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Effects of sludge retention time on oxic-settling-anoxic process performance: biosolids reduction and dewatering properties

Galilee U. Semblante
University of Wollongong, gus193@uowmail.edu.au

Faisal I. Hai
University of Wollongong, faisal@uow.edu.au

Heriberto A. Bustamante
Sydney Water Corporation, heri.bustamante@sydneywater.com.au

William E. Price
University of Wollongong, wprice@uow.edu.au

Long D. Nghiem
University of Wollongong, longn@uow.edu.au

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Abstract

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Keywords

dewatering, reduction, biosolids, performance, process, properties, anoxic, effects, settling, oxic, time, retention, sludge

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Galilee U. Semblante [a], Faisal I. Hai [a]*, Heriberto Bustamante [b], William E. Price [c], 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] Sydney Water, Parramatta, NSW 2124, Australia
[c] 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

In this study, the effect of sludge retention time (SRT) on oxic-settling-anoxic (OSA) process was determined using a sequencing batch reactor (SBR) attached to external aerobic/anoxic reactors. The SRT of the external reactors were varied from 10 to 40 d. Increasing SRT from 10 to 20 d enhanced volatile solids destruction in the external anoxic reactor as evidenced by the release of nutrients, however, increasing the SRT to 40 d did not enhance volatile solids destruction further. Relatively short SRT (10-20 d) favoured the conversion of destroyed solids into inert products. The application of an intermediate SRT (20 d) of the external reactor showed the highest sludge reduction performance (>35%). Moreover, at the optimum SRT, OSA improved sludge dewaterability as demonstrated by lower capillary suction time and higher dewatered cake solids content.

Keywords: biosolids reduction, capillary suction time, dewatered cake, oxic-settling-anoxic process, sludge yield.

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1. Introduction

Conventional activated sludge (CAS) is the most widely used process for wastewater treatment. However, CAS produces a large amount of sludge that is inherently difficult to stabilise and dewater (Mowla et al., 2013). Sludge management is an expensive exercise and excessive sludge production can result in high operating cost in wastewater treatment plants (WWTPs). Therefore, it is desirable to reduce sludge production in order to minimise the costs associated with downstream processing of sludge (*e.g.*, dewatering and digestion). Various methods have been employed to control sludge production. They include the manipulation of operation conditions such as dissolved oxygen (DO) and sludge retention time (SRT) of the aeration tank, the addition of chemicals to minimise biomass growth, and the use of advance oxidation processes to destroy biomass. Some of these methods require significant capital investment and operating cost and/or only result in a marginal biosolids reduction (Foladori et al., 2010). A promising alternative is the oxic-settling-anoxic (OSA) process, which modifies CAS by placing external anoxic reactor/s in the return activated sludge (RAS) loop. OSA allows RAS to be partially biodegraded in the external reactor, which has low DO and substrate concentration, before it is returned to the aeration tank. The interchange of sludge between conditions that are rich (the aeration tank) and deficient (the external anoxic reactor/s) in oxygen and substrate results in net excess sludge reduction. The appeal of OSA is in its simple configuration, which can be readily set up in existing or new plants with minimal capital and operating cost (Semblante et al., 2014).

Despite its potential, the wide-scale use of OSA is hindered by inconsistent performance which is evident in the literature. Laboratory-scale OSA operated using synthetic wastewater reportedly achieves over 40% sludge reduction (Chon et al., 2011b; Saby et al., 2003). However, these high sludge reduction values are rarely observed in pilot- or full-scale systems or when real sewage is used as the feed (Coma et al., 2013). This is probably because of poor operational control stemming from knowledge gaps about the mechanisms governing sludge reduction (Khursheed et al., 2015). It is only recently that laboratory-scale studies using domestic sewage demonstrated the key steps occurring in OSA. For example, Semblante *et al.*, (2015) showed that OSA causes destruction of volatile solids in the external anoxic reactor/s as well as a decline in the sludge yield (i.e., mass of biomass produced per mass of substrate consumed) of the main bioreactor.

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Previous research suggests that sludge reduction by OSA may be mainly due to its long SRT. The addition of external reactor/s that temporarily hold RAS results in an increase of the total SRT of activated sludge (Semblante et al., 2014). SRT is inversely proportional to sludge yield due to the diversion of energy towards cell maintenance rather than synthesis (Liu & Tay, 2001). However, contradicting reports have been reported regarding the relationship of SRT and OSA performance. For example, Saby *et al.,* (2003) observed that biosolids reduction (23-58%) was directly proportional to the SRT of the external anoxic reactor of OSA (11-17 d). On the contrary, Ye *et al.,* (2008) found that biosolids reduction (14-33%) had an inverse relationship with the SRT of the external anoxic reactor, although the range of SRTs investigated was much shorter (5.5-11.5 h) than that of Saby *et al.,* (2003). These studies were conducted with synthetic wastewater, and furthermore the SRTs reported were scattered, ranging from very short (*e.g.,* less than 1 day) (Ye et al., 2008) to significantly long (*e.g.,* 70-80 d) (Novak et al., 2007). It is difficult to establish a correlation between SRT and OSA performance based on the available literature, especially since the reports are based on varying wastewater, operation conditions, and methods of quantifying sludge reduction.

In addition to reducing biosolids, there is evidence that OSA may affect sludge properties. For example, some studies that used either synthetic (Saby et al., 2003; Ye et al., 2008) or real wastewater report that OSA decreased sludge volume index (SVI) and improved sludge settleability (Coma et al., 2013). The impact of OSA on sludge dewaterability has not been reported in literature. Sludge dewatering, which is one of the most challenging downstream processes associated with biosolids treatment, is influenced by several factors including the concentration and composition of extracellular polymer substances (EPS) that serve as the framework of sludge flocs (Mowla et al., 2013). OSA causes disintegration of EPS in the external reactor/s (Chon et al., 2011a; Semblante et al., 2015) and therefore may have implications on sludge dewatering characteristics, but this is yet to be studied systematically.

This study aims to determine the impact of SRT of the external anoxic reactors on biosolids reduction in an OSA system fed with real wastewater. Volatile solids reduction and associated other biological reactions, namely, release and fate of nutrients in the external reactors were closely monitored. Additionally, this study compares the dewaterability of waste activated sludge (WAS) with and without OSA. A systematic investigation concentrating on these topics has not

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been reported in literature. The results of this study will shed light on the underlying mechanisms in OSA, and will provide critical information on how OSA performance can be improved.

2. Materials and methods

2.1 Wastewater characteristics

Domestic unsettled sewage was collected from the beginning of the primary sedimentation channel of Wollongong WWTP fortnightly and stored at 4 °C prior to use. The properties of domestic sewage are provided in Supplementary Table S1. The use of domestic sewage ensures the cultivation of biomass possessing realistic properties.

2.2 Reactor configuration and operation

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). The control system consisted of $SBR_{control}$ (5 L) attached to a single-pass aerobic digester (2 L) (Figure 1b).

[**Figure 1**]

$SBR_{control}$ and SBR_{OSA} were fed with domestic sewage (Section 2.1). They were operated at 4 cycles/day and a HRT of 12 hours. Each cycle comprised of 15 min of filling, 5 hours and 30 min of aeration, 1 hour of settling, and 15 min of decanting. The SRT of both SBRs was maintained at 10 d by regular sludge wastage (W) (Figure 1). The average pH, DO concentration, and ORP of $SBR_{control}$ were 6.8±0.6 (n=62), 5.9±2.4 mg/L (n=62), and 117.7±20.5 mV (n=34), respectively, while those of SBR_{OSA} were 6.8±0.8 (n=62), 5.4±1.7 mg/L (n=62) and 129.7±28.2 mV, respectively. These measurements were taken during the aeration period.

The aerobic/anoxic reactor of the OSA system (Figure 1a) was intermittently aerated (*i.e.*, 8/16 hours aeration on/off) using an air diffuser placed at the bottom of the reactor. The anoxic reactor was kept airtight using a silicone-lined cap with inlet and outlet ports. The pH of the aerobic/anoxic reactor was 6.7±0.5 (n=62), whereas its DO concentration 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 aerobic/anoxic reactor was fed with sludge from SBR_{OSA} thickened to 5-10 g/L (q_1) by centrifugation (Beckman Coulter, USA) at 3728xg and 25 ºC for 10 min. Thirty-three percent

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(33%) of sludge from the aerobic/anoxic reactor was transferred to the anoxic reactor (q_2), and 67% was discharged (q_3). A sufficient amount was discharged from the external aerobic/anoxic reactor to vary the total SRT of the external reactors according to the following sequence: 20, 40, 20, and 10 d. The sludge discharged from the aerobic/anoxic reactor was thickened to 16-24 g/L by centrifugation (Beckman Coulter, USA) at 3728xg and 25 ºC for 10 min. 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 of the control system (Figure 1b) was continuously aerated using an air diffuser. The pH and DO concentration were 6.0±1.7 (n=62) and 6.2±0.19 mg/L (n=62), respectively. The SRT of this digester was varied by regular sludge wastage (Q_{out}) according to the following sequence: 20, 40, 20, and 10 d. The aerobic digester was fed with sludge from $SBR_{control}$ (Q_{in}) thickened to 5-10 g/L by centrifugation (Beckman Coulter, USA) at 3728xg and 25 ºC for 10 min. The supernatant produced by the thickening step was discarded.

2.3 Calculation of sludge yield

Sludge reduction was determined by comparing the sludge yield of the SBRs under parallel operation conditions. The experimental sludge yield (*Y*) of the SBRs was defined as

$$Y = \frac{P}{C} = \frac{g\ MLVSS}{g\ tCOD} \qquad \text{Equation 1}$$

wherein *P* is the sludge produced in terms of mixed liquor volatile suspended solids (MLVSS) and *C* is the substrate consumed in terms of tCOD. Sludge yield was derived from the slope of the linear regression of the cumulative sludge produced versus the cumulative substrate consumed. Cumulative values were obtained by incrementing the variations in sludge production and substrate consumption in previous sampling intervals (Chon et al., 2011b), and is discussed in detail in Supplementary Table S2.

Sludge reduction was calculated as the difference in sludge yield of $SBR_{control}$ and SBR_{OSA}:

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$$Sludge\ reduction\ (\%) = \frac{Y_{SBR_{control}} - Y_{SBR_{OSA}}}{Y_{SBR_{control}}} \times 100 \qquad \text{Equation 2}$$

Additionally, the sludge yield of the control (combined $SBR_{control}$ and aerobic digester) and OSA (combined SBR_{OSA} and external aerobic/anoxic and anoxic reactors) systems were calculated (Supplementary Table S2).

2.4 Assessment of sludge dewaterability

To assess the effect of OSA on sludge dewaterability, two different techniques were used. First, the capillary suction time (CST) of unconditioned sludge samples from the control and OSA systems were determined. CST was measured by placing 5 mL of the sample in Type 304M CST meter (Triton Electronics Limited, UK). CST was the time (s) taken by water to permeate through a specific interval in a standard filter paper. The time was monitored using two electrodes that detected the water front. To eliminate the effect of solids concentration on filtration, the specific CST was obtained by dividing CST by the MLSS of the sample. Second, the dewatered cake total solids (TS) concentration of WAS from the control ($WAS_{control}$) and OSA systems (WAS_{OSA}) were also determined using a previously described by To *et al.*, (2016). $WAS_{control}$ was the sludge discharged from $SBR_{control}$, whereas WAS_{OSA} was the sludge discharged from the external aerobic/anoxic reactor of OSA (Section 2.2), therefore, comparing these two parameters helped determining the impact of applying the OSA configuration on the WAS dewaterability. WAS samples were conditioned by adding thickening polymer (Zetag8169, BASF, Australia) at the concentration of 7.5 g polymer/kg MLSS followed by manual stirring for five minutes. The conditioned sludge samples were placed on top of a filter paper (Whatman No. 4) secured inside a modified centrifuge tube, and then centrifuged (Beckman Coulter, USA) at 3728xg and 25 ºC for 15 min. The filterable fraction was forced through the filter paper and settled at the bottom of the centrifuge tube. The TS of the dewatered cake, which was the pellet scraped from the filter paper after centrifugation, was analysed as described in Section 2.5.

2.5 Analytical techniques

Total and volatile suspended solids (TSS and VSS) of influent and effluent, MLSS and MLVSS of sludge from the reactors (Figure 1), and total solids (TS) of dewatered cake were measured

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according to APHA Standard Method 2540 (Eaton et al., 2005). The sludge volume index (SVI) was measured using 1000 mL of sludge according to APHA Standard Method 2710-D (Eaton et al., 2005). The tCOD of the influent and effluent was measured using Hach low range (LR) digestion vials that were heated in Hach DBR200 COD Reactor, and then analysed using Hach DR/2000 spectrophotometer (program number 430 COD LR) according to US-EPA Standard Method 5220. The sCOD of the influent and effluent was obtained using the same approach as that of tCOD measurement, except that the samples were initially passed through 1 μm filter paper. The influent, effluent and sludge samples were centrifuged (Beckman Coulter, USA) at 3728xg and 25 ºC for 10 min to remove large solids and then filtered using 1 μm filter paper. The ammonia and phosphate concentration of such filtered samples were measured using flow injection analysis (Lachat Instruments, USA) following the APHA Standard Method 4500 (Eaton et al., 2005). The nitrite and nitrate concentration of filtered samples were measured using ion chromatography (Shimadzu, Japan) with Ionpac AS23 anion-exchange column. The DO concentration of sludge was measured using a DO meter (YSI, USA). The pH and ORP of sludge were measured a pH/ORP meter (TPS, Australia).

3. Results and discussion

3.1 Wastewater treatment performance

The performance of the SBRs was assessed by monitoring influent and effluent tCOD, sCOD, ammonia, and phosphate concentrations (Figure 2 and 3). The tCOD concentration of the influent (560±292 mg/L; number of samples n=61) had a large variation due to temporal changes in weather patterns (e.g., dilution of wastewater by rainwater). Nonetheless, the tCOD concentration of the effluent of SBR_{OSA} (89±69 mg/L; n=61) and $SBR_{control}$ (82±71 mg/L; n=61) were similar to each other during the entire operation period. Likewise, SBR_{OSA} and $SBR_{control}$ effluents had similar ammonia and phosphate concentrations (Figure 3). SBR_{OSA} and $SBR_{control}$ both exhibited nitrification, removing approximately 90% of influent ammonia. Biological nitrate and phosphate removal were not observed in any of the SBRs probably because of the lack of a sufficient anaerobic phase. This shows that OSA would leave the performance of the aeration tank unchanged, which is consistent with previous studies (Chen et al., 2003; Semblante et al., 2015) but the current study confirms this over a broader range of influent strength. Nevertheless, this needs to be validated in full scale plant.

[Figure 2]

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[Figure 3]

3.2 Effect of SRT on OSA

3.2.1 Reduction of sludge yield

In this study, the SRT of both $SBR_{control}$ and SBR_{OSA} (hereafter denoted as SRT_{SBRs}) was maintained at 10 d, whereas the SRT of the external reactors ($SRT_{ext.reactors}$), *i.e.*, the single-pass aerobic digester appended after $SBR_{control}$ and the external aerobic/anoxic and anoxic reactors attached with SBR_{OSA}, were varied (10, 20, and 40 d). Results in Table 1 show that increasing $SRT_{ext.reactors}$ from 10 to 20 d resulted in sludge yield reduction in SBR_{OSA} from 16 to over 35%. Further increase of the $SRT_{ext.reactors}$ to 40 d did not achieve any additional sludge reduction (Table 1). In fact the $SRT_{ext.reactors}$ of 40 d increased MLVSS concentration in both external aerobic/anoxic (from 1 to 5 g/L) and anoxic (from 0.75 to 3.5 g/L) reactors (Figure 4). The sludge yield of the control (combined $SBR_{control}$ and aerobic digester) and OSA (combined SBR_{OSA} and external aerobic/anoxic and anoxic reactors) systems were also compared (Supplementary Table S3), and a similar trend emerged: sludge reduction increased when external reactor SRT was increased from 10 to 20 d, but did not improve when SRT was further increased to 40 d. These findings suggest the SRT of 40 d results in OSA system failure and thus increases the volatile solids fraction of WAS. In other words, increasing the $SRT_{ext.reactors}$ beyond 20 d is counterproductive to sludge reduction in OSA. As discussed further in Section 3.2.2, apparently at an intermediate $SRT_{ext.reactors}$ (20 d) the performance of this particular OSA configuration is maximised.

[Table 1]

[Figure 4]

Interestingly, a further advantage of the OSA over the control system was observed upon comparison of their final sludge residue. The MLVSS of WAS of the OSA system discarded from the aerobic/anoxic reactor (Figure 4) was up to 65% lower than that of the WAS of the control system discarded from the aerobic digester (Supplementary Figure S4). In other words, sludge produced by OSA is potentially more amenable to stabilization (Tchobanoglus et al.,

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2003) and may produce less odour (Semblante et al., 2015) than the sludge produced by the anaerobic digestion.

The optimum $SRT_{ext.reactors}$ revealed in this study (20 d) agrees with those found in literature (*e.g.*, 17.4 d reported by Saby *et al*., (2003)). However, unlike the study of Saby et al. (2003) that simultaneously changed the SRTs of the main (5.6-8.7 d) and external reactors (11-17.4 d) at relatively small increments, this study focused on the effect of external reactor SRT on OSA performance and featured a wider range of experimental conditions ($SRT_{ext.reactors}$ =10-40 d) that ensured a systematic investigation. Furthermore, the range of SRTs investigated in this study was significantly broader than those previously reported. For instance, Coma *et al.*, (2013) operated a pilot-scale anaerobic/anoxic/aerobic reactor (SRT=23.5 d) attached to an external anoxic reactor (SRT=0.2-2.3 h), and observed the greatest sludge reduction when external reactor SRT was 0.2 h. Ye *et al.*, (2008) operated a laboratory-scale SBR (SRT not reported) attached to an external anoxic reactor (SRT=5.5-11.5 h), and observed that an intermediate SRT of 7.5 h minimised the sludge production rate. Both Coma *et al.,* (2008) and Ye *et al.,* (2008) reported that the best OSA performance occurred when external reactor SRT was kept low (in the range of a few hours), but did not offer an explanation for their observation. A direct comparison of this study and previous studies is not possible due to variation in operation conditions and system configurations. Nonetheless, this study clearly demonstrates that decreasing $SRT_{ext.reactors}$ to 10 d did not favour sludge reduction. The impact of SRT on the mechanism of OSA is discussed in detail in Section 3.2.2.

3.2.2 Mechanism of sludge reduction

A previous study have demonstrated that sludge reduction in this particular OSA configuration (Figure 1) is due to the destruction of volatile solids in the external anoxic reactor, followed by the conversion of destroyed solids into inert products via nitrification/denitrification in the external aerobic/anoxic reactor (Semblante et al., 2016). In this study, the nutrient concentration (Figure 5) and ORP (Figure 6) of the external reactors were monitored to provide insight into the effect of SRT on the aforementioned biochemical reactions.

[Figure 5]

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[Figure 6]

The fact that increasing the SRT from 10 to 20 d increased volatile solids destruction in the external anoxic reactor but further increasing it to 40 d did not cause any improvement is evident in the release of phosphate and ammonia into the mixed liquor supernatant (Figure 5). Phosphate and ammonia concentrations in the sludge supernatant of $SBR_{control}$ (q_1) and anoxic reactor (q_4) fed into the aerobic/anoxic reactor (Figure 1) were determined on a fortnightly basis. The ratios of the average concentrations of phosphate and ammonia in the external anoxic reactor over that of feed sludge doubled when $SRT_{ext.reactors}$ was increased from 10 to 20 d (Table 2). The increase in volatile solids destruction can only be due to the enhancement of cell lysis and organic matter biodegradation. However, the ratios were comparable when $SRT_{ext.reactors}$ was 20 and 40 d (Table 2). This suggests that the maximum autolysis of sludge under oxygen- and substrate-deficient conditions in the external anoxic reactor occurred at the $SRT_{ext.reactors}$ of 20 d. Beyond this SRT, further degradation of the biodegradable fraction cannot take place due to limited availability of electron donors. This is supported by the fact that the ORP of the external anoxic reactor was stable at -400±50 mV (n=60) (Figure 6), which suggests that oxidizing agents were always rapidly consumed regardless of SRT.

[Table 2]

Previous studies noted that SRT plays a major role in volatile solids destruction during anaerobic digestion of sludge (de la Rubia et al., 2006; Maharaj & Elefsiniotis, 2001; Xiong et al., 2012). In those studies, optimum SRT ranges were reported based on the enhanced hydrolysis of particulate matter in sludge, resulting in the reduction of volatile solids (de la Rubia et al., 2006; Maharaj & Elefsiniotis, 2001; Xiong et al., 2012). This optimum SRT varies depending on other factors such as digestion temperature and sludge properties, and is usually determined by an empirical approach (de la Rubia et al., 2006; Maharaj & Elefsiniotis, 2001; Xiong et al., 2012). Generally, the SRT that maximizes hydrolysis in anaerobic digestion is relatively short (10 d or less), and therefore hydrolytic reactors are operated under such conditions (Xiong et al., 2012). However, there is very little information in literature about the relationship of external reactor SRT and volatile solids destruction in OSA, and the current study fills in that crucial gap.

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Nitrification/denitrification reactions occurred in the external aerobic/anoxic reactor when external reactor SRT was 10 and 20 d, but they were deactivated at the $SRT_{ext.reactors}$ of 40 d (Figure 5). Nitrification efficiency, calculated as the difference in the average ammonia concentrations of the feed sludge and the external aerobic/anoxic reactor (Table 3), was higher when $SRT_{ext.reactors}$ was 10 d (76%) than when it was 20 d (60-62%). However, as evidenced by the accumulation of ammonia (up to 120 mg/L) in the external aerobic/anoxic reactor, nitrification did not occur when $SRT_{ext.reactors}$ was 40 d (Figure 5). Nitrification involves two reactions: the conversion of ammonia into nitrite by ammonia-oxidizing bacteria (AOB) and the conversion of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). NOB has lower specific growth rate than AOB, and therefore reactors must be operated longer than the "minimum SRT" to facilitate the growth of NOBs and to achieve complete nitrification (Tchobanoglus et al., 2003). The external reactors in this study comprised an intermittently aerated reactor and another anoxic reactor. High nitrification was achieved even at the $SRT_{ext.reactors}$ of 10 d. However, nitrification declined when external $SRT_{ext.reactors}$ was increased to 40 d (Figure 5). This can be attributed to the increase in MLSS concentration (Figure 4) and oxygen consumption under the longer SRT, which resulted in the decrease of oxygen availability for nitrification. The required oxygen/ammonia-nitrogen (mg/mg) ratio for ammonia removal is 1.71 (Daigger, 2014). In this study, the oxygen/ammonia-nitrogen ratio at the $SRT_{ext.reactors}$ of 40 d was only 0.8. On the other hand, this ratio was 2.2 and 1.5 when $SRT_{ext.reactors}$ was 10 and 20 d, respectively, indicating that there was greater availability of oxygen for nitrification under those conditions. This finding further suggests that the addition of an aerobic phase in the external reactors facilitated the conversion of destroyed volatile solids to inert materials in OSA, but an appropriate $SRT_{ext.reactors}$ needs to be maintained to materialize that advantage.

[Table 3]

Denitrification in the external aerobic/anoxic reactor decreased when $SRT_{ext.reactors}$ was increased from 20 to 40 d (Figure 5). Denitrification efficiency was calculated as the difference in the average nitrate concentrations of the sludge fed from the SBR_{OSA} to the the external aerobic/anoxic reactor and the sludge within that reactor (Table 3). The occurrence of denitrification largely depends on the capacity of the preceding nitrification to produce nitrate, and therefore the efficiencies of the two reactions were related. In a previous OSA study,

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denitrification efficiency in the external aerobic/anoxic reactor declined due to insufficient biodegradable COD (Semblante et al., 2016). In this study, the soluble COD/nitrate-nitrogen ratio at different external reactor SRTs were similar to each other (Table 3) and were consistently close to the theoretical value of 3.7 (Chiu & Chung, 2003), which suggests that soluble COD would have been available for denitrification throughout the operation period. Therefore, the decline in denitrification at high $SRT_{ext.reactors}$ was not due to substrate deficiency. Rather, it is more closely associated with failure of the preceding nitrification reaction in the same tank. Other reasons could be the decay of denitrifying organisms at high SRT (Han et al., 2005) or the increase in MLVSS in the external aerobic/anoxic reactor (Figure 4) that hindered the mass transfer of electron acceptor and carbon sources in sludge (Li & Wu, 2014).

ORP is a key parameter for regulating sludge reduction in OSA when the sludge is interchanged between aerobic and anoxic conditions. Lower ORP has been associated with greater sludge reduction. For instance, Saby *et al.,* (2003) reported that increasing the SRT of an external anoxic reactor caused its ORP to decrease from +100 to -250 mV, which helped decrease bacterial count measured using 4',6-diamidino-2-phenyl indole (DAPI) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) staining techniques. However, in this study, the ORP of the external anoxic reactor was maintained at -400±50 mV (n=60) irrespective of the operation SRT (Figure 6). The ORP of the external anoxic reactor remained at a low level because nitrification and denitrification was completed, which is corroborated by the fact that there was minimal ammonia and nitrate in the reactor (see Figure 5). However, SRT clearly affected the ORP of the external aerobic/anoxic reactor during the anoxic phase (i.e., when aeration was turned off), which increased from -150 to +50 mV when $SRT_{external\ reactors}$ was decreased from 40 to 10 d (Figure 6). This indicates that an OSA configuration involving external aerobic phase that results in an intermediate ORP range (-50 mV) can facilitate sludge reduction.

The results of this study demonstrate that in contrast to previous hypothesis in the literature (Novak et al., 2007; Semblante et al., 2014), an extended SRT value in the external reactors is not the key mechanism responsible for sludge reduction in OSA. Increasing $SRT_{ext.reactors}$ from 10 to 40 d enhanced volatile solids destruction in the external anoxic reactor as evidenced by the release of degradation products (phosphate and ammonia) into the mixed liquor supernatant. However, an intermediate SRT (20 d) was necessary to convert products of cell lysis into inert

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products via nitrification/denitrification. Therefore, an intermediate SRT (20 d) maximizes the dynamics of the aforementioned reactions. Operation under this relatively short SRT has the additional advantage of minimizing aeration requirements in the external aerobic/anoxic reactor.

3.3 Impact of OSA on sludge settleability and dewaterability

SBR_{OSA} and $SBR_{control}$ had similar SVI throughout the operation period (Supplementary Figure S5). This indicates that under the operation conditions of this study neither the implementation of OSA nor the manipulation of external reactor SRT deteriorated the settleability of sludge in the main bioreactor. The dewaterability of sludge was additionally assessed under conditions that facilitated sludge reduction, that is, when external reactor SRT was 10 and 20 d. Results show that under optimum conditions (SRT=20 d), sludge from the OSA system had greater dewatering potential than sludge from the control system. The specific CST of the unconditioned sludge from SBR_{OSA} was lower than that of $SBR_{control}$ (Table 4). Likewise, the specific CST of unconditioned WAS_{OSA} was lower than that of unconditioned $WAS_{control}$ (Table 4). CST characterizes the filterability of slurry-type materials. The rate at which the filtrate is extracted from the slurry is dependent on its resistance, and is inversely proportional to the ease by which moisture can be extracted from the slurry (Scholz, 2005). The data indicate that it was easier to filter the supernatant from WAS produced by the OSA system than that of the control system.

[Table 4]

Results also provide evidence that exposing sludge to alternating redox conditions could increase dewatered sludge solids content. Under optimum conditions (*i.e.*, $SRT_{ext.reactors}$=20 d) the dewatered cake TS concentration of WAS_{OSA}, which was the final residue of aerobic/anoxic interchange, was significantly higher than that of $SBR_{control}$, which was solely exposed to aerobic conditions (Table 4). Additionally, the dewatered cake TS concentration of WAS_{OSA} was higher than that of the sludge from the aerobic digester placed after $SBR_{control}$ (Table 4). By contrast, when the external reactor SRT was 10 d, only a marginal difference in the dewatered cake TS concentration of sludge from the OSA and control systems was observed (Table 4). Improvement of sludge dewatering by manipulating $SRT_{ext.reactors}$ is an important finding of this study because such improvement entail savings in energy and resources for downstream sludge processing and handling.

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4. Conclusion

Under the optimum SRT, OSA reduces sludge by facilitating volatile solids destruction in the external anoxic reactor and nitrification/denitrification in the external aerobic/anoxic reactor. Increasing $SRT_{ext.reactors}$ facilitated autolysis of sludge under oxygen- and substrate-deficient conditions. However, beyond the optimum SRT (20 d), further biodegradation of sludge did not occur, rather a decrease in nitrification/denitrification efficiency in the external aerobic/anoxic reactor and consequently deteriorated OSA performance was observed. Furthermore, this study showed that aerobic/anoxic sludge interchange helps increase the dewatered cake solids content and reduce the CST value of unconditioned sludge when an optimum $SRT_{ext.reactors}$ was applied.

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Table 3. The removal of ammonia and nitrate in the external aerobic/anoxic reactor at different $SRT_{ext.reactors}$.

Table 4. Sludge concentration, CST, and TS after dewatering when SRT_{SBR} was 10 days and $SRT_{ext.reactors}$ was 10 and 20 days.

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Table 1 Sludge yield when SRT_{SBRs} was 10 days and $SRT_{ext.reactors}$ was varied.

Phase	SRT_{SBRs}	$SRT_{ext.\ reactors}$	Total SRT	Influent tCOD concentration (mg/L)	Sludge yield (g MLVSS/g tCOD)				Sludge yield reduction (%)
					SBR_{OSA}	R^2	$SBR_{control}$	R^2	
I	10	20	30	231±125 (n=13)	0.00	0.85	0.51	0.84	100
II	10	40	50	527±154 (n=19)	0.13	0.84	0.13	0.77	0
III	10	20	30	478±254 (n=12)	0.09	0.69	0.14	0.80	35
IV	10	10	20	491±194 (n=11)	0.19	0.65	0.16	0.67	16

Table 2. The ratios of phosphate and ammonia concentration in the feed and external anoxic reactor at different $SRT_{ext.reactors}$

SRT (days)	10	20	40
$PO_4^{3-}{}_{anoxic}/PO_4^{3-}{}_{feed\ sludge}$	1.3	2.7-3.0	2.5
$NH_4^{+}{}_{anoxic}/\ NH_4^{+}{}_{feed\ sludge}$	1.2	2.5-3.2	3.3

Table 3. The removal of ammonia and nitrate in the external aerobic/anoxic reactor at different $SRT_{ext.reactors}$

SRT (days)	10	20	40
NH_4^+ removal (%)	76	60-62	None
NO_3^- removal (%)	62	15-37	6
mg O_2/mg NH_4^+-N	2.2	1.5	0.8
mg COD/mg NO_3^--N	2.9	3.5-3.9	3.6

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Table 4. Sludge concentration, CST, and TS after dewatering when SRT_{SBR} was 10 days and $SRT_{ext.reactors}$ was 10 and 20 days

$SRT_{ext.reactors}$ (days)	Sludge	MLSS (g/L)	MLVSS/ MLSS ratio	CST [c] (sec)	Specific CST [c] (sec·L/g MLSS)	Dewatered cake [c] TS (%)
10	SBR_{OSA}	1.47	0.70	7.2±0.3; n=3	4.9	29.2±9.6; n=2
	$SBR_{control}$	2.36	0.75	6.5±0.1; n=3	2.7	20.3±0.4; n=2
	WAS_{OSA} [a]	2.02	0.71	7.7±0.1;n=3	3.8	20.2±1.4
	$WAS_{aerobic\ digester}$ [b]	4.43	0.47	10.6±0.8; n=3	2.4	19.8±5.7; n=3
20	SBR_{OSA}	3.24	0.71	10.1±0.2; n=3	3.1	17.5±1.7; n=2
	$SBR_{control}$	3.22	0.75	12.3±0.2; n=3	3.8	7.2±6.3; n=2
	WAS_{OSA} [a]	3.05	0.71	10.6±0.5; n=3	3.5	18.6±3.1; n=4
	$WAS_{aerobic\ digester}$ [b]	6.87	0.74	49.0±1.8; n=3	7.1	8.1±5.0; n=4

[a] WAS from the external aerobic/anoxic reactor. This was compared with the sludge from $SBR_{control}$

[b] From the single-pass aerobic digester appended to $SBR_{control}$

[c] CST of unconditioned sludge was measured

[d] TS of dewatered cake was measured. Dewatered cake was produced after conditioning and centrifugation of sludge.

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Figure 1 Schematic diagram of (a) the OSA system comprised of SBR_{OSA} attached to intermittently aerated (*i.e.*, aerobic/anoxic) and anoxic reactors, and (b) the control system (Blank) comprised of $SBR_{control}$ attached to a single-pass aerobic digester.

Figure 2. tCOD of SBR_{OSA} and $SBR_{control}$ when SRT_{SBRs} was 10 days and $SRT_{ext.\ reactors}$ was varied. The dashed line indicates different $SRT_{ext.reactors}$.

Figure 3. Ammonia and orthophosphate concentration of $SBR_{control}$ and SBR_{OSA} when SRT_{SBRs} was 10 days and $SRT_{ext.reactors}$ was varied. The dashed line indicates different $SRT_{ext,reactors}$.

Figure 4. MLVSS concentration in the OSA system reactors when SRT_{SBRs} was 10 days and $SRT_{ext.\ reactors}$ was varied. The dashed line indicates different $SRT_{ext.reactors}$.

Figure 5. Ammonia, phosphate, and nitrate concentration in the filtered supernatant of the feed sludge and external aerobic/anoxic and anoxic reactors at different $SRT_{ext.\ reactors}$. The box plot represents the average, median, maximum and minimum values when SRT was varied in the following sequence: 20 (number of samples n=13), 40 (18), 20 (16), and 10 (11) days.

Figure 6. ORP in the reactors of the OSA system at different $SRT_{ext.reactors}$. The dashed line indicates different $SRT_{ext.reactors}$.

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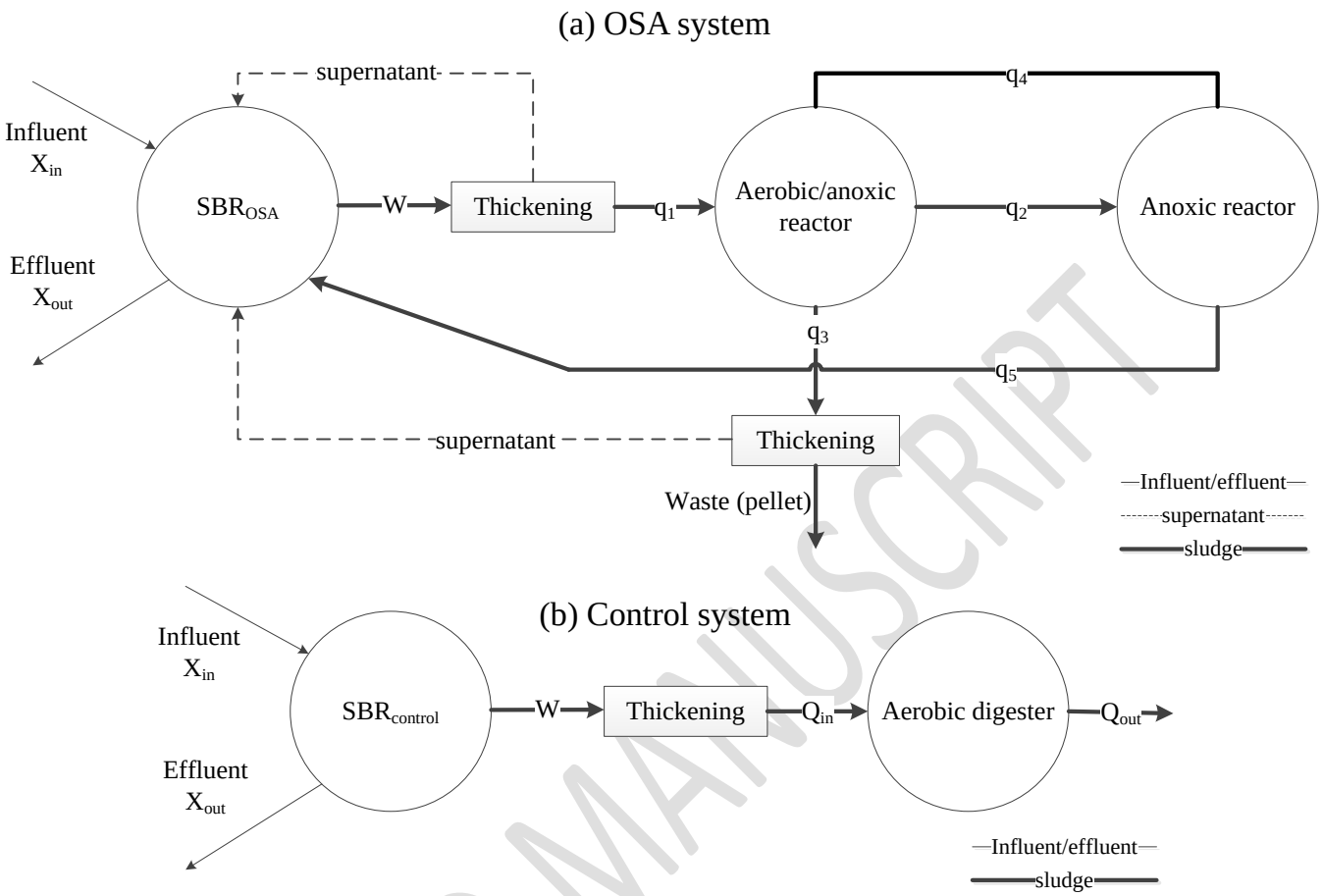


Figure 1

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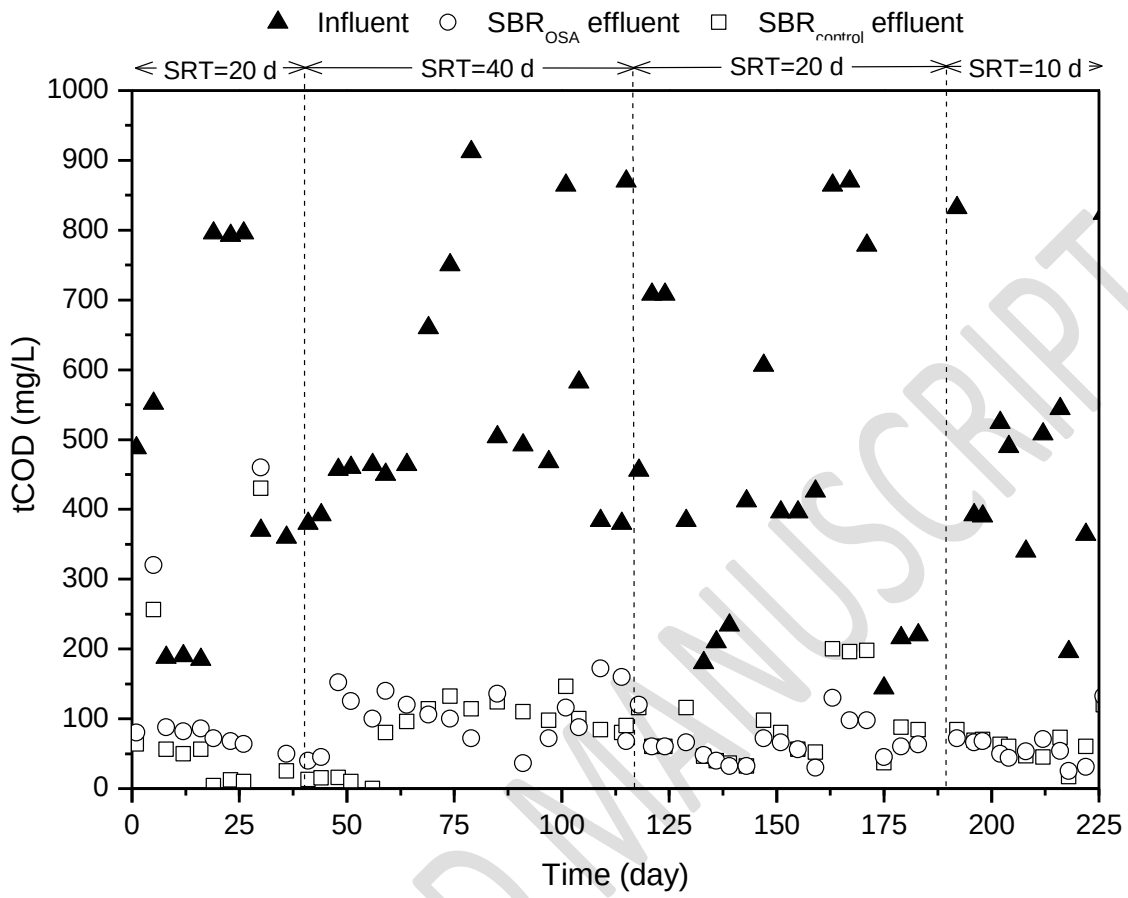


Figure 2

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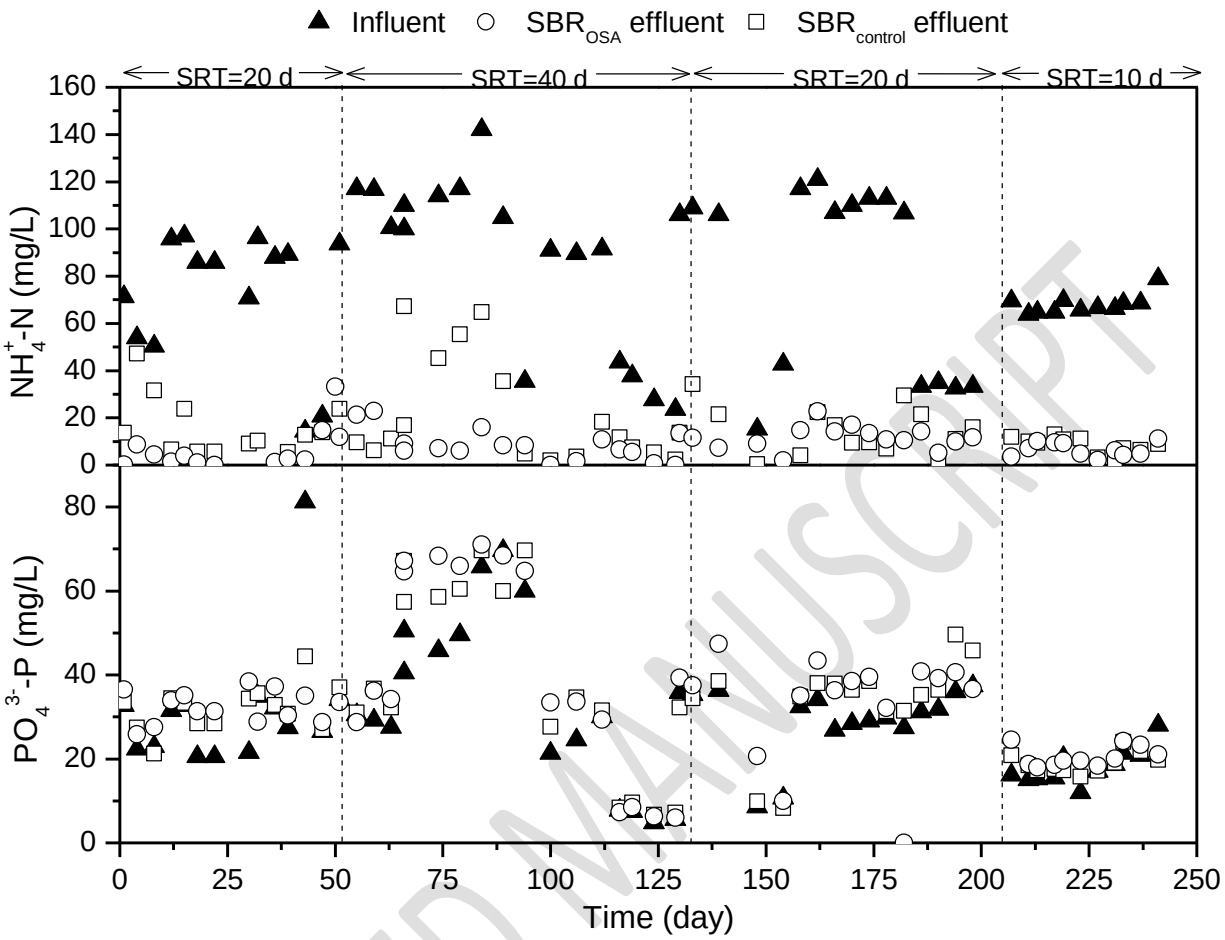


Figure 3

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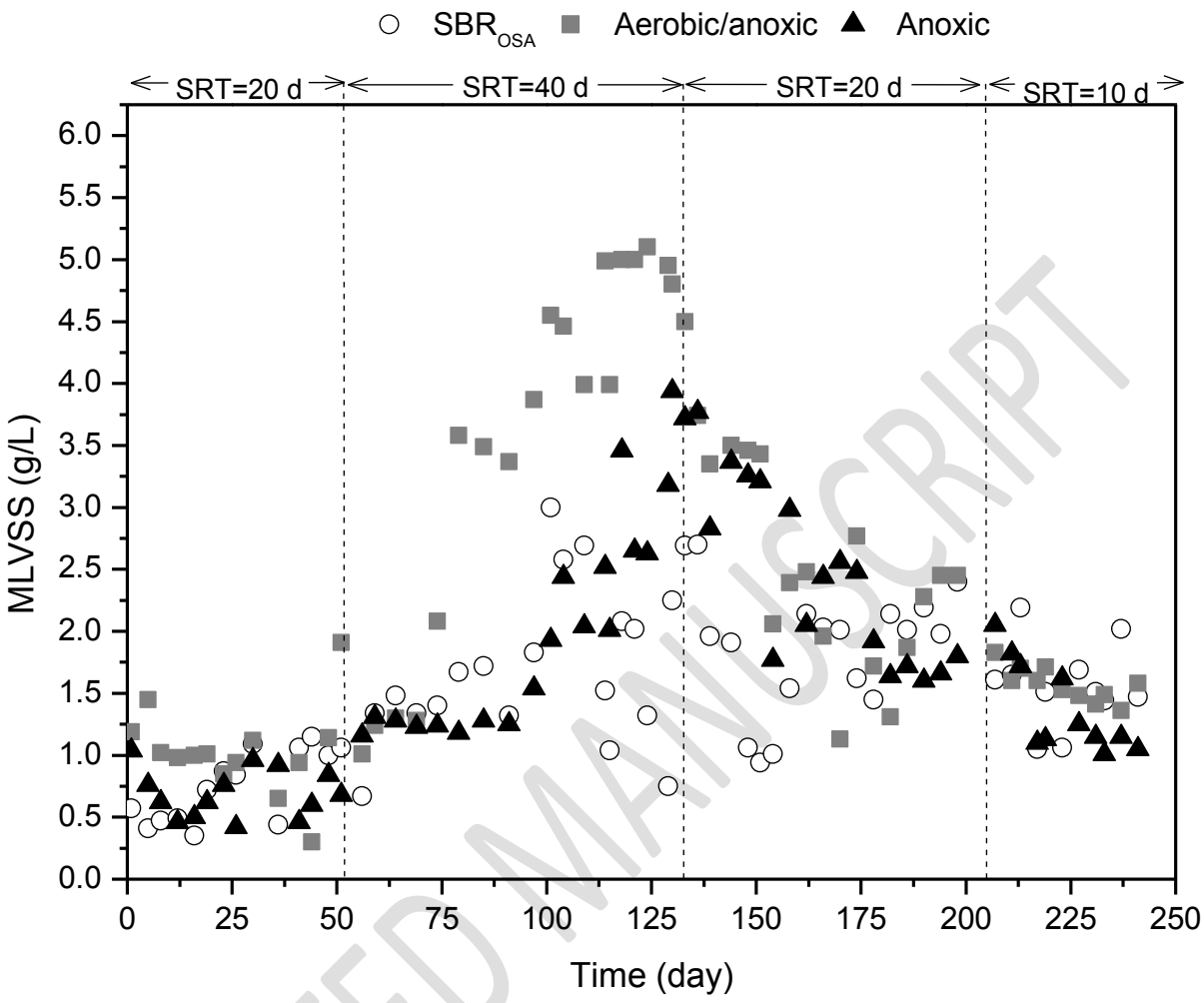


Figure 4

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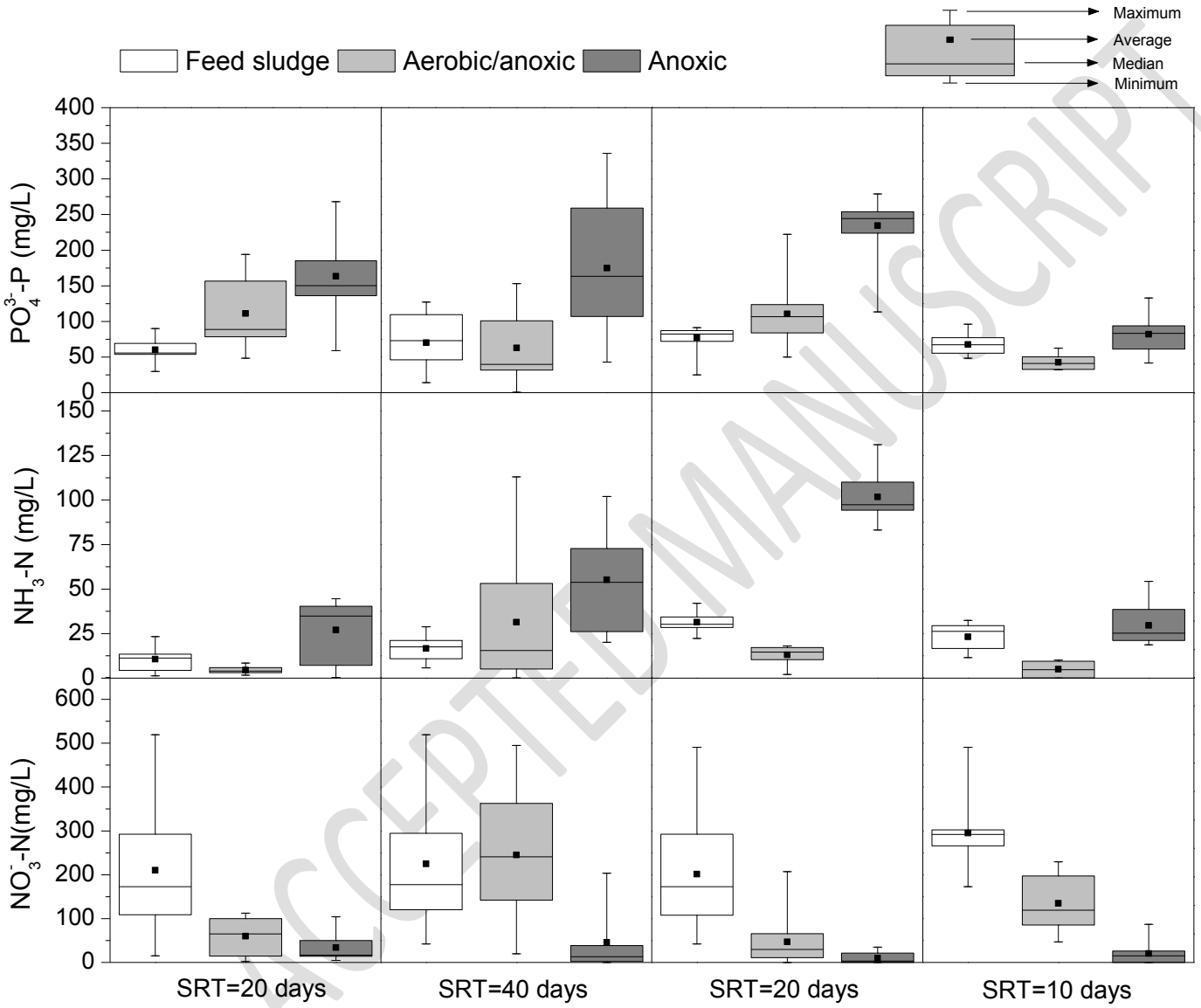


Figure 5

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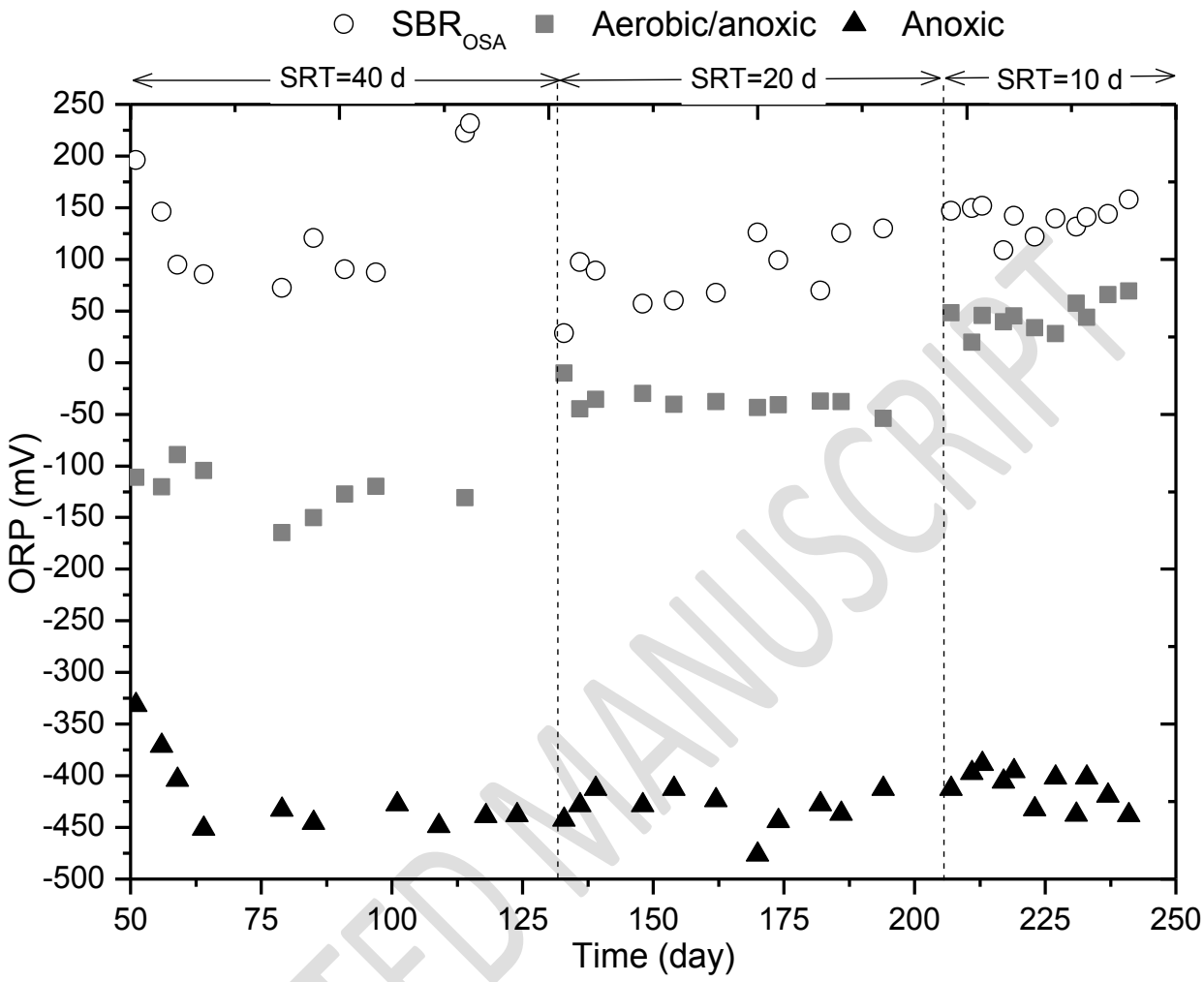


Figure 6

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Effects of sludge retention time on oxic-settling-anoxic process performance: biosolids reduction and dewatering properties

Supplementary Data submitted to
Bioresource Technology

July 2016

Galilee U. Semblante [a], Faisal I. Hai [a]*, Heriberto Bustamante [b], William E. Price [c], 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] Sydney Water, Parramatta, NSW 2124, Australia
[c] 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

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Table S1. Properties of domestic sewage where n=number of samples

Property	Average	*n*
tCOD	474±292 mg/L	72
sCOD	101±54 mg/L	72
TOC	47.2±23.5 mg/L	70
TN	45.0±11.1 mg/L	70
NH_4^+-N	78.4±32.1 mg/L	69
PO_4^{3-}-P	30.3±14.7 mg/L	69
Total P	21.3±14.5 mg/L	28
pH	7.2±0.5	61

Table S2. Calculation of sludge yield

The sludge yield *Y* of $SBR_{control}$ and SBR_{OSA} was determined as the slope of the linear regression of the cumulative sludge produced (g mixed liquor suspended solids, MLVSS) versus cumulative substrate consumed (g total chemical oxygen demand, tCOD). The MLVSS produced by SBR_1 (P_{SBR1}) and SBR_2 (P_{SBR2}) at a given time interval were quantified using a mass balance of biomass and shown in Equation S.1 and S.2, respectively:

$$P_{SBR1} = \Delta MLVSS_{SBR1} \times V_{SBR1} + (VSS_{e1} \times X_{out} + MLVSS_{SBR1} \times W - VSS_i \times X_{in}) \times \Delta t \quad \text{Equation S.1}$$

$$P_{SBR2} = \Delta MLVSS_{SBR2} \times V_{SBR2} + (VSS_{e2} \times X_{out} + MLVSS_{SBR2} \times W - VSS_i \times X_{in} - MLVSS_{ANX} \times q_5) \times \Delta t \quad \text{Equation S.2}$$

wherein $MLVSS_{SBR1,\ SBR2\ or\ ANX}$ is the biomass concentration of the SBRs and the anoxic reactor, $V_{SBR1\ or\ SBR2}$ is the effective reactor volume, VSS_i is the volatile suspended solids concentration of the influent, $VSS_{e1\ or\ e2}$ is volatile suspended solids concentration of the effluent, $X_{in\ or\ out}$ is flow rate of the influent or effluent, *W* is the flow rate of sludge wasted from the SBRs, q_5 is the flow rate of sludge returned from the anoxic reactor to SBR_2 (Section 2.2), and *t* is time. Notably, VSS_i is deducted from the calculation of P_{SBR1} and P_{SBR2} to discount the significant amount of volatile solids carried by real wastewater (e.g. 0.1-0.5 g/L), and $MLVSS_{ANX}$ is deducted from the calculation of P_{SBR2} to discount the biomass that was only recycled back to SBR_2 from OSA.

The amount of substrate consumed *C* by individual SBRs was calculated according to Equation S.3:

$$C = (tCOD_i - tCOD_{e1or e2}) \times X_{in} \times \Delta t \quad \text{Equation S.3}$$

wherein $tCOD_i$ and $tCOD_{e1/e2}$ are the tCOD of the influent and effluent, respectively.

The *Y* of the control and OSA systems were additionally determined. The synthesis of cells in the aerobic digester and OSA may occur even under limited substrate conditions when

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microorganisms consume products of cell lysis (Hao et al., 2010), so those reactors may also contribute to MLVSS production of the whole system. The MLVSS production of the control ($P_{Control}$) and OSA (P_{OSA}) systems were calculated using Equations S.4 and S.5, respectively:

$$P_{control} = \Delta MLVSS_{SBRcontrol} \times V_{SBRcontrol} + \Delta MLVSS_{AE} \times V_{AE} + (VSS_{e1} \times X_{out} + MLVSS_{AE} \times Q_{out} - VSS_i \times X_{in}) \times \Delta t \quad \text{Equation S.4}$$

$$P_{OSA} = \Delta MLVSS_{SBR_{OSA}} \times V_{SBR_{OSA}} + \Delta MLVSS_{AE/ANX} \times V_{AE/ANX} + \Delta MLVSS_{ANX} \times V_{ANX} + (VSS_{e2} \times X_{out} + MLVSS_{AE/ANX} \times q_3 - VSS_i \times X_{in}) \times \Delta t \quad \text{Equation S.5}$$

wherein $MLVSS_{AE/ANX\ or\ ANX}$ is the sludge concentration of the aerobic/anoxic and anoxic reactors, $V_{AE,\ AE/ANX\ or\ ANX}$ is the effective digester or reactor volume, Q_{out} is the flow rate of sludge wasted from the aerobic digester, and q_3 is flow rate of sludge wasted from the aerobic/anoxic reactor. Notably, the sludge interchanged within the external reactors and between SBR_{OSA} and external reactors were retained in the system hence it is not necessary to deduct those sludge flows from the calculation of P_{OSA}. The net substrate consumption of the system was calculated using Equation S.3.

Table S3. Sludge yield of control (combined $SBR_{control}$ and aerobic digester) and OSA systems (combined SBR_{OSA} and external aerobic/anoxic,and anoxic reactors) when SRT_{SBRs} was 10 days and $SRT_{ext.reactors}$ was varied.

Phase	SRT_{SBRs}	$SRT_{ext.\ reactors}$	Total SRT	Influent tCOD concentration (mg/L)	Sludge yield (g MLVSS/g tCOD)				Sludge yield reduction (%)
					Control system	R^2	OSA system	R^2	
I	10	20	30	231±125 (n=13)	0.08	0.64	0.02	0.67	75
II	10	40	50	527±154 (n=19)	0.17	0.92	0.15	0.88	11
III	10	20	30	478±254 (n=12)	0.18	0.78	0.14	0.72	22
IV	10	10	20	491±194 (n=11)	0.05	0.71	0.07	0.53	None

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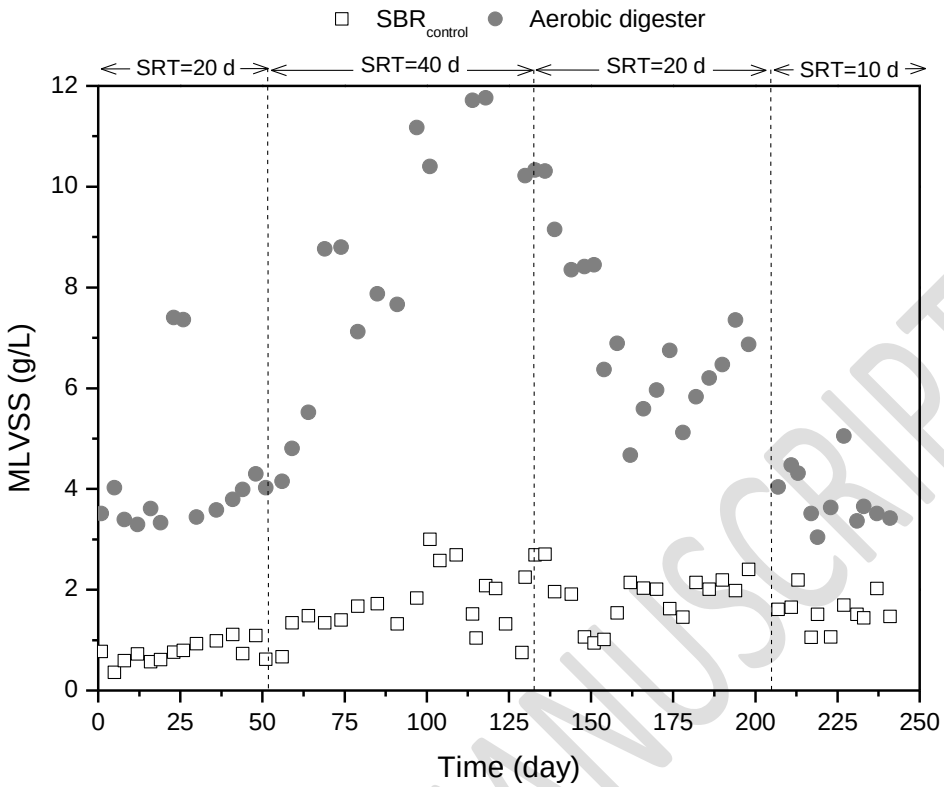
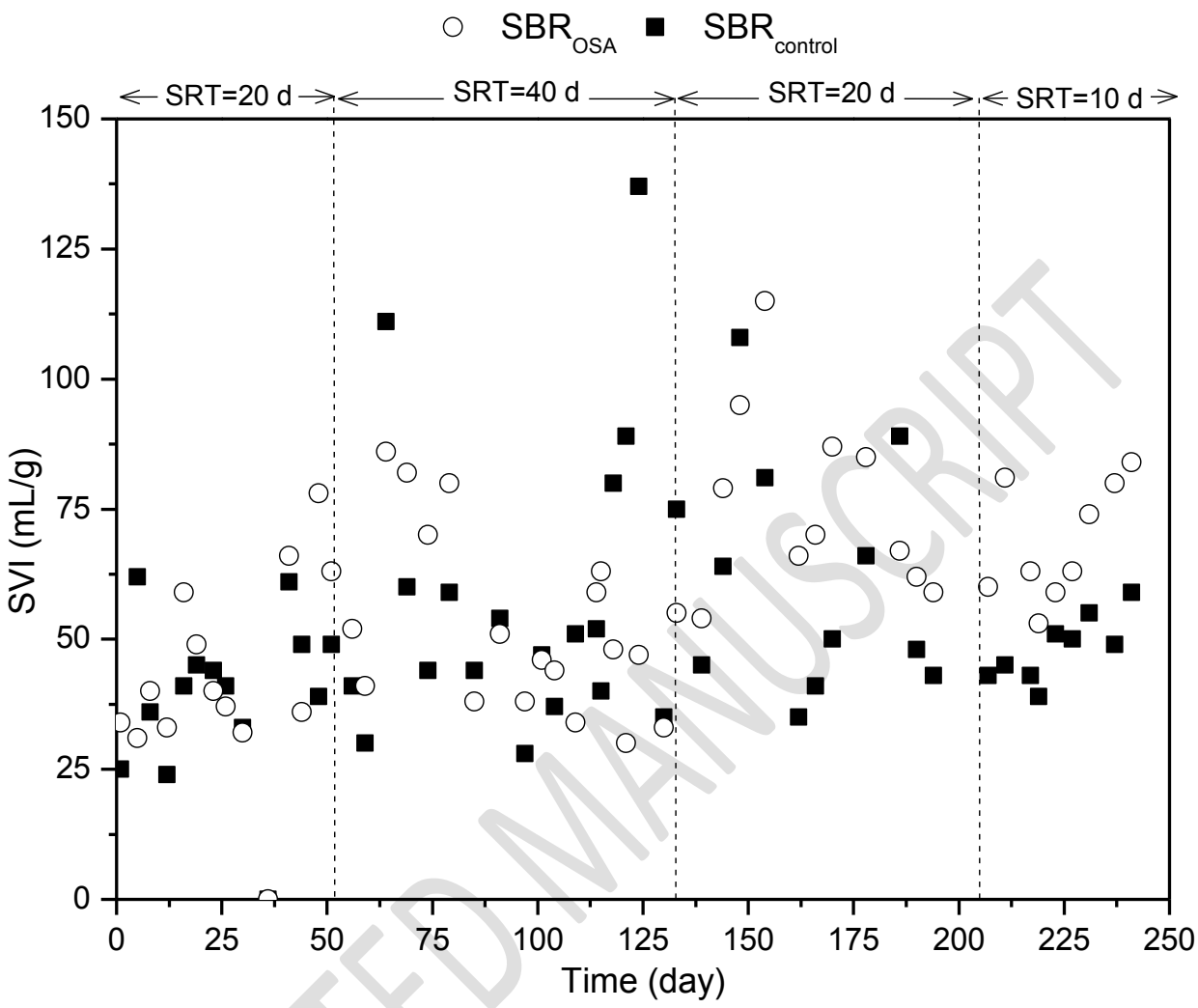


Figure S4. MLVSS concentration in the control system reactors when SRT_{SBRs} was 10 days and $SRT_{ext.\ reactors}$ was varied. The dashed line indicates different $SRT_{ext.reactors}$

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Supplementary Figure S5. SVI of SBR_{OSA} and $SBR_{control}$ when SRT_{SBRs} was 10 days and $SRT_{ext.\ reactors}$ was varied. The dashed line indicates different $SRT_{ext.reactors}$.

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