Fate of trace organic contaminants in oxic-settling-anoxic (OSA) process applied for biosolids reduction during wastewater treatment

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Galilee U. Semblante [a], Faisal I. Hai [a*], James McDonald [b], Stuart J. Khan [b], Mark Nelson [c], Duu-Jong Lee [d], William E. Price [e], and Long D. Nghiem [a]

[a] Strategic Water Infrastructure Laboratory, School of Civil, Mining and Environmental Engineering, University of Wollongong, Wollongong, NSW 2522, Australia

[b] Water Research Centre, The University of New South Wales, Sydney NSW 2052, Australia

[c] School of Mathematics and Applied Statistics, University of Wollongong, Wollongong, NSW 2522, Australia

[d] Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan

[e] Strategic Water Infrastructure Laboratory, School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia

* Corresponding author:

Email: faisal@uow.edu.au, Ph: +61 2 4221 3054

Abstract

This study investigated the fate of trace organic contaminants (TrOCs) in an oxic-settling-anoxic (OSA) process consisting of a sequencing batch reactor (SBR) with external aerobic/anoxic and anoxic reactors. OSA did not negatively affect TrOC removal of the SBR. Generally, low TrOC removal was observed under anoxic and low substrate conditions, implicating the role of co-metabolism in TrOC biodegradation. Several TrOCs that were recalcitrant in the SBR (*e.g.*, benzotriazole) were biodegraded in the external aerobic/anoxic reactor. Some hydrophobic TrOCs (*e.g.,* triclosan) were desorbed in the anoxic reactor possibly due to loss of sorption sites through volatile solids destruction. In OSA, the sludge was discharged from the aerobic/anoxic reactor which

contained lower concentration of TrOCs (*e.g.*, triclosan and triclocarban) than that of the control aerobic digester, suggesting that OSA can also help to reduce TrOC concentration in residual biosolids.

Keywords

biosolids yield reduction; biodegradation; municipal wastewater; oxic-settling-anoxic process; sorption; trace organic contaminants

1. Introduction

Wastewater treatment plants (WWTPs) commonly use the conventional activated sludge (CAS) process to treat municipal and industrial wastewater. CAS involves the conversion of soluble organic matter in wastewater into settleable biomass called "sludge" in an aeration tank. The removal of organic matter is affected by the food-to-microorganism ratio, which is maintained by wasting excess sludge (Tchobanoglus et al., 2003). Previously, sludge was dumped into the ocean but this approach has been banned due to adverse impact on marine life (Foladori et al., 2010). Currently, sludge is treated to reduce volatile solids and water content and then landfilled or incinerated (Semblante et al., 2014; Tchobanoglus et al., 2003). These approaches have high energy requirements and significantly increase the overall cost of sludge management (Semblante et al., 2014). Sludge is also converted into nutrient-rich "biosolids" applied on agricultural land – an approach that facilitates the recovery of carbon, nitrogen, and phosphorous (Foladori et al., 2010; Semblante et al., 2014). However, reducing the volatile solids and pathogen concentration of biosolids to levels that meet regulatory standards can be a technically challenging and expensive exercise involving multiple processes (*e.g.*, anaerobic digestion, composting, heat treatment, and others) (Lue-Hing et al., 1992; Tchobanoglus et al., 2003). Moreover, biosolids may accumulate heavy metals that can be transferred to soil and then taken up by plants or propagated to groundwater (Bai et al., 2012; Silveira et al., 2003). Other factors, such as soil chemistry, odourous emissions, and cost of transportation can also restrict the applicability of biosolids (Lu et al., 2012; Semblante et al., 2015b;

Xie et al., 2016). Clearly, to alleviate the cost of sludge treatment and disposal, sludge production in the aeration tank must be reduced. Various strategies have been proposed such as the (a) manipulation of operating conditions such as dissolved oxygen (DO) concentration and sludge retention time (SRT) (Wei et al., 2003), (b) addition of chemicals to inhibit microbial propagation (Foladori et al., 2010; Wei et al., 2003), (c) microbial predation (Silveira et al., 2003), (d) destruction of sludge by advanced oxidation processes (Foladori et al., 2010; Wei et al., 2003). Thus far, certain strategies have only resulted in marginal biosolids reduction (*e.g.*, 25% reduction when DO is manipulated) (Wei et al., 2003). Others have high process efficiency (*e.g.*, 100% reduction when sludge is destroyed by ozonation) but require high capital investment and operation cost (Foladori et al., 2010).

The oxic-settling-anoxic (OSA) process is an emerging approach to decrease sludge production. OSA involves the insertion of one or more external anoxic reactors in the return activated sludge loop of CAS. Compared to other sludge reduction schemes, OSA has low capital investment and energy requirement. OSA cycles sludge between conditions that are rich (aeration tank) and deficient (external anoxic reactor/s) in oxygen and substrate (Semblante et al., 2014). Consequently, volatile solids are destroyed and converted into inert forms in the external anoxic reactor/s. Using real sewage as feed, recent studies have shown that OSA can reduce the sludge yield (mass of sludge produced/mass of substrate consumed) of sequencing batch reactors (SBRs) by more than 35% depending on factors such as influent characteristics, sludge interchange rate, and SRT (Semblante et al., 2015a; Semblante et al., 2016). Additionally, they have shown that OSA does not have any negative impact on wastewater treatment efficiency regarding basic water parameters such as chemical oxygen demand and sludge properties such as settleability (Coma et al., 2013; Semblante et al., 2015a).

Trace organic contaminants (TrOCs) are pharmaceuticals, pesticides, personal care products, hormones, and other compounds that are commonly found in trace concentrations in the environment (Luo et al., 2014). At a sufficient level of exposure, certain TrOCs can damage the endocrine system, which govern the physiological development and reproduction of animals and humans (Clarke & Cummins, 2015). Their behavior during

wastewater treatment is dependent on chemical properties (Hai et al., 2011b). For example, non-biodegradable and hydrophilic compounds are unaffected by wastewater treatment and thus persists in the effluent in their original form. Meanwhile, non-biodegradable and hydrophobic TrOCs bind to the surface of sludge flocs and accumulate in biosolids (Semblante et al., 2015a). The occurrence of TrOCs either in the effluent or biosolids could result in the propagation of these contaminants to receiving soil and water bodies (Clarke & Cummins, 2015). Because of this, research efforts have been devoted to determine the fate of TrOCs in full-scale wastewater treatment systems (Janssen et al., 2015; Phan et al., 2015; Trinh et al., 2016). TrOC sorption and biodegradation are affected by operational conditions, such as redox regimes (*e.g.*, aerobic, anoxic, or anaerobic), SRT, and others (Semblante et al., 2015a). However, the fate of TrOC in OSA has not been investigated.

This study aims to determine the sorption and biodegradation of TrOCs in OSA operated using real wastewater. The TrOC concentrations in the effluent and sludge of an OSA system were compared to that of a control system to gain insight on the effects of sludge interchange between different redox regimes on the fate of TrOCs. Furthermore, the fate of TrOCs were determined at different external reactor SRT (SRT_{ext}), i.e. aerobic/anoxic and anoxic reactors and control aerobic digester. The findings of this study are relevant to the assessment of the TrOC discharge from OSA and in the future development of TrOC mitigation or treatment approaches.

2. Materials and methods

2.1. Municipal wastewater

Municipal wastewater was obtained from the beginning of primary sedimentation tank of Wollongong WWTP fortnightly and stored at 4 ºC prior to use to minimise chemical reactions and microbial activity. The basic properties of the municipal wastewater are listed in Supplementary Table S1.

2.2. Reactor configuration and operation

The reactors used in this study were previously described (Semblante et al., 2016). Briefly, the OSA system consisted of a sequencing batch reactor, SBR_{OSA} (5 L), attached to external aerobic/anoxic (2 L) and anoxic reactors (2 L) (Figure 1a). Meanwhile, the control system consisted of $SBR_{control}$ (5 L) attached to a single-pass aerobic digester (2 L) (Figure 1b).

[Figure 1]

SBR_{OSA} and $SBR_{control}$ were fed with municipal wastewater (Section 2.1). They were operated at 4 cycles/day and HRT of 12 hours. Each cycle comprised of 15 min of filling, 4 hours and 30 min of aeration, 1 hour of settling, and 15 min of decanting. The temperature of both SBRs was maintained at 25 ºC using a water bath. The SRT of both SBRs (SRT_{SBR}) was maintained at 10 d by regular sludge wastage (W) (Figure 1) throughout the experimental period (Table 1).

[Table 1]

The aerobic/anoxic reactor of the OSA system was intermittently aerated (*i.e.*, aeration was turned on for 8 h and then turned off for 16 h) using an air diffuser placed at the bottom of the reactor (Figure 1a). The DO concentration of the reactor (measured as described in Section 2.3.2) when aeration was turned on and off was 4.6±1.0 mg/L (n=62) and 0.4±0.2 mg/L (n=62), respectively. The anoxic reactor was kept airtight using a silicone-lined cap with inlet and outlet ports. The temperature of both external reactors was maintained at 25 ºC using a water bath.

The aerobic/anoxic reactor was fed with sludge from SBR_{OSA} thickened by centrifugation (Beckman Coulter, USA) to 5-10 g/L (q_1). Thirty-three percent (33%) of sludge from the aerobic/anoxic reactor was transferred to the anoxic reactor (q_2) and 67% was wasted (q_3). The total SRT of the external reactors (SRT_{ext}) was varied from10-40 d (Table 1) by adjusting sludge wastage (q_3). The wasted sludge was thickened to 16-24 g/L by centrifugation (Beckman Coulter, USA) for 10 min at 3,267 *g*. The supernatant was returned to SBR_{OSA}, and the

pellet was discarded. Sludge from the anoxic reactor was returned to the aerobic/anoxic reactor (q_4) and SBR_{OSA} (q_5).

The aerobic digester (Figure 1b) was continuously aerated using an air diffuser. The DO was 6.2±0.19 mg/L (n=62) and the temperature was maintained at 25 ºC using a water bath. The SRT of the aerobic digester (SRT_{ext}) was varied from 10-40 d (Table 1) by adjusting sludge wastage (Q_{out}). The aerobic digester was fed with sludge from $SBR_{control}$ (Q_{in}) that has been thickened to 5-10 g/L by centrifugation (Beckman Coulter, USA) for 10 min at 3,267 *g*. The supernatant produced after thickening was discarded to eliminate the potential impact of return flow on sludge production and/or substrate consumption of SBRcontrol. This facilitated the comparison of two SBRs (SBR_{OSA} vs. $SBR_{control}$) with and without sludge interchange.

2.3 Analytical techniques

2.3.1 Wastewater analysis

The total and volatile suspended solids (TSS and VSS) of influent and effluent were measured according to APHA Standard Method 2540 . The tCOD of the influent and effluent was measured using Hach digestion vials that were heated in Hach DBR200 COD Reactor, and then analysed using Hach DR/2000 spectrophotometer according to the APHA Standard Method 5220. Ammonia and orthophosphate were measured using flow injection analysis (Lachat Instruments, USA) following the APHA Standard Method 4500 .

2.3.2 Sludge analysis

The MLSS and MLVSS of sludge were measured according to APHA Standard Method 2540. The sludge volume index (SVI) was measured using 1000 mL of sludge according to APHA Standard Method 2710-D. The DO concentration of sludge was measured using a DO meter (YSI, USA). The pH and ORP of sludge were measured by a pH/ORP meter (TPS, Australia).

2.3.2 TrOC extraction and analysis

Duplicate measurements of the TrOC concentration of the influent (municipal wastewater), effluent, and sludge were obtained at the end of each operation period (SRT of the external reactors=10, 20, and 40 d), which corresponded to summer (December 2015), spring (October 2015), and winter (July 2015) seasons (Table 1). Details of sample preparation, solid phase extraction (SPE), and TrOC analysis are described in Supplementary Table S2. The concentration of TrOCs were determined using high performance liquid chromatography (Agilent 1200, USA) coupled with tandem triple quadrupole mass spectrometry (API 4000, Applied Biosystems, USA) as previously described by Phan *et al.* (2015).

2.4 Calculations

2.3.1 Sludge reduction

Sludge reduction was determined as described in a previous study (Semblante et al., 2016). Briefly, sludge reduction was the difference in sludge yield of SBR_{OSA} and $SBR_{control}$:

$$Sludge\ reduction\ (\%) = \frac{Y_{SBR_{control}} - Y_{SBR_{OSA}}}{Y_{SBR_{control}}} \times 100 \qquad \text{Equation 1}$$

The experimental sludge yield (Y) of the SBRs was defined as:

$$Y = \frac{P}{C} = \frac{g\ MLVSS}{g\ tCOD} \qquad \text{Equation 2}$$

where P is the sludge produced in terms of mixed liquor volatile suspended solids (MLVSS) and C is the substrate consumed in terms of total chemical oxygen demand (tCOD). Sludge yield was derived from the slope of the linear regression of the cumulative sludge produced versus the cumulative substrate consumed. The cumulative values were obtained by incrementing the variations in sludge production and substrate consumption in previous sampling intervals.

2.3.2 TrOC concentration in external reactor sludge

To gain further insight in the sorption of and biodegradation of TrOCs, the TrOC concentration of sludge (in ng/L) entering the external reactors i.e., the aerobic/anoxic and anoxic reactors of OSA and the aerobic digester of the control system were estimated as described in Supplementary Table S4.

3. Results and discussion

3.1 Sludge reduction by OSA

Sludge reduction by OSA at different SRT_{ext} has been reported elsewhere (Semblante et al., 2016). Briefly, increasing the SRT from 10 to 20 d enhanced sludge autolysis in the external reactors. However, increasing SRT from 20 to 40 d did not increase sludge autolysis further. Additionally, maintaining relatively low SRT (10 and 20 d) facilitated the conversion of destroyed sludge into inert products through denitrification and nitrification reactions. Therefore, an intermediate SRT_{ext} (20 d) favoured sludge reduction in OSA (Table 1). Furthermore, regardless of the SRT_{ext}, SBR_{OSA} and $SBR_{control}$ had similar tCOD and ammonia concentration in the effluent (Supplementary Figures S5 and S6). This suggests that OSA did not affect the overall wastewater treatment efficiency of the main aeration tank (SBR_{OSA}).

3.2 TrOC concentration in the influent

The sampling campaigns at different SRT_{ext} fell at different seasons (Table 1). Sludge reduction was estimated by comparing the performance of $SBR_{control}$ and SBR_{OSA} during a certain operation regime, and thus was not affected by variation in influent wastewater characteristics. On the other hand, sampling at different seasons helped to obtain a comprehensive profile of TrOCs in the influent (municipal wastewater) in the study site. A total of 52 TrOCs were detected throughout the operation period (Figure 2). Thirty-four (34) out of 45 target TrOCs were detected during the winter sampling campaign, whereas 45 out of 60 target TrOCs were detected during the spring and summer sampling campaigns (Supplementary Figure S7a). The detected TrOCs had a

wide range of concentrations (10-100,000 ng/L), and included food products, pharmaceuticals, personal care product ingredients, hormones, pesticides and industrial chemicals (Figure 2).

[Figure 2]

Among the detected TrOCs, the highest influent concentration was observed for salicylic acid, caffeine, paracetamol, and ibuprofen (Figure 2). Caffeine is a stimulant added to food and beverages, while the other compounds are ingredients of over-the-counter ointments and medicines (Luo et al., 2014). These compounds have also been found in high concentrations in the influent of other WWTPs in Australia (Phan et al., 2015; Trinh et al., 2016) probably because of the similarity in human consumption in these areas.

The influent concentration of several TrOCs changed at different sampling campaigns (Supplementary Figure S7a). The maximum concentration of certain food products, pharmaceuticals, and personal care products was approximately 100,000 ng/L in summer and spring, whereas it was only approximately 40,000 ng/L in winter. This was probably due to variation in human consumption at different seasons (Chiu & Westerhoff, 2010; Yu et al., 2013). Meanwhile, some endogenous hormones and metabolic products (estriol, androstenedione, etiocholanolone, and 17β-estradiol) had similar concentration in the influent at different seasons. There was also similar concentration of ethinylestradiol, a synthetic estrogen that is commonly used in contraceptive pills and hormone replacement therapy, at different seasons. The discharge of these hormones by humans is probably unaffected by seasonal changes. Trinh *et al.*, (2016) also observed that the influent concentration of these hormones in Bega Valley, Australia was not affected by seasonal changes.

3.3 TrOC concentration in the SBR effluent

[Figure 3]

The concentrations of all the TrOCs detected in the influent, effluent, and solid phase are presented in Supplementary Figure S7. Selected TrOCs representing highly biodegraded (caffeine, ketoprofen, and

paracetamol), partially biodegraded (sulfamethoxazole and bisphenol A), and poorly biodegraded (benzotriazole, carbamazepine, verapamil, amitriptyline, estrone, oxybenzone, triclosan, and triclocarban) contaminants are presented in Figure 3. Among the selected non-biodegradable compounds, benzotriazole, carbamazepine, and estrone (Figure 3a) were detected mostly in the effluent whereas verapamil, amitriptyline, triclosan and triclocarban (Figure 3b) were detected mostly in sludge.

3.3.1 SBR_{OSA} effluent

Hydrophilic TrOCs (log D<3; pH=7; 25 ºC) such as caffeine, ketoprofen, paracetamol (Figure 3), naproxen, ibuprofen, estriol, androstenedione, and propylparaben (Supplementary Figure S7) were biodegraded by SBR_{OSA} by more than 80%. In other words, the combined quantity of these TrOCs in both the effluent and sludge solid phase was less than 80% compared to the influent loading. Previous studies also reported high biodegradation of these compounds (Radjenović et al., 2009; Tadkaew et al., 2011; Trinh et al., 2016). On the other hand, hydrophilic TrOCs such as benzotriazole, carbamazepine (Figure 3), TCEP, sucralose, trimethoprim, dilantin, diclofenac, diuron, and diazepam (Supplementary Figure S7) were biodegraded by less than 1%and mostly found in the effluent. With the exception of sucralose, all the aforementioned non-biodegradable compounds possess electron-withdrawing groups (EWG) that decrease the electron density of the aromatic ring and consequently inhibit electrophilic attack by oxygenases, which is the potential first step in aerobic biodegradation (Hai et al., 2011a; Tadkaew et al., 2011). Sucralose, a non-caloric artificial sweetener, generally has low biodegradation under aerobic conditions (Torres et al., 2011).

Several hydrophilic TrOCs were only partially biodegraded in SBR_{OSA}. Indeed, sulfamethoxazole (Figure 3), atenolol, aspartame, salicylic acid, saccharin, primidone, triamterene, and gemfibrozil (Supplementary Figure S7) had varying biodegradation (20-90%) at different sampling campaigns. They were only partially removed from the influent and varying concentration in SBR_{OSA} effluent and sludge solid phase. Among these compounds, only saccharin and sulfamethoxazole have EWGs in the form of amide and sulfonamide (Supplementary Table S3), respectively, which helps explain poor biodegradation. The rest have electron-

donating groups (EDG) (Supplementary Table S3). EDGs are expected to enrich the electron density of the aromatic ring and facilitate electrophilic attack (Tadkaew et al., 2011). However, the complete biodegradation of these EDG-bearing compounds did not occur probably due to the relatively low SRT (10 d) of SBR_{OSA}.

Among the hydrophobic TrOCs (log D>3; pH=7; 25 ºC), phenylphenol, levonorgestrel, butylparaben, diazinon, etiocholanolone, androsterone, ethynylestradiol, 17-α-estradiol, and 17-β-estradiol were biodegraded by more than 95% (Supplementary Figure S7). Previous studies have reported that these compounds had moderate to high biodegradation under aerobic conditions (Deng et al., 2015; Phan et al., 2015; Tadkaew et al., 2011; Trinh et al., 2016). Other hydrophobic TrOCs including estrone, oxybenzone, triclosan, triclocarban (Figure 3), benzophenone, clozapine, 4-tert-octylphenol, and nonylphenol (Supplementary Figure S7) were biodegraded by less than 10%. Among them, only estrone had significant concentration in SBR_{OSA} effluent (Figure 3). Estrone may accumulate in the aqueous phase due to its moderate hydrophobicity (log D = 3.13; pH 7; 25 ºC) resulting in relatively poor sorption (Verlicchi et al., 2012) or the oxidation of 17β-estradiol or partial conjugation of other hormones by the bacterial β-glucuronidase (D'Ascenzo et al., 2003). The rest of the compounds (oxybenzone, triclosan, triclocarban, and others) had tendency to accumulate in the sludge solid phase possibly due to hydrophobic interactions.

3.3.2 Comparison of SBR_{OSA} and $SBR_{control}$ effluent

The effect of OSA on effluent quality is an important criterion to evaluate its effectiveness as a sludge reduction strategy. Previous studies based on both synthetic (Goel & Noguera, 2006) and real wastewater (Semblante et al., 2016) showed that OSA did not have deleterious effect on the organic or nutrient removal of CAS. This study additionally confirms that OSA did not impact TrOC sorption and biodegradation in the main aeration tank (SBR_{OSA}). With a few exceptions (Supplementary Table S8), there was minimal difference (<10-20%) in the effluent TrOC concentrations of SBR_{OSA} and $SBR_{control}$, and the two SBRs had identical biodegradation efficiencies (Figure 3; Supplementary Figure S7).

3.4 TrOC concentration in SBR sludge

The concentrations of selected TrOCs in the solid phase of sludge are presented in Figure 3b. Results showed that sorption of TrOCs on sludge depended on electrostatic and hydrophobic interactions, and the integration of the external reactors did not impact TrOC sorption on SBR_{OSA} sludge.

3.4.1 SBR_{OSA} sludge

Despite their moderately hydrophilic nature, verapamil and amitriptyline (log D=2.08 and 2.28, respectively; pH 7; 25 ºC) preferentially sorbed on sludge (Figure 3) possibly due to electrostatic interactions. These two compounds are positively-charged whereas the sludge surface is negatively-charged under normal environmental conditions (Stevens-Garmon et al., 2011). High sorption of verapamil and amitriptyline on sludge has been previously reported (Stevens-Garmon et al., 2011). The current results indicate that electrostatic binding was an auxiliary sorption mechanism since other positively-charged but highly hydrophilic compounds (*e.g.*, atenolol, log D = -2.09; pH 7; 25 ºC) had low sorption. In other words, sorption through electrostatic interactions did not occur for TrOCs with high hydrophilicity.

Among the hydrophobic TrOCs (log D>3; pH 7; 25 ºC), triclosan, triclocarban (Figure 3b), and clozapine (Supplementary Figure S7b) had the greatest concentration in the sludge of SBR_{OSA}. These compounds have EWGs (*e.g.*, –Cl) that potentially contributed to their low biodegradation. The positive charge of clozapine at neutral pH probably perpetuated its sorption (Stevens-Garmon et al., 2011). Triclosan and triclocarban had the highest log D values among the TrOCs analysed in this study, and thus they sustained the highest concentration (>500 ng/kg) in SBR_{OSA} sludge (Figure 3b).

3.4.2 Comparison of SBR_{OSA} and $SBR_{control}$ sludge

Most TrOCs had nominal variation (<10%) in SBR_{OSA} and $SBR_{control}$ sludge at different SRT_{ext}, indicating that OSA did not affect the sorption of TrOCs in SBR (Figure 3). The volume of sludge interchanged between

SBR_{OSA} and the external reactors and the change in reactor medium was relatively low (Supplementary Table S9), and thus dramatic change in the TrOC profile of sludge was not observed (Supplementary Table S7).

Most of the TrOCs that showed a significant difference (>30%) between SBR_{OSA} and $SBR_{control}$ sludge (Supplementary Table S10) were non- or partially biodegradable (*e.g.*, TCEP, benzophenone, and others) (Section 3.3.1), which explains why they were detected varying amounts in the sludge. Highly biodegraded compounds such as caffeine, paracetamol, and ibuprofen also showed different concentrations in SBR_{OSA} and $SBR_{control}$ sludge because the residual sludge concentration of these compounds was negligible compared to the influent load (1,000-80,000 ng/L). In contrast, highly biodegraded compounds like ketoprofen and naproxen did not have high concentration in sludge and no significant variation between the two SBRs was detected.

3.5 Impact of redox regimes in OSA external reactors

The potential impact of additional redox regimes on the fate of TrOCs in OSA was assessed. The aerobic/anoxic reactor received sludge from SBR_{OSA} and the anoxic reactor. It had ORP of 120±20 mV (n=34) and 40±20 (n=34) when aeration was turned on and off, respectively. Also, it was deficient in substrate because biodegradable COD has already been consumed in the preceding reactors. Meanwhile, the anoxic reactor received sludge only from the aerobic/anoxic reactor. It had an ORP of -450±20 (n=34) and was deficient in substrate, which resulted in volatile solids destruction (Semblante et al., 2016).

3.5.1 Aerobic/anoxic reactor

To determine the fate of TrOCs in the external aerobic/anoxic reactor, its aqueous and solid phase TrOC concentrations were compared with that of SBR_{OSA} and anoxic reactor at each SRT (Supplementary Figure S11). The concentrations of selected TrOCs are presented in Figure 4. To assess TrOC sorption and biodegradation, the concentration of individual TrOCs entering the aerobic/anoxic reactor ($Y_{in\text{-}ae/anx}$) was estimated (Section 2.3.2) and compared with the actual concentrations detected in the reactor (Supplementary Figure S12).

[Figure 4]

A few compounds that were poorly biodegraded in SBR_{OSA} (i.e., benzotriazole, estrone, and oxybenzone) were biodegraded by 75-100% in the aerobic/anoxic reactor (Figure 4). Benzotriazole was probably biodegraded due to the increase in reaction time under aerobic condition brought about by sludge recirculation. It has been found that this compound is not biodegraded under anoxic or anaerobic conditions but long aerobic treatment (40-50 d) can result in near complete biodegradation (Herzog et al., 2014). The current study shows that the removal of benzotriazole can be improved through the addition of external reactors in the return activated sludge loop. Meanwhile, estrone and oxybenzone were probably biodegraded due to anoxic phase in the external reactors. It has been observed that estrone is transformed to 17β-estradiol through the reduction of its ketone group under anoxic condition (Shi et al., 2013). Since 17β-estradiol was not detected in either aqueous or solid phase of the aerobic/anoxic reactor, further biodegradation in either aerobic or anoxic phases could be inferred. Meanwhile, a previous study showed that oxybenzone is removed through aerobic-anoxic recirculation of sludge (Phan et al., 2014). Apart from benzotriazole, estrone, and oxybenzone, the majority of the TrOCs had varying but generally low biodegradation in the aerobic/anoxic reactor compared to SBR_{OSA} (Supplementary Figure S12). Poor biodegradation at substrate-deficient conditions suggests that co-metabolism is the primary mechanism involved in the biotransformation of these TrOCs. In other words, many TrOCs cannot stand as primary carbon source for microbial maintenance. Instead, these are catabolised only when other carbon sources are available (Semblante et al., 2015a).

The sludge concentration of a few recalcitrant and sorbing TrOCs such as verapamil, amitriptyline, triclosan and triclocarban was higher in the aerobic/anoxic reactor (Figure 4) compared to SBR_{OSA} (Figure 3b). For example, the concentrations of triclosan (266-1,477 ng/g MLSS) and triclocarban (1,886-8,384 ng/g MLSS) in the aerobic/anoxic reactor sludge were three and sixteen times greater than in SBR_{OSA}/$SBR_{control}$. The implications of these findings on TrOC discharge from OSA are discussed in Section 3.7.

3.5.2 Anoxic reactor

Generally, there was poor biodegradation of TrOCs in the anoxic reactor relative to the aerobic/anoxic reactor or SBR_{OSA} (Supplementary Figure S12 and S13). A few TrOCs such verapamil, amitriptyline, carbamazepine (Figure 4), TCEP, and clozapine (Supplementary Figure S13) had some biodegradation (*e.g.*, 20-30%) especially when SRT was increased from 10 to 40 d (to be discussed in Section 3.5.3). The rest of the TrOCs were recalcitrant under anoxic treatment.

The aqueous phase concentration of paracetamol, carbamazepine, bisphenol A, triclosan (Figure 4) sucralose, ibuprofen, and diclofenac (Supplementary Figure S13) in the anoxic reactor was greater than that of the aerobic/anoxic reactor and the incoming sludge. These originally partitioned in the solid phase of the aerobic/anoxic reactor, but were released to the supernatant of the anoxic reactor. Previous research demonstrated that the key sludge reduction mechanism of OSA is sludge autolysis in the anoxic reactor (Semblante et al., 2016). The destruction of solids probably resulted in the loss of TrOC sorption sites which led to the desorption of contaminants that were sorbed on sludge. The desorption of TrOCs, such as estrogens and nonylphenol, as a direct result of solids destruction during biological or advanced oxidation treatment has been reported in literature (Chawla et al., 2014; Semblante et al., 2015b). Nonetheless, this is the first report showing the desorption of TrOCs from sludge during application of a biological sludge reduction strategy. Notably, in this particular OSA configuration, sludge is discharged from the aerobic/anoxic rather than the anoxic reactor (Figure 1a) where TrOC desorption occurs. Therefore, this configuration helps minimise the discharge of TrOCs in the aqueous phase.

3.5.3 Impact of SRT_{ext} on TrOC biodegradation in external reactors

The biodegradation of certain TrOCs exhibited dependence on SRT_{ext}. The biodegradation of caffeine (Figure 4) and primidone (Supplementary Figure S12) in the aerobic/anoxic reactor was enhanced when SRT_{ext} was increased from 10 to 40 d, although a complete biodegradation of either compound was not observed. These

compounds can be biodegraded in both anoxic (Bradley et al., 2007) and aerobic/anoxic conditions (Phan et al., 2014). The improved degradation of caffeine and primidone in this study could be attributed to longer reaction time under anoxic condition.

The biodegradation of some compounds such as verapamil, amitriptyline, bisphenol A (Figure 4), atenolol, gemfibrozil, and clozapine (Supplementary Figure S12) increased slightly in the aerobic/anoxic reactor when SRT_{ext} was increased from 10 to 20 d, but decreased when SRT_{ext} was 40 d. In a previous study, SRT_{ext} of 20 d favoured nitrification/denitrification in the aerobic/anoxic reactor and helped facilitate the cycle of sludge autolysis in OSA (Semblante et al., 2016). Consistent with recent reports (Phan et al., 2014; Tran et al., 2014), the results here point to a linkage between TrOC biodegradation and nitrification/denitrification. However, the lack of substrate in the aerobic/anoxic reactor explains why TrOC biodegradation was generally poor relative to SBR_{OSA} and further emphasizes the relevance of co-metabolic pathways in TrOC biodegradation.

A few TrOCs, namely, TCEP, verapamil, amitriptyline, carbamazepine, and clozapine exhibited a slight increase in biodegradation in the anoxic reactor with increasing SRT_{ext} (Supplementary Figure S13). The biodegradation of verapamil and amitriptyline increased as nitrifying/denitrifying efficiency improved. This suggests that anoxic treatment was conducive to their biodegradation. Unlike this study, high biodegradation of amitriptyline, carbamazepine, and clozapine has been reported in an anaerobic MBR (ORP=-200 mV) which was not deficient in substrate and had high methanogenic activity (Wijekoon et al., 2015). In this study, the ORP of the anoxic reactor (-450±30 mV; n= 34) was low but there was no methanogenic activity (indicated by biogas production) due to substrate deficiency. Although a relationship between biodegradation and SRT was observed for the aforementioned compounds, the majority of the load from the incoming sludge was not biodegraded probably because co-metabolic degradation pathways were not activated in the absence of substrate. The residues partitioned in varying concentrations in the aqueous and/or solid phase of anoxic sludge (Supplementary Figure S13).

3.6 *$SBR_{control}$ vs. aerobic digester: Impact of substrate deficiency*

Aerobic digestion involves the treatment of sludge in a completely mixed aerated reactor. The fate of TrOCs in the aerobic digester was investigated to assess TrOC discharge from a conventional sludge treatment unit (Figure 5). Furthermore, $SBR_{control}$ and aerobic digester were both aerobic reactors, but the former was fed with influent (municipal wastewater) with relatively high concentration of TrOCs and the latter was fed with sludge containing low concentration of readily biodegradable sCOD and reduced concentration of TrOCs (Section 2.2). Thus comparison of SBR_{OSA} and the aerobic digester helps determining the impact of substrate deficiency in TrOC removal (Supplementary Figure S14 and S15).

Generally, with a few exceptions (Section 3.4.1), treatment in $SBR_{control}$ resulted in (i) up to 80% biodegradation of hydrophilic TrOCs especially those with EDG and, (ii) poor biodegradation of hydrophobic TrOCs especially those with EWG. On the contrary, only estrone (a hydrophobic TrOC that was poorly biodegraded in $SBR_{control}$, Section 3.3) was consistently biodegraded at different SRT_{ext} in the aerobic digester. Additionally, a few TrOCs (e.g., caffeine, naproxen, and gemfibrozil, discussed in Section 3.5) were highly biodegraded in the aerobic digester at high SRT_{ext} only (40 d). This demonstrates that the biodegradation of many TrOCs under aerobic condition occurs only when primary substrate is available (Semblante et al., 2015b).

[Figure 5]

3.7 Insights on the TrOC discharge from OSA

TrOC discharge from the particular OSA configuration used in this study was assessed by comparing TrOC concentrations in SBR_{OSA}, the aerobic/anoxic reactor (where sludge is discharged from the OSA system, Section 2.2), and the control aerobic digester (where sludge is discharged from the control system, Section 2.2). The aerobic/anoxic reactor (Figure 4) generally showed lower concentration of many TrOCs in both aqueous and solid phases than SBR_{OSA} (Figure 3) given that the majority of the contaminants have already been biodegraded in the main aeration tank.

The aerobic/anoxic reactor also enhanced the biodegradation of estrone, oxybenzone, and benzotriazole (Section 3.5.1). However, non-biodegradable TrOCs (*e.g.*, triclosan and triclocarban) accumulated in the aerobic/anoxic reactor and therefore the solid phase concentration was higher than that of SBR_{OSA} (Section 3.5.1). In other words, treatment of sludge in the external reactors enhanced the biodegradation of some TrOCs (*e.g.*, benzotriazole, Figure 4a) but resulted in the accumulation of others (*e.g.*, triclosan, Figure 4b) especially those that are hydrophobic and non-biodegradable in either aerobic or anoxic condition.

Notably, this particular OSA configuration discharges sludge from an aerobic/anoxic reactor rather than an anoxic reactor, which is commonly found in literature (Goel & Noguera, 2006; Semblante et al., 2014). The current study revealed that the aerobic/anoxic treatment results in greater biodegradation of TrOCs than the anoxic treatment (Section 3.5.1). Moreover, the destruction of volatile solids in the anoxic reactor caused desorption of some TrOCs (*e.g.* paracetamol, sucralose, and bisphenol A) from the solid phase of sludge and consequently increased TrOC concentration in the aqueous phase (Section 3.5.2). This is an indication that the current OSA configuration has potential to have lower TrOC discharge than others involving a single external anoxic reactor.

Generally, the aerobic/anoxic and anoxic reactors of OSA resulted in the biodegradation of a greater number of TrOCs than the aerobic digester. The superior performance of the aerobic/anoxic reactor can be attributed to the variation in redox conditions, which gave rise to nitrifying/denitrifying bacteria that potentially facilitated the biodegradation of some recalcitrant TrOCs (Section 3.5). Furthermore, the concentration of highly sorbing TrOCs (*e.g.*, triclosan and triclocarban) in the aerobic digester (406-10,413 ng/g MLSS) was higher than that of the aerobic/anoxic reactor of OSA (266-8,384 ng/g MLSS). This shows that OSA has potential to yield higher quality biosolids compared to aerobic digestion.

4. Conclusion

OSA did not affect the effluent TrOC concentration of the SBR. However, the biodegradation of estrone, benzotriazole, and benzophenone was enhanced in the aerobic/anoxic reactor. Generally, aerobic/anoxic condition favoured TrOC biodegradation than anoxic condition. Some TrOCs underwent desorption from sludge due to volatile solids destruction under anoxic condition. The concentration of highly sorbing and recalcitrant TrOCs (*e.g.*, triclosan) in the aerobic/anoxic reactor was lower than that of the control aerobic digester. This suggests that the final sludge residue generated by OSA have potential to have lower TrOC content than that of CAS paired with aerobic digestion.

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6. Reference

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List of figures

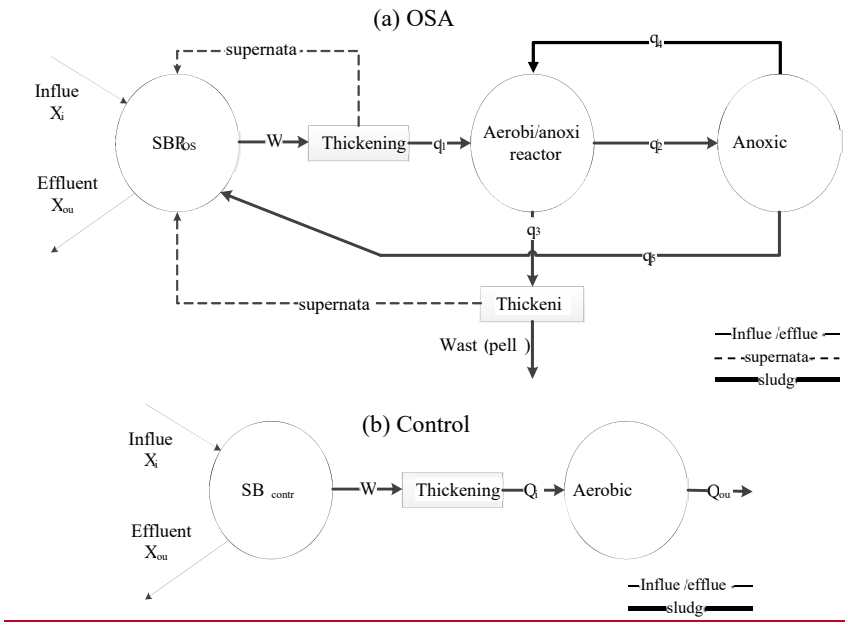
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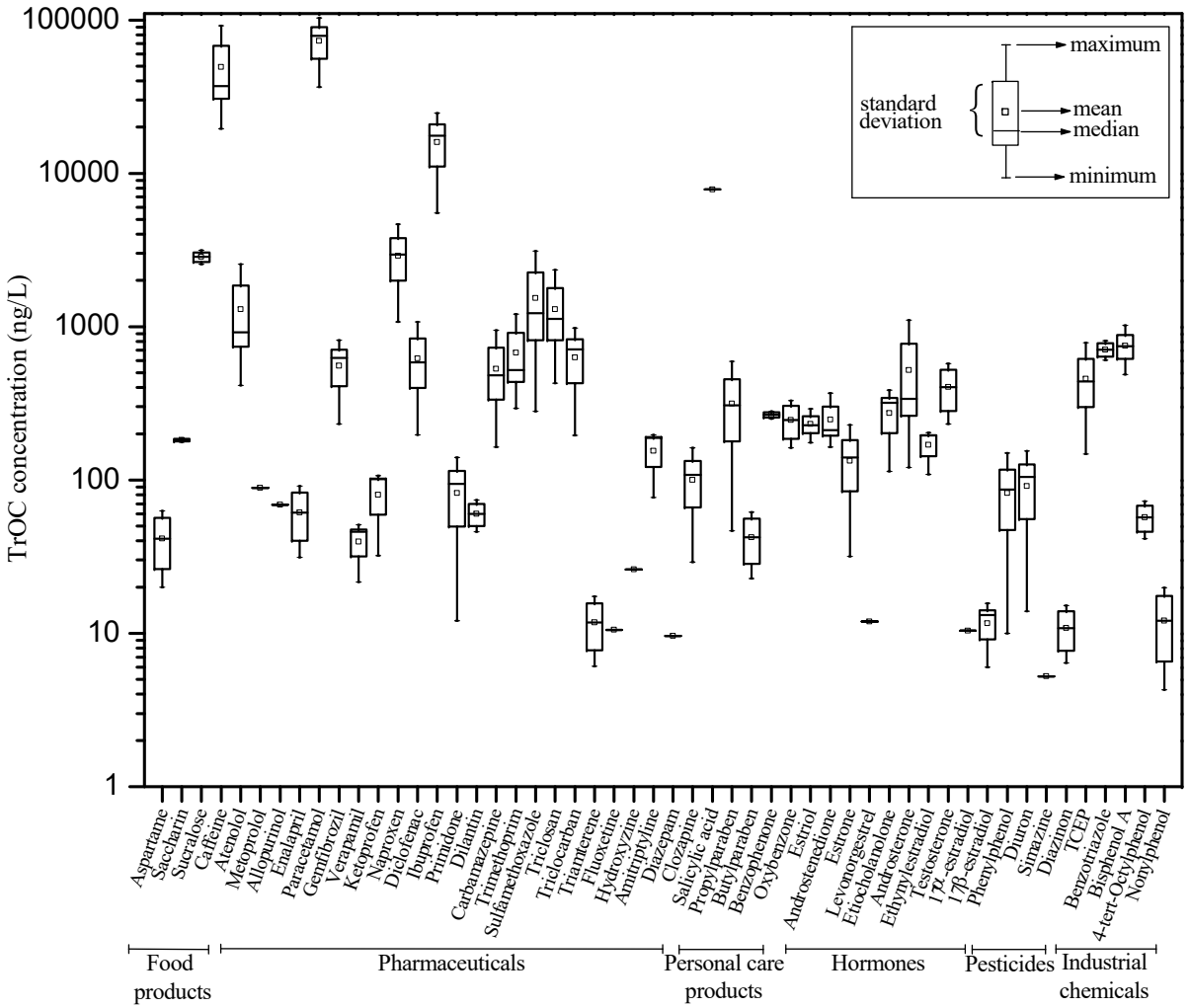
Figure 1



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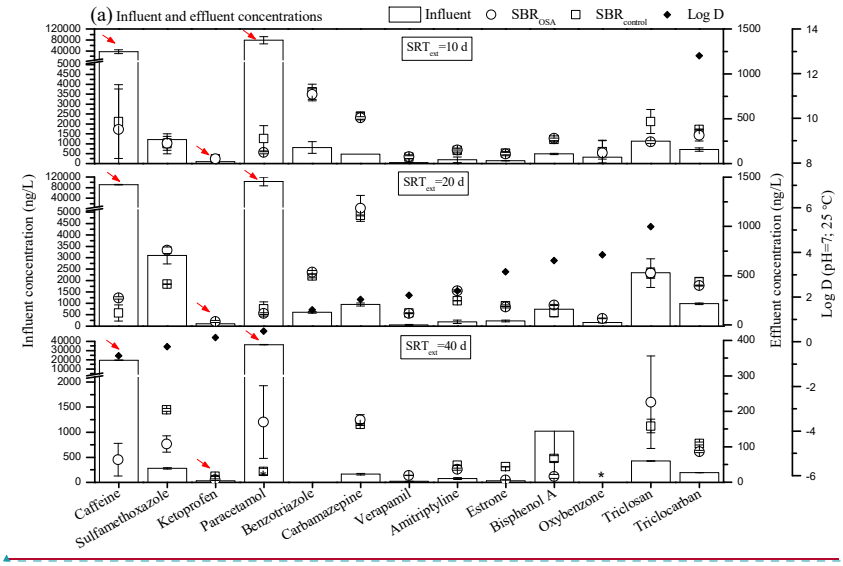

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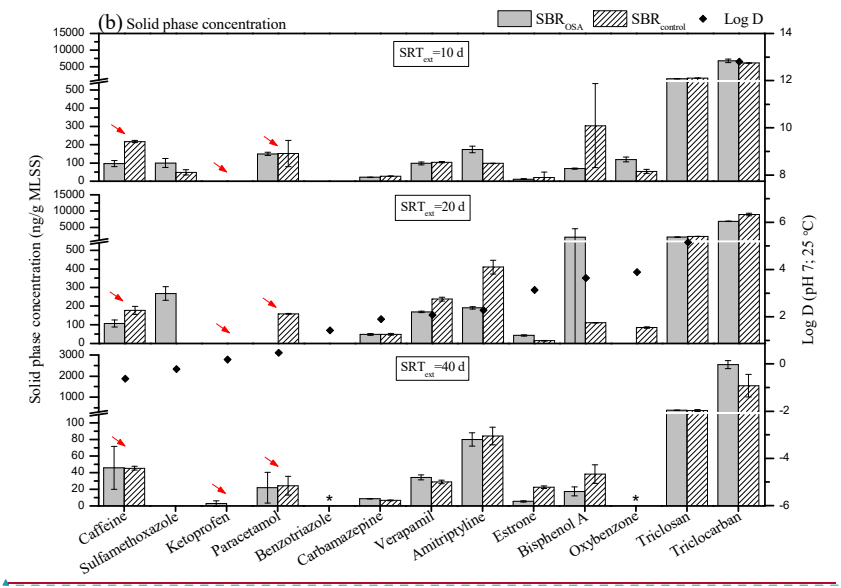

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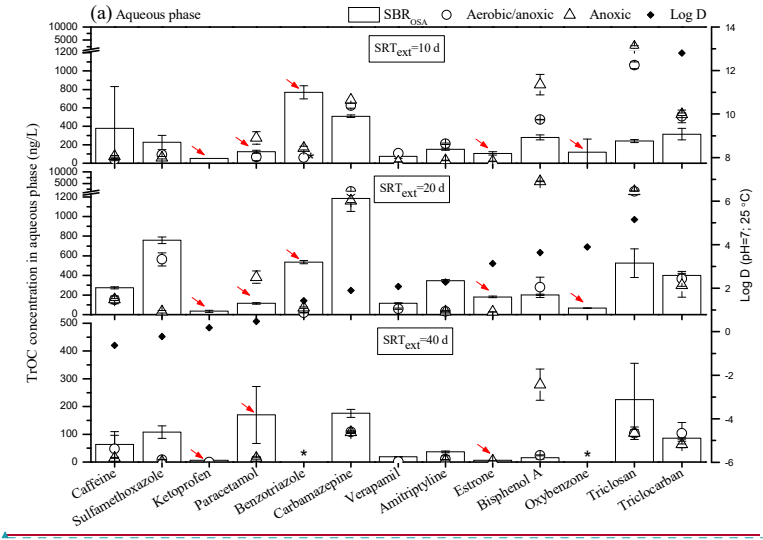

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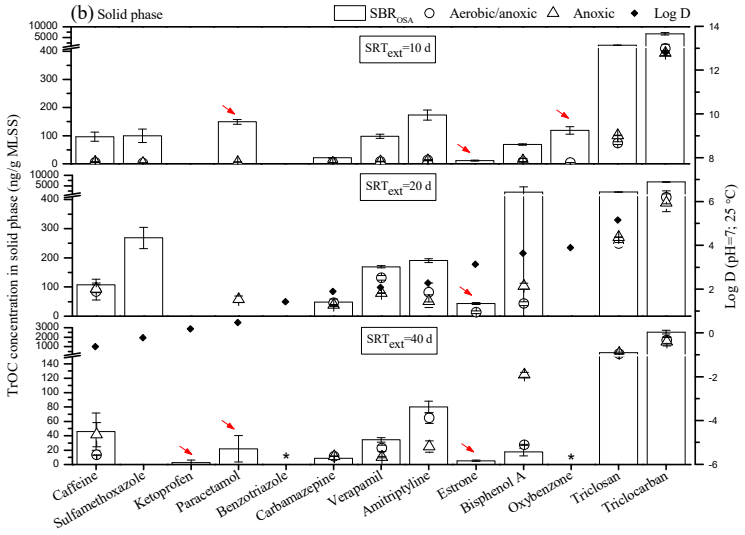

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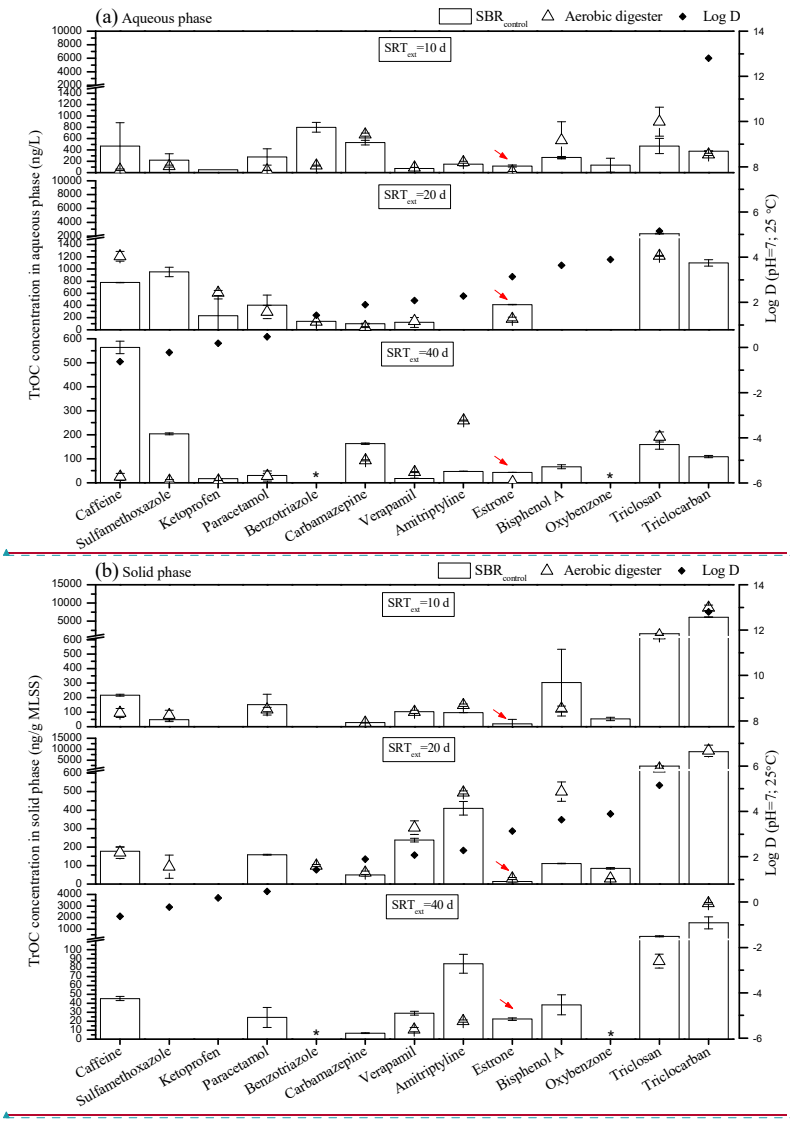

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Figure 5

List of Tables

Table 1 Summary of operation conditions and sludge reduction by OSA (mean ± standard deviation; *n*=number of samples)

SRT_{SBR} (d)	SRT_{ext} (d)	Season	Influent tCOD (mg/L)	Sludge yield (g MLVSS/g tCOD)		Sludge reduction (%)
				SBR_{OSA}	$SBR_{control}$	
10	10	Summer	527±154 (n=19)	0.13	0.13	0
10	20	Spring	478±254 (n=12)	0.09	0.14	35
10	40	Winter	491±194 (n=11)	0.16	0.19	16