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Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal by an anoxic-aerobic membrane bioreactor (MBR)

Hop Phan

University of Wollongong, vhp997@uowmail.edu.au

Faisal Ibney Hai

University of Wollongong, faisal@uow.edu.au

Jinguo Kang

University of Wollongong, jkang@uow.edu.au

Hoa Dam

University of Wollongong, hoa@uow.edu.au

Ren Zhang

University of Wollongong, rzhang@uow.edu.au

See next page for additional authors

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Abstract

Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal during wastewater treatment by an integrated anoxic-aerobic MBR was examined. A set of 30 compounds was selected to represent TrOCs that occur ubiquitously in domestic wastewater. The system achieved over 95% total organic carbon (TOC) and over 80% total nitrogen (TN) removal. In addition, 21 of the 30 TrOCs investigated here were removed by over 90%. Low oxidation reduction potential (i.e., anoxic/anaerobic) regimes were conducive to moderate to high (50% to 90%) removal of nine TrOCs. These included four pharmaceuticals and personal care products (primidone, metronidazole, triclosan, and amitriptyline), one steroid hormone (17 β -estradiol-17-acetate), one industrial chemical (4-tert-octylphenol) and all three selected UV filters (benzophenone, oxybenzone, and octocrylene). Internal recirculation between the anoxic and aerobic bioreactors was essential for anoxic removal of remaining TrOCs. A major role of the aerobic MBR for TOC, TN, and TrOC removal was observed.

Keywords

Trace organic contaminant (TrOC), Anoxic membrane bioreactor, Biosorption, Aerobic biodegradation, Redox conditions, GeoQuest, CMMB

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Authors

Hop Phan, Faisal Ibney Hai, Jinguo Kang, Hoa Dam, Ren Zhang, William Price, Andreas Broeckmann, and Long Nghiem

Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal by an anoxic—aerobic membrane bioreactor (MBR)

Hop V. Phan^{a,b}, Faisal I. Hai^{a*}, Jinguo Kang^{a,c}, Hoa K. Dam^d, Ren Zhang^b, William E. Price^c, Andreas Broeckmann^e, Long D. Nghiem^a

^aStrategic Water Infrastructure Lab, School of Civil, Mining and Environmental Engineering, University of Wollongong, Australia.

^bMolecular Biology Lab, School of Biological Sciences, University of Wollongong, Australia.

^cStrategic Water Infrastructure Lab, School of Chemistry, University of Wollongong, Australia.

^dDecision Systems Lab, School of Computer Science and Software Engineering, University of Wollongong, Australia.

^e GHD Australia.

*Corresponding author: Faisal I. Hai (E-mail: faisal@uow.edu.au; Phone: +61-2-4221-3054)

Research Highlights

- Simultaneous nitrogen and TrOC removal by an anoxic-aerobic MBR was demonstrated
- Low ORP (anoxic/anaerobic) regimes are conducive to biodegradation of some TrOCs
- Without anoxic-aerobic internal mixing, low ORP regime may only aid biosorption
- Stable sludge phase concentrations confirm biodegradation as main removal mechanism
- Aerobic degradation plays a major role in TrOC removal

Abstract

Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal during wastewater treatment by an integrated anoxic-aerobic MBR was examined. A set of 30 compounds was selected to represent TrOCs that occur ubiquitously in domestic wastewater. The system achieved over 95% total organic carbon (TOC) and over 80% total nitrogen (TN) removal. In addition, 21 of the 30 TrOCs investigated here were removed by over 90%. Low oxidation reduction potential (i.e., anoxic/anaerobic) regimes were conducive to moderate to high (over 50% and up to 90%) removal of nine TrOCs. These include four pharmaceuticals and personal care products (primidone, metronidazole, triclosan, and amitriptyline), one steroid hormone (17 β -estradiol-17-acetate), one industrial chemical (4-tert-octylphenol) and all three selected UV filters (benzophenone, oxybenzone, octocrylene). Internal recirculation between the anoxic and aerobic bioreactors was essential for anoxic removal of remaining TrOCs. A major role of the aerobic MBR for TOC, TN and TrOC removal was observed.

Keywords: trace organic contaminants (TrOC); anoxic membrane bioreactor; biosorption; aerobic biodegradation; redox conditions

1. Introduction

Trace organic contaminants (TrOCs) have been widely detected in sewage and sewage impacted water bodies at concentrations of up to several $\mu\text{g/L}$. Depending on their usage and toxicological effects, TrOCs can be classified into several groups including pharmaceuticals and personal care products, steroid hormones, industrial chemicals, pesticides, phytoestrogens, and UV filters. The widespread occurrence of these TrOCs in the environment raises significant concern regarding the potential detrimental effects on human and other biota. While TrOCs can be introduced into the environment via different pathways, inefficient treatment performance of wastewater treatment plants (WWTPs) has been identified as the major route of release of TrOCs to natural waters (Alexander et al., 2012; Hai et al., 2014; Luo et al., 2014).

Membrane bioreactors (MBR) that combine biodegradation by activated sludge with direct solid-liquid separation using membrane filtration are an advancement over the conventional activated sludge (CAS) process for bulk organics and nutrient removal in a single-step, compact process.

Although a number of studies have reported better and more stable removal of the moderately biodegradable TrOCs by MBR than CAS, little improvement is generally reported in case of hydrophilic and resistant TrOCs (Boonyaroj et al., 2012; Radjenović et al., 2009; Tadkaew et al., 2011). In order to find avenues to enhance TrOC removal by MBR, the effect of different operating parameters such as sludge retention time (SRT) and hydraulic retention time (HRT) (Fernandez-Fontaina et al., 2012; Zeng et al., 2013), mixed liquor pH (Urase et al., 2005) and temperature (Hai et al., 2011c) have been studied. Several studies (e.g., (Dytczak et al., 2008; Hai et al., 2011a; Zwiener et al., 2000)) have investigated the impact of dissolved oxygen concentration (DO) and/or redox conditions (i.e., oxidation reduction potential, ORP). However, a clear consensus has not been reached to date.

Biodegradation processes can possibly be induced under aerobic (in the presence of molecular oxygen), anoxic (in the absence of molecular oxygen but in the presence of nitrate) or anaerobic conditions (in the absence of both molecular oxygen and nitrate). Different redox conditions may promote the growth of different microbial consortia leading to the excretion of diverse enzymes, and therefore, achieving varying degree of TrOC biodegradation. Additionally, redox conditions can significantly influence the properties of sludge, which govern biosorption of TrOCs. The mechanisms of biological nutrient (i.e., nitrogen and phosphorous) removal under different redox conditions are well understood and have been successfully applied in full-scale WWTPs. However, the same cannot be claimed in the case of TrOC removal. Recent studies highlight the TrOC removal performance of aerobic nitrifying reactors (Dorival-García et al., 2013; Suarez et al., 2010). However, compared to aerobic conditions, fewer studies have been conducted on TrOC removal under anaerobic and/or anoxic conditions. Therefore, to date understanding of TrOC degradation under anaerobic and/or anoxic conditions remains rather limited. Furthermore, the performance of combined anaerobic and/or anoxic and aerobic reactors has been the focus of only a limited number of recent investigations, and contradictory reports can often be seen in the literature. For example, Li et al. (2011) reported biodegradation of both natural (17β -estradiol) and synthetic (17α -ethinylestradiol) estrogens under all three redox conditions in a lab-scale anaerobic-anoxic-aerobic activated sludge system. By contrast, estrogens were only degraded under nitrifying conditions in a combined nitrification (aerobic) and denitrification (anoxic) system (Suarez et al., 2012). Differences in results from recent studies may originate from the

variation in operating conditions. Systematic studies under controlled operating regimes with a broad set of TrOCs are required to elucidate the contribution of the individual reactors (facilitating different redox conditions) in combined nitrifying and denitrifying systems, but such attempts have been scarce to date.

In line with the aforementioned research gaps, the aim of this study is to investigate the removal and fate of a set of 30 TrOCs by a laboratory scale anoxic-aerobic MBR. Insights into the influence of anoxic and aerobic conditions on the removal of these compounds from both aqueous and sludge phases along with nitrogen removal are presented.

2. Materials and Methods

2.1 Model TrOCs and synthetic wastewater

A set of 30 compounds representing five major groups of TrOCs, namely pharmaceuticals and personal care products, pesticides, steroid hormones, industrial chemicals, phytoestrogens and UV filters were used in this study. These TrOCs were selected based on their widespread occurrence in domestic sewage and their diverse physicochemical properties (Supplementary Data Table 1). The compounds were purchased from Sigma-Aldrich (Australia) with a purity of 99% or higher. A combined stock solution of TrOCs was prepared in pure methanol and stored at -20 °C in the dark. Once a stable MBR operation had been achieved (See Section 2.3), TrOCs were continuously spiked into the synthetic wastewater to achieve a final concentration of approximately 5 µg/L of each selected compound.

A synthetic wastewater was used to provide a source of carbon, nitrogen, phosphorus and trace metal ions for the growth of the microbes. The synthetic wastewater was prepared fresh each day by dissolving the chemicals into deionized water to obtain a final concentration of 100 mg/L glucose, 100 mg/L peptone, 17.5 mg/L KH₂PO₄, 17.5 mg/L MgSO₄, 10 mg/L FeSO₄, 225 mg/L CH₃COONa and 35 mg/L urea (Wijekoon et al., 2013).

2.2 Laboratory scale MBR set-up

A laboratory scale anoxic-aerobic MBR (Supplementary Data Figure S2) with a 13.8 L anoxic reactor and an 11.7 L aerobic reactor with an immersed membrane module was used. The

membrane module used was a hollow fibre ultrafiltration membrane (Zeweed-10) supplied by Zenon Environmental (Ontario, Canada). This membrane had a nominal pore size of $0.04\ \mu\text{m}$ with an effective membrane surface area of $0.93\ \text{m}^2$. Peristaltic pumps (Masterflex L/S, USA) were used for feeding, recirculation and effluent extraction. The permeate withdrawal pump connected with the membrane was operated using an 8 min on and 2 min off cycle. The on/off time aimed to provide relaxation time to the membrane module. The influent flow rate was adjusted to be the same as the effluent flow rate to maintain a constant water level inside the reactors. A certain volume of the media was constantly recirculated from the aerobic to the anoxic reactor. The ratio of the media recirculation flow rate to the feed flow rate (denoted internal recirculation (IR) henceforth) governed the overflow of media from the anoxic tank to the aerobic tank (See Section 2.3). The mixed liquor in the upper quarter of the anoxic tank was intermittently (1 min on and 15 min off) mixed by a mixer (200 rpm) to ensure that the sludge transferred from the aerobic tank did not get trapped within the anoxic reactor. An air pump was employed to continuously aerate the (aerobic) reactor via a diffuser located at the bottom of the tank. Another air pump was intermittently operated to provide air flow through the membrane module to reduce cake layer fouling. A high resolution ($\pm 0.1\ \text{kPa}$) pressure sensor (SPER scientific, Extech equipment Pty. Ltd, Victoria, Australia) connected to a computer for data recording was utilized to continuously monitor the transmembrane pressure (TMP). The *in-situ* air scrubbing was found adequate to keep the TMP stable at below $5\ \text{kPa}$, and no chemical cleaning was required over the whole operation period. The total hydraulic retention time (HRT) was set at 24 h (i.e., 13 h in anoxic tank and 11 h in aerobic tank), corresponding to a permeate flux of $1.23\ \text{L}/\text{m}^2\cdot\text{h}$. The mixed liquor pH was stable at 7.25 ± 0.75 . Dissolved oxygen concentration (DO) was maintained at above $3\ \text{mg}/\text{L}$ and approximately $0.1\ \text{mg}/\text{L}$ for the aerobic and the anoxic reactors, respectively. The ORP remained relatively stable at $141\pm 18\ \text{mV}$ ($n=55$) in the aerobic reactor. In the low DO reactor, the ORP varied from $-122\pm 22\ \text{mV}$ ($n=40$) at an IR ratio of 3 to $-230\pm 75\ \text{mV}$ ($n=15$) in absence of IR (See Section 2.3). Throughout the period of investigation, the MBR system was covered with aluminium foil to avoid any exposure to sunlight to prevent possible photolysis of the TrOCs.

2.3 MBR operation protocol

The MBR system was initially seeded with activated sludge from the biological nutrient removal unit of the Wollongong Sewage Treatment Plant (Wollongong, Australia). It was operated for total 305 d (Supplementary Data Table S3). For the initial 180 d, the MBR was operated without any planned sludge withdrawal except for sludge sampling. Under this regime, the MBR was first operated for 125 d for sludge acclimatization and stabilization of TOC and TN removal by fine-tuning the IR ratio (0.5-3) between the anoxic and the aerobic reactor. Following this, TrOCs were introduced to the synthetic wastewater that was continuously fed to the MBR. This part of the study spanned 55 d (Day 126-170) and was conducted with an IR ratio of 3. During this period, the mixed liquor suspended solids (MLSS) concentration increased for both the anoxic (from 8.12 g/L to 10.4 g/L) and the aerobic reactors (from 7.38 g/L to 8.75 g/L). However, MLVSS/MLSS ratios were stable at 0.71 ± 0.02 and 0.70 ± 0.01 for the anoxic and the aerobic reactors, respectively (Supplementary Data Figure S4).

The MBR was operated under a fixed SRT of 25 d for the rest of the period (Day 181-305). At the beginning of this trial, the MBR system was operated for a period of 55 d without any addition of TrOCs to the synthetic wastewater. This run was conducted to ensure stable biological performance (e.g., TOC and TN removal) following the change in SRT. TrOC spiking to the synthetic wastewater was resumed from Day 226. The MBR was hence run for 40 d at an IR ratio of 3 and MLSS concentration of 5.12 ± 0.18 g/L and 3.78 ± 0.23 for the anoxic and the aerobic reactors, respectively. The MBR was operated for further 35 d without IR to assess the impact of recirculation of media from the aerobic to the anoxic reactor. In this paper, the low DO reactor has been generally described as an ‘anoxic’ reactor except for during the operation without IR when it was described as an ‘anaerobic’ reactor due to the absence of nitrate.

2.4 Analytical methods

2.4.1 Basic parameters

Total organic carbon (TOC) and total nitrogen (TN) were analyzed using a TOC/TN-V_{CSH} analyzer (Shimadzu, Japan). Ammonia and orthophosphate concentrations were measured using flow injection analysis (Lachat instruments, Milwaukee, USA) following the standard methods (Eaton et al., 2005). For ammonia, the analysis comprised production of the blue indophenol dye from the Berthelot reaction, intensification of this blue color by the addition of nitroferricyanide

and then measurement of absorbance at 630 nm (Standard method: 4500-NH₃ H). In ortho-phosphate analysis, the reaction between ortho-phosphate with ammonium molybdate and antimony potassium tartrate under acidic conditions formed a complex. The reduction of this complex with ascorbic acid led to the formation of a blue complex that absorbs light at 880 nm (Standard method: 4500-P G.) Ion Chromatography (IonPac[®] AS23 Anion-Exchange Column, Dionex Corporation, USA) was applied to quantify anions such as nitrate and nitrite. The anions were separated on a strongly basic anion exchanger and converted to their highly conductive acid forms. The separated anions in their acid forms were measured by conductivity. The analysis of other basic parameters was also carried out according to the standard methods (Eaton et al., 2005).

2.4.2 TrOC analysis

The concentration of the selected TrOCs in the (i) feed, (ii) supernatant of the anoxic bioreactor and the (iii) aerobic MBR permeate, was determined using a gas chromatography-mass spectrometry (GC-MS) method described by Hai et al. (2011c). Duplicate samples (500 mL) were concentrated and extracted by solid phase extraction (SPE) using Oasis[®] HLB 6cc cartridges (Water Corporation, Milford, Massachusetts, USA). The TrOCs were eluted and derivatized before being subjected to GC-MS analysis via a Shimadzu GC-MS QP5000 system, equipped with a Shimadzu AOC 20i autosampler, using a PhenomenexZebron ZB-5 (5% diphenyl-95% dimethylpolysiloxane) capillary column (30 m x 0.25 mm ID, $d_f = 0.25 \mu\text{m}$).

TrOC concentration in sludge was determined using a previously reported method (Wijekoon et al., 2013). The sludge sample was freeze-dried using an Alpha 1-2 LD plus Freeze Dryer (Christ GmbH, Germany). The dried sludge (0.5 g) was extracted successively with 5 mL methanol and 5 mL dichloromethane and methanol (1:1) by ultrasonic solvent extraction. The solvent was then evaporated using nitrogen gas and the extracts were diluted to 500 mL with Milli-Q water for SPE. The samples were then analyzed as described above.

Because a microfiltration membrane was utilized, membrane rejection was not expected to be significant for the TrOCs in this study. Accordingly, the performance of anoxic and aerobic

TrOC removal was compared, taking into consideration the TrOC concentration in the supernatant of the anoxic bioreactor and that in MBR permeate.

3. Results and Discussion

The operation of the integrated anoxic-aerobic MBR was initiated with no sludge withdrawal as a reference; however the main focus was on the performance of the system under an SRT of 25d, which is a more realistic value considering the present day full-scale MBRs. Systematic changes in IR ratio were made to verify its effect on bulk organics, nutrient and TrOC removal and to identify the role of anoxic/aerobic conditions on TrOC degradation. The operation protocol has been detailed in Section 2.3 but the important steps are worth reiterating here: (i) fine-tuning IR ratio (0.5-3) during start-up of the MBR; (ii) addition of TrOC to the synthetic wastewater after achievement of high and stable TOC/TN removal at an IR ratio of 3, (iii) change of SRT to 25 d, (iv) operation without IR to identify the impact of anoxic/aerobic conditions on TrOC removal as well as verify the role of IR.

3.1 Bulk organics and nutrient removal

A high and stable (up to 99%) overall TOC removal was achieved throughout the operation period (Figure 1). Notably, irrespective of the level of TOC in the supernatant of the anoxic reactor, the aerobic MBR served as an efficient post treatment step and accordingly a similar level of overall TOC removal was achieved irrespective of the IR ratio (Figure 1).

Biological nitrogen removal necessitates an activated sludge system allowing internal sludge recirculation between aerobic and anoxic regimes to facilitate nitrification (oxidation of ammonia and nitrite) and denitrification (reduction of nitrate to nitrogen gas). While nitrification is carried out by autotrophic bacteria under aerobic conditions, denitrification takes place under anoxic conditions. In this study, $\text{NH}_4^+\text{-N}$ in the supernatant of the aerobic reactor was below the detection limit ($0.7 \mu\text{g N/L}$ as NH_3) (Supplementary Data Figure S5), which implies complete nitrification. The results confirm that an SRT of 25 d (as applied from Day 181 to 305) was adequate to support proliferation of both heterotrophic and slow-growing nitrifying microorganisms that sustain high organics removal, and particularly nitrification. Previous

studies also noted that WWTPs operating at SRTs longer than 10 d can induce high removal efficiencies of bulk organics and nutrients (Zeng et al., 2013).

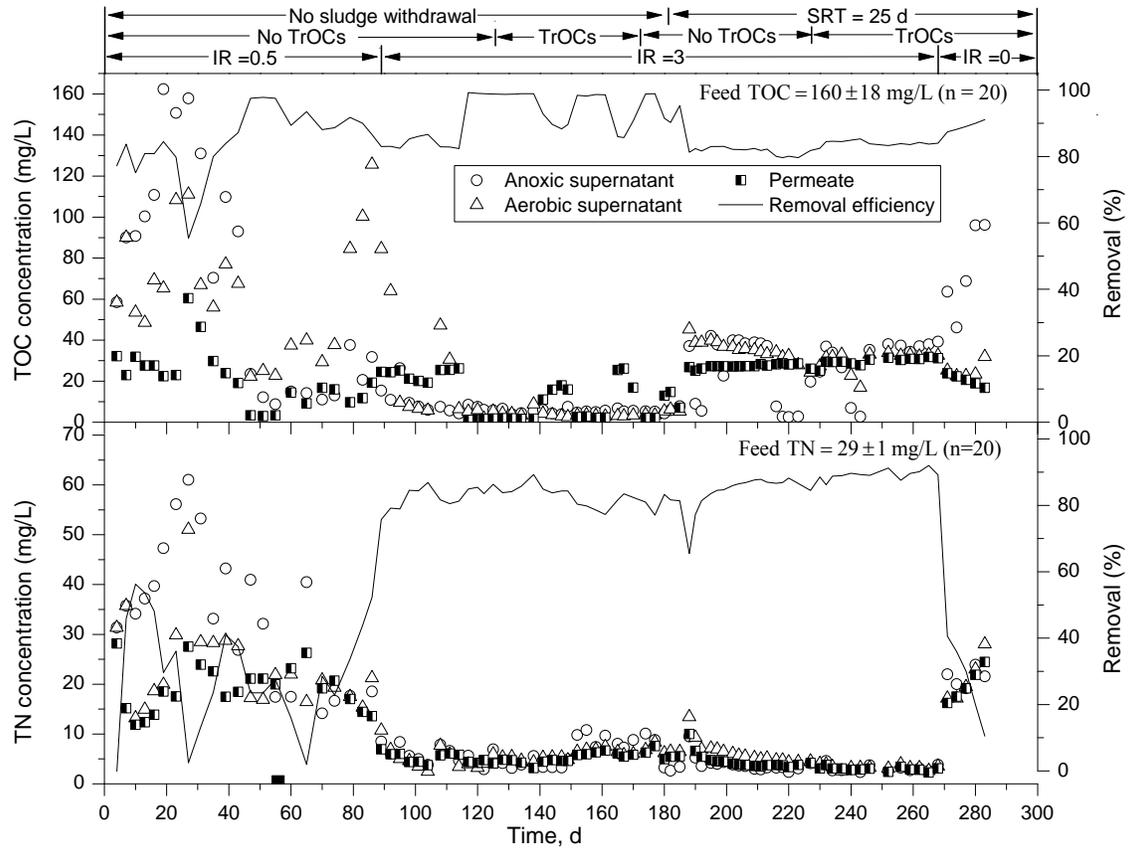


Figure 1: TOC/TN concentration and removal efficiency profiles over the entire operation period of the anoxic-aerobic MBR.

In contrast to nitrification, TN removal (which is governed by denitrification) varied depending on the IR, which controlled the supply of nitrate to the anoxic bioreactor (Figure 1). High fluctuations in TN removal were observed during the initial 90 d when the MBR system was run under an IR ratio of 0.5. Similarly, during the operation without IR (over the last 35 d), lack of exposure of nitrate to the low ORP environment led to a rapid decline in TN removal (Figure 1). By contrast, over 80% TN removal (corresponding to a permeate TN concentration of less than 3 mg/L) was achieved consistently at an IR ratio of 3 (Day 91 to 265). A further enhanced TN removal may have been achieved by applying a higher IR ratio, however, that was not attempted because practically a higher IR means requirement of higher pumping and aeration energy (Baeza et al., 2004; Kim et al., 2010).

It is also interesting to note that more than 90% phosphate removal (Supplementary Data Figure S5) was achieved during operation at an SRT of 25 d and an IR ratio of 3 although the system was not specifically designed for phosphorous removal (i.e., a strictly anaerobic reactor was not used). This may be attributed to the relatively low phosphorous concentration in the synthetic wastewater (about 4 mg/L as P) as well as the role of the phosphorus accumulating organisms (PAOs). Under anaerobic conditions, PAOs assimilate fermentation products (i.e., volatile fatty acids) into storage products within the cells with the concomitant release of phosphorous from stored polyphosphates. Conversely, in the aerobic zone, energy is produced by the oxidation of storage products and polyphosphate storage within the cell increases. As a portion of the biomass is wasted, the stored phosphorous is removed from the bioreactor for ultimate disposal with the waste sludge (Kim et al., 2010). Therefore, integral to biological phosphorous removal are IR and sludge withdrawal. The role of PAOs in the current study is evident from the significant accumulation of phosphorus in the anoxic reactor (Supplementary Data Figure S5) in absence of either sludge withdrawal or IR (Day 0-124 and 266-305, respectively).

The introduction of TrOCs to feed wastewater did not show any discernible impact on the basic biological performance of the MBR system including TOC and TN removal (Figure 1) and the ratio of MLVSS/MLSS (Supplementary Data Figure S4). This observation is consistent with several previous studies (Abegglen et al., 2009; Dorival-García et al., 2013). At trace concentrations, TrOCs may induce impact on oxygen uptake rate of microorganisms but not hinder the overall performance of the system (Hai et al., 2014).

3.2 Overall aqueous phase TrOC removal

It is worth reiterating that, in this study, TrOCs were introduced to the influent continuously over three intervals (Supplementary Data Table S3): (i) Day 126-170 (no sludge withdrawal, IR ratio =3), (ii) Day 226- 265 (SRT=25 d, IR ratio =3), and (iii) Day 266-305 (SRT=25 d, no IR). This section provides an overview of the TrOC removal depending on the compound categories during Stage (i) and (ii) (Figure 2). Discussion on the comparative removal by the anoxic and aerobic bioreactors along with the critical impact of IR (i.e., Stage (ii) vs. Stage (iii)) has been conducted in Section 3.3 and 3.4, while the relative contribution of biodegradation and biosorption has been elucidated in Sections 3.5 and 3.6.

Over 90% removals of all five steroid hormones, three industrial compounds and three UV filters were observed in this study (Figure 2). It is noteworthy that these compounds possess significant hydrophobicity ($\log D > 3$), which may explain the similarities of their aqueous phase removal efficiencies (Joss et al., 2004; Suarez et al., 2012; Wijekoon et al., 2013; Xue et al., 2010). On the other hand, despite low hydrophobicity ($\log D < 3$), significant removal of the phytoestrogens was achieved, possibly due to the presence of $-OH$ (Supplementary Data Table S1), which is a strong electron donating functional group (EDG), in their structure. The presence of EDG increases the biodegradability of TrOCs (Hai et al., 2011b; Tadkaew et al., 2011).

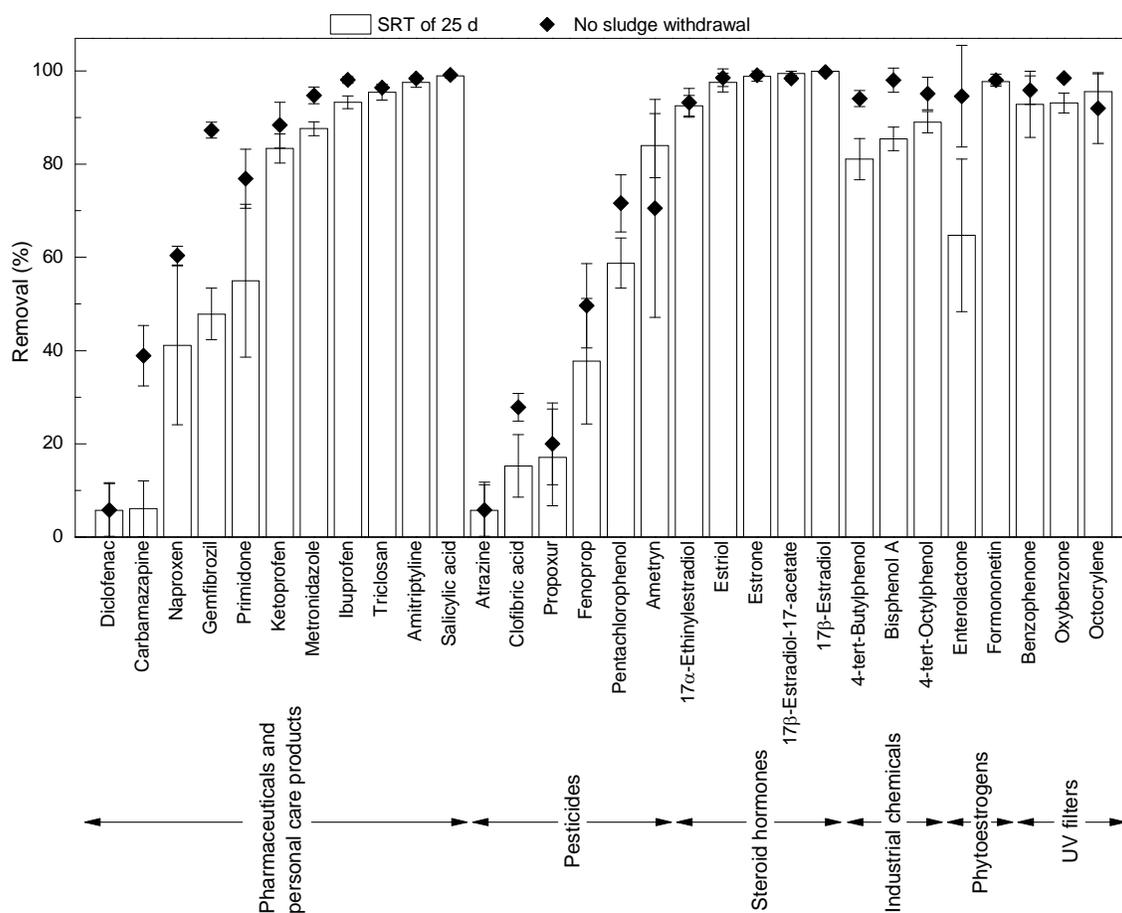


Figure 2: TrOC removal by the MBR with no sludge withdrawal and at an SRT of 25 d. Error bars represent the standard deviation of duplicate samples taken once a week over the operation period.

All pharmaceuticals and personal care products (except triclosan and amitriptyline), and all pesticides (except pentachlorophenol) investigated in this study were hydrophilic, and, therefore, no generalizations can be inferred for their aqueous phase removal based on hydrophobicity i.e, log D (Figure 2). Given the considerable dissimilarity in the molecular structure among these TrOCs (Supplementary Data Table S1), differences in their removal efficiencies can be expected. Among the pesticides, atrazine, clofibric acid, fenoprop and pentachlorophenol contain one or more –Cl group which is a strong electron withdrawing group (EWG). Of these four pesticides, pentachlorophenol was well removed, possibly because it contains –OH, which is a strong EDG, in addition to being a hydrophobic compound (Methatham et al., 2011). Atrazine and ametryn are both triazine compounds, but only ametryn was well removed (Figure 2), possibly because of the presence of –Cl (strong EWG) in atrazine but not in ametryn.

Of the 11 pharmaceuticals and personal care products selected in this study, five, namely, diclofenac, carbamazepine, naproxen, gemfibrozil and primidone showed significantly lower removal efficiencies (negligible to 60%), particularly at an SRT of 25 d (Figure 2). These compounds are hydrophilic and their molecules possess strong EWGs such as –CONH₂ and -Cl or are devoid of any strong EDGs (Supplementary data Table S1). Thus, the low removal efficiency could be attributed to a combined impact of low hydrophobicity and resistance to biodegradation (Tadkaew et al., 2011; Wijekoon et al., 2013).

Assessing the impact of SRT was beyond the scope of this study and the TrOC removal during the operation without sludge withdrawal (Day 126-170) was intended to serve as a reference. Nevertheless, it is worth noting that TrOC removal trend during this period was generally similar to that during the 25 d SRT operation (Day 226- 265), and, furthermore, the removal was significantly better for two compounds, namely, carbamazepine and gemfibrozil (Figure 2). In a lab-scale anaerobic/anoxic/aerobic-activated sludge treatment study by Zeng et al. (2013), no significant effect of SRT on the removal of natural estrogens over a range of 10-25d was observed, but the removal of synthetic estrogen increased with SRT. Recently, Maeng et al. (2013) achieved effective removal of seven pharmaceutical and personal care products and two natural estrogens (17 β -estradiol and estrone) at an SRT of 8 d, while the removal efficiency of gemfibrozil, ketoprofen, clofibric acid and 17 α -ethinylestradiol increased when the SRT was

increased from 20 to 80 d. The removal of resistant compounds may improve at comparatively longer SRTs, although controversies regarding this observation exist in the literature (Hai et al., 2014; Luo et al., 2014) . Nevertheless, long term operation of an MBR under an extremely long SRT is associated with operational problems including inefficient mixing and increased aeration demand for the biological metabolism and membrane cleaning. Accordingly, further discussion on TrOC removal focuses on the operation at an SRT of 25 d, which is more relevant to present day MBRs.

3.3 TrOC removal by the anoxic bioreactor

As discussed in Section 3.1, not only the inclusion of an anoxic bioreactor (low DO and ORP environment) but also the application of an appropriate IR ratio (=3) between the anoxic and the aerobic bioreactors was essential to achieve a significant level of denitrification (Figure 1). Notably, because of the significant exchange of the mixed liquor between the bioreactors at an IR ratio of 3, the TrOC concentrations in the supernatant of these two reactors were generally similar (Figure 3). Therefore, with IR between the reactors, the impact of different redox conditions (anoxic or aerobic) *vs.* the impact of exchange of sludge in between the bioreactors could not be demonstrated. Accordingly, TrOC removal in the absence of IR (Day 266-305) was additionally observed.

3.3.1 Role of low DO and ORP regimes

Before discussing the impact of additional factors other than redox conditions, it is worth noting that, under both anoxic (IR ratio =3) and anaerobic (no IR) conditions, moderate to high removal (over 50% and up to 90%) was consistently achieved for the following TrOCs: four pharmaceutical and personal care products (primidone, metronidazole, triclosan, and amitriptyline), one steroid hormone (17 β -estradiol-17-acetate), one industrial chemical (4-tert-octylphenol) and all selected UV filters (benzophenone, oxybenzone, and octocrylene) (Figure 3). This observation implies that these TrOCs are removed under low DO and ORP conditions.

The observation made here regarding benzophenone, octocrylene and 4-tert-octylphenol removal is consistent with several previous studies. Liu et al. (2013) reported the degradation of six UV filters including benzophenone and octocrylene under aerobic and anaerobic conditions (nitrate,

sulphate or iron as the electron acceptor). Similarly, Liu et al. (2008) reported anaerobic degradation of 4-tert-octylphenol by granular sludge. However, the current study shows for the first time the removal of 17 β -estradiol-17-acetate and the pharmaceuticals and personal care products such as primidone, metronidazole, triclosan and amitriptyline under low DO and ORP conditions. As triclosan, amitriptyline and 17 β -estradiol-17-acetate are hydrophobic compounds ($\log D > 3.2$, Supplementary Data Table S1), they can be removed by sorption and/or biodegradation (See Section 3.5). On the other hand, primidone and metronidazole are hydrophilic, but they were removed under low DO and ORP conditions and then eliminated well overall. No prior work on the assessment of anaerobic biodegradation of primidone could be found for comparison; one possible explanation is that the reducing condition may induce the ring cleavage of primidone (such as an attack of nucleophilic form of hydride at 2-position) to form phenylethylmalonamide. Conversely the data presented here differs from the previous reports on negligible anaerobic/anoxic removal of metronidazole (Ingerslev et al., 2001; Kümmerer et al., 2000). The reason for this discrepancy could not be resolved but it is possible that microbial community composition is an important factor, which can be influenced by other operating parameters in addition to the redox conditions. Dorival-García et al. (2013) reported that the removal of the selected antibiotics under different redox conditions (i.e., aerobic, nitrifying and anoxic conditions) depended significantly on the bacterial composition of the sludge. Assessment of the microbial community is an important research gap; however, this is beyond the scope of this study.

3.3.2 Impact of IR

The similar removal efficiencies under both anoxic (IR ratio =3) and anaerobic (no IR) conditions for the aforementioned nine compounds indicate the suitability of low DO and ORP regimes for their removal. However, IR appeared to exert a significant impact on the anoxic (anaerobic) removal efficiency (Figure 3) and sorption onto sludge (Section 3.5) of the rest of the compounds. Particularly, 11 TrOCs including three pharmaceuticals and personal care products (ketoprofen, ibuprofen, and salicylic acid), all steroid hormones except 17 β -estradiol-17-acetate, one pesticide (ametryn), two industrial chemicals (4-tert-butylphenol and bisphenol A), and one phytoestrogen (formononetin) showed moderate to very high removal under the anoxic regime (IR=3), whereas these compounds had no or very low removal under the anaerobic regime (no

IR). The discrepancy between removal in absence and presence of IR in this study suggests that the TrOC removal by an anoxic bioreactor is governed not only by the specific redox conditions (i.e., low DO or ORP) but also by other conditions arising from sludge exchange with the aerobic bioreactor.

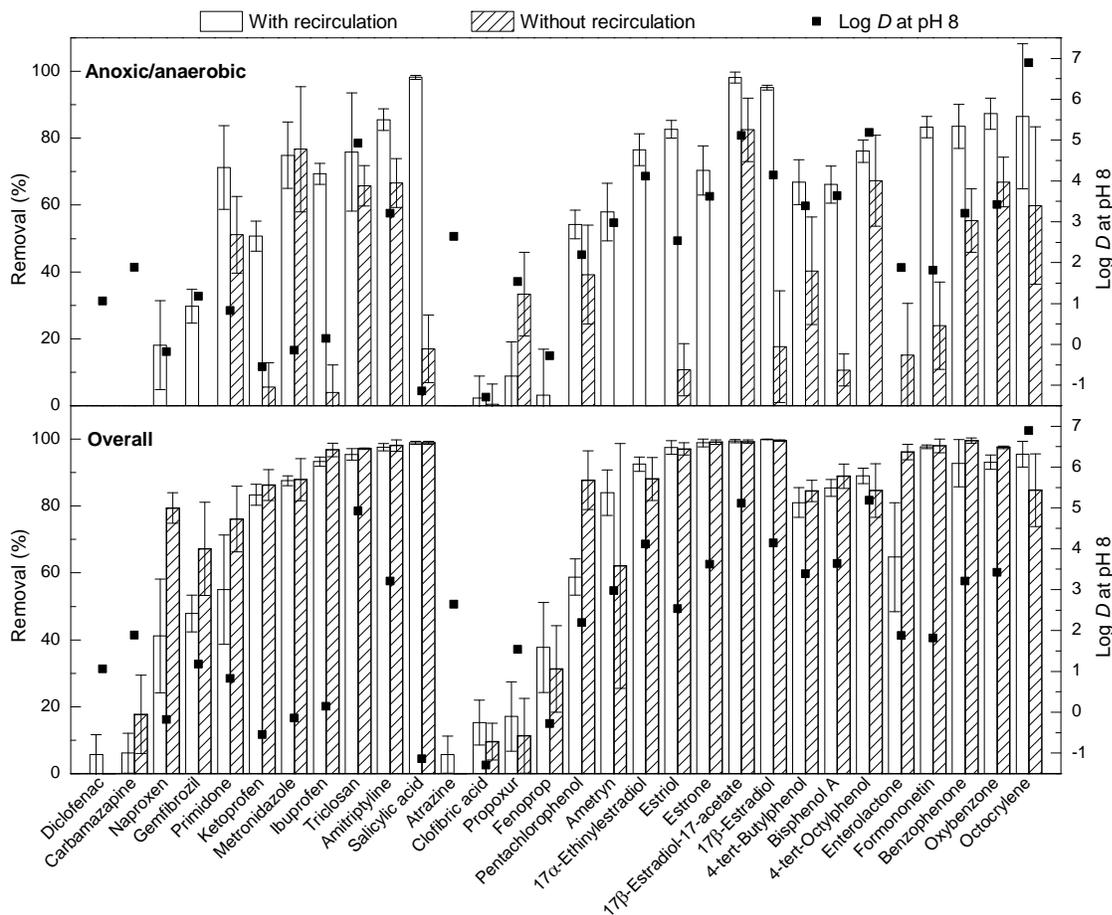


Figure 3: TrOC removal by the anoxic reactor as compared to the overall removal (SRT of 25 d; with and without IR). Error bars represent the standard deviation of duplicate samples taken once a week for six weeks.

IR from the aerobic to anoxic bioreactor may lead to the following: (i) dilution of the media, (ii) improved mixing/ mass transfer, (iii) supply of nitrate, and (iv) transfer of a portion of DO from the aerobic tank, potentially facilitating some extent of aerobic degradation even within the anoxic reactor (Andersen et al., 2003; Suarez et al., 2010; Suarez et al., 2012; Xue et al., 2010). Another possible factor is the impact on development of bacterial community. A shared

bacterial community that is highly functionalized for contaminant removal may flourish due to sludge exchange between two redox conditions during long-term operation of an MBR. While all these factors may be relevant, there is particularly strong evidence regarding the role of the presence of nitrate in anaerobic TrOC degradation. For example, Zeng et al. (2009) reported two distinct modes of anaerobic 17 α -ethinylestradiol removal depending on the presence or absence of nitrate: in the presence of nitrate, biodegradation was the dominant process, while in the absence of nitrate, the removal was simply a result of sorption onto activated sludge. Similarly, Xue et al. (2010) reported that an anaerobic reactor (in absence of nitrate) may achieve significant TrOC removal, but mostly due to enhanced biosorption. Therefore, low ORP corresponding to anoxic/anaerobic regimes may enhance the degradation of certain TrOCs, but the application of IR between the bioreactors (facilitating phenomenon such as presence of nitrate) is an important prerequisite to that. Further discussion in this line is furnished in Section 3.5 in relation to biosorption.

3.4 Importance of the aerobic bioreactor

Despite the significantly different removal of certain TrOCs in the preceding bioreactor (depending on the IR), the permeate quality of the subsequent aerobic MBR did not vary significantly (Figure 3), indicating an important role of the aerobic bioreactor for TrOC removal. The crucial role of aerobic conditions in promoting the overall TrOC degradation has been consistently reported in the literature (Andersen et al., 2003; Li et al., 2011; Suarez et al., 2012; Xue et al., 2010). However, to date this aspect has been studied in relation to only a few compounds. For example, Dytczak et al. (2008) reported similar removal of natural (estrone and 17 β -estradiol) and synthetic (17 α -ethinylestradiol) estrogens under aerobic and alternating anoxic/aerobic conditions. Joss et al. (2004) investigated 17 α -ethinylestradiol degradation kinetics under different redox conditions, and reported that it was removed at a significant rate only under aerobic conditions. A similar observation regarding 17 α -ethinylestradiol degradation was made by Andersen et al. (2003) in combined anoxic/aerobic treatment plants. McAvoy et al. (2002) and Chen et al. (2011) observed better biodegradation of triclosan under aerobic than anoxic or anaerobic conditions. Recently, Dorival-Carcia et al. (2013) reported a much higher biodegradation of six quinolones under nitrifying than anoxic conditions. The originality of this

study is that the data presented here confirms the importance of aerobic biodegradation under same operating conditions with a broader set of TrOCs than the above examples.

3.5 TrOC sorption on sludge

In addition to biodegradation, TrOCs can be removed from the aqueous phase by mechanisms such as biosorption, volatilization and photolysis. In this study, photolysis was prevented by covering the bioreactors (Section 2.2). Given the vapor pressure or Henry's law constant of the TrOCs investigated (Supplementary Data Table S1), volatilization could also be considered negligible. However, biosorption was monitored to clarify the impact of different operational regimes on the removal of the TrOCs, particularly the impact of IR which was observed to significantly influence the aqueous phase removal by the anoxic (anaerobic) reactor (Section 3.3).

Two important observations regarding sludge adsorption were made in this study (Figure 4): (i) TrOC adsorption on sludge within the anoxic and aerobic reactors was similar due to the significant mixing of the mixed liquor at an IR ratio of 3, however, mostly higher sorption on anaerobic sludge than aerobic sludge was observed in absence of IR, and (ii) For certain TrOCs sorption on sludge in the anaerobic reactor was much higher in the absence of IR than with IR.

The higher sorption within the anaerobic tank is evident by the accumulation of some TrOCs (e.g., amitriptyline, benzophenone, triclosan, 4-tert-octylphenol and octocrylene) in the sludge phase and their high removal from the aqueous phase by the anaerobic reactor (no IR). It is hypothesized that the anaerobic/anoxic conditions can facilitate their sorption to sludge (Li et al., 2011; Suarez et al., 2010; Zeng et al., 2009), however, these TrOCs are degraded only if an electron acceptor such as nitrate (with IR) is available. The sludge adsorption data reaffirms the point noted in Section 3.3.2 that IR between the anoxic and aerobic bioreactors is an important prerequisite to anoxic biodegradation.

In this study, higher concentration of hydrophobic compounds such as amitriptyline, benzophenone, triclosan, 4-tert-octylphenol and octocrylene in sludge under anaerobic conditions demonstrated high sorption capacity of anaerobic sludge. Two other hydrophobic compounds,

namely, oxybenzone and 17 β -estradiol-17-acetate were removed efficiently without significant accumulation in sludge. This can be explained by the presence of EDGs (e.g., -OH and -CH₃) in their structure. Probably, these TrOCs are quickly absorbed to the sludge and subsequently biodegraded/biotransformed under the anaerobic/anoxic regimes.

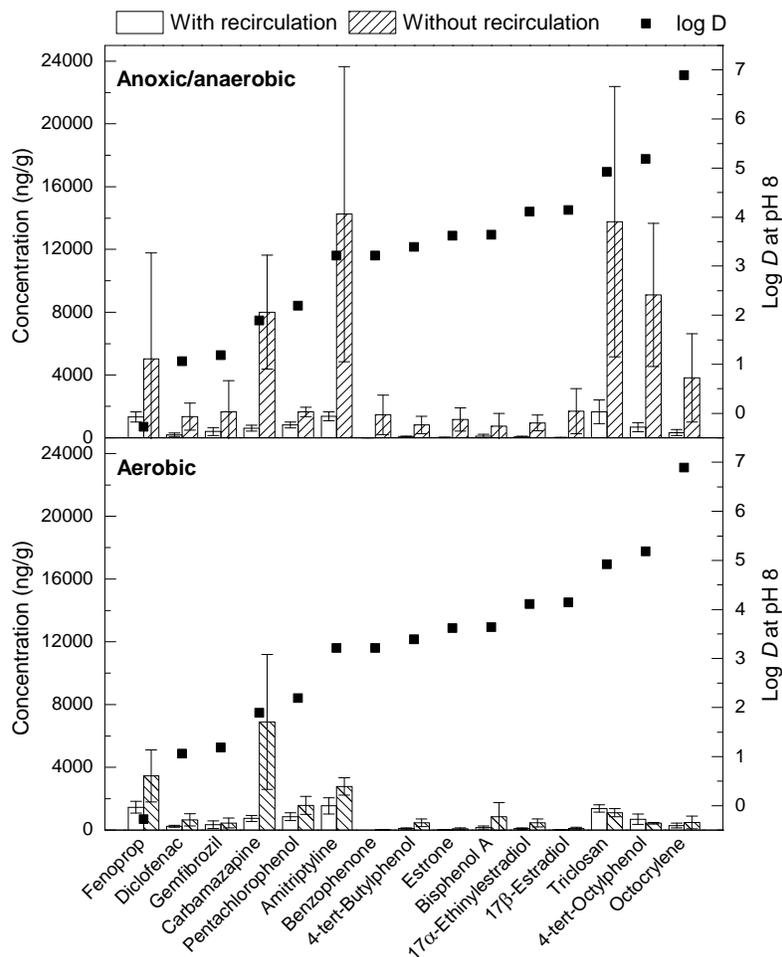


Figure 4: Concentration of TrOCs showing significant adsorption on sludge in anoxic and aerobic reactors of the MBR system (SRT of 25 d; with and without IR). Error bars represent the standard deviation of samples taken once a week for six weeks. Large standard deviation in case of some TrOCs is due to their progressive accumulation in sludge.

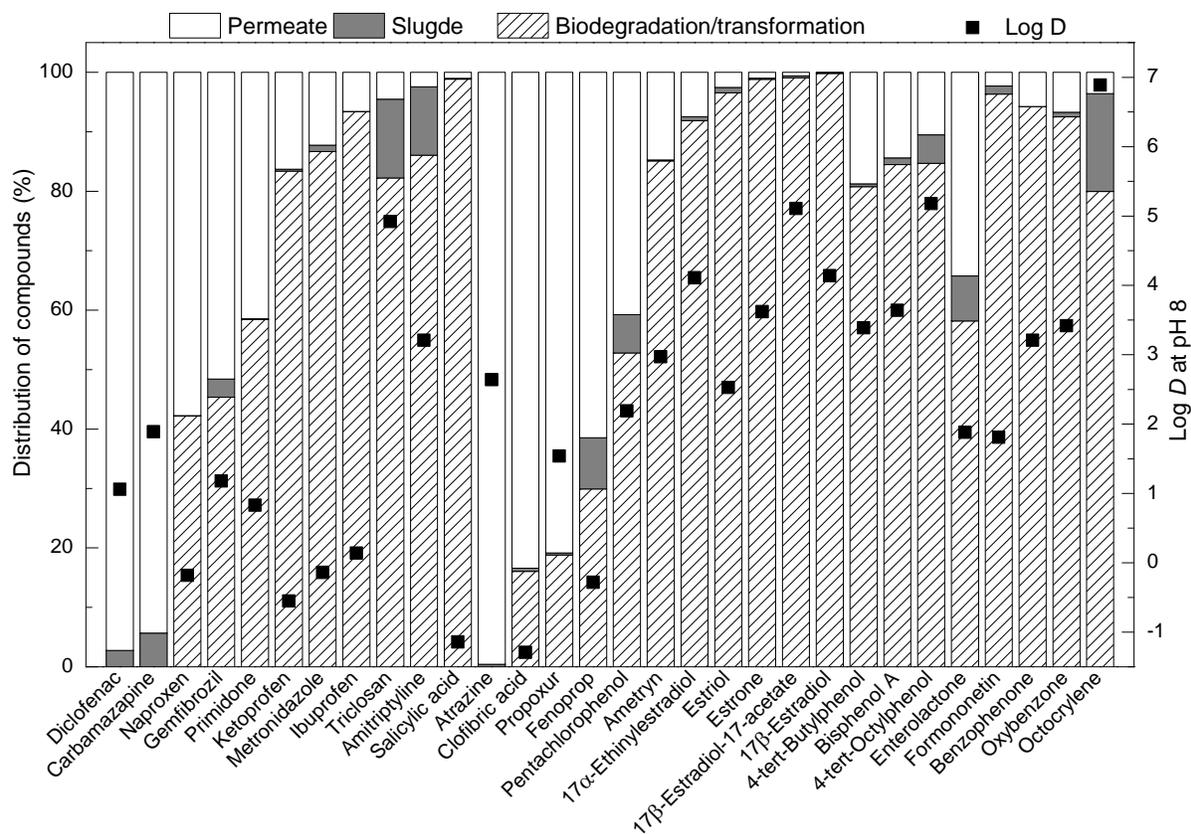


Figure 5: Fate of the TrOCs during MBR treatment (SRT of 25 d; with IR).

3.6 Overall fate of the TrOCs

In this section, insights into the fate of the TrOCs during MBR treatment is provided focusing on the period of steady state operation at an SRT of 25 d and an IR ratio of 3 (Day 226 to 265, Supplementary Data Table S3). A mass balance based on the total amount of TrOCs in the feed, permeate and sludge during that period was conducted (Figure 5). TrOC removal from wastewater by bioreactors is the result of a dynamic equilibrium between biosorption and biodegradation, which occur simultaneously. Apart from the poorly removed compounds, stable concentrations of most TrOCs were observed in both liquid and solid phases during the steady state operation at an SRT of 25 d and an IR of 3 (Figure 3 and 4). For the well removed compounds, in line with contemporary reports (Abegglen et al., 2009; Wijekoon et al., 2013), mass balance (Figure 5) confirms biodegradation/transformation as the predominant removal mechanism for most TrOCs during MBR treatment.

Among the compounds showing significant sorption (Figures 4 and 5), octocrylene, amitriptyline, triclosan and 4-tert-octylphenol are hydrophobic compounds, which can explain their high distribution in the solid phase. A low distribution of other hydrophobic compounds in sludge can be attributed to their high biodegradability. The significant distribution in sludge of certain hydrophilic compounds, namely, carbamazepine and fenprop can be attributed to their recalcitrant structure (Wijekoon et al., 2013). Results presented here highlight the combined influence of intrinsic properties of TrOCs (Section 3.2) and operational parameters such as redox conditions and IR (Section 3.3).

4. Conclusion

Long-term operation of an integrated anoxic-aerobic MBR revealed that low DO or ORP (i.e., anoxic/anaerobic) regimes are conducive to biodegradation of some TrOCs. However, an important prerequisite to anoxic biodegradation of TrOCs is internal recirculation (IR) between the anoxic and aerobic bioreactors, in absence of which anoxic/anaerobic regimes alone may only enhance biosorption. Dependence of TN removal on IR that controls the supply of nitrate to the anoxic reactor was also evident. Despite the significantly different removal of certain TrOC by the preceding anoxic bioreactor (depending on the IR), TrOC concentration in effluent from the aerobic MBR was stable.

Acknowledgment

A PhD scholarship to Hop V. Phan from the University of Wollongong, Australia and the Thanh Hoa provincial government (Viet Nam) is greatly appreciated. Zenon Environmental Inc., Canada is thanked for the provision of the membrane module. A Research Partnership Grant from Shoalhaven Water (NSW) is gratefully acknowledged.

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Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal by an anoxic—aerobic membrane bioreactor (MBR)

Hop V. Phan^{a,b}, Faisal I. Hai^{a*}, Jinguo Kang^{a,c}, Hoa K. Dam^d, Ren Zhang^b, William E. Price^c, Andreas Broeckmann^e, Long D. Nghiem^a

^aStrategic Water Infrastructure Lab, School of Civil, Mining and Environmental Engineering, University of Wollongong, Australia.

^bMolecular Biology Lab, School of Biological Sciences, University of Wollongong, Australia.

^cStrategic Water Infrastructure Lab, School of Chemistry, University of Wollongong, Australia.

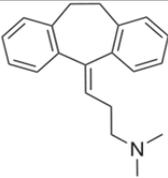
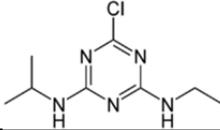
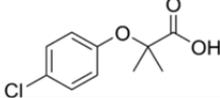
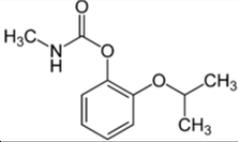
^dDecision Systems Lab, School of Computer Science and Software Engineering, University of Wollongong, Australia.

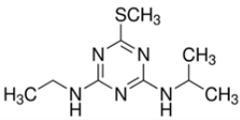
^e GHD Australia.

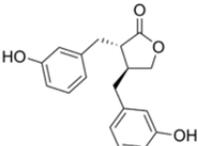
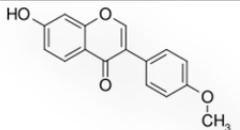
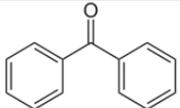
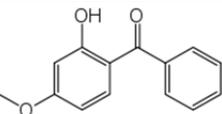
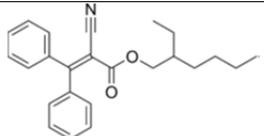
*Corresponding author: Faisal I. Hai (E-mail: faisal@uow.edu.au; Phone: +61-2-4221-3054)

Table S1: Physicochemical properties of the selected trace organic contaminants (TrOC).

Category	Chemical formula	Molecular weight (g/mol)	logD (pH 8) ^a	Henry's Law constant at 25°C (atm m ³ /mol) ^a	Molecular structure
Pharmaceuticals and personal care products	Diclofenac (C ₁₄ H ₁₁ Cl ₂ NO ₂)	296.15	1.06	2.69 × 10 ⁻¹¹	
	Carbamazepine (C ₁₅ H ₁₂ N ₂ O)	236.27	1.89	9.41 × 10 ⁻¹²	
	Naproxen (C ₁₄ H ₁₄ O ₃)	230.30	-0.18	6.08 × 10 ⁻¹²	
	Gemfibrozil (C ₁₅ H ₂₂ O ₃)	250.30	1.18	1.83 × 10 ⁻¹¹	
	Primidone (C ₁₂ H ₁₄ N ₂ O ₂)	218.25	0.83	1.16 × 10 ⁻¹⁴	
	Ketoprofen (C ₁₆ H ₁₄ O ₃)	254.30	-0.55	1.92 × 10 ⁻¹³	
	Metronidazole (C ₆ H ₉ N ₃ O ₃)	171.15	-0.14	2.07 × 10 ⁻¹²	
	Ibuprofen (C ₁₃ H ₁₈ O ₂)	206.30	0.14	5.54 × 10 ⁻¹⁰	

	Triclosan (C ₁₂ H ₇ Cl ₃ O ₂)	287.50	4.92	9.49 × 10 ⁻⁶	
	Amitriptyline (C ₂₀ H ₂₃ N)	277.40	3.21	1.24 × 10 ⁻¹⁰	
	Salicylic acid (C ₇ H ₆ O ₃)	138.12	-1.14	1.42 × 10 ⁻⁸	
Pesticides	Atrazine (C ₈ H ₁₄ ClN ₅)	215.68	2.64	5.22 × 10 ⁻⁸	
	Clofibric acid (C ₁₀ H ₁₁ ClO ₃)	214.64	-1.29	2.91 × 10 ⁻¹⁰	
	Propoxur (C ₁₁ H ₁₅ NO ₃)	209.24	1.54	5.26 × 10 ⁻⁷	
	Fenoprop (C ₉ H ₇ Cl ₃ O ₃)	269.51	-0.28	4.72 × 10 ⁻¹²	
	Pentachlorophenol (C ₆ HCl ₅ O)	266.38	2.19	1.82 × 10 ⁻⁷	

	Ametryn (C ₉ H ₁₇ N ₅ S)	227.33	2.97	3.67 × 10 ⁻⁹	
Steroid hormones	17 α -Ethinylestradiol (EE2) (C ₂₀ H ₂₄ O ₂)	296.48	4.11	3.74 × 10 ⁻¹⁰	
	Estriol (E3) (C ₁₈ H ₂₄ O ₃)	288.40	2.53	1.75 × 10 ⁻¹¹	
	Estrone (E1) (C ₁₈ H ₂₂ O ₂)	270.36	3.62	9.61 × 10 ⁻¹⁰	
	17 β -Estradiol-17-acetate (E2Ac) (C ₂₀ H ₂₆ O ₃)	314.42	5.11	2.15 × 10 ⁻⁹	
	17 β -Estradiol (E2) (C ₁₈ H ₂₄ O ₂)	272.38	4.14	1.17 × 10 ⁻⁹	
Industrial chemicals	4-tert-Butylphenol ((CH ₃) ₃ CC ₆ H ₄ OH)	150.22	3.39	7.51 × 10 ⁻⁶	

	Bisphenol A (C ₁₅ H ₁₆ O ₂)	228.29	3.64	9.16 × 10 ⁻¹²	
	4-tert-Octylphenol (C ₁₄ H ₂₂ O)	206.33	5.18	8.67 × 10 ⁻⁶	
Physoestrogens	Enterolactone (C ₁₈ H ₁₈ O ₄)	298.33	1.88	8.07 × 10 ⁻¹³	
	Formononetin (C ₁₆ H ₁₂ O ₄)	268.26	1.81	2.91 × 10 ⁻¹⁰	
UV filters	Benzophenone (C ₁₃ H ₁₀ O)	182.22	3.21	1.31 × 10 ⁻⁶	
	Oxybenzone (C ₁₄ H ₁₂ O ₃)	228.24	3.42	1.22 × 10 ⁻⁸	
	Octocrylene (C ₂₄ H ₂₇ N)	361.48	6.89	3.38 × 10 ⁻⁹	

^a Source: SciFinder database <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>

Log *D* is logarithm of the distribution coefficient which is the ratio of the sum of concentrations of all forms of the compound (ionised and unionised) in octanol and water at a given pH.

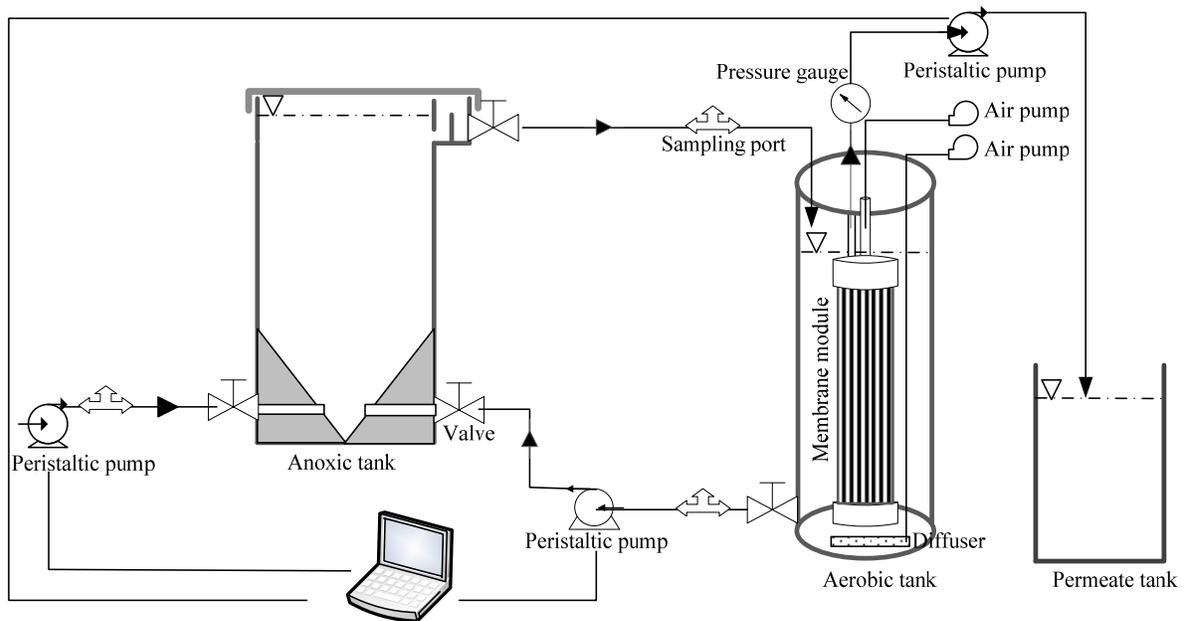


Figure S2: A schematic diagram of the laboratory scale anoxic-aerobic MBR

Table S3: Schedule of continuous operation of the anoxic—aerobic MBR

Day	SRT	Internal recirculation (IR) ratio	TrOC added	Operation mode
0-90	Infinite ^a	0.5	No	MBR start- up period (without trace organics in feed)
91-125	Infinite ^a	3	No	
126-170	Infinite ^a	3	Yes	Operation with TrOCs in feed
171-180	Infinite ^a	3	No	MBR run without TrOCs in feed
181-225	25 d	3	No	Stabilization period for SRT of 25 days
226-265	25 d	3	Yes	Operation with TrOCs in feed.
266-305	25 d	0	Yes	Operation with TrOCs in feed.

^aNo sludge withdrawal except sampling, resulting in a theoretical SRT of >1000 d.

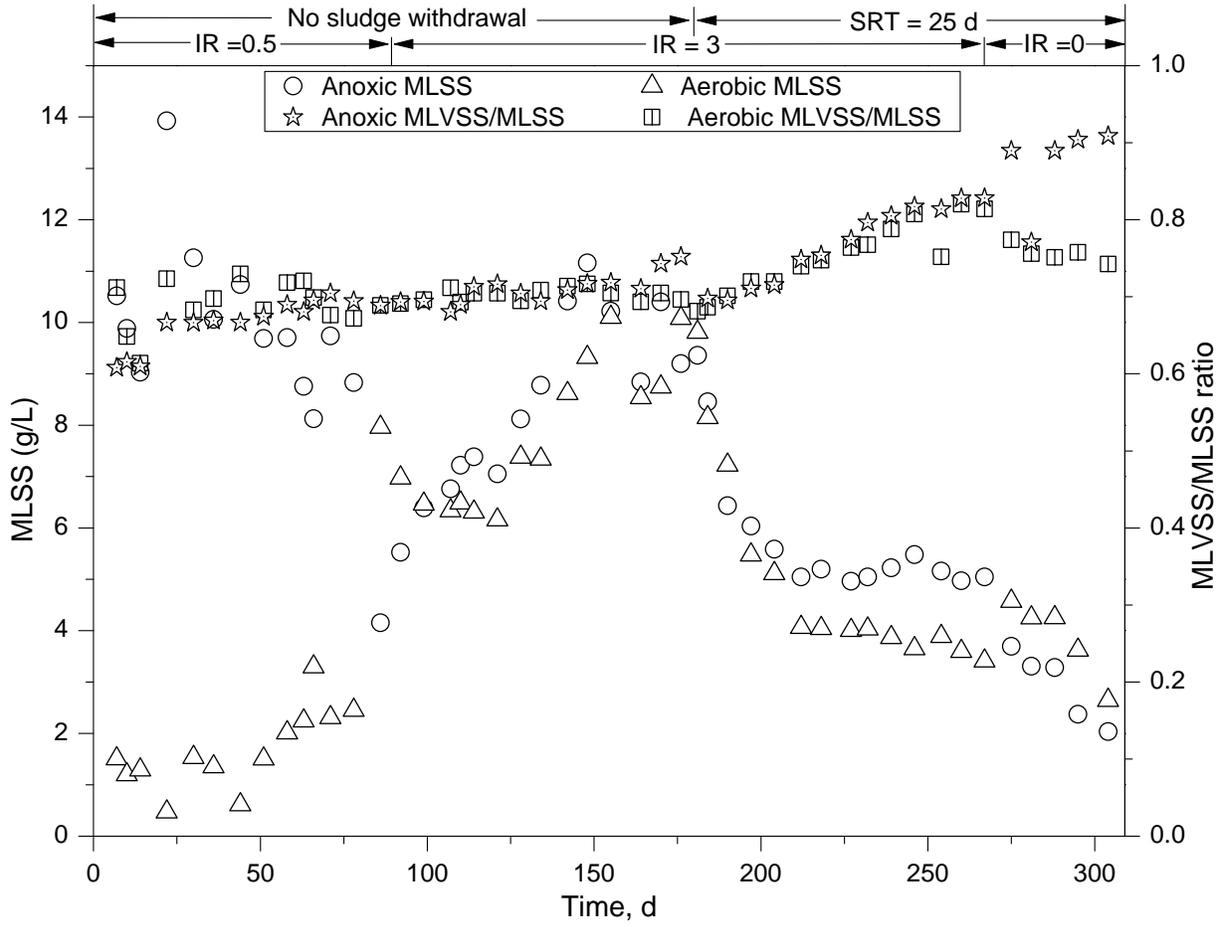


Figure S4: MLSS and MLVSS profiles in anoxic and aerobic reactors over the entire operation period.

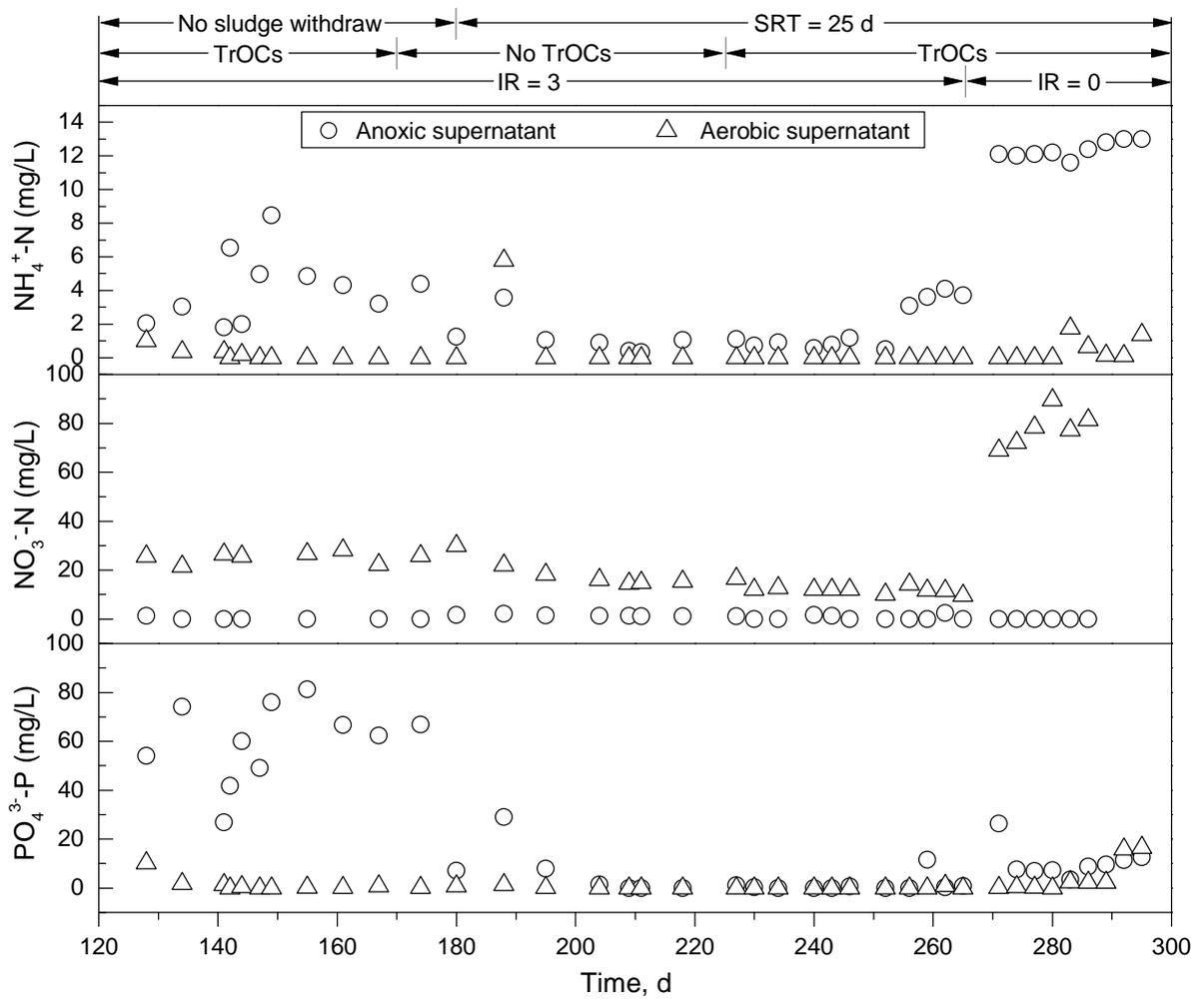


Figure S5: $\text{NH}_4^+ - \text{N}$ / $\text{NO}_3^- - \text{N}$ / $\text{PO}_4^{3-} - \text{P}$ concentrations in the supernatant of the anoxic and aerobic reactors. Data has been plotted from Day 120 (start of stable TN removal).