) on day 247.

4.3.2.2 Sewage Pumping System

The wastewater was taken from a 48” diameter sewer main running under the University of Cincinnati west campus in Cincinnati, Ohio, USA. Upstream of the connection were several restaurants, dorms, classroom buildings, and office buildings as well as runoff collection drains. All of these sources were assumed to have contributed to the sewage stream, though the exact contributions of each are unknown and most likely varied widely over time.

The pumping system consisted of a large storage tank, a primary settling tank, and several pumps. Wastewater was pumped from the sewer main into the storage tank and, subsequently, up to the lab and into the settling tank. Within the settling tank, the sewage was allowed to separate, with the solids and grit settling to the bottom and the fats and oils floating to the surface. A port located just below the middle of the tank was used to supply the sewage to the reactor.
4.3.2.3 Reactor Startup and Operation

The BCR was seeded with biomass from the aeration tank of a local municipal wastewater treatment plant. Approximately 4 L of mixed liquor was added to the reactor along with deionized tap water. The reactor start-up period lasted 170 days. The time needed to attain steady state was lengthy due to operational problems and perceived limited denitrification in the anoxic zone. Initially, we attributed this limited denitrification to either poor mixing in the anoxic section or short-circuiting between the aerobic and anoxic compartments. We later realized that the poor nitrogen removal was due to variations in feed Chemical Oxygen Demand (COD) strength and periods where the feed biodegradable COD was insufficient to achieve the desired level of denitrification (see Discussion section). Since fortifying the wastewater would defeat the goals of this work, we did not alter its quality. After addressing these problems, we started the 137-d experimental period.

The membrane was cleaned four times per week using a jet of recycled mixed liquor from the aerobic section of the reactor. This process helped prevent buildup in-between the pleats of the membrane where the scouring intensity of the air bubbles was insufficient to keep the surface clean. The system was initially run without a buffer; however, the pH in the system varied significantly and was often below 7, which is not optimum for nitrification. Consequently, on day 136, a Na₂CO₃ solution (16 mg/L) was pumped into the aerobic section at a flow of ~1 L / d to maintain a pH between 7.4 and 8. Alkalinity was adjusted as needed thereafter. The system was operated until the head differential across the membrane exceeded 2.5 cm, above which the membrane was replaced. The fouled module was chemically cleaned, first soaking for 24 hours in 10% nitric acid solution followed by a 10% Clorox solution.

4.3.2.4 Sampling Collection

During the start-up period grab samples were collected twice per week from the influent, effluent, and aerobic and anoxic mixed liquors. On day 102, a composite sampler was put in place so that influent and effluent were monitored over 24-hour periods by collecting 20 mL of each stream every 15 minutes. The
apparatus was placed in a refrigerator at 4°C to limit further biological activity. Every 24 hours, the sampling bottles were replaced and kept at 4°C until analysis could be performed. Mixed liquor samples were grabbed from the 2 BCR compartments and analyzed along with the stored composite samples 3 times per week.

4.3.2.5 Analytical Methods

Each composite influent and effluent sample was split into aliquots to measure the concentrations of COD, ammonia, nitrate, nitrite, and total Kjeldahl nitrogen (TKN). Influent and both mixed liquors were also analyzed for total suspended solids (TSS) and volatile suspended solids (VSS). All samples were analyzed in triplicate following Standard Methods (APHA, 1998). These methods were COD method 8000 by Hach, ammonia method 4500 (Orion 9512HPBNWP Ammonia Electrode), nitrate and nitrite method 4110B (Dionex DX 500 Ion Chromatograph), TKN method 4075 by Hach, and TSS/VSS method 2540D (Hach, 1992). The pH (Oakton WD-35801-00 pH Electrode) of the aerobic and anoxic mixed liquors was measured daily.

4.3.3 Results and Discussion

During the startup period, the wastewater was high in ammonia, between 20 and 40 mg/L as NH₃-N, and had the expected amount of COD ranging from 150 to 400 mg/L (shown in the Appendix, Figures 6.3-1 through 6.3-4). However, less than half of that COD was readily biodegradable, which is not entirely unexpected when working with real wastewater (Grady et al., 2011). The low BOD plus the higher than expected ammonia meant that denitrification was carbon-limited and almost never reached the expected removal. Confirmation of this carbon limitation came when a large, one-day spike in the COD on day 204 (up to ~700 mg/L) reduced the effluent nitrate concentration to the approximately the expected value.

4.3.3.1 Chemical Oxygen Demand

COD was measured in the influent and effluent (see Figure 4.3-1). The influent COD was generally low with a few high spikes when compared to a typical municipal wastewater. When compared to the data...
collected during startup, the COD was much lower because of a drastic change in sewage production: the end of the spring school term at the University of Cincinnati occurred 2 days before the experimental period began and the autumn term did not begin until day 290. Activity on campus dropped significantly during summer, highlighted by the vacancy of most of the dorms and apartments that would have contributed to the wastewater stream. The restaurants and classrooms also generated significantly lower usage, leaving the majority of the flow to come from the office buildings and rainfall runoff. Erratic spikes in the COD are understandable given that the sewage comes from a small area and could be influenced very easily by a number of different factors. On day 290, the new school year began and an increasing trend in the influent COD can be seen in the days just prior to classes starting.

![Figure 4.3-1: Influent and effluent COD.](image)

Throughout the entire period of observation, the effluent COD was consistently below 20 mg/L with an average of 7.3 ± 1.5 mg/L, regardless of the corresponding influent concentration. The consistency of this removal highlights the flexibility of the BCR in handling a varying-strength waste stream. Other MBRs have shown comparable results when dealing with variations in the waste stream. Using a similar, low strength wastewater, Ueda and Hata (1999) reported achievement of COD removal down to 3.7 mg/L,
while Rosenberger et al. (2002) reported a COD reduction from a high strength wastewater to below 40 mg/L. In both cases, influent fluctuations had little impact on the effluent quality.

The average COD removal rate was approximately 93%. This rate is consistent with the results of Scott et al. (2013) and other MBR systems, which range from 80 to 99% (Ueda and Hata, 1999; Rosenberger et al., 2002; Kimura et al., 2008). The switch to real waste from the synthetic feed used in the previous BCR research was expected to have caused a decrease in the removal efficiency because of potentially non-degradable components in the wastewater. The high removal efficiency indicated that either the wastewater did not contain large quantities of non-degradable COD or that the BCR was effective in retaining this fraction of the COD. MBRs have been shown to be effective in retaining non-degradable or slowly degrading COD, allowing the increased diversity of the culture in the reactor to eventually remove it (Rosenberger et al., 2002). Two minor increases in effluent COD were observed on days 190 and 204. The increase on day 190 was the result of contamination in the effluent line, which was purged after it was discovered. This buildup only occurred in the tubing used in the composite sampler and was likely due to a combination of contamination of the tubing prior to installation, the large surface area of the tubing, and the reduced flow of the composite sampler. Contamination of a membrane system is common anytime nutrients and a surface for attachment are available (Flemming, 1997; Matin et al., 2011). The contamination was not observed prior to the composite sampler installation and was not reported in any of the previous BCR studies. As a precaution, the tubing was flushed every 3-4 weeks afterward. The increase on day 204 was likely due to the large spike in the influent COD coupled with the failure of the membrane.

4.3.3.2 Nitrogen

Several forms of nitrogen were monitored in the influent and effluent: ammonia, nitrate and nitrite, and TKN. The aerobic section was intended for nitrification, while denitrification took place in the anoxic stage. The NH$_3$-N results are shown in Figure 4.3-2(a). The influent NH$_3$-N fluctuated between 5 and 60
mg/L with an average of 20.8 ± 9.6 mg/L. This fluctuation did not correlate to changes in the influent COD, but the ammonia did follow the same pattern as the COD in relation to the changes in the wastewater source. During the period of observation, the effluent NH$_3$-N concentration did not exceed 0.6 mg/L and was almost always below 0.1 mg/L. Furthermore, variations in influent NH$_3$-N concentration had no apparent effect on the effluent concentration, even when the influent surged to 60 mg/L. Influent and effluent TKN concentration averaged 25.4 ± 8.9 and 1.2 ± 0.4 mg/L, respectively, which resulted in an overall organic nitrogen removal efficiency of 95% (Figure 4.3-2(b)). Ammonia represented 80% of the total TKN load.

![Figure 4.3-2: Influent and effluent ammonia (a) and TKN (b).](image)

The effluent concentration of NO$_3$-N was 13.7 ± 7.0 mg/L while the NO$_2$-N was consistently below 0.1 mg/L. The NO$_3$-N removal was limited compared to the removal effectiveness observed for ammonia and TKN. As previously discussed, the combination of low influent COD, which had a small fraction of readily biodegradable carbon, and high influent NH$_3$-N led to less than expected denitrification. Figure 4.3-3 presents three data sets: effluent NO$_3$-N, theoretical maximum effluent NO$_3$-N based on influent
TKN and no denitrification, and theoretical effluent NO$_3$-N assuming complete denitrification of TKN input. The theoretical concentrations were determined using Eqns. 4.3-1 and 4.3-2 (Metcalf and Eddy, 2003):

$$NO_x = TKN_{in} - NH_{3,eff} - 0.035 \times \frac{P_x}{Q} \quad (4.3-1)$$

$$IR = \frac{NO_x}{NH_{3,eff}} - 1 \quad (4.3-2)$$

where all the forms of nitrogen were expressed as N; P$_x$, Q, and IR represent the daily wasted biomass, daily flow rate, and internal recycle ratio, respectively; and 0.035 g N/g VSS is substituted for 0.12 g N/g VSS based on TKN digestion of the biomass. These calculated concentrations represent upper and lower boundaries for NO$_3$-N, and, as shown in the figure, measured values remained between the two margins, being closer to the upper, or nitrification only, limit. Observed data are below the nitrification only values, which points to denitrification limited by the readily biodegradable COD.

![Figure 4.3-3: Effluent nitrate, theoretical effluent nitrate, and theoretical nitrate produced.](image)
We compared NO\textsubscript{3}-N removed to influent COD in Figure 4.3-4; clearly surges in COD led to higher NO\textsubscript{3}-N removal. While other factors may affect NO\textsubscript{3}-N disappearance, this graph provides reasonable evidence that the COD heavily influenced nitrate metabolism and was likely the limiting factor. The recycle ratio between the anoxic and aerobic zones was changed on day 247 from 3Q to 5Q. The intention was to evaluate whether nitrogen removal affected the BCR performance. Because of the limited COD concentrations, reactor hydraulics did not affect denitrification.

![Nitrate removal v. influent COD](image)

**Figure 4.3-4: Nitrate removal v. influent COD.**

The total nitrogen removed was calculated using Eqn.4.3-3:

\[
\%N_{removed} = \frac{(TKN_{inf} - NH_{3,eff} - NO_{3,eff} - NO_{2,eff})}{TKN_{inf}} \times 100
\]

where all the forms of nitrogen were expressed as N. The average removal was 46.3 ± 17.1%, ranging from 10 to 90%. The wide range and high standard deviation are consequences of the low COD and fluctuating influent concentrations. Such nitrogen elimination is low for a nitrifying/denitrifying process and is more in line with a purely nitrifying system (Scott et al., 2013; Kimura et al., 2008).
4.3.3.3 Total and Volatile Suspended Solids

The TSS and VSS were measured in both the aerobic and anoxic sections as well as in the influent. Influent solids were monitored to verify that the settling/degritting tank was working properly and that large amounts of non-biodegradable material were not fed into the system. The TSS concentration from both sections and the total biomass in the reactor are shown in Figure 4.3-5. As expected, solids followed the COD trend, since biomass depends on organic matter to grow. The figure clearly indicates a drop in TSS and total biomass during the low COD period, with a recovery exhibited after the COD started to increase again around day 285. The VSS was 77% of the TSS, which is consistent with reported values for systems operated on municipal wastewater (Metcalf and Eddy, 2003). Influent TSS was only about 100 mg/L with 60% being VSS, which suggests good particle separation.

![Figure 4.3-5: Total suspended solids of the aerobic and anoxic mixed liquor and the influent (a) and total biomass in the reactor (b).](image)

The aerobic and anoxic sections of the BCR had similar concentrations of TSS during most of the period of study. Large differences were observed only at the beginning and end of the run. Such differences can
be attributed to changes in the influent COD concentration during the study. High COD supported a
greater amount of denitrification, which results in a lower growth rate compared to aerobic COD
utilization, but also provided more COD for aerobic utilization (Metcalf and Eddy, 2003; Grady et al.,
2011). Hence, solids rose in the aerobic section compared to the anoxic section. With low influent COD,
however, the majority of the COD was used for denitrification, limiting any aerobic COD utilization. As a
result, the growth rates converged and the two sections had similar TSS.

4.3.3.4 Observed Yield

The observed biomass yield for the experimental period is displayed in Figure 4.3-6. The yield is
typically determined based on the cumulative COD removed and cumulative VSS wasted, but this method
assumes the reactor is at steady-state. Because the influent COD fluctuated significantly, the VSS never
reached steady-state and the fluctuations in VSS needed to be added into the calculations to determine the
yield more accurately. The VSS difference, from the figure, was defined as the VSS wasted intentionally
on a given day plus the change in VSS that occurred in the 24 hours since the previous wasting (wasting
was conducted daily). The graph reveals three distinct rates that correspond to the periods of high and low
COD described earlier. The yields were 0.22, 0.15, and 0.24 g VSS/g COD removed for the three
periods, which are all at the lower end for a nitrification/denitrification process (Metcalf and Eddy, 2003;
Grady et al., 2011). These results are likely due to a combination of the high SRT and the carbon-limited
denitrification process. Scott et al. (2013) also observed a decrease in yield as the SRT increased when
comparing nitrifying and hybrid processes. The periods prior to 1,750 g of COD removed and after 2200
g of COD removed contained higher amounts of COD, allowing for growth to occur in both aerobic and
denitrifying conditions. During the low influent COD period (between 1750 and 2200 g of COD
eliminated), the COD was utilized primarily in the denitrifying compartment, resulting in a lower yield.
4.3.4 Conclusion

The BCR was assessed for treating municipal wastewater and was shown to effect excellent removal of carbon and ammonia-nitrogen. Operated by gravity flow at less than 2.5 cm of head, the large pore-size, depth filtration membrane completely separated the solids from the treated water. On average, the COD and NH$_3$-N were reduced by 93 and 99%, respectively. Total nitrogen removal was not as effective, averaging approximately 46%, but the carbon content of the influent wastewater limited the system. The results exceeded conventional activated sludge treatment systems and compared favorably with other MBR systems. Overall, the BCR proved flexible, capable of handling large fluctuations in the wastewater stream without compromising effluent quality.
5 Bibliography


# Appendix

## 6.1 Wastewater Components

### Table 6.1-1: Target Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
<td>Caffeine</td>
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<tr>
<td>Carbamazepine</td>
<td><img src="image" alt="Carbamazepine structure" /></td>
</tr>
<tr>
<td>Testosterone</td>
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</tr>
<tr>
<td>Progesterone</td>
<td><img src="image" alt="Progesterone structure" /></td>
</tr>
<tr>
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</tr>
<tr>
<td>Triclosan</td>
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</tr>
<tr>
<td>NP*</td>
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</tr>
<tr>
<td>Atrazine</td>
<td><img src="image" alt="Atrazine structure" /></td>
</tr>
<tr>
<td>NP1EO*</td>
<td><img src="image" alt="NP1EO structure" /></td>
</tr>
<tr>
<td>NP2EO*</td>
<td><img src="image" alt="NP2EO structure" /></td>
</tr>
</tbody>
</table>

*EE2, ethinylestradiol; NP, nonylphenol; NP1EO, nonylphenol ethoxylate; NP2EO, nonylphenol diethoxylate.
<table>
<thead>
<tr>
<th>Component</th>
<th>Final Concentration, mg/L</th>
</tr>
</thead>
<tbody>
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<td><strong>Organic feed</strong></td>
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</tr>
<tr>
<td>Casein</td>
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</tr>
<tr>
<td>Tryptone</td>
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</tr>
<tr>
<td>Starch</td>
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<td>Sodium acetate</td>
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<tr>
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<td>Potassium phosphate</td>
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</tr>
<tr>
<td>Sodium molybdate</td>
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</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.13</td>
</tr>
<tr>
<td>Zinc chloride</td>
<td>0.23</td>
</tr>
<tr>
<td>Iron chloride</td>
<td>0.42</td>
</tr>
<tr>
<td>Cobalt chloride</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td></td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>249</td>
</tr>
<tr>
<td>Sodium sulfite</td>
<td>162</td>
</tr>
</tbody>
</table>
6.2 Reactor Designs

Figure 6.2-1: Synthetic Hybrid and Aerobic BCR Designs.
Figure 6.2-2: Real wastewater BCR design.
6.3 Real wastewater BCR Startup Data

Figure 6.3-1: Solids startup data for the real wastewater BCR.

Figure 6.3-2: Influent and effluent COD startup data for the real wastewater BCR.
Figure 6.3-3: Influent and effluent ammonia and TKN startup data for the real wastewater BCR.

Figure 6.3-4: Effluent nitrate startup data for the real wastewater BCR.