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# Removal of polycyclic musks by anaerobic membrane bioreactor: biodegradation, biosorption, and enantioselectivity

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# Removal of polycyclic musks by anaerobic membrane bioreactor: biodegradation, biosorption, and enantioselectivity

## **Abstract**

This study aims to investigate the performance of anaerobic membrane bioreactor (AnMBR) for removing five polycyclic musks (PCMs), which are common active ingredients of personal care and household cleaning products. A laboratory scale AnMBR system was used in this investigation. Concentrations of the PCMs in both the liquid and biosolids phase were measured to conduct a mass balance analysis and elucidate their fate during AnMBR treatment. The AnMBR was effective for removing PCMs from the aqueous phase by a combination of biotransformation and sorption onto the biosolids. However, bio- transformation was observed to be the dominant removal mechanism for all five PCMs. Enantioselective analysis of the PCMs in influent, effluent and biomass samples indicated that there was negligible enanti-oselectivity in the removal of these PCMs. Accordingly, all enantiomers of these PCMs can be expected to be removed by AnMBR with similar efficiency.

## **Disciplines**

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## **Publication Details**

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# 1 **Removal of Polycyclic Musks by Anaerobic Membrane Bioreactor:** 2 **Biodegradation, biosorption, and enantioselectivity**

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18 samples indicated that there was negligible enantioselectivity in the removal of these PCMs.  
19 Accordingly, all enantiomers of these PCMs can be expected to be removed by AnMBR with similar  
20 efficiency.

21 **Keywords:** Anaerobic membrane bioreactor (AnMBR), polycyclic musks (PCMs), biodegradation,  
22 enantioselectivity, sorption.

## 23 **1 Introduction**

24 Reclaimed municipal effluent is an increasingly important water resource used in many countries for a  
25 diverse range of applications including agricultural irrigation, industrial processes, non-potable usage  
26 and even to supplement potable water supplies. As a consequence, there has been an increasing  
27 attention to the elimination of trace organic chemicals (TrOCs) during the wastewater treatment and  
28 reclamation processes. Conventional wastewater treatment processes were not specifically developed  
29 for removing TrOCs (Le-Minh et al., 2010b; Rivera-Utrilla et al., 2013). Thus, the removal of some  
30 TrOCs can be quite low or highly variable. In recent years, membrane bioreactors (MBRs) have been  
31 shown to improve the removal of refractory trace chemicals as a consequence of extended biosolids  
32 retention times and high biomass concentrations (Alturki et al., 2010; Le-Minh et al., 2010a; Le-Minh

33 et al., 2010b). Many studies have shown the effective removal of TrOCs including pharmaceuticals  
34 and personal care products (PPCPs), pesticides, and endocrine disrupting chemicals by MBRs  
35 (Coleman et al., 2009; Nghiem et al., 2009; Tadkaew et al., 2011; Trinh et al., 2012). In particular,  
36 MBRs have been shown to achieve improved removal of some contaminants, which have otherwise  
37 been considered to be relatively persistent and recalcitrant compounds during treatment (Clara et al.,  
38 2005; De Wever et al., 2007; Radjenovic et al., 2009; Sipma et al., 2010; Tambosi et al., 2010).

39 In addition to the more established aerobic MBR systems, there is a growing interest in of the  
40 deployment of anaerobic MBR (AnMBR) systems for municipal wastewater treatment (Lew et al.,  
41 2009). Compared to aerobic MBR, AnMBR can be much more energy efficient but can also maintain  
42 a high effluent quality suitable for environmental discharge and water reuse. Other advantages of  
43 AnMBRs include the reduction in chemical consumption and sludge production. In addition, AnMBR  
44 can convert the organic content in wastewater to biogas, which is a renewable fuel (Visvanathan and  
45 Abeynayaka, 2012).

46 Several studies have previously been conducted to investigate the removal efficiencies of  
47 micropollutants using AnMBRs (Xu et al., 2008; Monsalvo et al., 2014). Most of these have focused  
48 on high strength organic industrial wastewater such as alcohol-distillery and brewery wastewater  
49 (Choo and Lee, 1998; Ince et al., 1998). More recently, there has been a focus on the use of AnMBRs  
50 for treating municipal wastewater at centralised (Saddoud et al., 2007; Baek et al., 2010; Martinez-  
51 Sosa et al., 2011) and decentralised (Wen et al., 1999; Lew et al., 2009) facilities. The potential to  
52 apply AnMBR for municipal wastewater treatment is the development in sewer mining, in which,  
53 clean water is extracted from the sewer at source (Butler and MacCormick, 1996; Xie et al., 2013).  
54 The remaining wastewater is of much higher wastewater strength and is suitable for anaerobic  
55 treatment. However, while information about the removal of TrOCs by AnMBRs is still limited, little  
56 is known about the fate of polycyclic musks (PCMs) during AnMBR treatment. PCMs are commonly  
57 used ingredients in personal care and household cleaning products. They have been reported to be  
58 resistant to biodegradation under aerobic conditions, which has led to their detection at high  
59 concentrations in wastewater treatment plant effluents and in effluent impacted water bodies (Ricking  
60 et al., 2003; Yang and Metcalfe, 2006; Clara et al., 2011; Wang and Khan, 2014).

61 Most PCMs are chiral chemicals. For examples, tonalide (AHTN), phantolide (AHDI), and cashmeran  
62 (DPMI) have one chiral centre. Some PCMs such as galaxolide (HHCB) and traseolide (ATII) have  
63 two chiral centres. As such, AHTN, AHDI and DPMI may occur in two enantiomeric forms, while  
64 HHCB and ATII have four stereoisomers. However, commercial formulations of ATII tend to  
65 produce only the 'trans' configurations (Gatermann et al., 2002). Consistent with this, only two  
66 enantiomers of ATII were detected in analytical standards and in environmental samples. Our  
67 previous research has shown that these chemicals are used and occur in municipal wastewater as an

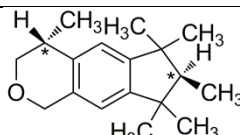
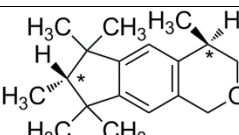
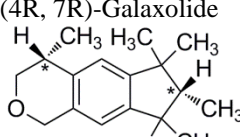
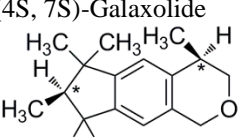
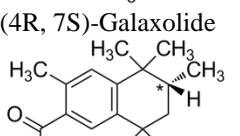
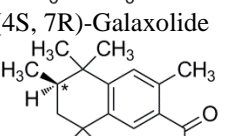
68 even composition of each of the possible enantiomers (Wang and Khan, 2014). However, it is known  
 69 that the enantiomeric fractions (EF) of some chiral chemicals may be changed during biological  
 70 wastewater treatment processes (Hashim and Khan, 2011; Hashim et al., 2011). Accordingly, this  
 71 investigation was undertaken using an enantiospecific analytical method to enable observation of any  
 72 changes in EF during AnMBR treatment.

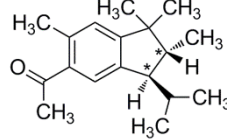
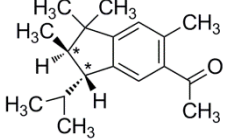
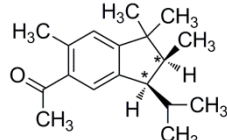
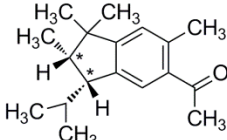
## 73 2 Materials and Methods

### 74 2.1 Materials

75 Five chiral PCMs were investigated in this study. Their molecular structures are shown in Table 1 and  
 76 the chiral centre is marked by an asterisk. Analytical standards of synthetic PCMs including HHCB,  
 77 AHTN, DPMI, AHDI and ATII, as well as isotope-labelled internal standard AHTN-d3 were  
 78 purchased from Dr.Ehrenstorfer GmbH (Augsburg, Germany). Ethyl acetate (anhydrous spectroscopy  
 79 grade) was purchased from Sigma Aldrich, Australia. HPLC grade methanol was purchased from  
 80 Ajax Finechem (Taren Point, NSW, Australia). Kimble culture tubes (13mm I.D.×100mm) and a  
 81 Thermo Speedvac™ concentrator (Model No. SPD121P) were purchased from Biolab (Clayton, Vic,  
 82 Australia). Oasis Hydrophilic-lipophilic balance (HLB) solid phase extraction cartridges (6cc, 500mg)  
 83 were purchased from Waters (Rydalme, NSW, Australia). Whatman Grade 1 filter papers (0.75 μm  
 84 particle retention) were purchased from Millipore, Australia. Ultrapure water was produced by a  
 85 Driec-Q™ filtering system, which is also from Millipore.

86 Table 1: Chemical name, common trade names and molecular structures of five PCMs

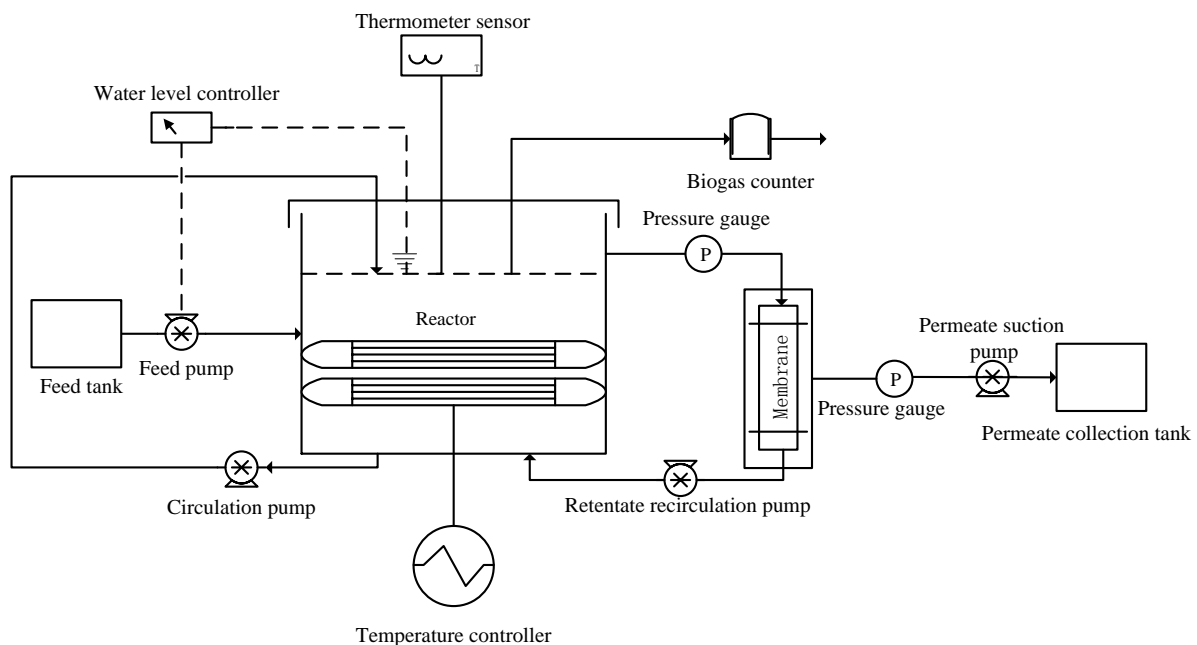
Abbreviation	Chemical name	Trade name	Structure	
HHCB	4,6,6,7,8,8-hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[ <i>g</i> ]isocromene	Galaxolide, Abbalide		
			(4R, 7R)-Galaxolide	(4S, 7S)-Galaxolide
AHTN	7-acetyl-1,1,3,4,4,6-hexamethyl-tetraline	Tonalide, Fixolide		
			(4R, 7S)-Galaxolide	(4S, 7R)-Galaxolide
AHDI	5-acetyl-1,1,2,3,3,6-hexamethylindane	Phantolide		
			(2R)-Phantolide	(2S)-Phantolide

ATII	5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane	Traseolide	 (2R, 3R)- Traseolide	 (2S, 3S)- Traseolide
DPMI	1,1,2,3,3,-pentamethyl-1,2,3,5,6,7-hexahydro-4H-inden-4-one	Cashmeran	 (2S)-Cashmeran	 (2R)-Cashmeran

87

## 88 2.2 Anaerobic MBR (AnMBR) system

89 A laboratory scale AnMBR system was used to assess the fate of PCMs. This system consists of a 10  
 90 L steel feed container, 30 L stainless steel reactor chamber, four pumps including an feed pump, a  
 91 sludge circulation pump, an retentate recirculation pump and a permeate suction pump, a temperature  
 92 control unit, and an external ceramic membrane filtration unit (see Figure 1). A singular tubular  
 93 ceramic membrane module with a nominal pore size of 0.1 $\mu$ m and an effective membrane surface  
 94 area of 0.09 m<sup>2</sup> was used for these experiments.



95  
96

Figure 1: Diagram of the laboratory scale AnMBR

97 Peristaltic pumps (Masterflex L/S, USA) were used for influent feed, recirculation, and effluent  
 98 extraction. The feed pump was connected to a water level controller to maintain the working volume

99 in the reactor at 20 L. The retentate recirculation pump was operated in a 15 min on and 1 min off  
 100 cycle to provide relaxation time to the membrane module for reducing the fouling. A peristaltic  
 101 suction pump was used to drive MBR permeate across the membrane. An industrial grade peristaltic  
 102 hose pump (ProMinent, Australia) with higher working power was used for circulating sludge. The  
 103 temperature controller (Thermo Electron Corporation, Australia) was used to maintain the reactor at  
 104 35 °C. The effluent flow rate was adjusted to be the same as the influent flow rate to maintain a  
 105 constant reactor volume. Chemical cleaning of the ceramic membrane was conducted once per month.

### 106 2.3 AnMBR experimental protocol

107 Synthetic wastewater was used in this study to facilitate precise compositional control and to avoid  
 108 pathogen exposure risks to personnel. The synthetic wastewater solution was prepared according to  
 109 the composition shown in Table 2 based on a previous publication (Hashim et al., 2011).  
 110 Concentrated synthetic wastewater was stored in a refrigerator at 4 °C. The reactor was seeded with  
 111 sludge from an anaerobic digester of the Wollongong Sewage Treatment Plant (NSW, Australia).  
 112 NaHCO<sub>3</sub> was used as buffer during acclimatisation to stabilise the reactor pH of 7 ± 0.1. The pH of  
 113 the mixed liquor was monitored using an Orion 4 Star Plus portable pH/conductivity meter (Thermo  
 114 Scientific, Waltham, MA).

115 Table 2: Composition of AnMBR synthetic wastewater

Chemical	Chemical formula	Concentration (mg L <sup>-1</sup> )
Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	4000
Peptone	-	750
Potassium dihydrogen phosphate	KH <sub>2</sub> PO <sub>4</sub>	175
Magnesium sulphate	MgSO <sub>4</sub>	175
Sodium acetate	CH <sub>3</sub> COONa	2250
Urea	CO(NH <sub>2</sub> ) <sub>2</sub>	135
FeCl <sub>2</sub> .4 H <sub>2</sub> O	FeCl <sub>2</sub> .4 H <sub>2</sub> O	112
Nickel chloride	NiCl <sub>2</sub> .6H <sub>2</sub> O	21
Cobalt chloride	CoCl <sub>2</sub> .6H <sub>2</sub> O	13
Ammonium molybdate	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	8

116

117 After seeding, an initial system start-up and stabilisation process was undertaken for approximately 40  
 118 days. Following this period, a small quantity of biomass was regularly wasted from the reactor to  
 119 establish and maintain a solids retention time (SRT) of approximately 150 days. The mixed liquor  
 120 suspended solids (MLSS) concentration in the reactor was maintained at 10 g L<sup>-1</sup>. The hydraulic  
 121 retention time was set at 4 days, corresponding to permeate flux of 5 L d<sup>-1</sup> (2.36 L m<sup>-2</sup> h<sup>-1</sup>). The reactor

122 temperature was kept constant at  $35.0 \pm 0.1$  °C. Performance of the system with regard to basic water  
123 quality parameters was then monitored for assessment of the stability of the system. The measured  
124 parameters included total organic carbon (TOC) removal, total nitrogen (TN) removal, chemical  
125 oxygen demand (COD) removal and concentration of methane in biogas. TOC and TN were analysed  
126 using a TOC/TN-VCSH analyser (Shimadzu, Japan). COD was analysed using the dichromate  
127 method according to Standard Methods for the Examination of Water and Wastewater (Eugene W.  
128 Rice, 2012). Biogas composition was measured using a portable biogas analyser (Biogass 5000,  
129 Geotech, UK) following a protocol described elsewhere (Nghiem et al., 2014).

130 Once stable TOC removal and biogas production had been achieved, all five PCMs ( $10 \text{ mg L}^{-1}$  in  
131 ethyl acetate 1 mL) was added to the feed solution to obtain a concentration of approximately  $2 \text{ } \mu\text{g L}^{-1}$   
132 of each compound. The feed solution was kept in a stainless steel reservoir in an air-conditioned  
133 laboratory maintained at a temperature of  $20 \pm 2$  °C. Following the introduction of PCMs to the  
134 reactor feed, a further stabilisation time of three times the HRT (a total of 12 days) was enacted,  
135 during which no samples were collected. After day 12, duplicate feed (500 mL), permeate (500 mL)  
136 and MLSS (250 mL) samples were collected once per week over four weeks. The four weekly  
137 sampling events were indicated by S1, S2, S3 and S4.

#### 138 2.4 Biomass sample extraction

139 Biomass extraction was undertaken using an adaptation of a method previously reported for extracting  
140 sewage sludge samples (Ternes et al., 2005; Coleman et al., 2009). The mixed liquor samples from the  
141 anaerobic reactor were centrifuged and the wet solid biomass was then stored in a china container and  
142 frozen for at least 24 h at  $-25$  °C. The frozen biomass samples were then freeze dried for 36h using a  
143 ModulyoD freeze dryer (Thermo Electron Corporation, Australia). The freeze dried samples were  
144 then subjected to ultrasonic solvent extraction. Firstly, the freeze dried samples were finely grounded  
145 using mortar and pestle. Duplicate samples (0.5 g for each) were weighted into 13 mL glass culture  
146 tubes. The internal standard AHTN-d3 ( $50 \text{ } \mu\text{L}$ ,  $1 \text{ } \mu\text{g mL}^{-1}$ ) was added to the glass tube. 5mL ethyl  
147 acetate was then added and the solution was thoroughly mixed for 3 mins using a vortex mixer. Each  
148 sample was then ultrasonicated for 10 mins at  $40$  °C (Unisonics, Australia). The samples were  
149 centrifuged at 3000rpm for 5 mins and the supernatant was collected into glass culture tubes. Ethyl  
150 acetate (5 mL) was added to the remaining biomass. The whole process of mixing, ultrasonic solvent  
151 extraction and centrifugation was repeated and the supernatant was mixed together with the  
152 supernatant from the first step. The combined supernatants were diluted with MiliQ water (500 mL)  
153 into glass bottles for solid phase extraction (SPE). To determine the recoveries of individual PCMs,  
154 0.5 g freeze dried and finely grounded biomass were spiked with 50 ng, 200 ng and 1000 ng of PCMs,  
155 together with 50 ng of internal standard and then subjected to the method described above. The  
156 method recoveries are presented in Table 3.



157 Table 3: Method recoveries of analytes in biomass from a spiking concentration of 50 ng L<sup>-1</sup>, 200 ng  
 158 L<sup>-1</sup> and 1000 ng L<sup>-1</sup>,  $\mu(\pm\sigma)\%$ , n=3

Analyte	50 ng L <sup>-1</sup>	200 ng L <sup>-1</sup>	1000 ng L <sup>-1</sup>
DPMI	88(±7)	86(±8)	94(±6)
AHDI	91(±3)	94(±6)	99(±2)
ATII	97(±4)	96(±4)	97(±5)
AHTN	96(±7)	95(±4)	102(±6)
HHCB	93(±3)	93(±7)	101(±4)

### 159 2.5 Aqueous sample extraction

160 Influent and permeate (500 mL) samples were filtered with a 0.75  $\mu\text{m}$  filter paper and then spiked  
 161 with 50 ng AHTN-d3. All the liquid samples were extracted using solid phase extraction by loading  
 162 the samples onto the HLB cartridges conditioned with 5 mL ethyl acetate, 5 mL methanol and 5 mL  
 163 MiliQ water. A full method validation is presented in previously published paper (Wang et al., 2013).  
 164 After concentrating to 1 mL, eluted samples were subjected to gas chromatography tandem mass  
 165 spectrometry (GC-MS/MS) analysis.

### 166 2.6 GC-MS/MS analysis

167 Chromatographic separations of all the samples were performed on an Agilent 7890A gas  
 168 chromatograph, equipped with a dual-column configuration of a chiral heptakis (2,3- di-*O*-methyl-6-  
 169 *O*-butyl dimethylsilyl)- $\beta$ -cyclodextrin column coupled with a (non-chiral) HP-5MS column. Mass  
 170 spectral detection was undertaken with an Agilent 7000B triple quadrupole mass spectrometer. Mass  
 171 spectrometric ionisation was undertaken in electron ionisation (EI) mode with an EI voltage of 70 eV.  
 172 Multiple reaction monitoring (MRM) was used to identified target PCMs. Detailed information about  
 173 instrument, method and quality assurance and control is available elsewhere (Wang et al., 2013).

### 174 2.7 Calculation of PCM mass balances and sorption coefficients $K_d$

175 Mass balance calculation was conducted after 12 days of spiking PCMs until the system reached the  
 176 equilibrium. Equilibrium was confirmed by the observation of steady-stage biosolids concentrations  
 177 of PCMs after this time. The concentration of PCMs in influent ( $C_{in}$  (ng L<sup>-1</sup>)), biomass ( $C_{bio}$  (ng g<sup>-1</sup>)),  
 178 and effluent ( $C_{eff}$  (ng L<sup>-1</sup>)), MLSS ( $C_{MLSS}=10\text{g L}^{-1}$ ), the volume of the MLSS taken out from the  
 179 system every week (750 mL/week) and the volume of influent and effluent every day (5 L) as well as  
 180 the experimental time (21 days) were used to calculate the overall PCMs mass balances. The overall  
 181 mass balance of each PCM during the experimental period was calculated for the whole system using  
 182 the Eq.A.1:

183 Influent load= effluent load + wasted biomass load + biotransformation load (Eq.A.1)

184 Influent load (ng) =  $5\text{L day}^{-1} \times C_{\text{in}} (\text{ng L}^{-1}) \times 21 \text{ days}$

185 Wasted biomass load (ng) =  $(C_{\text{MLSS}} (\text{g L}^{-1}) \times (0.75 \text{ L/week} \times 3 \text{ weeks})) \times C_{\text{bio}} (\text{ng g}^{-1})$

186 Effluent load (ng) =  $5\text{L day}^{-1} \times C_{\text{eff}} (\text{ng L}^{-1}) \times 21 \text{ days}$

187 The calculation of sorption coefficients  $K_d$  in the anaerobic reactor was performed according to (Joss  
188 et al., 2005).  $K_d$  was defined as:

189  $K_d = C_s/C_w$ .

190 Where  $K_d$  is the sorption coefficient ( $\text{L KgSS}^{-1}$ ),  $C_s$  is the sorbed concentration per amount of  
191 suspended solids ( $\text{ug KgSS}^{-1}$ ),  $C_w$  is the measured concentration of effluent ( $\text{ng L}^{-1}$ ).

## 192 **3 Results and discussion**

### 193 3.1 Basic performance of the AnMBR system

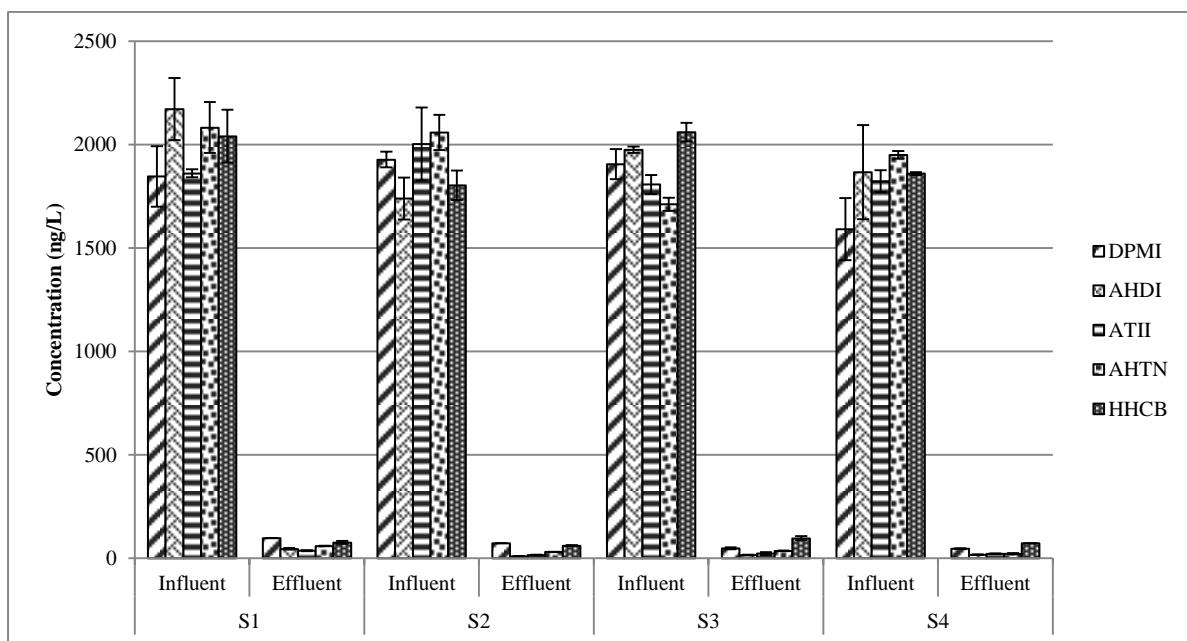
194 After 40 days of system acclimatization, the AnMBR achieved a stable performance for TOC removal  
195 ( $77\pm 4\%$ ), TN removal ( $77\pm 3.5\%$ ) and COD removal ( $84\pm 2\%$ ). The biogas production and mixed  
196 liquor alkalinity were stable at  $0.16 \text{ L CH}_4/\text{gCOD}_{\text{removed}}$  and  $2100 \pm 124 \text{ mg CaCO}_3/\text{L}$  during the  
197 experimental period.

### 198 3.2 Removal of PCMs by AnMBR

199 The total concentrations of individual PCMs in influents and effluents are shown in Figure 2. The  
200 total concentrations of individual PCMs in influent were measured between  $1.6\text{-}2.3 \mu\text{g L}^{-1}$  for all five  
201 PCMs. The overall removal efficiency from influent to effluent was stable at over 95% for each PCM.  
202 The concentration of PCMs in effluent ranged from  $9.1 \text{ ng L}^{-1}$  to  $97 \text{ ng L}^{-1}$ . The performance of  
203 AnMBR for removing of trace organics has previously been reported to be strongly related to the  
204 properties of the chemicals (Monsalvo et al., 2014). Hydrophobic and easily degradable compounds  
205 typically show high removal efficiencies by sorption to biomass and biotransformation.

206 The removal efficiencies of PCMs by full scale and laboratory scale aerobic MBR are usually  
207 moderate (50%) to high and sorption onto the biomass is expected to be the predominant mechanism  
208 of eliminating these compounds (Joss et al., 2005; Ternes et al., 2005; Kupper et al., 2006). Compared  
209 with aerobic MBRs, the performance of eliminating PCMs by this laboratory scale AnMBR is very  
210 good. The reason for the relatively high removal efficiency by the system may be attributed to the  
211 long HRT and SRT applied. The high sludge age achieved by the long SRT may facilitate an adaption  
212 of microorganisms responsible for less biodegradable PCMs. Previous investigations have also

213 indicated that high sludge retention time enhances biological transformation in aerobic MBRs for  
 214 pharmaceuticals (Abegglen et al., 2009). It might also contribute to the transformation of PCMs in  
 215 this anaerobic system. It has also been shown that certain PPCPs are better removed under anaerobic  
 216 conditions (e.g., antibiotics, naproxen, diatrizoate, estrogens and musk fragrances), while others are  
 217 more effectively-treated aerobically (e.g. ibuprofen and bezafibrate) (Joss et al., 2004; Ternes et al.,  
 218 2005). Redox conditions were found to be playing a very important role in PPCPs removal by Drewes  
 219 et al. (Drewes et al., 2001), who investigated the removal of absorbable organo-iodine (AOI) in  
 220 laboratory soil-column system under different redox condition. They found that the unsaturated  
 221 aerobic conditions did not lead to significant biotransformation of AOI, saturated anoxic conditions  
 222 produced about 20% removal, while the anaerobic conditions increased the removal to 57%.



223  
 224 Figure 2: Concentration of individual PCMs in influent and effluent in the four sampling events (S1-  
 225 S4). Error bars represent the observed range of duplicate samples.

226 3.3 Mass balance, biotransformation and sorption coefficients  $K_d$

227 The concentrations of PCMs in dry biomass was calculated to be  $44 \pm 13 \text{ ng g}^{-1}$  (DPMI),  $129 \pm 46 \text{ ng g}^{-1}$   
 228  $^1$  (AHDI),  $412 \pm 30 \text{ ng g}^{-1}$  (ATII),  $284 \pm 128 \text{ ng g}^{-1}$  (AHTN) and  $1187 \pm 161 \text{ ng g}^{-1}$  (HHCB) during the  
 229 sampling period. Although some variation is evident from these figures, it is notable that no general  
 230 increasing or decreasing trend was observed over the experimental period. This indicates that the  
 231 PCMs were either being generally accumulated or released from the biomass during these  
 232 experimental times. The mass balance of the PCMs in the AnMBR system is shown in Table 4. The  
 233 removal of influent concentrations by biotransformation during the AnMBR process was 96% for  
 234 DPMI, 97% for AHDI, 94% for ATII and 95% for AHTN, respectively, while the removal of HHCB

235 by biotransformation was somewhat lower (83%). These biotransformation rates for AHTN under  
 236 anaerobic conditions are somewhat greater than reported in a number of previous studies investigating  
 237 full scale and pilot scale aerobic MBR (Joss et al., 2005; Ternes et al., 2005; Xue et al., 2010) and full  
 238 scale conventional activated sludge (CAS) wastewater treatment (Kupper et al., 2006). There is no  
 239 information regarding to the biotransformation rates of DPMI, AHDI and ATII in the open literature.  
 240 Though sorption onto biosolids has been reported to contribute most to the removal of PCMs in  
 241 aerobic systems, biotransformation is also non-negligible since transformation products including  
 242 HHCB-lactone have been identified (Hühnerfuss et al., 2001; Kupper et al., 2004). Other authors have  
 243 observed higher biotransformation for PCMs during aerobic MBR and CAS treatment. For example,  
 244 Clara et al. (Clara et al., 2005) reported over 95% removal of HHCB and AHTN by biotransformation  
 245 in both CAS and MBR treatment.

246 The calculated average sorption coefficients  $K_d$  for each of the PCMs are presented in Table 4. Highly  
 247 variable  $K_d$  values have been previously reported for PCMs in wastewater treatment reactors, with the  
 248 differences possibly related to the type of reactor. For example,  $K_d$ 's were determined to be  
 249  $4920 \pm 2080 \text{ L kg}^{-1}$  for HHCB and  $5300 \pm 1900 \text{ L kg}^{-1}$  for AHTN in primary sludge and  $1810 \pm 530 \text{ L}$   
 250  $\text{kg}^{-1}$  for HHCB and  $2400 \pm 960 \text{ L kg}^{-1}$  for AHTN in secondary sludge (Ternes et al., 2004). However,  
 251 values 2 to 3 orders of magnitude higher were estimated for HHCB ( $10,040 \text{ L kg}^{-1}$ ) and AHTN  
 252 ( $15,400 \text{ L kg}^{-1}$ ) from activated sludge according to the published  $\text{Log } K_{ow}$  values (Simonich et al.,  
 253 2002).

254 Table 4: Mass balance and, biotransformation and sorption coefficients  $K_d$

	DPMI	AHDI	ATII	AHTN	HHCB
Influent load ( $\mu\text{g}$ )	191	203	197	205	204
Effluent load ( $\mu\text{g}$ )	7	2	9	6	3
Biomass waste load ( $\mu\text{g}$ )	1	3	9	6	3
Biotransformation ( $\mu\text{g}$ )	183	198	185	195	169
Removal by biomass waste (%)	1	1	5	3	13
Removal in effluent (%)	4	1	1	2	4
Biotransformation (%)	96	97	94	95	83
$K_d (\text{L KgSS}^{-1})$	749	7138	19242	16148	15560

255

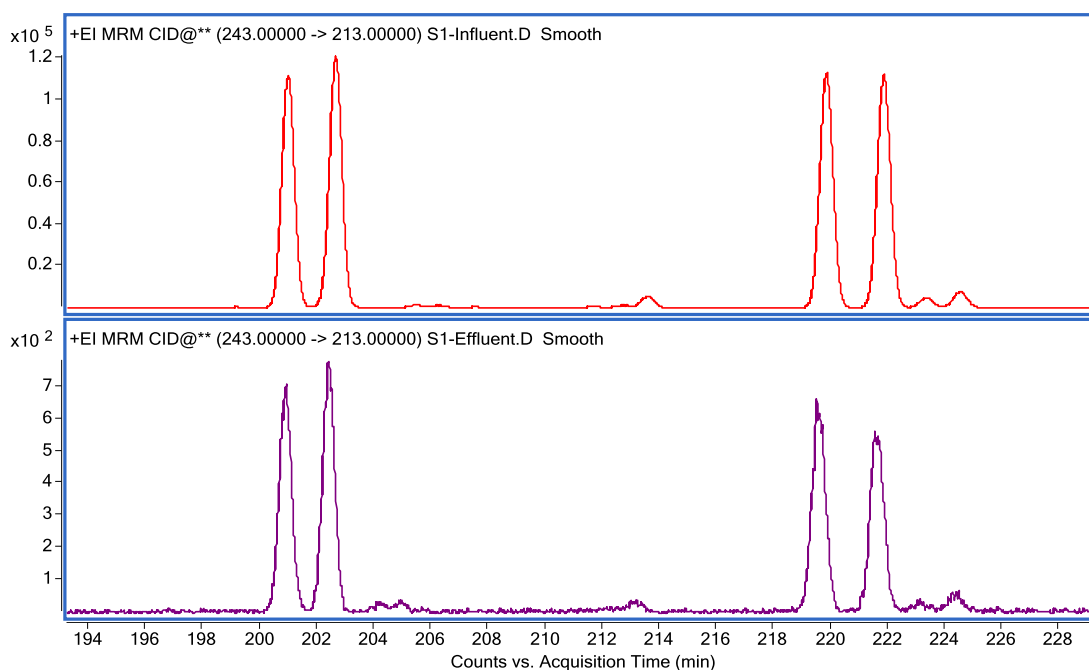
### 256 3.4 Enantioselective fate of PCMs in AnMBR

257 Enantioselective transformation of PCMs was investigated for the first time in this study. Designation,  
 258 quantification and calculation of the enantiomeric composition of single enantiomers has been  
 259 previously reported (Wang et al., 2013). The EF for each of the enantiomers of DPMI, AHDI, ATII,

260 and AHTN was calculated as the relative fraction of the first eluted enantiomer and this was  
261 designated as EF1. For example, the EF1 for DPMI was calculated as  $EF1 =$   
262  $[DPMI1]/([DPMI1]+[DPMI2])$ . Since HHCB has four stereoisomers, full description of the  
263 stereoisomeric proportions requires the determination of the stereoisomeric fraction (SF) for three  
264 peaks (the fourth being implied). SF1, SF2 and SF3 were calculated as the relative fraction of the first,  
265 second and third eluted stereoisomer of HHCB, respectively. Accordingly, these were determined as  
266  $SF1 = [HHCB1]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4])$ ,  
267  $SF2 = [HHCB2]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4])$  and  
268  $SF3 = [HHCB3]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4])$ . EF analysis of these analytical  
269 standards at concentrations of 50 ng mL<sup>-1</sup> and 500 ng mL<sup>-1</sup> in ethyl acetate confirmed that EF1 was  
270 measured as 0.50 for DPMI, AHDI, ATII and AHTN, and SF1, SF2, SF3 and SF4 for HHCB were all  
271 measured as 0.25. Specific enantiomeric compositions of the first eluted enantiomer of DPMI, AHDI,  
272 ATII and AHTN in the influent were determined to have an EF1 = 0.50 as racemic mixture. Most of  
273 them stayed as equal or nearly equal mixtures of enantiomers throughout the experiments and both in  
274 the biomass and effluent. No significant EF changes were detected for DPMI, AHDI, ATII and  
275 AHTN.

276 The elution order of the four stereoisomers ((4S, 7S)-HHCB, (4S, 7R)-HHCB, (4R, 7S)-HHCB, (4R,  
277 7R)-HHCB) have been described in the literature (Biselli, 1999). The first eluted (4S, 7S)-HHCB and  
278 the fourth eluted (4R, 7R)-HHCB are the *trans*-enantiomer pair, and the second and third are the *cis*-  
279 enantiomer pair. The first eluted two diastereomers of HHCB (4S configuration) are responsible for  
280 the significant musky odour. The average stereoisomeric compositions of the four stereoisomers of  
281 HHCB were all 0.25 in influent. Very minor SF changes were observed for HHCB in effluent and  
282 biomass, with average effluent SF values over the four sampling events being 0.27, 0.27, 0.25 and  
283 0.21 for the four sequentially eluted stereoisomers and average biomass SF values being 0.27, 0.27,  
284 0.24, and 0.23. This change can be observed by comparing the chromatographic peak sizes of the first  
285 and fourth eluted stereoisomers of HHCB in Figure 4. These results suggest that the third and fourth  
286 eluting stereoisomers of *cis*- and *trans*-HHCB may have been preferentially metabolised, leading to  
287 the small change in stereochemical composition.

288 There is only limited information about the enantioselective transformation and degradation of PCMs  
289 in the environment and during wastewater treatment (Franke et al., 1999; Gatermann, 1999;  
290 Hühnerfuss, 1999; Gatermann et al., 2002; Berset et al., 2004; Bester, 2005). However, each of these  
291 existing reports do suggest that there is potential minor enantioselective transformation of some PCMs  
292 under environmental and wastewater treatment conditions.



293

294 Figure 4: MRM chromatogram of HHCB in influent and effluent on the first sampling event

#### 295 4 Conclusion

296 This study investigated the fate of five PCMs with a specific focus on individual stereoisomers of  
 297 each PCM. Aqueous and biomass phases were both analysed to facilitate a full mass-balance for the  
 298 removal of PCMs during AnMBR treatment. The AnMBR system showed high performance for  
 299 elimination of PCMs from synthetic wastewater, with removal efficiencies of over 95% for all the  
 300 analysed PCMs. Mass balance calculations indicate that biotransformation was the dominant removal  
 301 pathway for PCMs by this AnMBR. Over 94% of DPMI, AHDI, ATII and AHTN were removed  
 302 through biotransformation and 83% for HHCB. The sorption coefficients  $K_d$  showed that these are  
 303 hydrophobic compounds and significantly partitioned onto the biosolids phase in the anaerobic reactor.  
 304 This strong partitioning to biomass is likely to have facilitated the observed biotransformational  
 305 removal. Enantioselective analysis of these PCMs revealed negligible enantioselectivity for removal  
 306 in most cases. Only very minor stereochemical compositional changes were observed for HHCB  
 307 between influent and effluent samples. The results of this work indicate that AnMBR may be an  
 308 effective treatment process for the removal of PCMs from wastewater and that all PCM stereoisomers  
 309 can be expected to be removed with similar efficiency.

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