Fate and removal of selected antibiotics in an osmotic membrane bioreactor

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GRAPHICAL ABSTRACT

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ABSTRACT

The fate and removal behavior of 12 antibiotics from 5 classes were investigated in an osmotic membrane bioreactor (OMBR), along with their effects on the system performance. High overall removal of all the antibiotics (77.7–99.8%) was observed, resulting from their rejection by the forward osmosis membrane (> 90%). Biodegradation (ranging from 16.6% to 94.4%) was a significant removal pathway for all the antibiotics except ofloxacin, ciprofloxacin and roxithromycin. Sulfathiazole, enrofloxacn, and chlortetracycline showed the highest removal via biodegradation at 94.4%, 90.2%, and 78.9% respectively, followed by trimethoprim (68.2%), lomefloxacn (57.1%) and norfloxacn (53.2%). Sorption contributed to varying extent to their removal (at 2.0–30.1%); the highest was observed for ofloxacin and roxithromycin. No evident change was observed in the pollutant removal performance of the bioreactor even after 40 days of continuous exposure to these antibiotics (at 500 ng/L), with the overall TOC and NH₄⁺-N removal maintained > 98% and > 97%, respectively.

1. Introduction

The occurrence and fate of pharmaceutical and personal care products (PPCPs) in the water environment has become a topic of worldwide interest in the past few years. PPCPs encompass a wide range of pollutants, including antibiotics, hormones, cosmetics, fragrances, pesticides, and endocrine disrupting compounds (EDC). US EPA has labeled PPCPs as contaminants of emerging concern (CEC) and a database with 200 CECs has been made publicly available since 2010 [1]. Within these compounds, antibiotics are of special concern, due not only to their potential adverse effects on aquatic life and human, but the fact that their increased release into the environment may promote the development and dissemination of antibiotic resistance [2]. Increasing occurrences of antibiotics have been observed in water, wastewater, and aquatic environment including municipal wastewater, hospital wastewater, surface water, and drinking water, which have resulted from their increased usage due to advancements in medicine, as well as their refractory nature [2,3]. Wastewater treatment...
processes, which are the last barrier in stopping antibiotics from entering into the water environment, are of extreme importance in eliminating these emerging pollutants. Unfortunately, studies have shown that most of the antibiotics (predominantly from the sulphonamide, trimethoprim, tetracycline, quinolone and macrolide groups) are not well removed in conventional activated sludge (CAS), and even membrane bioreactor (MBR) processes [4,5], largely due to their relatively low concentration (typically at ng/L-μg/L level) and their resistance to biodegradation. These pollutants pass through the wastewater treatment processes, enter into the aquatic environment and subsequently threaten aquatic ecology, drinking water security and human health. Additional risk is also posed to water sustainability, especially in regions which face water scarcity problems and have high demand for water reuse [6].

Forward osmosis (FO) is an osmotic process that uses a semi-permeable membrane to effect separation of water from dissolved solutes. Unlike hydraulic pressure-driven membrane processes (e.g. reverse osmosis, RO or nanofiltration, NF), the permeation of water is driven by osmotic pressure difference across the membrane, which results in potentially reducing energy consumption and membrane fouling in the FO process. The absence of hydraulic pressure in FO facilitates its direct incorporation into an biological wastewater treatment process to form a novel MBR process, known as the osmotic membrane bioreactor (OMBR) [7-9]. The use of dense FO membranes in an OMBR confers the process a high rejection capacity to both ionic and neutral trace or- ganic contaminants. This unprecedented advantage of OMBR has been explored for enhanced wastewater treatment, water reclamation and facilitated nutrients recovery [9-11]. Researchers have focused their attention on investigating the capability of OMBR in the removal of a variety of emerging pollutants from wastewater [12,13]. Studies suggest an interplay of both size exclusion and electrostatic repulsion in the rejection of charged trace organics while the rejection of neutral compounds are largely due to their physical-chemical properties (molecular weight (MW), water solubility, acid dissociation constant (pKa) values, charges at pH = 8.0, octanol-water partition coefficient (logKow) values and their distribution-coefficient (log D) values) are provided in Supplementary Materials Table S1.

2. Materials and methods

2.1. Antibiotics

Twelve antibiotics belonging to five different classes, i.e. two sulphonamides (sulfathiazole and sulfamethazine), five fluoroquinolones (norfloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and ofloxacin), three tetracyclines (tetracycline, oxytetracycline and chlorotetraacycline), one macrolide (roxithromycin) and trimethoprim were used in this study. All the antibiotics used were purchased from Sigma-Aldrich (Singapore). Standard stock solutions (8 mg/L) for each antibiotic were prepared in methanol and were stored at ~20 °C in amber glass bottles and used within a month. More details about the identity of the antibiotics and their physical-chemical properties (molecular weight (MW), water solubility, acid dissociation constant (pKa) values, charges at pH = 8.0, octanol-water partition coefficient (logKow) values and their distribution-coefficient (log D) values) are provided in Supplementary Materials Table S1.

2.2. OMBR system

A lab-scale OMBR system was used in the experiment (Fig. 1). The system consisted of a bioreactor with an operating volume of 5.03 L and a submerged FO membrane module with an effective membrane area of 0.036 m². Cellulose triacetate (CTA) FO membrane (obtained from Hydration Technologies Inc., Oregon, USA) was used for the experiment. The bioreactor was continuously aerated at 15.0 L/min to provide oxygen for biological activities and to control FO membrane fouling. 49.0 g/L NaCl (corresponding to an osmotic pressure of 4.0 MPa at 23.5 °C) was used as draw solution (DS) to drive the FO membrane. The concentration of the DS was maintained by a feedback conductivity controller (Thermo Scientific, USA) connected to a peristaltic pump (Cole-Parmer, USA) which dosed concentrated NaCl (4.0 M) into the DS tank whenever the conductivity dropped below the set point. The extraction of water from the bioreactor through FO results in continuous increase in the volume of the DS. The overflow from the DS tank was collected in a DS effluent tank and water samples were
collected from the tank (as DS effluent) for the analyses of TOC, NH₄⁺-N and antibiotics.

Synthetic wastewater was prepared daily as influent to the system. The wastewater contained 600 mg/L glucose, 151.4 mg/L NH₄Cl, 35.12 mg/L KH₂PO₄, 71 mg/L MgSO₄ 7H₂O, 19.3 mg/L CaCl₂ 2H₂O, 17.4 mg/L FeSO₄ 7H₂O, 0.07 mg/L CuCl₂ 2H₂O, 0.13 mg/L MnCl₂ 4H₂O, 0.13 mg/L ZnSO₄ 7H₂O, 0.03 mg/L Na₂MoO₄ 2H₂O, 0.025 mg/L H₂BO₃ and 0.033 mg/L KI (giving COD, NH₄⁺-N, and PO₄³⁻-P values of around 600 mg/L, 50.0 mg/L and 8.0 mg/L respectively). The pH of the feed was adjusted to 8.0 using 1.0% NaOH before it was fed into the bioreactor. The liquid level in the bioreactor was maintained using an overflow trough with its bottom connected to the bioreactor [9]. Activated sludge collected from an MBR plant in Singapore was used as the seed sludge from the system and a MLSS concentration of around 5.0 g/L was maintained during the operation. The system was operated for 60 days to achieve a relatively stable performance before antibiotics were added to the influent wastewater (from Day 61 onwards). A stock solution containing 12 antibiotics was spiked into the freshly prepared feed wastewater to produce an environmentally relevant concentration of 500 ng/L for each antibiotic. During the experiment, a sludge retention time (SRT) of 50 days was maintained by a routine daily discharge of 100 ml of mixed liquor. An additional 250 ml of mixed liquor was withdrawn from the bioreactor daily and was allowed to settle for 30 min, from which 150 ml of clarified supernatant was removed for the control of salt accumulation. The rest of the mixed liquor was returned to the bioreactor. The mixed liquor pH in the bioreactor was maintained at around 8.0 by adding 1.0% NaOH solution. The hydraulic retention time (HRT) of the system was 16.18 h initially and eventually increased to and stabilized at 27.15 h due to the water flux decline.

2.3. Analytical methods

Water samples were collected daily for the influent, the bioreactor supernatant, and the DS tank. NH₄⁺-N and TOC concentrations were analyzed according to standard methods [14]. To determine the concentrations of the antibiotics, all the samples were analyzed using high-performance liquid chromatography-tandem mass spectrophotometry (UPLC-MS/MS) [15].

To evaluate the biosorption behaviors of antibiotics onto the activated sludge, sludge samples were collected weekly and freeze dried immediately. The antibiotics were then extracted according to Xu et al. [16]. Approximately 0.5 g of sludge was added to 10 ml extraction buffer (2:1:1 ratio of methanol, 0.1 M citric acid adjusted to pH 4.0 with 1.0 M NaOH and 4% Na₂EDTA adjusted to pH 4.0 with 1.0 M H₂SO₄). The suspension was then vortexed for 1 min and sonicated for 15 min before centrifuging at 3000 g for 10 min. The supernatant was then filtered through a 0.45 µm glass fiber membrane. The extraction was then repeated and the extracted filtrates were combined and diluted with ultrapure water to 250 ml before SPE.

To analyse the antibiotics in the water and sludge extracts, chromatographic separation was performed using an Acquity UPLC System (Waters) with a BEH C₁₈ column (130 Å, 1.7 µm, 2.1 mm × 50 mm). Mass spectrometry was performed using a Bruker Q-TOF micro MS (Bruker Daltonik, Bremen, Germany). A previously established method for simultaneous multi residue analysis of pharmaceuticals was used for the analyses [15]. More details on the SPE, and the UPLC-MS/MS analysis are provided in the Supplementary Materials.
3. Results and discussion

3.1. Effects of antibiotics on the system performance

3.1.1. Water flux and salt accumulation

Fig. 2 shows the changes in the permeate flux and the mixed liquor conductivity in the bioreactor over a period of 100 days. Pseudo steady state was reached after 60 days of operation. The water flux dropped from 8.64 L/m²/h (LMH) initially to a relative stable value of around 5.15 LMH in 60 days, as the mixed liquor conductivity increased from its initial value of 1.41 mS/cm to 27.9 mS/cm. This increase in the mixed liquor salinity was attributed to the rejection of solutes from the feed by the FO membrane and the reverse diffusion of NaCl from the DS, which resulted in the gradual reduction in the trans-membrane osmotic pressure driving force, and the decrease in the water flux [9,17,18]. The water permeate flux and the mixed liquor conductivity (which remained at 5.15 LMH and 27.89 mS/cm respectively) did not show any evident changes after the addition of antibiotics from Day 61 onwards, thus suggesting negligible effects of antibiotics on the membrane performance.

3.1.2. Pollutant removal performance

The overall TOC removal was above 98% and was stable throughout the experimental run even after the spiking of the antibiotics from Day 61 (Fig. 3a). An increase in the TOC concentration from 5.45 mg/L to 57.85 mg/L was observed in the bioreactor supernatant at the beginning of the operation; this may be attributed to the high rejection property of the FO membrane, which effective rejects non-biodegradable organic matter and resulted in their accumulation within the bioreactor [19]. The supernatant TOC concentration remained largely unchanged after the addition of antibiotics. The TOC concentration in the DS remained below 5 mg/L throughout the operation, again indicating an insignificant effect of the antibiotics.

Fig. 3b shows the removal of NH₄⁺-N in the OMBR system. Salt accumulation in the reactor resulted in a mild deterioration of the nitrifying activity at the beginning of operation, which led to the accumulation of NH₄⁺-N (to 7 mg/L) in the first 10 days. This phenomenon has been attributed to the acclimatization of the slow growing and sensitive nitrifying bacteria to the elevated saline conditions [9,10]. The nitrifying activity was restored after the bacteria has acclimatized to the high salinity conditions and the NH₄⁺-N concentration in the reactor decreased to around 1.0 mg/L after 20 days. Nitrification was unaffected by the addition of the antibiotics and the NH₄⁺-N removal efficiency remained > 97% with the concentration in the DS consistently below 1.0 mg/L. This negligible effect of antibiotics on the performance of the OMBR corroborates with previous studies on MBR and OMBR with trace organic chemicals including pharmaceuticals and synthetic musk compounds [4].

Antibiotics were typically found to show inhibition effects to aquatic and/or activated sludge bacteria with concentrations in the higher range of µg/L or up to the mg/L level. Using Economic Co-operation and Development (OECD) closed bottle test [20], Al-Ahmad and co-workers evaluated the biodegradation and toxicity of some selected β-lactamase, sulfonamide, fluoroquinolone antibiotics. The most toxic compounds were found to be fluoroquinolones, with 50% growth inhibition on Pseudomonas putida observed at a concentration of 80 µg/L and 10 µg/L for ciprofloxacin and ofloxacin, respectively [21,22]. Campos et al. [23] also showed that oxytetracycline did not show remarkable inhibition effects on nitrification at concentrations up to 100 mg/L, which agreed with Gómez et al. (1996) [24] who reported that oxytetracycline at up to 250 mg/L had no effect on either biomass production or ammonia oxidation for mixed nitrifying sludge in batch assays. Halling-Sørensen [25] also reported an EC₅₀ value of 4.0 mg/L for tetracycline in a growth inhibition test of Nitrosomonas europaea. As for sulfonamide antibiotics, 20% inhibition of microbial activity in activated sludge was reported at concentrations of 10–400 mg/L [26]. Even for the very susceptible anammox bacteria, studies have shown that their activity was not substantially affected with short-term exposure to oxytetracycline (IC₅₀ = 1100 mg/L) and sulfathiazole (IC₅₀ = 650 mg/L) at concentration of 100 mg/L. Continuous exposure to the same concentration, however, have resulted in activity decreases to 75% and 50% of that of unexposed culture for oxytetracycline and sulfathiazole, respectively [27]. These results suggest that antibiotics at environmentally-relevant concentrations (in ng/L, and lower µg/L ranges) are not considered sufficiently high to cause noticeable effects on wastewater treatment processes, and may also explain why relatively stable COD and NH₄⁺-N removal performance were observed in this work.

Antibiotics from different groups are active via different functional mechanisms and a combined action of these antibiotics is expected when present in a mixture. Indeed, additive and synergistic effects have been observed in mixtures of antibiotics. When Ghosh et al. [28] tested Fig. 2. Water flux and salt accumulation profile in the OMBR system. The vertical line represents the spiking of antibiotics on Day 61.

Fig. 3. (a) TOC and (b) NH₄⁺-N removal in the OMBR system. The vertical line represents the spiking of antibiotics on Day 61.
the effect of antibiotics on bacterial ammonia oxidation, determined by oxygen uptake rate, no significant effect was observed for each single antibiotic (enrofloxacin, sulfamethoxazole, tetracycline; trimethoprim and clarithromycin), below 0.05 mg/L. However, when present as a mixture at the same concentration, 25% inhibition was observed. These combined effects, however, was not evident for COD and NH4⁺-N removal in this study with concentrations of 0.5 µg/l for each of the 12 antibiotics.

### 3.2. Overall system removal of antibiotics

The overall removal of antibiotics is calculated from the concentrations in the feed and the DS. In the system, the overall removal of all the antibiotics was always > 75%, with sulfamethazine showing the highest overall removal (up to 100%) and chlorotetracycline the lowest (77.6%) (Fig. 4). The antibiotics which showed > 90% overall removal include sulfathiazole, trimethoprim, norfloxacin, lomefloxacin, ofloxacin, and roxithromycin. Among the fluoroquinolones, ciprofloxacin and enrofloxacin are the only two antibiotics which showed lesser than 90% removal. Tetracycline and oxytetracycline showed better overall removal efficiencies when compared to chlorotetracycline from the same class of antibiotics. Generally, these observed overall removal of antibiotics was higher than that reported for conventional activated sludge (CAS) and traditional membrane bioreactor systems [4,6]. For instance, Sahar et al. [29] compared the removals of several macrolide, sulfonamide and trimethoprim antibiotics from raw sewage in a full-scale CAS system coupled with ultrafiltration (CAS-UF) and a pilot scale MBR. The elimination of trimethoprim, sulfamethoxazole and erythromycin was 99%, 70%, 61% in the MBR system, and 45%, 52% and 71% in the CAS-UF system, respectively. Leung et al. [30] also showed that, although relatively higher removal efficiencies (> 70%) were observed for cefalexin, cefotaxime, amoxicillin, sulfamethoxazole and chloramphenicol during secondary treatment in municipal wastewater treatment plants in Hong Kong, removal efficiencies of norfloxacin, trimethoprim and tetracycline were moderate, with values of 52%, 43%, and 69%, respectively. Even lower removal (0–20%) was observed for macrolides such as erythromycin and roxithromycin. The relatively high removal observed in this work was attributed to the use of dense FO membranes, which effectively rejected these compounds, thus conferring their prolonged retention in the bioreactors [18,31], which benefited their removal via either biodegradation and/or biosorption.

Additionally, it should be noted that the removal of antibiotics even within the same class does not always follow similar trends. This was expected since the removal of the antibiotics in the OMBR system involved mechanisms apart from membrane rejection, such as biodegradation and biosorption, which are largely determined by the physiochemical properties of the antibiotics (molecular weight, molecular structure, hydrophobicity, and biodegradation rate constants) [4]. The fate and removal behaviors of the individual antibiotics examined in this research are further discussed in the next section.

### 3.3. Removal behaviors of antibiotics

#### 3.3.1. Membrane rejection

The removal efficiencies of the antibiotics by the FO membrane are determined from the concentrations of the antibiotics in the supernatant and the effluent. Fig. 5 shows the membrane rejection of the antibiotics with increasing molecular weight. In all instances, the FO membrane showed high rejection (> 90%) of the antibiotics. These results are consistent with several studies which reported the rejection of several antibiotics including fluoroquinolones, sulfonamides, tetracyclines, and trimethoprim (up to 99%) found in large scale RO and NF processes [26,32] and are also in agreement with previous investigations on trace organics [10,33]. As shown in Fig. 5, the membrane rejection did not strictly increase with increasing molecular weight of the antibiotics, implying that size exclusion is not the only factor influencing the rejection efficiency. It was suggested that key rejection mechanisms of FO membranes include steric hinderance (size exclusion), electrostatic interactions (attractive or repulsive), and hydrophobic interactions (diffusion and partitioning) between compounds and the membrane [26,32]. Studies have shown that hydrophobicity and
charge of the compounds play an important role in the membrane rejection performance, since the hydrophobicity and the surface charge of the membrane affect the sorption capacity and the electrostatic status of the membrane which in turn affects their interactions with different compounds [33]. Like most NF/RO membranes, the CTA FO membrane used in this study are negatively charged under neutral conditions due to the presence of carboxylic functional groups [32,33] while most of the antibiotics used in this study are amphoteric substances and may be positively or negatively charged (or neutral) depending on the pH of the solution, in relation to their $p_Ka$ values. The charge status of the antibiotics in the bioreactor (i.e. at pH 8) are given in Table S1 (Supplementary Material). Negatively charged compounds are rejected mainly via electrostatic repulsion, while positively charged species are removed by combination of size exclusion, and attractive electrostatic interaction with the membrane [26]. As for the hydrophobicity, octanol-water coefficient ($\log Kow$) of a compound is commonly used as a measure of its hydrophobicity; a $\log Kow \geq 2$ deemed hydrophobic and a $\log Kow < 2$ deemed hydrophilic [35]. The log Kow values of the antibiotics studied are also shown in Table S1 (Supplementary Material).

Contributions of the charge status and hydrophobicity of the compounds on their rejection behaviors has been observed in this study. For instance, although having low molecular weights, sulfonamide compounds, namely sulfathiazole (MW 255.32 Da) and sulfamethazine (MW 278.33 Da), were rejected by the FO membranes at 96.1% and almost 100%, respectively. This may be attributed to their negative charged at pH 8 (with $p_Ka$ values of around 2 and 7) as well as their hydrophilic nature ($\log Kow < 2$). Similarly, negatively charged hydrophilic compound trimethoprim also showed high membrane rejection (nearly 97%) although with a relatively low molecular weight (MW 290.3). Comparatively, fluoroquinolones, which includes antibiotics (norfloxacin, ciprofloxacin, lomefloxacin, enrofloxacin, and ofloxacin) in the molecular weight range of 300 Da–400 Da, showed membrane rejection of 97.8–99.9%. These antibiotics are neutral in charge at pH = 8.0 ($p_Ka$ values approximately 3–4, 6, 7.5–9 and 10–11), and their electrostatic interaction with the membrane was minimal. The observed high rejection of these compounds may be attributed to their relatively high molecular weights, which led to a high size exclusion by the FO membrane. Among the tetracyclines (with $p_Ka$ values of approximately 3, 7 and 9), the moderately hydrophobic chlortetracycline showed lower membrane rejection (91.44%) compared to hydrophilic tetracycline (94.22%) and oxytetracycline (96.42), although chlortetracycline has the highest molecular weight, thus suggesting the role of hydrophobicity in membrane rejection. The antibiotic with the highest

![Figure 6](image-url)
molecular weight used in this study, roxithromycin (MW 837.05 Da) which is positively charged (pKa 9.17 ± 0.30) showed the highest FO membrane rejection (99.75%); both the high molecular weight and the charges would have contributed to the high rejection. Previous research suggested that charged species of trace organics (both positive and negative) showed higher rejection compared to neutral species. It has also been observed that hydrophobic neutral trace organics showed increased rejection with increased molecular weights [33]. The similar trend observed for the antibiotics investigated in this work suggests a combined role of the charge, molecular weight and hydrophobicity of the compounds in its rejection by FO membrane.

Additionally, studies have suggested that fouling on FO membranes may affect the rejection of micropollutants [34]. It has been observed that except for hydrophilic compounds, the presence of a fouling layer increases the rejection of micropollutants, attributed to an increased hydrophilicity of the fouled membrane compared to the virgin membrane. The FO membrane used in our study was inspected for membrane fouling at the end of operation and a foulant layer, though minimal, was nevertheless present. The formation of the fouling layer on the FO membrane may also have contributed to the higher rejection of antibiotics in this work.

### 3.3.2. Biological removal

Fig. 6(a) shows the percentage removal of antibiotics through biosorption and biodegradation. Except for oxytetracycline, chlorotetracycline and sulfamethazine, all the antibiotics showed significant removal via sorption onto the activated sludge. Ofoxacin (log D = −0.82) and roxithromycin (log D = 2.51) showed the highest biosorption behaviors, with their concentrations in the activated sludge at 194.87 µg/gMLSS and 154.91 µg/gMLSS respectively at the end of the operation. Roxithromycin which belongs to the class of macrolides, is known to show significant sorption and low biodegradability due to its hydrophobicity and complex structure. Studies have indicated that biosorption is a major removal pathway for macrolides due to their high solid-water distribution coefficients (LogD) [4,26]. In activated sludge processes, the solid-water distribution coefficient is defined as the partition of a compound between the sludge and the water phase. Taking into consideration both K OW and pKa, Log D has been proposed as a relatively accurate indicator of sorption behavior [4]. However, the lack of correlation between log D and sorption on the sludge has also been observed previously, and the other sorption mechanisms including electrostatic interactions has been suggested [36,37]. This might explain some anomalous cases noted in this study. For instance, ofloxacin showed high sorption to the sludge (154.91 µg/gMLSS) compared to the other three fluoroquinolone antibiotics (i.e. norfloxacin, ciprofloxacin and lomefloxacin) although with a slightly lower Log D value. The high biosorption behavior of ofloxacin is believed to be due to the lower deprotonation tendency of the amide group in ofloxacin as opposed to the other three fluoroquinolones. At pH = 8, a reduced extent of amide deprotonation in ofloxacin is expected, leaving a higher degree of positively charged protonated amide in the molecule. The higher extent of the cationic charged moiety could result in stronger binding strength driven by ionic interactions between protonated amide groups and negatively charged activated sludge particles [30], thus the high biosorption of ofloxacin observed in this study was probably due to the interaction of the positively charged amide group and the activated sludge. Sulfathiazole, tetracycline, and trimethoprim were adsorbed on the sludge at 12.86 µg/gMLSS, 38.48 µg/gMLSS, and 44.04 µg/gMLSS respectively. The low sorption of sulfonamides and trimethoprim is probably due to their low log D values [26]. Additionally, since they are negatively charged at pH values around 8 (as in the bioreactor), their binding to biomass via cation exchange with anionic sites or by metal complexation are also likely to be minimal [26]. Tetracycline is quite soluble in aqueous solutions and is poorly adsorbed onto biomass as indicated by their low Log D values, which explains its relatively low sorption in the bioreactor.

Antibiotics were generally found to be resistant to biodegradation except for certain classes (such as β-lactams), mainly due to their recalcitrant nature and low concentrations in WWTPs. For instance, the biodegradation of selected β-lactamase, sulfonamide, fluoroquinolone antibiotics were investigated by Al-Ahmad et al. [21] based on OECD closed bottle tests. Except for penicillin G, all other antibiotics showed limited biodegradation (< 10%) in a 40-days testing period. Kim et al. [38] also observed no evidence of biodegradation for tetracycline; sorption was found to be the principal removal mechanism in activated sludge process. Biodegradation experiments performed by Halling-Sorensen et al. also showed the strong persistence of trimethoprim in activated sludge batch reactors [39]. However, nitrifying bacteria was suspected to be capable of co-metabolizing trimethoprim. The removal efficiency of trimethoprim appeared to be enhanced by long SRT, which is beneficial to nitrification [4].

In this study, except for ofloxacin, ciprofloxacin and roxithromycin, biodegradation was found to be a significant removal mechanism amongst the 12 compounds examined (Fig. 6(a) and (b)). Of the sulfonamide compounds, sulfathiazole shows the highest removal efficiency by biodegradation (94.4%) followed by sulfamethazine (16.5%). All three tetracycline compounds, namely oxytetracycline, tetracycline, and chlortetracycline, showed significant biodegradation (> 25%), with the highest removal (up to 80%) for chlortetracycline. These results corroborate the work of Loftin et al. where higher degradation of chlortetracycline than oxytetracycline and tetracycline was also observed [40]. Tetracycline constitutes the base structure for this group of compounds, with chlortetracycline and oxytetracycline having a chlorine atom and hydroxyl group substitution, respectively. It was suggested that their degradation may be affected by the presence or absence of the substitutions, where changes in the acidic or basic nature of the reactive site on the molecule induce steric hindrance, or enhance or detract from the cross-conjugated systems present in the tetracycline class of antibiotics, thus resulting in their different removal behaviors [40]. Trimethoprim was biologically degraded efficiently at 69.3%, as mentioned above; its relatively higher degree of removal was most probably related to the high nitrification activities observed in the bioreactor. Fluoroquinolones, with the exception of ciprofloxacin (which showed no evident biodegradation) and ofloxacin (1.68%), also showed good removal via biodegradation (> 50%). In a more general sense, higher degrees of biodegradation were observed for most of the antibiotics compared to the findings in WWTPs [26], which were attributed to the high membrane rejection against these compounds, and which resulted in their prolonged retention in the bioreactor [18,19]. Their biodegradation was thus promoted. Additionally, pseudo first-order degradation kinetics was observed for most of the antibiotics with concentrations down to ng/L levels [4]. FO membrane rejection resulted in their enrichment in the bioreactor, and increased degree of biodegradation is thus expected.

Nevertheless, even though the removal and fate of the antibiotics may be explained individually, there was not a very obvious trend for the removal of the antibiotics within the same class or among classes. Researchers have also attempted to obtain correlations between the removals of the compounds with their log D, hydrophilicity, and molecular size characteristics [3,4,33]. However, it has been shown to be difficult to generalize the removal of trace organics by classes. Although antibiotics from the same class may have similar skeletal structures, the derivatization with different functional groups rendered them different from each other in terms of solubility, polarity, charge and hydrophobicity [3,40]. Their characteristics in turn affects the partitioning behaviors and their bioavailability, which further resulted in their distinct removal behaviors.

### 4. Conclusions

The presence of 12 antibiotics at a concentration of 500 ng/L each has negligible effects on the overall performance of an OMBR system;
the overall removal of TOC and NH₄⁺-N remained at > 98% and > 97% after 40 days continuous exposure to the antibiotics. > 75% overall removal of all the antibiotics was observed, which resulted from the high rejection properties of the FO membrane towards these antibiotics (> 90%). All the antibiotics except for ciprofloxacin and roxithromycin showed biodegradation as a significant removal pathway. Ofloxacin and roxithromycin showed the highest biosorption onto the activated sludge. No remarkable biosorption was observed for oxytetracycline, chlorotetracycline and sulfamethazine. Overall, OMBR was an effective process for the removal of antibiotics from different classes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcej.2017.10.026.

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