Removal of endocrine disrupting chemicals and microbial indicators by a decentralised membrane bioreactor for water reuse

T. Trinh, B. van den Akker, H. M. Coleman, R. M. Stuetz, P. Le-Clech and S. J. Khan

ABSTRACT

Submerged membrane bioreactors (MBRs) have attracted a significant amount of interest for decentralised treatment systems due to their small footprint and ability to produce high quality effluent, which is favourable for water reuse applications. This study provides a comprehensive overview of the capacity of a full-scale decentralised MBR to eliminate 17 endocrine disrupting chemicals (EDCs) and five indigenous microbial indicators. The results show that the MBR consistently achieved high removal of EDCs (>86.5%). Only 2 of the 17 EDCs were detected in the MBR permeate, namely two-phenylphenol and 4-tert-octylphenol. Measured log10 reduction values of vegetative bacterial indicators were in the range of 5–5.3 log10 units, and for clostridia, they were marginally lower at 4.6 log10 units. Removal of bacteriophage was in excess of 4.9 log10 units. This research shows that MBRs are a promising technology for decentralised water reuse applications.

INTRODUCTION

In regional and rural communities where connection to centralised sewer networks is not possible or is economically unfeasible, decentralised wastewater treatment systems (or package plants) are becoming the preferred option for sewage treatment. Recently, submerged membrane bioreactors (MBRs) have attracted a significant amount of interest for decentralised treatment systems due to their small footprint and ability to produce high quality effluent, which is favourable for water reuse applications (Coleman et al. 2009; Le-Minh et al. 2010).

In Australia, implementation of water recycling processes such as MBRs requires validation to demonstrate that the process is capable of achieving the required water quality objectives (Australian Guidelines for Water Recycling 2008). Validation is most frequently based on characterising the removal of contaminants with health effects associated with acute or single dose exposures and therefore the majority of research on MBRs has focused on the removal of human pathogens or their surrogates (e.g. faecal coliforms, bacterial spores and bacteriophage).

Over the past decade, interest in the ability of MBRs to eliminate trace organic chemicals, such as endocrine disrupting chemicals (EDCs), has increased – particularly for water reclamation schemes that have potential for chronic human exposure (e.g. direct or indirect potable reuse). In contrast to microbial constituents, the efficiency of MBR technology as a barrier for EDCs is less clear and most of the data available have been derived from pilot- or laboratory-scale MBRs (e.g. Chen et al. 2008; Tadkaew et al. 2011). These studies show high removal of EDCs within the order of 90.4–99.5%; however, without complementary research at the field scale, it can only be assumed that these values reflect the performance of larger-scale systems.
Accordingly, the aim of this study was to investigate the removal of EDCs through a full-scale package MBR plant treating municipal wastewater in New South Wales, Australia. The removal of microbial indicators was also characterised in parallel to provide a comprehensive overview of the MBR’s overall capacity to remove key contaminants of concern. The selected EDCs included seven natural and synthetic steroidal estrogens (17α-estradiol, 17β-estradiol, estrone, mestranol, 17α-ethynylestradiol, levonorgestrel, estriol), five steroidal androgens (testosterone, androsterone, etiocholanolone, dihydrotestosterone, androstenedione) and five xenoestrogens (bisphenol A, nonylphenol, 2-phenylphenol, propylparaben, 4-tert-octylphenol). As such, they represent the full suite of EDCs that have been subject to most environmental concerns internationally. Five indigenous microbial indicators were monitored: total coliforms, Escherichia coli, enterococci, sulphite-reducing clostridia (SRC) and F-RNA bacteriophage. These microbial indicators were selected because they are commonly used as surrogates for estimating the removal of pathogens in wastewater treatment systems (Wen et al. 2009).

MATERIALS AND METHODS

Description of the decentralised MBR

Samples were collected from a decentralised full-scale MBR plant (800 equivalent persons) located in Wolumla, Bega Valley, New South Wales, Australia. A schematic diagram of the MBR is presented in Figure 1, which summarises the key components, flow direction and sample sites. The treatment process comprises a fine screen (3 mm), a bioreactor tank, two parallel-submerged membrane modules and a medium pressure ultra-violet (UV) disinfection unit. The sludge retention time of the bioreactor was 10–15 d, the hydraulic retention time was 1 d and the mixed liquor suspended solids concentration was 7.5–8.5 g L⁻¹. The bioreactor tank was intermittently aerated in 10 min cycles (dissolved oxygen set-point of 1 mg L⁻¹) to achieve simultaneous nitrification and denitrification. The submerged membrane modules were made of hollow fibre membranes (Koch Puron), which have an effective pore size of 0.1–0.2 μm and a surface area of 235 m² (each). For cleaning, scour air was applied to the membranes using a positive displacement blower and backwashing occurred for a period of 60 s every 360 s. Chemical backwashing occurred automatically every 3 weeks, in accordance with the manufacturer’s recommendations, to maintain a transmembrane pressure of <20 kPa. The membrane unit was designed to achieve an average flux of 25 L m⁻² h. All of the final effluent is used for irrigation. The water quality values in the raw sewage and MBR permeate are presented in Table 1.

Analysis of EDCs

Sample collection

Daily composite aqueous samples of raw sewage (0.5 L) and MBR permeate (1 L) were taken in triplicate over a 5-day period in March 2011 (giving a total of 15 raw sewage samples and 15 MBR permeate samples). After collection, raw sewage was immediately filtered through 0.7 μm

Table 1 | Quality of raw sewage and MBR permeate

<table>
<thead>
<tr>
<th>Quality parameters</th>
<th>Raw sewage range (mean) (n=5)</th>
<th>MBR permeate range (mean) (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>106.7–120.8 (114)</td>
<td>12.5–13.8 (13.2)</td>
</tr>
<tr>
<td>NH₃ (mg L⁻¹)</td>
<td>35.7–50.7 (43.2)</td>
<td>0–0.2 (0.1)</td>
</tr>
<tr>
<td>Total N (mg L⁻¹)</td>
<td>77.3–92.5 (81.5)</td>
<td>3.1–6.2 (4.5)</td>
</tr>
<tr>
<td>Total P (mg L⁻¹)</td>
<td>Unavailable</td>
<td>5.0–7.4 (6.2)</td>
</tr>
<tr>
<td>pH</td>
<td>6.8–7.2 (7.0)</td>
<td>7.7–8.1 (7.9)</td>
</tr>
</tbody>
</table>

DOC: dissolved organic carbon.
Millipore glass fibre prefilters. All samples were then spiked with isotopically labelled standards of trace chemicals of interest for accurate isotope dilution quantification. The samples were stored in ice and extracted on site using solid phase extraction (SPE) within 24 h of collection. The SPE procedure was reported in a previous publication (Trinh et al. 2011b).

**LC/MS-MS analysis**

The concentrations of nonylphenol, 2-phenylphenol, bisphenol A, 4-tert-octylphenol and propylparaben in the samples were analysed by LC-MS/MS method using negative mode electrospray ionisation, following an adaptation of a previous published method (Vanderford & Snyder 2006). Direct isotopically labelled analogues were used for nonylphenol (D4-nonylphenol), 2-phenylphenol (phenylphenol-13C6-1) and bisphenol A (D6-bisphenol A). No direct isotopically labelled compound is available for 4-tert-octylphenol and propylparaben, therefore D17-n-octylphenol was used for quantification of 4-tert-octylphenol and D6-bisphenol A was used for quantification of propylparaben.

**Trimethylsilyl derivatisation and GC/MS-MS analysis**

After analysis by LC-MS/MS, the same samples were processed for GC-MS/MS analysis of steroidal hormones using a previously published method (Trinh et al. 2011a). The physicochemical properties of the EDCs are presented in Table 2.

**Analysis of microbial indicators**

Refrigerated time-proportional composite sampling of the raw sewage and membrane permeate (pre-UV disinfection) was performed to assess the MBR's overall capacity to remove microbial indicators. Slanetz and Bartley Agar plates (Oxoid CM0377) were used to enumerate enterococci and incubated at 44°C for 44 h. Brilliance agar (Oxoid CM1046) was used to enumerate both E. coli and total coliforms, which were incubated at 37°C for 24 h.

### Table 2 | Physicochemical properties of the EDCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS number</th>
<th>Formula</th>
<th>Molecular weight (g/mol)</th>
<th>Partition coefficient Log $K_{ow}$</th>
<th>Distribution coefficient Log $D$ pH − 8</th>
<th>pH$_{ka}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-Estradiol</td>
<td>57-91-0</td>
<td>C$<em>{18}$H$</em>{24}$O$_{2}$</td>
<td>272.4</td>
<td>4.13</td>
<td>4.13</td>
<td>10.27</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>50-28-2</td>
<td>C$<em>{18}$H$</em>{24}$O$_{2}$</td>
<td>272.4</td>
<td>4.13</td>
<td>4.13</td>
<td>10.27</td>
</tr>
<tr>
<td>17α-Ethynylestradiol</td>
<td>57-63-6</td>
<td>C$<em>{20}$H$</em>{24}$O$_{2}$</td>
<td>296.4</td>
<td>4.52</td>
<td>4.52</td>
<td>10.24</td>
</tr>
<tr>
<td>Estriol</td>
<td>50-27-1</td>
<td>C$<em>{18}$H$</em>{24}$O$_{3}$</td>
<td>288.4</td>
<td>2.94</td>
<td>2.94</td>
<td>10.25</td>
</tr>
<tr>
<td>Estrone</td>
<td>53-16-7</td>
<td>C$<em>{18}$H$</em>{22}$O$_{2}$</td>
<td>270.4</td>
<td>3.69</td>
<td>3.68</td>
<td>10.25</td>
</tr>
<tr>
<td>Lenovogestrel</td>
<td>797-63-7</td>
<td>C$<em>{21}$H$</em>{28}$O$_{2}$</td>
<td>312.4</td>
<td>Unavailable</td>
<td>3.37</td>
<td>13.09</td>
</tr>
<tr>
<td>Mestranol</td>
<td>72-33-3</td>
<td>C$<em>{21}$H$</em>{26}$O$_{2}$</td>
<td>310.4</td>
<td>Unavailable</td>
<td>4.94</td>
<td>13.10</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>63-05-8</td>
<td>C$<em>{19}$H$</em>{26}$O$_{2}$</td>
<td>286.4</td>
<td>2.90</td>
<td>2.90</td>
<td>8.78</td>
</tr>
<tr>
<td>Etocholanolone</td>
<td>53-42-9</td>
<td>C$<em>{19}$H$</em>{30}$O$_{2}$</td>
<td>290.4</td>
<td>3.75</td>
<td>3.75</td>
<td>15.13</td>
</tr>
<tr>
<td>Androsterone</td>
<td>53-41-8</td>
<td>C$<em>{19}$H$</em>{30}$O$_{2}$</td>
<td>290.4</td>
<td>3.93</td>
<td>3.93</td>
<td>15.14</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>521-18-6</td>
<td>C$<em>{19}$H$</em>{30}$O$_{2}$</td>
<td>290.4</td>
<td>Unavailable</td>
<td>3.95</td>
<td>15.08</td>
</tr>
<tr>
<td>Testosterone</td>
<td>58-22-0</td>
<td>C$<em>{19}$H$</em>{28}$O$_{2}$</td>
<td>288.4</td>
<td>3.47</td>
<td>3.47</td>
<td>15.06</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>80-05-7</td>
<td>C$<em>{13}$H$</em>{16}$O$_{2}$</td>
<td>228.3</td>
<td>3.43</td>
<td>3.43</td>
<td>9.73</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>104-40-5</td>
<td>C$<em>{15}$H$</em>{24}$O$_{2}$</td>
<td>220.4</td>
<td>Unavailable</td>
<td>6.19</td>
<td>10.14</td>
</tr>
<tr>
<td>2-Phenylphenol</td>
<td>90-43-7</td>
<td>C$<em>{12}$H$</em>{10}$O$_{2}$</td>
<td>170.2</td>
<td>Unavailable</td>
<td>3.29</td>
<td>10.00</td>
</tr>
<tr>
<td>Propylparaben</td>
<td>94-13-3</td>
<td>C$<em>{10}$H$</em>{12}$O$_{3}$</td>
<td>180.2</td>
<td>Unavailable</td>
<td>2.70</td>
<td>8.23</td>
</tr>
<tr>
<td>4-Tert-octylphenol</td>
<td>140-66-9</td>
<td>C$<em>{14}$H$</em>{22}$O$_{2}$</td>
<td>206.3</td>
<td>4.93</td>
<td>4.93</td>
<td>10.15</td>
</tr>
</tbody>
</table>

Source: SciFinder Scholar (2011); Tadkaew et al. (2011).
indicators were selected because they are commonly used as surrogates for estimating the removal of pathogenic bacteria in wastewater treatment systems (Wen et al. 2009). SRC were enumerated using the tryptose sulphite cycloserine agar for Clostridium perfringens (Oxoid CM0587), and incubated anaerobically at 35°C for 24 h. F-RNA bacteriophage were quantified using the double agar layer technique as per the method of Noble et al. (2004), using E. coli F-amp (ATCC No. 700891) as the host and MS2 bacteriophage as the positive control. SRC and F-RNA bacteriophage were included because they are widely used as surrogates for measuring the inactivation of protozoa and enteric human viruses respectively (Wen et al. 2009; van den Akker et al. 2011).

All bacterial indicators measured within the permeate were quantified using membrane filtration (Standard Methods for the Examination of Water and Wastewater 1992), whereby a desired quantity of sample (typically 5, 50 and 100 mL) was filtered through a 47 mm diameter, 0.45 μm gridded filter membrane (Millipore, S-Pak, type HA). The filter membrane was then transferred onto the surface of a well-dried plate of selective agar.

RESULTS AND DISCUSSION

Endocrine disrupting chemicals

Levels of EDCs in raw sewage

Concentrations of EDCs in raw sewage are presented in Table 3. The main components of the contraceptive pill (17α-ethynylestradiol, mestranol and levonorgestrel) and the breakdown product of the chemical used in detergents and personal care products (nonylphenol) were not detected. Natural estrogenic hormones detected include 17α-estradiol, 17β-estradiol and its metabolised products estrone and estriol. The androgenic hormone, testosterone and its androgenic metabolised products androsterone, etiocholanolone, androstenedione and dihydrotestosterone were also detected. The levels of androgenic hormones detected were higher than those of estrogenic hormones, which may be due to the higher excretion rates of androgens compared to estrogens in humans (Le-Minh et al. 2010). Generally, the levels of steroidal hormones within the sewage were comparable to

<p>| Table 3 | Concentrations and removals of the EDCs by the MBR (samples taken in triplicate over a 5-day period) |</p>
<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Raw sewage range (mean) (ng L⁻¹)</th>
<th>MBR permeate range (ng L⁻¹)</th>
<th>Removal range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-Estradiol</td>
<td>3.7–6.5 (5.0)</td>
<td>&lt;0.5</td>
<td>&gt;86.5–92.3</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>26.5–41.7 (32.6)</td>
<td>&lt;0.7</td>
<td>&gt;97.4</td>
</tr>
<tr>
<td>17α-Ethynylestradiol</td>
<td>&lt;1.2</td>
<td>&lt;0.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Estriol</td>
<td>291–1,053 (574)</td>
<td>&lt;1.5</td>
<td>&gt;99.5</td>
</tr>
<tr>
<td>Estrone</td>
<td>88–173 (127)</td>
<td>&lt;0.4</td>
<td>&gt;99.6</td>
</tr>
<tr>
<td>Lenovorgestrel</td>
<td>&lt;7.0</td>
<td>&lt;3.5</td>
<td>n/a</td>
</tr>
<tr>
<td>Mestranol</td>
<td>&lt;1.2</td>
<td>&lt;0.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>99–465 (216)</td>
<td>&lt;2.8</td>
<td>&gt;97.2</td>
</tr>
<tr>
<td>Etioccholanolone</td>
<td>6,884–9,162 (7,682)</td>
<td>&lt;3.2</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Androsterone</td>
<td>2,090–2,565 (2,360)</td>
<td>&lt;0.7</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>450–1,453 (716)</td>
<td>&lt;7.5</td>
<td>&gt;98.3</td>
</tr>
<tr>
<td>Testosterone</td>
<td>88–541 (215)</td>
<td>&lt;3.0</td>
<td>&gt;96.6</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>453–1,200 (842)</td>
<td>&lt;10.0</td>
<td>&gt;97.8</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>&lt;1.0</td>
<td>&lt;0.5</td>
<td>n/a</td>
</tr>
<tr>
<td>2-Phenylphenol</td>
<td>2,150–4,290 (3,057)</td>
<td>11.2–15.6</td>
<td>99.5–99.6</td>
</tr>
<tr>
<td>Propylparaben</td>
<td>2,270–5,260 (4,053)</td>
<td>&lt;0.5</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>4-tert-Octylphenol</td>
<td>2,170–8,190 (5,175)</td>
<td>18.0–33.8</td>
<td>99.2–99.6</td>
</tr>
</tbody>
</table>

n/a: not applicable.
values reported in previous Australian research (Coleman et al. 2009, 2010; Le-Minh et al. 2010), with the exception of testosterone and dihydrotestosterone, which were found to be one to two orders of magnitude higher in the current study. This may be due to the higher sensitivity of the analytical method used here compared to other studies (Coleman et al. 2009, 2010; Le-Minh et al. 2010).

The detected estrogenic phenolic compounds include bisphenol A, 2-phenylphenol and 4-tert-octylphenol. Bisphenol A is used to produce polycarbonate plastic and epoxy resins (Staples et al. 1998) and 2-phenylphenol is used as an agriculture fungicide and household disinfectant (Tumah 2005). 4-tert-octylphenol is the breakdown product of octylphenol ethoxylate, which is widely used in detergents, emulsifiers, solubilisers, wetting agents and dispersants (Staples et al. 1999). The level of bisphenol A detected was comparable with previous studies (Lee et al. 2005; Cases et al. 2011) while the level of 4-tert-octylphenol detected was one order of magnitude higher than values reported previously (Coleman et al. 2009; Cases et al. 2011). This may again be due to the highly sensitive method used for analysis. Literature on the level of 2-phenylphenol in raw sewage is still limited but a previous study reported similar values to those found in this study (Lee et al. 2005).

Propylparaben is a preservative typically found in many water-based cosmetics, such as creams, lotions and some bath products. This compound was detected at concentrations of 2,270–5,260 ng L\(^{-1}\) which is comparable with previous reported values in the raw sewage (Regueiro et al. 2009).

**Removal of EDCs by the MBR**

The percentage removal of the EDCs investigated are presented in Table 3. The results show that the MBR removed the studied EDCs effectively with most removal rates being close to 100%. 2-phenylphenol and 4-tert-octylphenol were the only chemicals detected in the MBR permeate at concentrations of 11.2–15.6 and 18.0–33.8 ng L\(^{-1}\) respectively. However, removal efficiencies were still high (99.2–99.6%). The concentrations of 2-phenylphenol and 4-tert-octylphenol were two to three orders of magnitude lower than Australian guideline values for water recycling (Australian Guidelines for Water Recycling 2008). All other studied EDCs were undetectable in the MBR permeate. This indicates that MBRs are extremely promising for water reuse applications in terms of removal of EDCs.

These excellent removal efficiencies of steroidal hormones are consistent with previous studies on MBRs (Coleman et al. 2009; Le-Minh et al. 2010). The mechanisms responsible for removing these steroidal hormones in MBR plants typically include a combination of particulate adsorption and biodegradation (Cirja et al. 2008; Abegglen et al. 2009; Coleman et al. 2009). The estrogenic hormones are classified as having moderate hydrophobicity to high hydrophobicity with log \(D_{pH=8}\) values of 2.9 to 4.9 therefore having medium to high sorption potential to biomass (Rogers 1996; Cirja et al. 2008). Information on fate and removal of androgenic hormones through treatment processes is limited compared to that of estrogenic hormones. However, the log \(D_{pH=8}\) values of androgenic hormones suggesting that these compounds are moderately to highly absorbed to the biomass (Liu et al. 2009).

The high removal efficiencies of bisphenol A and 4-tert-octylphenol were comparable with other studies on MBRs (Coleman et al. 2009; Tadkaew et al. 2010, 2011; Cases et al. 2011). A previous study found high concentrations of 4-tert-octylphenol in biomass which indicated that adsorption to biomass was the main pathway of removal for this compound (Coleman et al. 2009). This can be explained by its hydrophobicity with high distribution coefficient (log \(D_{pH=8}=4.93\)) (Tadkaew et al. 2011). In contrast, bisphenol A has been found at low concentration in the biomass suggesting that biodegradation is the main mechanism responsible for the removal of this compound (Chen et al. 2008) since bisphenol A is a moderately hydrophobic compound with log \(D_{pH=8}=3.43\) (Tadkaew et al. 2011).

This is the first reported study to investigate the removal of 2-phenylphenol and propylparaben by MBRs, which was >99%. Limited data concerning the removal of these compounds through wastewater treatment processes are available, with the exception of Regueiro et al. (2009) who reported removal efficiencies above 90% by a conventional wastewater treatment process.

**Microbial indicator organisms**

The numbers of indicators in the raw sewage and permeate including their reductions are summarised in Table 4.
The mean log_{10} reduction values of all microbial indicators are comparable to those reported in pilot-scale studies (Otto-
son et al. 2006; Zhang & Farahbakhsh 2007; Marti et al. 2011). The log_{10} reduction of SRC (4.6 log_{10} units) was marginally
lower than all vegetative bacterial indicators (5.0–5.3 log_{10} units) and may be viewed as a useful worst-case performance
benchmark. Removal values for F-RNA phage reached >5.7 log_{10} units; however, a reliable estimate of their removal
was not obtained because they were not detected in the permeate. The failure to detect F-RNA phage within the
permeate can be attributed to a combination of: (i) poor sen-
sitivity of the assay, which was constrained by the low sample
volume (10 mL); and (ii) low density within the sewage.

### CONCLUSIONS

This study provides a comprehensive overview of a full-scale package MBR’s ability to remove 17 different types of EDCs
and five microbial indicators. The results of chemical analy-
sis show that MBR treatment was highly effective in
removing all of the studied EDCs. Of the 17 studied
EDCs, only 2-phenylenol and 4-tert-octylphenol were
detected in the MBR permeate. The removal of all microbial indicators was in the range of 4.6–5.3 log_{10} units. This study
highlights the applicability of MBRs as decentralised sys-
tems for water reuse.

### ACKNOWLEDGEMENTS

This work was supported by the Australian Research Council Linkage Project LP0989365 (with industry
support from MidCoast Water, Bega Valley Council,
Hunter Water and NSW Health) and Water Quality
Research Australia. In particular, we thank Ken McLeod,
Chris Scharf and Tony Brown from Bega Valley Council
for their support during the sampling period. The authors
also thank Dr James McDonald for his technical support
with the undertaking of this work and Dr David Halliwell
for his helpful comments on the manuscript.

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of endocrine disrupting chemicals and pharmaceuticals.


SciFinder Scholar 2011 Data calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 @ 1994–2011 ACD/Labs.


First received 13 January 2012; accepted in revised form 4 March 2012