Membrane Aerated Biofilm Reactor: A Correlation between Biofilm Thickness and Removal of Pharmaceuticals and Endocrinal Disruptor Compounds

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Abstract Removal of thirteen pharmaceuticals and endocrinal disruptor compounds via membrane aerated biofilm reactor was tested. MABR system demonstrated good management of nitrogenous nutrients. An increase in biofilm thickness enhanced the removal of analgesics, hormones, and disinfectants.

Keywords: biodegradation, organic micropollutants, biofilm, nitrification

1. Introduction

The presence of organic micropollutants (OMPs) such as pharmaceuticals, antibiotics, and endocrinal disruptor compounds (EDCs) in natural water bodies is an increasing concern due to the risk that their occurrence represents for the ecosystems, human health, and their high potential to enter drinking water sources. One of the main sources of water pollution by OMPs is the discharge of effluents from wastewater treatment plants (WWTPs) based on conventional activated sludge (CAS), which not only have poor efficiency in removing such recalcitrant OMPs but also incurs high energy cost due to aeration.

Membrane aerated biofilm reactor (MABR) is a technology that is gaining momentum as a retrofit system for WWTP. MABR employs hollow-fiber membranes, not as a filtration system, but to supply bubble-less aeration at high efficiencies (up to 100% oxygen transfer efficiency) (Perez-Calleja et al. 2017; Syron, Semmens, and Casey 2015). MABR supports biofilm growth in a substrate co-counter diffusion principle, hence allowing simultaneous nitrification-denitrification (SND) and COD removal in one vessel, in turn reducing the footprint of the treatment process (Aybar et al. 2014; Syron and Casey 2008).

MABR was mainly designed for the management of nitrogenous nutrients (i.e., ammonia). In addition, several studies have shown a strong correlation between nitrification and the biotransformation of organic micropollutants, primarily due to co-oxidation catalyzed by ammonia monooxygenase enzyme (AMO) (Men et al. 2017; Xu, Yuan, and Ni 2016). Therefore, this study proposes the use of an MABR system to enhance the removal of a cocktail of OMPs, including pharmaceuticals and EDCs. The main objective is to obtain a correlation between biofilm thickness with removal of OMPs to propose an optimal biofilm thickness that would allow the highest OMPs removals without compromising the degradation of organic carbon and nitrogenous nutrients.

2. Methodology

2.1. Setup

Zeelung membranes provided by Suez, US, were used for experimentation. 170mL bench-scale zeelung MABRs system was built on a glass tube, integrated by Three Zeelung cords (85cm^2 membrane surface area) were glued into an air-supply manifold at the bottom and opened at the top. This is to avoid back diffusion of gases. Air was supplied at 10 psi pressure at the lumen of the membrane. MABRs were operated in continuous; influent was pumped at 0.14 mL min^{-1}. The bulk liquid was continuously recirculated at 150 mL min^{-1} to ensure homogeneity inside the system. After 20 h of hydraulic retention time (HRT), treated effluent was pumped out of the system and collected in amber glass bottles.

2.2. Feed wastewater

Municipal wastewater was taken from KAUST WWTP, Thuwal, Saudi Arabia (≈300±10 mg COD L^{-1} and ≈25±3 mg L^{-1} ammonium) and spiked with 50 µg L^{-1} OMPs cocktail. This solution was used as influent for MABR systems. Analytical grade (>98 %) acetaminophen, diclofenac, ibuprofen, ketoprofen, mefenamic acid, gemfibrozil, carbamazepine, 17α-ethinyl estradiol and bisphenol-A purchased (Sigma-Aldrich); primidone and, estrone (MP Biomedical LLC); and naproxen and triclosan (USP reference standards), were used to prepare the OMPs cocktail in methanol.

2.3. Analytical methods

Biofilm thickness and removal of nitrogenous nutrients, organic carbon, and OMPs were monitored through time. Samples were pre-filtered through a 0.45 µm syringe PES filter (Sterlitech Corporation) and used for COD, ammonia, nitrite, and nitrate analysis, performed through HACH® kits.

100 mL influent and effluent samples were spiked with 100µL of internal standard (IS) stock solution at 700 $\mu g L^{-1}$, containing Acetaminophen 13-2C (Cambridge Isotope Laboratories), Ibuprofen-d3 (Fluka), Naproxen-d3 (Toronto Research Chemicals Inc.), and Bisphenol A-d16 and Gemfibrozil-d6 (2,2-dimethyl-d6) (CDN Isotopes). Solid-phase extraction was performed using 500 mg Oasis HLB cartridges supplied by Waters (Ireland) and Dionex® Autotrace 280 apparatus. Eluted samples were evaporated to dryness and reconstituted on 50 µL BSTFA+1% TMCS solution (Sigma Aldrich) and 50 µL pyridine (Fisher Scientific) to allow silylation. All reagents used were HPLC grade.

Samples were injected in the Agilent Technologies 7890A gas chromatograph system coupled with an Agilent 5975C TAD inert XL EI/CI MSD high vacuum pump with a triple-axis detector. Chromatographic separation was performed with a J&W GC DB-5MS column with a 0.25 µm pore size (60 m x 0.25 mm). Injection volume was 1 µL in splitless mode and oven temperature program as follows: 80°C for 1 min, ramping up from 80 °C to 260 °C from minute 1 to 13 (15 °C min^{-1}), then from 260 °C to 300 °C from minute 13 to 22 (at 4.4 °C min^{-1}) and held constant at 300°C until minute 30. Helium (99.999%) was used as carrier gas at a constant flow of 0.8 mL min^{-1}. GC-MS quantitative analysis was based on a six-point calibration curve (20-1500 $\mu g L^{-1}$). The instrument's linearity based on the relative responsive factor (RF) showed a correlation higher than 0.989 for all analytes.

Optical coherence tomography (OCT) (Thorlabs GANYMEDE GmbH, Dachau, Germany) with a central wavelength of 930nm and equipped with a 5X telecentric scan lens (Thorlabs LSM 03BB) was used to investigate the biofilm formation over time and measure biofilm thickness. Image stack resolution was 512x256x1022 pixels. OCT images were processed using ThorImageOCT 5.2.1.

3. Results

3.1. Ammonia and COD removal

High hydraulic retention time in the MABR system allowed observable removals of nitrogenous nutrients and organic carbon after only 2 weeks of inoculation, although the biofilm presented an average thickness of 0.09 ± 0.06 µm (n=6). 18% ammonia removal and 34% COD removal were achieved at this stage. After 30 days from inoculation, biofilm had double its thickness to 0.22 ± 0.16 µm, allowing over 50% removal for both ammonium and COD. At average biofilm thickness of 0.48 µm, nitrification was enhanced after 45 days from inoculation, reaching over 70% removal. Biofilm continued developing, reaching a thickness of 0.95 ± 0.19, 1.25 ± 0.18, 3.64 ± 0.32, and 4.48 ±0.28 µm at 60, 75, 90 and 105 days, respectively. With the increase of biofilm thickness, nitrifying activity in the MABR was also enhanced, reaching a nitrification efficiency of 82% at day 60, 91% at day 75 and over 95% from day 90 until the end of experimentation. Full conversion of ammonia into nitrate and then into nitrogen gas was achieved as no nitrite nor nitrate were detected in the bulk liquid and effluent.

Removal of organic carbon (COD) did not correlate with biofilm thickness. After only 30 days from inoculation and a thin biofilm (0.22 µm), MABR allowed 60% COD removal efficiency. This efficiency increased linearly to almost 70% by day 60 (0.95 µm biofilm thickness), when removal reached a plateau and was maintained constant until the end of experimentation (69.1±1.5 % COD removal).

The final measurement of biofilm showed an average thickness of 4.59 µm. MABR maintained over 95% efficiency in ammonia removal for the control MABRs without OMPs spike, as well as in the MABRs spiked with 50 $\mu g L^{-1}$ OMPs cocktail. This suggests that there are no inhibitory effects to nitrifying bacteria caused by the presence of these OMPs at spiked concentration, nor occurrence of competition for ammonia monooxygenase enzyme to perform nitrification. COD removal was maintained around 60-70% efficiency for most stages of experimentation, meaning that further increase in the biofilm thickness would not have a significant effect on improving the organic carbon removal. Incomplete removal of COD could be explained due to oxygen limitation. Oxygen supply through the lumen of the membrane could have been utilized by nitrifying bacteria localized in the inner layers of the biofilm, closed to the membrane-biofilm interface, and depleted in the outer layers of the biofilm. An anoxic/anaerobic environment in the bulk liquid allowed complete denitrification. Thus, the increase of the air supply pressure at the lumen of the membrane might be required to allow further degradation of COD. However, this increase must be controlled so as not to compromise the efficiency of denitrification.

3.2. Removal of OMPs

Negligible transformation of selected OMPs (<10% removal) was observed after the first two weeks from inoculation, when average biofilm thickness was 0.09 µm.

The 13-targeted OMPs were divided into six groups according to their biodegradability (Table 1). Biofilm had a fast development of degradation capabilities for group 1. By day 30, MABR with biofilm thickness of 0.22 µm provided significant removals for acetaminophen (67±5.3%) and triclosan (70±1.2%). For the rest of the pollutants, minimal change in concentration was observed. Further increase of efficiency was achieved for group 1 compounds by day 45 (thickness 0.48 µm). Over 99% removal of triclosan, with concentrations of 4.8± 4.4 ng/L remaining in the effluent, was obtained. Removal of acetaminophen at this stage reached 90% and further

increased to 95 and 99% at average biofilm thickness of 1.25 and 4.98 µm, respectively. Acetaminophen and triclosan were demonstrated to have the highest biodegradability among the 13 OMPs studied. The fast transformation of these compounds might be correlated and dependent on the speed and amount of ammonia converting into nitrates (Cho and Chu 2015). In addition, studies with a tricking filter have shown that at high COD and nitrification rates, over 80% APAP was removed; however the decrease of the nitrification rates and COD removal had a negative impact in APAP removal, reaching under 60% efficiency; suggesting the contribution of nitrifiers and heterotrophs in the biotransformation of these pollutants (Park and Seungdae 2020).

MABR also showed important efficiency in removing the second group, which include natural and synthetic hormones, Estrone (E1) and Ethinyl Estradiol (EE2). High biotransformation was attained for E1 (46±4%) and EE2 (82.9±3.3%) starting on day 45, and reaching a final removal efficiency over 99% for E1 and over 90% for EE2 at day 105. High removal might have been achieved by the long HRT (20 h) and enrichment of the biofilm with estrogen degrading bacteria (e.g. *Nitrosomonas europaea*, or *Rhodococcus equi*, *Sphingobacterium* sp. JCR5) that supported biotransformation through cometabolism via AMO enzyme (Yi and Harper 2007); or through oxidation and cleavage of the aromatic-rings (Haiyan et al. 2007). However, a further microbial community analysis would need to be performed to verify this.

An aerated nitrifying fixed bed had previously demonstrated high removals of estrogens (96%) at 4.3 d HRT and volumetric loading rate of 11 µg $EE2L^{-1}$ d^{-1} (Forrez et al. 2009); thus, the MABR system tested in this study might be providing better operation conditions through its co-counter diffusion principle that accelerated the removal of these pollutants. E1 presented a higher removal rate in comparison to EE2, which could be explained based on its smaller size 270 g/mol and 296 g/mol, respectively; and less complex molecular structure. Lower initial removal of E1 registered at day 45 could have occurred due to the biological transformation of EE2 into E1, which has been previously reported (Haiyan et al. 2007).

Removal of OMPs from groups 3, 4, and 5 exhibited a high correlation with biofilm thickness but at different degrees. MABR showed limited removal of ibuprofen in the first 60 days of continuous operation (<50%), however after day 75, biofilm had a significant increase in its thickness, which allowed an increase in the ibuprofen removal, reaching over 98% removal at the end of experimentation at 4.59 µm biofilm thickness. Bisphenol-A (BPA) presented a similar trend. However, the increase in removal efficiency was not as abrupt as for ibuprofen. MABR allowed a constant increase of BPA removal; the effluent BPA concentration was 45 ± 1.49 µg BPA/L at 0.09 µm thickness, and it progressively decreased to 15.8 ± 0.32 and 2.52 ± 0.94 µg BPA/L at 0.95 and 4.59 µm thickness. The same trend, but in a lower degree, was observed for partially biodegradable naproxen and mefenamic acid, with removals reaching over 65%. For hardly-biodegradable Ketoprofen and Gemfibrozil, a maximum removal of only 35% and 20%, respectively, was achieved at the final measurement (4.59 µm thickness). Removal of these pollutants has been closely linked to nitrifying activity (Margot et al., 2015). Most likely, through hydroxylation after reactive oxygen from ammonia monooxygenase enzyme attack N–H bond, a C–H bond, or a double bond of these molecules (Velázquez and Nacheva 2017). Thus, the enrichment of the biofilm with ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), and balance between the biofilm thickness and oxygen supply (to avoid substrate limitation) are keys for the MABR to allow high OMPs biotransformation rates.

Finally, MABR did not show any capacity to remove highly recalcitrant pollutants like primidone, carbamazepine, and diclofenac. Even higher concentrations of these pollutants were found in the effluent, most likely due to their initial sorption inside biofilm, accumulation, and desorption back into the bulk liquid.

Results obtained in this study are comparable to those obtained for granular SBR with complete nitrification (Margot et al., 2015). High removals (over 90%) were registered for APAP, TCS, E1, BPA, and IBU, and in this study for EE2 as well. In addition, slightly higher removals were obtained for Naproxen and Mefenamic acid at the last stage with 4.59 µm biofilm thickness, which could be explained by the co-counter diffusion principle of the MABR that enhanced nitrification activity and cometabolism. Granular SBR with complete nitrification also presented negligible removal of CBZ and DCF.

Table 1. Targeted OMPs classified into six groups according to their biodegradability. Groups as follow: 1 Highly biodegradable; 2 Biodegradable; 3 Biodegradable dependent of biofilm thickness; 4 Partially biodegradable, dependent on biofilm thickness; 5 Hardly biodegradable, dependent on biofilm thickness; and 6 Highly recalcitrant.

No.	Compound	Abbrev.	CAS Number
1	Acetaminophen	APAP	103-90-2
	Triclosan	TCS	3380-34-5
2	Estrone	E1	53-16-7
	17a Ethinyl Estradiol	EE2	57-63-6
3	Ibuprofen	IBU	15687-27-1
	Bisphenol A	BPA	80-05-7
4	Naproxen	NPX	22204-53-1
	Mefenamic acid	MFA	61-68-7
5	Ketoprofen	KET	22071-15-4
	Gemfibrozil	GMZ	25812-30-0
6	Carbamazepine	CBZ	298-46-4
	Primidone	PMD	125-33-7
	Diclofenac	DCF	15307-86-5

4. Conclusions

MABR system demonstrated to be highly efficient in the nitrification-denitrification process, even at the early stages of experimentation, when biofilm was less than 0.2 µm thick. An increase in the biofilm thickness enhanced the nitrification-denitrification process. In addition, it was observed to result in an improvement in the removal of highly biodegradable and partially biodegradable OMPs like acetaminophen, triclosan, Ethinyl estradiol, Estrone, bisphenol A, ibuprofen and naproxen. Finally, it is crucial to maintain a balance between the biofilm thickness and oxygen supply, as oxygen limitation might slow the rate of COD removal and biotransformation of OMPs.

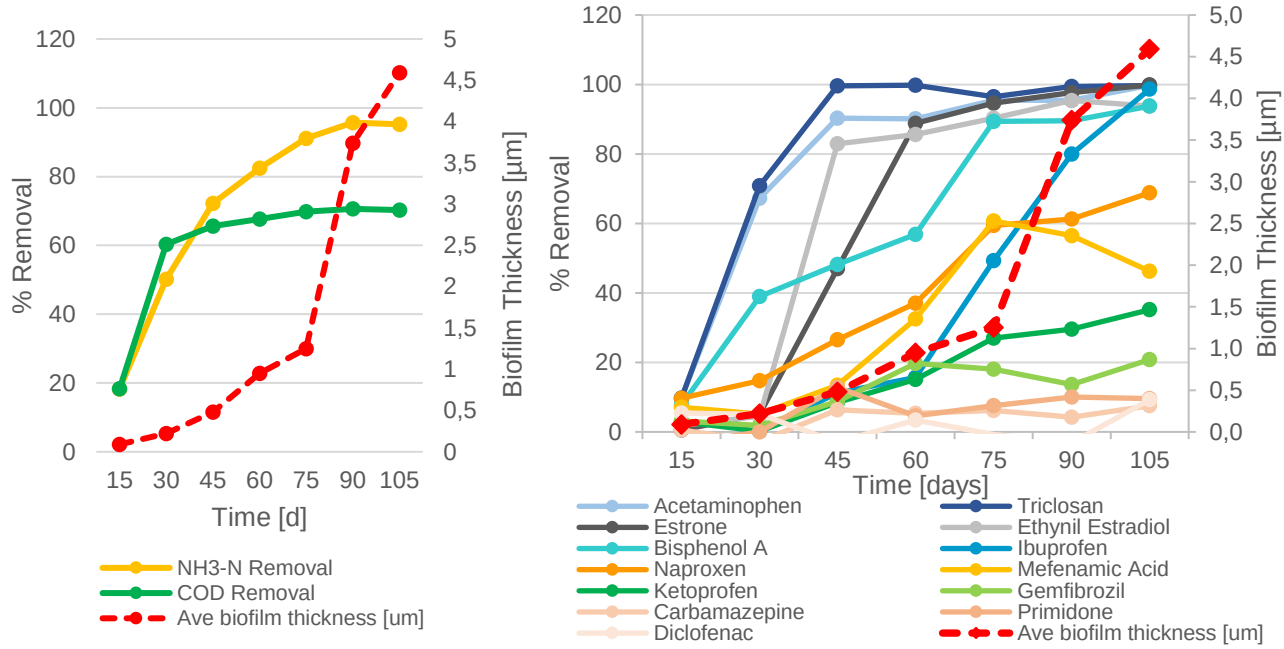

Figure 1. Correlation between biofilm thickness and right: COD and ammonia removal; left: OMPs removal

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