Micropollutants removal in an anaerobic membrane bioreactor and in an aerobic conventional treatment plant
M. R. Abargues, A. Robles, A. Bouzas and A. Seco

ABSTRACT

The paper expresses an attempt to tackle the problem due to the presence of micropollutants in wastewater which may be able to disrupt the endocrine system of some organisms. These kinds of compounds are ubiquitously present in municipal wastewater treatment plant (WWTP) effluents. The aim of this paper is to compare the fate of the alkylphenols–APs (4-(tert-octyl) phenol, t-nonylphenol and 4-p-nonylphenol and the hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) in a submerged anaerobic membrane bioreactor (SAMBR) pilot plant and in a conventional activated sludge wastewater treatment plant (CTP). The obtained results are also compared with the results obtained in a previous study carried out in an aerobic MBR pilot plant. The results showed that the APs soluble concentrations in the SAMBR effluent were always significantly higher than the CTP ones. Moreover, the analyses of the suspended fraction revealed that the AP concentrations in the SAMBR reactor were usually higher than in the CTP reactor, indicating that under anaerobic conditions the APs were accumulated in the digested sludge. The aerobic conditions maintained both in the CTP system and in the aerobic MBR favoured the APs and hormones degradation, and gave rise to lower concentrations in the effluent and in the reactor of these systems. Furthermore, the results also indicated that the degradation of APs under aerobic conditions was enhanced working at high solid retention time (SRT) and hydraulic retention time (HRT) values.

Key words | activated sludge plant, alkylphenols, endocrine disruptor, estrogenic hormones, membrane bioreactor

INTRODUCTION

Nowadays, society has changed its consumption habits, and large quantities of industrial products, drugs, and antibiotics are being produced. In developed countries, the increase in prescription and consumption of pharmaceutical products can be observed in the presence of these products in wastewater treatment plant (WWTP) streams, both in the sludge and in the effluent (Diaz et al. 2002; Kasprzyk-Hordern et al. 2008).

Among the great variety of non-natural substances that nowadays can be found in waters, the alkylphenol polyethoxylates (APEOs), their metabolites (alkylphenols–APs) and certain hormones are being widely studied, due to their potential to act as endocrine disrupting compounds (EDCs) affecting the normal function of endocrine systems of some organisms. These micropollutants and other EDCs have been studied in surface waters (Ying & Kookana 2003; Bouzas et al. 2011; Martí et al. 2011).

APEOs are a group of compounds widely used as nonionic surfactants with industrial, agricultural and domestic applications (Petrovic & Barcelo 2010). APEOs can be degraded during the wastewater treatment process to APs (Giger et al. 1984; Lu et al. 2008): 4-p-nonylphenol (4-NP, CAS Number 104-40-5), 4-(1,1,3,3-tetramethylbutyl)phenol (OP; CAS Number 140-66-9) and technical nonylphenol (t-NP, CAS Number 84852-15-3). These APs are more toxic and lipophilic than APEOs and are considered as EDCs. Table 1 shows the chemical structure and the log $K_{ow}$, which provides a measure of bioaccumulation for each analyte. The estrogenic activity observed for APs appeared to be confined to para- or 4-substituted compounds (Jobling & Sumpter 1993). The mentioned estrogenic activity becomes stronger with the increase in the number of the alkyl carbons. This activity is maximized with a nonyl chain (Tabira et al. 1999).

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Key words | activated sludge plant, alkylphenols, endocrine disruptor, estrogenic hormones, membrane bioreactor

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Elimination of APEOs has been studied by several authors in conventional activated sludge (CTP) or membrane bioreactor (MBR) operational configurations. González et al. (2007) studied the removal of APEOs using a microfiltration aerobic MBR configuration working in parallel with a CTP working in aerobic conditions. The removal rates obtained in the MBR showed better results than the ones reported for the CTP. Similarly, Clara et al. (2005) concluded that removal of APEOs in aerobic conditions in an ultrafiltration MBR system produces better results, but it is necessary to have an anaerobic step for a complete biodegradation of APEOs. Giger et al. (1984) studied the effect of APEOs on digested sludge concluding that alkylphenols mono- and di-ethoxylates are degraded to APs under anaerobic digestion and these metabolites are accumulated in the digested sludge. This finding was confirmed following the increase in the concentration of 4-NP and t-NP in anaerobic digested sludge, which indicates that anaerobic environments favour the accumulation of nonylphenols (Tan et al. 2008). As the degradation of APEOs into APs is promoted under anaerobic conditions, therefore the load of nonylphenols during anaerobic digestion is in most cases observed to increase in the aqueous phase (Janex-Habibi et al. 2009).

Among the hormones, it is important to highlight estrone (E1, CAS Number 53-16-7) and 17β-estradiol (E2, CAS Number 50-28-2), which are sexual female hormones, and 17α-ethynylestradiol (EE2, CAS Number 57-63-6), a synthetic estrogenic hormone, which are also considered as EDCs. 17β-estradiol and estrone are natural hormones derived from excreta of humans and livestock. 17α-ethynylestradiol is the main component of the oral contraceptive pill. The biodegradation of E1, E2 and EE2 in WWTP processes seems to be very effective in both aerobic and anaerobic conditions (Joss et al. 2004; Zeng et al. 2009; Xu et al. 2012).

European Commission (2000), also known as the Water Framework Directive (WFD), is probably the most significant international legislation introduced in the field of water for many years. WFD includes and protects different kinds of water in Europe (surface water, groundwater, transitional and coastal waters) with the aim of achieving and ensure a good quality for all of them. Moreover, European Commission (2008) lays down environmental quality standards (EQS) for priority substances and certain other pollutants as provided for in the WFD. These priority substances and other pollutants used to appear in waters at concentrations lower than µg/L. OP and 4-NP are included in the WFD as priority substances. European Commission (2008) set the extent permitted of OP and 4-NP in inland and other surface waters, expressed as an annual average value (EQS-AA) or as maximum allowable concentration (EQS-MAC). The EQS-AA value for OP is 0.1 µg/L and 0.01 µg/L for inland and other surface waters respectively. The EQS-AA value for 4-NP is 0.3 µg/L for inland and other surface waters and the EQS-MAC is 2.0 µg/L. So, attention must be paid to the fate of these substances in order to fulfill the WFD requirements. E2 and EE2 are being considered to be included as priority substances in the WFD.

The APs and hormones, described above, are hydrophobic organic pollutants, and in aquatic environments tend to accumulate on the solid phases such as sediments, underwater fauna or WWTP sludge (Liu et al. 2004; Zhang & Zhou 2008). However, the magnitude of this accumulation is dependent on analytes and solid phase properties. Therefore, not only the aqueous phase but also the solid phases must be considered in order to study the fate of these micropollutants. The uses of APs and hormones make them very likely to be found in municipal and industrial wastewaters. Therefore, the fate of these substances and their metabolites in the WWTP must be considered in order to analyse their biological or physical removal.

The aim of this work is to compare the fate of the APs (OP, 4-NP and t-NP) and the hormones (E1, E2 and EE2) in a submerged anaerobic membrane bioreactor (SAMBR) pilot plant and in a conventional activated sludge wastewater treatment plant (CTP). The obtained results are also compared with the results obtained in a previous study carried out in an aerobic MBR pilot plant.

**MATERIALS AND METHODS**

**Plant descriptions**

The three plants compared in this study (SAMBR, CTP and aerobic MBR) were located in Alboraya (Valencia, Spain),
and all of them received the same wastewater. The three plants were prepared for organic matter removal without nitrogen removal.

The CTP, with a capacity of 140,000 population equivalents, treated domestic wastewater combined with a small fraction of industrial wastewater. This CTP was operated at an average solid retention time (SRT) and hydraulic retention time (HRT) of 5 d and 4 h respectively. The CTP system mainly consists of primary sedimentation, an aerobic reactor (4,192 m$^3$, average suspended solids between 2 and 3 g/L) and secondary clarification.

The SAMBR consists of a rotary screening system (0.5 mm screen size), an anaerobic bioreactor (0.9 m$^3$ working volume, average suspended solids between 16 and 22 g/L) and two ultrafiltration membrane tanks (0.6 m$^3$ working volume each). Each membrane tank includes one industrial hollow-fibre ultrafiltration membrane module (PURON® Koch Membrane Systems (PUR-PSH31), 0.05 μm pore size). The maximum value of transmembrane pressure reached was 0.08 bar. Each module consists of 9 hollow-fibre bundles of 1.8 m length giving a total of 30 m$^2$ membrane surface. The pilot plant was operated at an average SRT of 80 d and at HRT of 8 h, from October to January (period I), and 25 h from February to May (period II).

The third plant analysed in this paper consists of an aerobic immersed MBR pilot plant (1 m$^3$ working volume, average suspended solids 15 g/L) with a submerged hollow-fibre ultrafiltration membrane module (Zenon, Zee-Weed® 500 module) with a total filtration surface of 46.5 m$^2$. The membrane module is continuously aerated to minimize the fouling process. During the experimental period, the SRT was kept at 100 d and the HRT was 9 h.

The SAMBR received degritted wastewater from the CTP while the aerobic MBR received settled wastewater from the primary settlers of the CTP. Table 2 shows the average wastewater characteristics of the influent to the three systems during the experimental period studied. This table highlights the significant sulphate concentration and the relatively low soluble chemical oxygen demand (COD) concentration of the influent, as well as the strong variability of the influent load as can be deduced from the high values of standard deviation associated with each parameter.

### Table 2 | Average influent wastewater characteristics. ‘s.d.’ indicates the standard deviation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>SAMBR Mean ± s.d.</th>
<th>CTP Mean ± s.d.</th>
<th>Aerobic MBR Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>mg TSS/L</td>
<td>330 ± 90</td>
<td>115 ± 30</td>
<td>120 ± 40</td>
</tr>
<tr>
<td>VSS</td>
<td>mg VSS/L</td>
<td>270 ± 80</td>
<td>97 ± 30</td>
<td>95 ± 30</td>
</tr>
<tr>
<td>Total COD</td>
<td>mg COD/L</td>
<td>600 ± 200</td>
<td>290 ± 30</td>
<td>310 ± 20</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>mg COD/L</td>
<td>68 ± 13</td>
<td>96 ± 13</td>
<td>135 ± 10</td>
</tr>
<tr>
<td>VFA</td>
<td>mg COD/L</td>
<td>9 ± 5</td>
<td>6 ± 3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>SO$_4$S</td>
<td>mg S/L</td>
<td>102 ± 9</td>
<td>112 ± 7</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>NH$_4$N</td>
<td>mg N/L</td>
<td>26 ± 6</td>
<td>32 ± 4</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>PO$_4$P</td>
<td>mg P/L</td>
<td>3.5 ± 1.5</td>
<td>5 ± 2</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td>Alk</td>
<td>mg CaCO$_3$/L</td>
<td>310 ± 80</td>
<td>350 ± 30</td>
<td>300 ± 40</td>
</tr>
</tbody>
</table>

### Sampling

The CTP and the SAMBR systems were studied from October 2010 to May 2011. In order to prevent systematic mistakes, collected samples were distributed randomly throughout the week during the studied period. The results of the aerobic MBR presented in this paper correspond to a previous work carried out from May to July 2009. A three-point sampling campaign was carried out in each plant in order to study the distribution and fate of APs and hormones.

In the SAMBR, three samples were collected: influent (after the rotary screener), anaerobic reactor and effluent (membrane permeate). In the CTP, the samples were collected after the primary sedimentation (influent), in the aerobic reactor and after the secondary clarification (effluent). Finally, for the aerobic MBR, the samples were taken after the CTP primary sedimentation (influent), in the MBR aerobic reactor and in the effluent (membrane permeate).

Influent and effluent samples were collected in glass bottles as 24 h composite samples while reactor ones were grab samples. Samples were centrifuged at 3,500 rcf for 10 min and filtered through 0.45 μm nylon paper filters (Millipore, Bedford, MA) to remove suspended solids in order to
separate the soluble fraction from the suspended fraction (excepting permeate samples). The soluble fraction was extracted by solid phase micro-extraction (SPME) and analysed by gas chromatography/mass spectrometry (GC/MS). The suspended fraction was dehydrated by freeze-drying, and later extracted with acetonitrile and analysed by GC/MS.

Reagents and solutions

All the reagents were of analytical grade. 4-p-nonylphenol (CAS Number 104-40-5) and technical nonylphenol (CAS Number 84852-15-3) were obtained from Riedel-de Haën (Seelze, Germany). 4-(tert-octyl) phenol (CAS Number 140-66-9), estrone (CAS Number 53-16-7) and 17β-estradiol (CAS Number 50-28-2) were purchased from Sigma-Aldrich (Steinheim, Germany). 17α-ethinylestradiol (CAS Number 57-63-6) was purchased from Fluka Biochemika (Steinheim). Methanol was purchased from Merck (Darmstadt, Germany). Pure water was obtained by means of a Milli-Q water purification system (Millipore, Bedford, MA, USA). Helium used as carrier gas was purchased from Carburos Metálicos (Barcelona, Spain). The stock solutions of standards were prepared in methanol up to a maximum concentration of 1,000 mg/L. The more dilute solutions were prepared from stock solutions directly in water up to a maximum concentration of 1 mg/L. All solutions were kept at 4°C until use.

Apparatus and chromatographic conditions

All analyses were performed on a GC/MS system 6,890 GC with 5,973 MSD (Agilent, San José, USA). The capillary column was a fused-silica HP-5 MS (30.0 m, 250 μm I.D., 0.25 μm film thickness) (Agilent, San José, USA). Helium was used as carrier gas with a flow of 1.0 mL/min. The transfer line was held at 280°C, and the ion source at 250°C. The MS worked in selected-ion-monitoring mode and the electron impact energy was set to 69.9 eV. The gas chromatograph was operated in splitless mode and the injection port temperature was held isothermally at 280°C. The temperature program used was as follows: initial temperature of 50°C, 30°C/min to 140, held for 1 min, 20°C/min to 280°C, held for 4 min, 30°C/min to 310, held for 2 min, for a total run time of 19 min.

A SPME holder with replaceable extraction fibre was used for the extraction of the analytes. The fibre used in the study was coated with 85 μm thickness polyacrylate. The SPME holder and the fibres were obtained from Supelco (Bellefonte, PA, USA). A magnetic stirrer and stir bars (VWR International Eurolab) were used for the extraction.

Extraction procedures

Different analytical methods were applied to determine the concentration of the analytes in the samples. The soluble fraction was analysed with direct immersion SPME with a polyacrylate fibre. Four millilitres of sample were placed in a 4 mL clear vial screw top with a stir bar sorptive (3 mm I.D., 6 mm width). The magnetic stirrer was set to 1,500 r.p.m. for 30 min. Afterwards, the SPME device was placed into the GC interface, set up at 280°C, and the analytes were desorbed from the fibre under static mode during 3 min. The dehydrated suspended fractions were analysed as described in detail in Campíns et al. (2008). Briefly, samples were extracted with a solid-phase extraction (SPE) procedure using acetonitrile as eluent. Then, the acetonitrile extract was mixed with an adequate volume of water and the same procedure used for the soluble fraction was followed. All experiments were performed in duplicate at room temperature.

Analytical parameters

The limits of detection (LOD) and quantification (LOQ) obtained for each micropollutant are presented in Table 3. The LOD was experimentally set as the concentration that produced a peak with a signal to noise ratio of 3. The LOQ was defined as the concentration that produced a peak with a signal to noise ratio of 10.

RESULTS AND DISCUSSION

The SAMBR and CTP systems were studied during the same period (October 2010 to May 2011), so the results of these

<table>
<thead>
<tr>
<th>Compound</th>
<th>Suspended Fraction (μg/kg)</th>
<th>Soluble Fraction (μg/L)</th>
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</thead>
<tbody>
<tr>
<td>OP</td>
<td>0.7</td>
<td>0.006</td>
</tr>
<tr>
<td>t-NP</td>
<td>7</td>
<td>0.05</td>
</tr>
<tr>
<td>4-NP</td>
<td>4</td>
<td>0.01</td>
</tr>
<tr>
<td>E1</td>
<td>45</td>
<td>0.25</td>
</tr>
<tr>
<td>E2</td>
<td>40</td>
<td>0.15</td>
</tr>
<tr>
<td>EE2</td>
<td>40</td>
<td>0.15</td>
</tr>
</tbody>
</table>

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plants are compared more extensively. The aerobic MBR study was performed in a previous period (May to July 2009), although influent wastewater to this aerobic MBR showed quite similar characteristics as the influent wastewater to the CTP (Table 2).

Table 4 and Table 5 show statistical data for the analytes determined in the different sampling points for the soluble and the suspended fractions, respectively. Hormones (E1, E2 and EE2) were below LOD in the soluble fraction of SAMBR, CTP and aerobic MBR systems. The effluent of the SAMBR and aerobic MBR systems is a permeate, so no suspended fraction was obtained from the sample. Also, the concentration of suspended solids in the CTP effluent was very low and it was not possible to analyse its suspended fraction.

Regarding the soluble fraction, as can be observed in Table 4, the SAMBR effluent concentrations of OP, t-NP and 4-NP were always higher than in the influent, while the CTP effluent concentrations were lower than the influent ones. On the one hand, the anaerobic conditions in the SAMBR seem to favour APEOs degradation into APs that remain in the system under these anaerobic conditions. On the other hand, the CTP aerobic conditions seem to favour APs degradation, removing them from the system. The results obtained in the aerobic MBR also indicated that APs were degraded under aerobic conditions. These results are in accordance with the previous literature (Giger et al. 1984; Clara et al. 2005; Tan et al. 2008; Janex-Habibi et al. 2009). Moreover, comparing both aerobic systems (CTP and MBR), the operational parameters, SRT and HRT, seem to be important factors in APs removal from the soluble fraction as higher SRT and HRT values in the aerobic MBR led to higher removal rates. The APs removal was between 64 and 78% for the CTP and 100% for the aerobic MBR. Considering the soluble fraction results, the use of high SRT values has a
lower influence on APs removal than the environmental conditions of the reactor. SAMBR and aerobic MBR systems worked at similar SRT and showed extremely different removal values (no removal in the SAMBR). Thus, the aerobic conditions are pointed out as the main factor in terms of APs reduction from the soluble fraction.

Among the APs studied, OP and t-NP showed the highest frequency of occurrence (92% for OP and 93% for t-NP) in the soluble fraction. The results obtained in the soluble fraction for OP and t-NP are depicted in Figure 1 and Figure 2, respectively. The OP and t-NP influent concentrations for both systems were quite similar during the whole period. As stated before, the SAMBR effluent concentrations were always significantly higher than the CTP ones for both analytes.

As can be seen in Table 5, the average suspended fraction concentrations in the SAMBR and CTP influent showed similar values. This result indicated that the different pretreatments for the wastewater (rotary screening and primary settling, respectively) did not significantly affect the concentration in the suspended fraction. t-NP and OP showed the highest concentrations in the suspended fractions both in the influent and in the reactor. In general, the corresponding concentrations for OP and t-NP in the reactor suspended fraction were higher in the SAMBR than in the CTP. This finding could be explained as under anaerobic conditions and high SRT, the APs were retained in the suspended fraction, while under aerobic conditions APs were degraded. In the aerobic MBR, only t-NP was found in the suspended fraction of the reactor, showing a maximum concentration of 8,000 μg/kg. Comparing both aerobic systems (CTP and MBR), the suspended fraction concentrations found in the aerobic MBR were quite a bit lower than the concentrations found in the CTP. This finding could be explained by attending to the SRT value in each system. The high SRT value in the aerobic MBR could have improved the APs degradation in the suspended fraction as APs were retained under aerobic conditions during a longer period. Despite no hormones being found in the soluble fraction, EE2 was found in the influent and in the reactor suspended fractions of CTP and SAMBR systems. No hormones were detected in the aerobic MBR, indicating that this configuration also favoured hormones degradation. Nevertheless, more data on aerobic MBR through a longer period should be necessary to confirm this result.

Among the APs studied, OP and t-NP showed the highest frequency of occurrence (83 and 100% for OP and t-NP respectively) in the suspended fraction. Figure 3 and Figure 4 show the results obtained for these two micropollutants.

In the SAMBR, it can observed that OP and t-NP concentrations during period I (October to January) were lower than in period II (February to May), except for 6th October 2010. This behaviour can be attributed to the HRT used in the SAMBR, 8 h (period I) and 25 h (period II). The longer HRT under anaerobic conditions allowed more APs to be released through APEOs degradation and
subsequently a greater retention in the sludge due to their high potential of bioaccumulation (log $K_{ow}$ between 4.9 and 5.8).

**CONCLUSIONS**

The fate of the APs (OP, 4-NP and t-NP) and the hormones (E1, E2 and EE2) in a SAMBR pilot plant, a conventional activated sludge wastewater treatment plant (CTP) and an aerobic MBR pilot plant has been studied in this paper.

The APs soluble concentrations in the SAMBR effluent were always significantly higher than the CTP values during all the studied period. The anaerobic conditions in the SAMBR seem to favour APEOs degradation into APs leading to an increase in the soluble concentrations obtained in the effluent. The aerobic conditions maintained both in the CTP
system and in the aerobic MBR favoured the APs degradation, and gave rise to low concentrations in the effluent of these systems. Moreover, comparing both aerobic systems (CTP and MBR), higher SRT and HRT values led to higher removal rates. These results suggest that an aerobic stage working at high SRT and HRT values should be required for the complete degradation of APs. None of the studied hormones were detected in the soluble fraction of the three systems studied.

The analyses of the suspended fraction showed that the concentrations of OP and t-NP in the SAMBR reactor were usually higher than in the CTP reactor. The anaerobic conditions which improve APs release as well as the high potential for bioaccumulation of APs led to a greater retention of these micropolllutants in the SAMBR than in the CTP. This accumulation under anaerobic conditions was enhanced by the use of high HRT values. The use of aerobic conditions and high SRT values seems to reduce the suspended fraction concentrations of APs and hormones.

More research is needed in order to analyse the behaviour of APs precursors (APEOs) and hormones metabolites in the three systems studied.

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