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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

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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, biotransformation 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 enantioselectivity in the removal of these PCMs. Accordingly, all enantiomers of these PCMs can be expected to be removed by AnMBR with similar efficiency.

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

1 Introduction

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

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

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

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

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

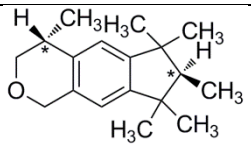
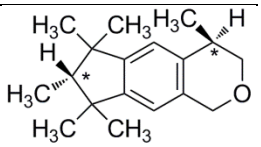
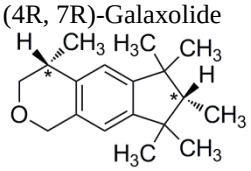
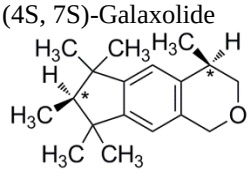
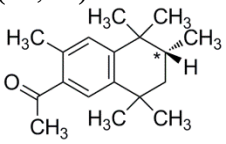
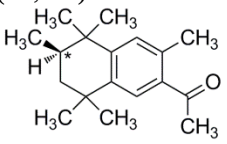
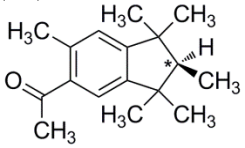
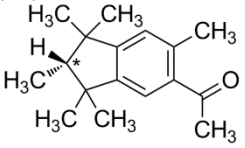
even composition of each of the possible enantiomers (Wang and Khan, 2014). However, it is known that the enantiomeric fractions (EF) of some chiral chemicals may be changed during biological wastewater treatment processes (Hashim and Khan, 2011; Hashim et al., 2011). Accordingly, this investigation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

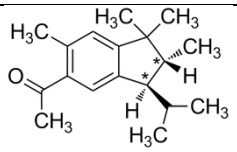
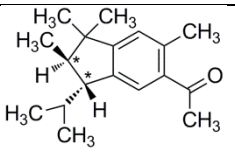
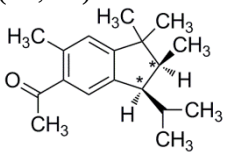
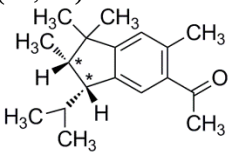
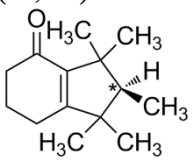
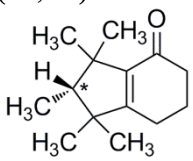
2 Materials and Methods

2.1 Materials

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

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

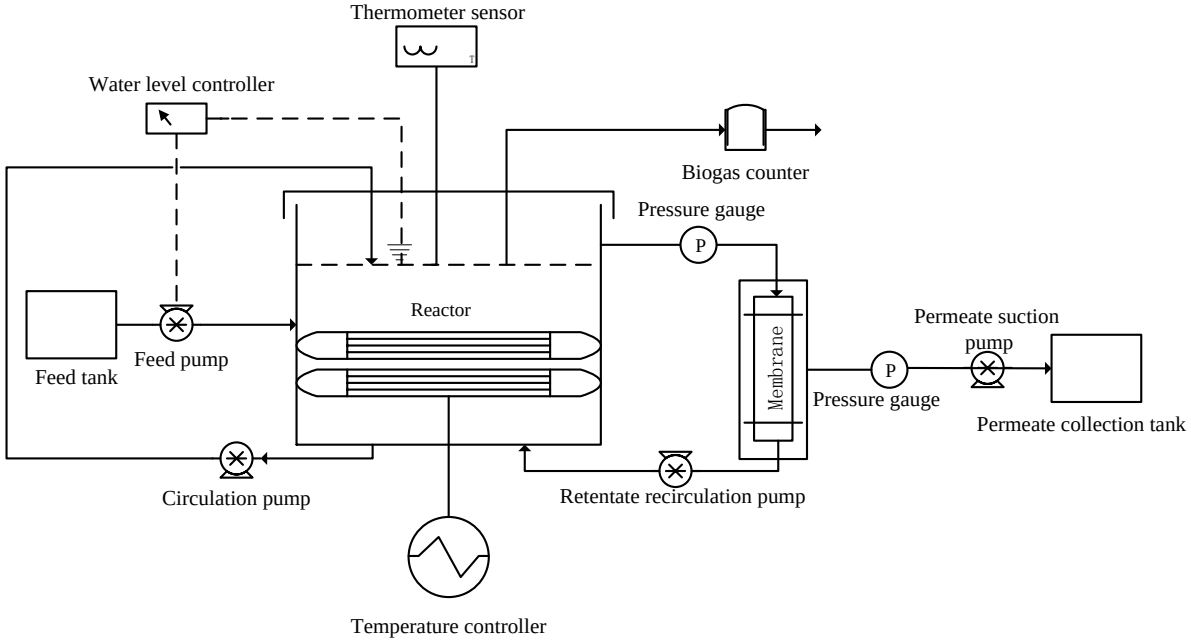
Abbreviation	Chemical name	Trade name	Structure	
HHCB	4,6,6,7,8,8-hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isocromene	Galaxolide, Abbalide	 (4R, 7R)-Galaxolide	 (4S, 7S)-Galaxolide
			 (4R, 7S)-Galaxolide	 (4S, 7R)-Galaxolide
AHTN	7-acetyl-1,1,3,4,4,6-hexamethyl-tetraline	Tonalide, Fixolide	 (3R)-Tonalide	 (3S)-Tonalide
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
			 (2S, 3R)- Traseolide	 (2R, 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 0.1μm and an effective membrane surface
94 area of 0.09 m^2 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

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

2.3 AnMBR experimental protocol

Synthetic wastewater was used in this study to facilitate precise compositional control and to avoid pathogen exposure risks to personnel. The synthetic wastewater solution was prepared according to the composition shown in Table 2 based on a previous publication (Hashim et al., 2011). Concentrated synthetic wastewater was stored in a refrigerator at 4 ^{o}C. The reactor was seeded with sludge from an anaerobic digester of the Wollongong Sewage Treatment Plant (NSW, Australia). $NaHCO_3$ was used as buffer during acclimatisation to stabilise the reactor pH of 7 ± 0.1. The pH of the mixed liquor was monitored using an Orion 4 Star Plus portable pH/conductivity meter (Thermo Scientific, Waltham, MA).

Table 2: Composition of AnMBR synthetic wastewater

Chemical	**Chemical formula**	**Concentration (mg L^{-1})**
Glucose	$C_6H_{12}O_6$	4000
Peptone	-	750
Potassium dihydrogen phosphate	KH_2PO_4	175
Magnesium sulphate	$MgSO_4$	175
Sodium acetate	CH_3COONa	2250
Urea	$CO(NH_2)_2$	135
$FeCl_2$.4 H_2O	$FeCl_2$.4 H_2O	112
Nickel chloride	$NiCl_2.6H_2O$	21
Cobalt chloride	$CoCl_2.6H_2O$	13
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$	8

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

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

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

2.4 Biomass sample extraction

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

Table 3: Method recoveries of analytes in biomass from a spiking concentration of 50 ng L^{-1}, 200 ng $L^{-1}$and 1000 ng L^{-1}, $\mu(\pm\sigma)$%, n=3

Analyte	50 ng L^{-1}	200 ng L^{-1}	1000 ng L^{-1}
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)

2.5 Aqueous sample extraction

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

2.6 GC-MS/MS analysis

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

2.7 Calculation of PCM mass balances and sorption coefficients K_d

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

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

Influent load (ng) = 5L day^{-1} × C_{in} (ng L^{-1}) × 21 days

Wasted biomass load (ng) = (C_{MLSS} (g L^{-1}) × (0.75 L/week ×3 weeks)) × C_{bio} (ng g^{-1})

Effluent load (ng) = 5L day^{-1} × C_{eff} (ng L^{-1}) × 21 days

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

$K_d = C_s/C_w$.

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

3 Results and discussion

3.1 Basic performance of the AnMBR system

After 40 days of system acclimatization, the AnMBR achieved a stable performance for TOC removal (77±4%), TN removal (77±3.5%) and COD removal (84±2%). The biogas production and mixed liquor alkalinity were stable at 0.16 L $CH4/g_{COD\ removed}$ and 2100 ± 124 mg $CaCO_3$/L during the experimental period.

3.2 Removal of PCMs by AnMBR

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

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

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

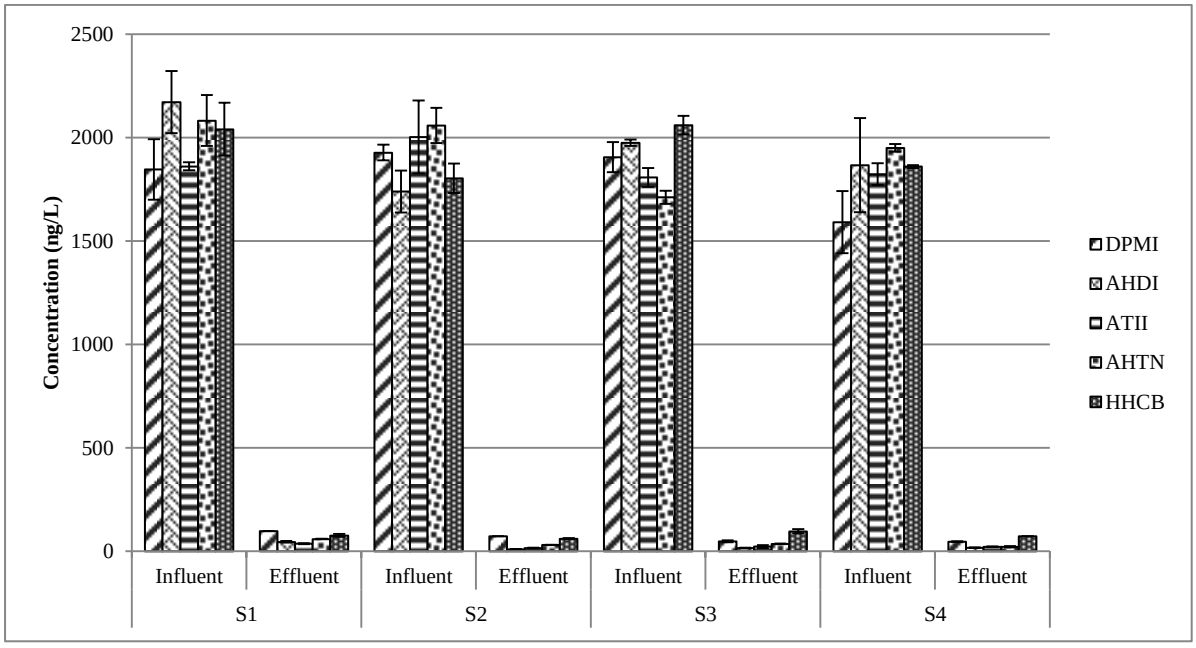


Figure 2: Concentration of individual PCMs in influent and effluent in the four sampling events (S1-S4). Error bars represent the observed range of duplicate samples.

3.3 Mass balance, biotransformation and sorption coefficients K_d

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

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

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

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

	DPMI	AHDI	ATII	AHTN	HHCB
Influent load (µg)	191	203	197	205	204
Effluent load (µg)	7	2	9	6	3
Biomass waste load (µg)	1	3	9	6	3
Biotransformation (µg)	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 (L $KgSS^{-1}$)	749	7138	19242	16148	15560

3.4 Enantioselective fate of PCMs in AnMBR

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

and AHTN was calculated as the relative fraction of the first eluted enantiomer and this was designated asEF1. For example, the EF1 for DPMI was calculated as EF1 = [DPMI1]/([DPMI1]+[DPMI2]). Since HHCB has four stereoisomers, full description of the stereoisomeric proportions requires the determination of the stereoisomeric fraction (SF) for three peaks (the fourth being implied). SF1, SF2 and SF3 were calculated as the relative fraction of the first, second and third eluted stereoisomer of HHCB, respectively. Accordingly, these were determined as SF1= [HHCB1]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4]), SF2=[HHCB2]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4]) and SF3=[HHCB3]/([HHCB1]+[HHCB2]+[HHCB3]+[HHCB4]). EF analysis of these analytical standards at concentrations of 50 ng mL^{-1} and 500 ng mL^{-1} in ethyl acetate confirmed that EF1 was measured as 0.50 for DPMI, AHDI, ATII and AHTN, and SF1, SF2, SF3 and SF4 for HHCB were all measured as 0.25. Specific enantiomeric compositions of the first eluted enantiomer of DPMI, AHDI, ATII and AHTN in the influent were determined to have an EF1 = 0.50 as racemic mixture. Most of them stayed as equal or nearly equal mixtures of enantiomers throughout the experiments and both in the biomass and effluent. No significant EF changes were detected for DPMI, AHDI, ATII and AHTN.

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

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

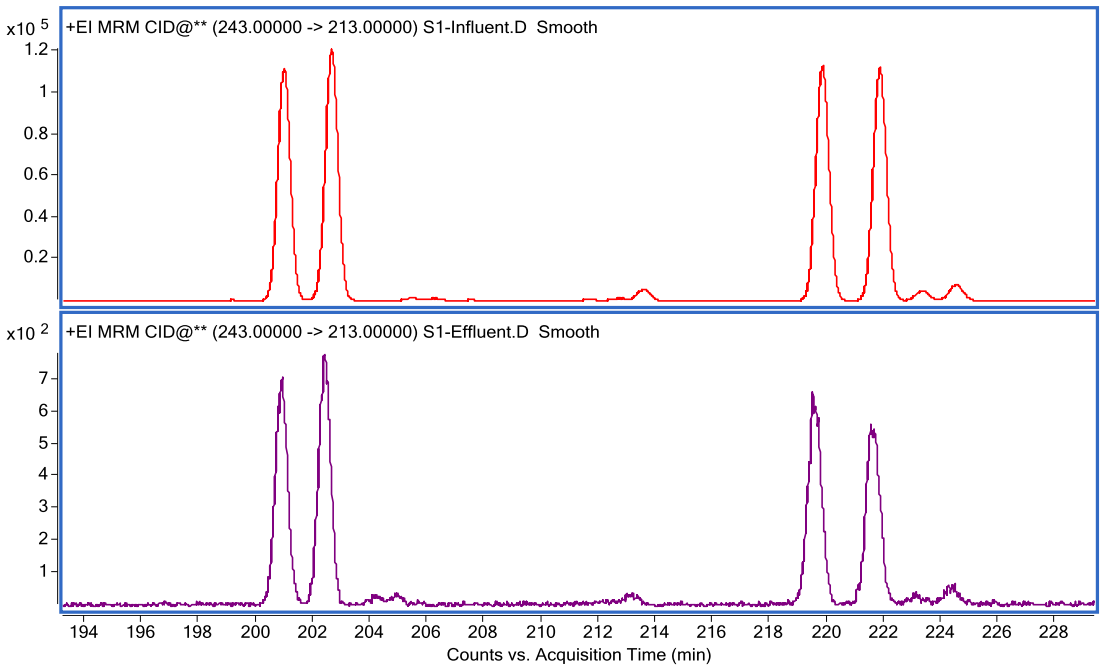


Figure 4: MRM chromatogram of HHCB in influent and effluent on the first sampling event

4 Conclusion

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

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