Removal mechanisms of 17β-estradiol and 17α-ethinylestradiol in membrane bioreactors
W. Yang, H. Zhou and N. Cicek

ABSTRACT
The fate and behavior of natural and synthetic estrogens in wastewater treatment processes is currently of increasing concern all over the world. In this study, the removal mechanisms of a natural estrogen, 17β-estradiol (E2), and a synthetic estrogen, 17α-ethinylestradiol (EE2) were investigated in membrane bioreactors (MBRs) with and without powdered activated carbon (PAC) addition. The experimental results showed that the average removal rates of E2 and EE2 by the MBR without PAC addition were 89.0 and 70.9%; PAC addition in the MBR increased the removal rate of E2 and EE2 by 3.4 and 15.8%, respectively. The greater impact of PAC dosing on EE2 removal was due to its greater hydrophobic property. Adsorption played a more important role in the removal mechanisms of EE2 than E2. Biodegradation was the dominant mechanism for the removal of E2 and EE2 in MBRs. Unlike their adsorption behavior, the biodegradation rates of both E2 and EE2 were not significantly different between the MBRs with and without PAC addition.

INTRODUCTION
Since the mid-1990s, occurrence and behavior of endocrine disrupting compounds (EDCs) in the environment, especially in wastewater treatment plants (WWTPs), has gained considerable attention worldwide. Some scientists have concluded that discharge from WWTPs is one of the largest sources of EDCs in the Canadian aquatic environment (Servos et al. 2005). Despite controversial opinions on the impact of EDCs on human beings and overall ecology, the adverse effects on aquatic species such as fish have been well documented. The negative impacts include the feminization due to hormonal imbalance and reduced reproductive success in fish and avian species (Rodgers-Gray et al. 2000). Although a number of studies have been done to understand the fate of EDCs in wastewater treatment processes, further research is needed to develop effective ways to remove them to environmentally acceptable levels.

Steroid estrogens, especially 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are EDCs of concern due to their adverse impact on the aquatic environment. Their removal mechanisms in WWTPs and their transport out into the environment have been shown to depend on the design and operational characteristics of the treatment plant. Conventional biological treatment processes, such as the activated sludge process, have been reported effective in reducing estrogens to certain levels in wastewater and sewage. The principal removal mechanisms of steroid estrogens in the activated sludge process are likely to be sorption and biodegradation (Johnson & Sumpter 2001). Field data suggested that conventional activated sludge processes could remove certain EDCs, including E2 and EE2, with a wide range of removal efficiencies (Johnson & Sumpter 2001; Servos et al. 2005; Cicek et al. 2007).

Over the past decades, membrane bioreactor (MBR) technology has been advancing rapidly around the world both in research and commercial applications (Yang et al. 2006). MBRs have been proven effective in removing both chemical and biological pollutants from water or wastewater streams, owing to their distinct advantages such as high sludge age, high biomass concentration and complete particle retention. Thus, it is reasonable to expect that MBRs achieve enhanced EDC removal. This hypothesis has been supported by previous studies on the removal of EDCs in MBRs (Joss et al. 2004; Chang et al. 2006). Table 1 presents a summary of the studies on the removal of E2 and EE2 in
MBRs during the past years. The majority of the studies were conducted by measuring the concentrations of EDCs in influents and effluents in pilot-scale MBRs. It is clear that neither E2 nor EE2 was completely removed by MBRs and they remained with fluctuating concentrations in MBR effluents. Further research is needed to reveal specific removal mechanisms of E2 and EE2 in MBRs and to investigate the performance of full-scale MBRs in removing E2 and EE2.

It has been reported that powdered activated carbon (PAC) addition could reduce membrane fouling in MBR systems (Seo et al. 2004). By adding PAC in the bioreactor, PAC would change the characteristics such as biodegradation and/or adsorption abilities of the sludge in the bioreactor (Yang et al. 2010a). A couple of studies have been conducted to test the efficiency of PAC in further eliminating estrogens in MBR. Wintgens et al. (2005) investigated the removal efficiencies of EDCs with different process configurations in landfill leachate treatment plants. The results showed that the final activated carbon (AC) adsorption, following the MBR, was able to remove half of the remaining micro-pollutants. In another study (Korner et al. 2001), it was reported that additional AC filtration was very efficient in further eliminating estrogenic activity (EA) from WWTP effluents. In contrast other investigators have shown that the estrogenic activity in the aqueous phase leaving an MBR system did not further decrease by polishing with either granular activated carbon (GAC) or PAC (Holbrook et al. 2002).

With the expansion of MBR installations for wastewater treatment and water reuse, the removal behavior and mechanisms of estrogens in MBRs need to be further studied. The objective of this study was to assess the impact of PAC addition on removal mechanisms of estrogens E2 and EE2 in bench-scale MBRs.

### MATERIAL AND METHODS

#### Experimental set-up

Two bench-scale MBRs, one without PAC addition (control MBR) and the other with PAC addition (PAC-MBR), were operated in parallel under similar experimental conditions. A schematic drawing of the experimental set-up can be found in a previous publication (Yang et al. 2010a). The working volume of each bioreactor was 3.0 L. A hollow fiber membrane module with a surface area of 0.08 m² was installed in each bioreactor. The specifications of the membrane modules are shown in Table 2. Coarse bubble air-diffusers at the bottom of the membrane module were used to provide dissolved oxygen (DO) for biomass growth and to introduce vigorous shear force on the membrane surface to reduce membrane fouling.

#### Table 1 | Summary of the studies on the removal of E2 and EE2 in MBRs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Scale</th>
<th>influent (ng/L)</th>
<th>effluent (ng/L)</th>
<th>Removal (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>Pilot</td>
<td>N.D.−5.74</td>
<td>N.D.−2.83</td>
<td>49.3–63.1</td>
<td>Hu et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Lab</td>
<td>N.D.−6.21</td>
<td>N.D.</td>
<td>60–66.5</td>
<td>Hu et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>N.A.</td>
<td>N.D.−1.1</td>
<td>N.A.</td>
<td>Spring et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>11−43</td>
<td>0.8a</td>
<td>94–99</td>
<td>Zuehlke et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>N.D.</td>
<td>N.D.</td>
<td>–</td>
<td>Lee et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>67−125</td>
<td>N.D.−6</td>
<td>–</td>
<td>Clara et al. (2005a)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>6.3 ± 1.3</td>
<td>≤ 0.5</td>
<td>≥ 98</td>
<td>Joss et al. (2004)</td>
</tr>
<tr>
<td>EE2</td>
<td>Lab</td>
<td>750, 000</td>
<td>–</td>
<td>58.4–97.7b</td>
<td>De Gussem et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>N.A.</td>
<td>0.9–1.6</td>
<td>N.A.</td>
<td>Spring et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Lab</td>
<td>100, 000</td>
<td>–</td>
<td>~80.0</td>
<td>Cirja et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>5−23</td>
<td>1.3a</td>
<td>82–94</td>
<td>Zuehlke et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>N.D.−38.6</td>
<td>N.D.</td>
<td>&gt; 71</td>
<td>Lee et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>3−20</td>
<td>N.D.−4</td>
<td>–</td>
<td>Clara et al. (2005a)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>1.6 ± 0.3</td>
<td>≤ 0.5</td>
<td>≥ 75</td>
<td>Joss et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Pilot</td>
<td>2,025–2,376</td>
<td>16–158</td>
<td>–</td>
<td>Clara et al. (2005b)</td>
</tr>
</tbody>
</table>

*aaverage; bHRT = 4 days; cN.D. – not detected; dN.A. – not available.

A schematic drawing of the experimental set-up can be found in a previous publication (Yang et al. 2010a).
μPAC with an average size of 100 μm was reported to shift the particle size distribution of the mixed liquor from bioreactors daily. The hydraulic retention time (HRT) of both MBRs was 5.8–6.0 h, depending on membrane flux. During the experimental period, transmembrane pressures (TMP) of the two MBR systems were monitored every day. Temperature, DO and pH in the bioreactors were measured on a regular basis. The operating conditions of the two MBRs can be found elsewhere (Yang et al. 2009).

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

Based on these results, the PAC with a relatively higher particle size range would be suitable for dosing into MBR systems.

### Operation of MBRs

The two MBR systems were operated at constant flux mode by withdrawing permeate through peristaltic pumps from the outside-to-inside hollow membrane fibers. The two MBR systems were fed with synthetic municipal wastewater spiked with 500 ng/L E2 or EE2. The average chemical oxygen demand (COD) and NH₄-N concentration of the feed were 475.6 and 25.5 mg/L, respectively. To control membrane fouling, a cyclic pumping mode with 10 min ON and 2 min OFF was used. Solids retention time (SRT) of both MBR and PAC-MBR remained 25 d by wasting a portion of the mix liquor from bioreactors daily. The hydraulic retention time (HRT) of both MBRs was 5.8–6.0 h, depending on membrane flux. During the experimental period, transmembrane pressures (TMP) of the two MBR systems were monitored every day. Temperature, DO and pH in the bioreactors were measured on a regular basis. The operating conditions of the two MBRs can be found elsewhere (Yang et al. 2009).

### Analytical methods

For wastewater and sludge samples, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), COD, soluble COD, color, and ammonia-nitrogen (NH₄-N) were measured according to standard methods (APHA-AWWA-WEF 1998). A yeast estrogen screen (YES) assay was used to quantify estrogen activity associated with E2 or EE2 in samples. Details of the YES assay have been described in previous studies (Routledge & Sumpter 1996; Yang & Cicek 2008). In brief, a recombinant yeast strain containing a human estrogen receptor (hER) and an expression plasmid carrying estrogen response elements, was grown in a selective growth medium. When the cells were incubated in the presence of estrogenic compounds in samples, the lac-Z product β-galactosidase is secreted into the medium and causes the chromogenic substrate chlorophenol red β-D-galactopyranoside (CPRG) to change color from yellow to red. This color change can be quantified by measuring absorbance at 540 nm and represents overall EA of the sample.

Prior to the YES assay, samples were pre-treated and then extracted by using cyclohexane extraction for the enrichment of estrogen concentrations. For waste activated sludge (WAS) samples, 100 ml sludge was filtrated by GF/C filters. The GF/C filters were pre-treated by heating in furnace at 550 °C for 1 h to avoid any estrogenic activity in the filters. The filtrates and wastewater samples were then handled as liquid samples. The solids retained by GF/C filters were handled as solid samples. Both liquid and solid samples were then extracted by cyclohexane extraction for the enrichment of estrogen concentrations. During extraction, a 20 ml liquid sample or solid sample obtained from 100 ml sludge was placed in a 125 ml conical flask along with 10 ml cyclohexane. The flasks were shaken for 3 h on a vertical shaker. After shaking, 4 ml of cyclohexane was extracted from each conical flask, dried down under N₂ stream and reconstituted in 0.2 ml absolute ethanol for the YES assay. The average extraction efficiencies of E2 from MBR and PAC-MBR sludge were 82.3 and 71.0%, respectively. The average extraction efficiencies of EE2 from MBR and PAC-MBR sludge were 76.7 and 64.2%, respectively.

### RESULTS AND DISCUSSION

#### Overall performance of MBRs

The control MBR and PAC-MBR were operated in parallel for over 6 months. At steady-state conditions, both MBRs
achieved great performance in removal of COD, ammonia and color (Yang et al. 2010b). Although there was no significant difference in effluent COD between the control MBR and PAC-MBR, the soluble COD of the mixed liquor in the PAC-MBR bioreactor was significantly lower than in the control MBR. This indicates that PAC was capable of enhancing the adsorption of soluble COD to the PAC-sludge.

Another benefit of PAC addition in the MBR was that PAC addition resulted in a slower rate of TMP increase in the PAC-MBR. In practice, a slower TMP increase rate could reduce the MBR operational and maintenance cost for membrane cleaning and/or membrane replacement. PAC addition at the dosage used in this study of 2.0 g/L could reduce annual cost for membrane maintenance by approximately 25% (Yang et al. 2010b).

Removal rates of E2 and EE2 in MBRs

In order to demonstrate the removal of estrogens in the MBR and PAC-MBR, a natural estrogen E2 and a synthetic estrogen EE2 were selected as target contaminants in this study. E2 and EE2 were chosen because they have been released from a wide variety of WWTPs and because they possess relatively high estrogenic activity (Cicek et al. 2007). The removal rates of E2 and EE2 in the control MBR and PAC-MBR were presented in Figure 1.

As shown in Figure 1, the average removal rate of E2 and EE2 by the MBR without PAC dosing was 89.0 and 70.9%, respectively. These removal efficiencies are consistent with the results from previous studies in activated sludge process (Cicek et al. 2007). The synthetic estrogen EE2 appeared to be removed to a lesser extent than E2, which is reasonable because of its more stable chemical structure. PAC addition increased the removal efficiencies of both E2 and EE2 in the MBR system. In comparison with the control MBR, the removal rate of E2 and EE2 in the PAC-MBR was increased by 3.4 and 15.8%, respectively. The impact of PAC dosing on EE2 removal was found to be greater than E2 removal. This observation will be discussed further in the following section.

Removal mechanisms of E2 and EE2 in MBRs

In order to reveal the removal mechanisms of E2 and EE2 in the control MBR and PAC-MBR, mass balances of E2 and EE2 in the two investigated MBRs were performed; this was done by multiplying the average concentrations with daily flow rates. Table 3 shows the results of the mass balance for E2 and EE2. It was found that an average of 11.0 and 7.6% of E2 in the influent was detected in the MBR effluent and PAC-MBR effluent, respectively. By wasting sludge every day, 1.3 and 3.0% of E2 in the influent was removed from the MBR and PAC-MBR, respectively. This resulted in approximately 88% of E2 removal via biodegradation or evaporation in both the MBR and PAC-MBR systems.

As shown in Table 3, an average of 29.1 and 13.3% of EE2 in the influent was detected in the MBR effluent and PAC-MBR effluent, respectively. By wasting sludge every day, an average of 4.1 and 17.1% of EE2 in the influent was removed from the MBR and PAC-MBR, respectively. This indicates that PAC addition increased the adsorption of EE2 to the wasted sludge by 13.0%, much higher than the increase rate of 1.7% for E2 by PAC addition. It was also found that approximately 68% of the EE2 was removed by the mechanisms of biodegradation or evaporation in the MBRs regardless of PAC addition. Because estrogens have very low Henry’s law constants, the removal of estrogens from wastewater by volatilization was likely to be negligible (Khanal et al. 2006). Therefore, biological degradation was the dominant mechanism for the removal of estrogens in the control MBR and PAC-MBR.

Effects of PAC addition on biodegradation of E2 and EE2 in MBRs

Based on the mass balance, an average removal of 87.7% E2 and 66.8% EE2 can be attributed to biological degradation in the control MBR, in contrast to 89.4% E2 and 69.6% EE2 in the PAC-MBR. Joss et al. (2004) have reported that biodegradation of estrogens at low concentrations followed up pseudo-first-order kinetics. By fitting the experimental data into the pseudo-first-order model, the biodegradation...
constant $k_{\text{bio}}$ for E2 and EE2 in the control MBR and PAC-MBR were estimated and compared in Figure 2. As shown, PAC addition had little impact on biodegradation constants of both E2 and EE2 in MBRs.

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

Effects of PAC addition on adsorption of E2 and EE2 to sludge

The data in Table 3 indicate that PAC addition had a greater impact on the removal of EE2 than E2 by means of adsorption to wasted sludge. Based on the concentrations of E2 and EE2 in liquid and solid phase, the observed adsorption coefficients $k_D$ for E2 and EE2 to the MBR and PAC-MBR sludge were estimated and presented in Table 4. The values of observed $k_D$ for E2 and EE2 to the MBR sludge were in good agreement with the results of Clara et al. (2005b). The difference of $k_D$ between E2 and EE2 might be due to their difference in hydrophobic properties, which significantly affect their adsorption capacity to the sludge and PAC. Natural estrogen E2 is considered to be
weakly hydrophobic. The hydrophobicity of EE2 is approximately 10 times greater than E2 (Lai et al. 2000). Therefore, removal of EE2 by sorption to sludge could play a more important role in the removal mechanisms of estrogens in biological wastewater treatment processes. The enhanced adsorption capacity of PAC-MBR sludge could increase the amount of EE2 being adsorbed onto the sludge.

It was reported that AC had very high adsorption capacities for estrogens, with a maximum adsorption constant 12.2 L/g for E2 (Zhang & Zhou 2005). Assuming that the PAC dosed in the MBR reached its maximum adsorption capacity 12.2 L/g-AC for E2, the amount of E2 removed by the adsorption to the PAC in the wasted PAC-MBR sludge should be 238.6 ng/d (81.5 ng/L × 0.24 g/d × 12.2 L/g). The calculated amount was much higher than the observed amount 108.1 ng/d, which was the difference of the removal amounts of E2 by WAS between the MBR and PAC-MBR (Table 4). The lower observed value was likely because AC had lower adsorption capacity for E2 in mixed liquor than in water. In the study by Zhang & Zhou (2005), the maximum adsorption capacities of AC were obtained from the experiments using aqueous solution of E2 in water. The complex composition of wastewater and mixed liquor could reduce the AC’s adsorption capacity (Ifelebuegu et al. 2006).

**CONCLUSIONS**

Two bench-scale MBRs, one without PAC addition and the other with PAC addition, were operated in parallel under similar experimental conditions to investigate the removal mechanisms of E2 and EE2 in MBRs. The following conclusions can be drawn from this study:

1. The average removal rate of E2 and EE2 by the MBR without PAC dosing was 89.0 and 70.9%; PAC dosing in the MBR increased the removal rate of E2 and EE2 by 3.4 and 15.8%, respectively. The synthetic estrogen EE2 appeared to be removed to a lesser extent than E2 in the two MBRs regardless of PAC dosing.

2. Biodegradation was the dominant mechanism for the removal of E2 and EE2 in MBRs; the biodegradation rates of both E2 and EE2 were not significantly different between the MBRs with and without PAC addition.

3. Adsorption played a more important role in the removal mechanisms of EE2 than E2. The greater impact of PAC addition on the removal of EE2 than E2 in MBRs was due to the greater hydrophobic property of EE2.

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