  Water Science & Technology | 79.6 | 2019

The effects of a full-scale anaerobic side-stream reactor on sludge decay and biomass activity

V. F. Velho, G. Andreottola, P. Foladori and R. H. R. Costa

ABSTRACT

A full-scale anaerobic side-stream reactor (ASSR) for sludge reduction was monitored in terms of sludge production and compared with the previous conventional activated sludge configuration (CAS). A detailed solid mass balance was calculated on the whole full-scale plant to estimate the sludge reduction associated with the ASSR. The activity of the biomass, which undergoes alternation of aerobic and anaerobic conditions, was investigated by the respirometric test. The ASSR promoted a reduction of heterotrophic biomass activity and the substrate consumption rate in the activated sludge implemented with ASSR (AS + ASSR) was 36% smaller than in the CAS period. The solid mass balance indicated a sludge reduction of 28%. During the 270-day operation, the observed sludge yield passed from 0.438 kgTSS/kgCOD in the CAS to 0.315 in the AS + ASSR configuration. The solubilization of chemical oxygen demand (COD), NH_4^+-N and orthophosphate were verified under anaerobic conditions. The results suggest that the possible mechanisms of sludge reduction were the increase of the system sludge retention time (SRT) by ASSR addition, and the reduction in heterotrophic biomass activity added to the organic compounds' hydrolysis.

Key words | biological substrate consumption, hydrolysis, sludge reduction, solids balance, wastewater treatment

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INTRODUCTION

The excess sludge from wastewater treatment plants (WWTP) is one of the most critical issues nowadays, because the micro-pollutants and the high organic content present a great potential for impacts in terms of public and environmental health. Despite the volume of excess sludge produced in a WWTP being only about 1% of the volume of influent wastewater to be treated, its management and disposal are complex and may reach 20–60% of the total cost of wastewater treatment (Liu & Tay 2001). The implementation of European Union Directive 91/271/EC (CEC 1991) forced the Member States to improve their wastewater collecting and treatment systems. As a result, a total amount of annual sewage sludge production of 10.9 million ton dry solids was recorded in 2005 (Kelessidis & Stasinakis 2012). Considering this production is set to rise during the following years, exceeding 13 million ton dry solids up to 2020 (Leonard 2011), it is necessary to develop technologies that are able to reduce the amount of solids to be disposed of. An alternative to solve the waste sludge problem would be the reduction of excess sludge production within the WWTP. The treatment should be cost effective and not affect other treatment characteristics such as effluent quality and settling properties (Foladori *et al.* 2010).

The anaerobic side-stream reactor (ASSR) or oxic-settling-anaerobic (OSA) process is a feasible technique for efficiently reducing excess sludge production at low cost. It presents a good stability of process operation and does not worsen effluent quality and sludge settleability (Foladori *et al.* 2010; Semblante *et al.* 2017; Ferrentino *et al.* 2019). The system modifies the conventional activated sludge system by inserting an anaerobic concentrated sludge tank in the sludge recycling line, obtaining an OSA process. The process has been confirmed to reduce sludge production by 20% to 70% (Foladori *et al.* 2010; Salehiziri *et al.* 2018; Ferrentino *et al.* 2019). The mechanisms involved in this process are not yet well understood. There are some hypotheses on the reduction of excess sludge such as uncoupling metabolism, cell lysis-cryptic growth, maintenance metabolism, predation on bacteria and endogenous decay. According to An & Chen (2008), the mechanism that

doi: 10.2166/wst.2019.104

seems to be at the basis of the OSA system is the sludge decay, due to the phenomena of lysis and hydrolysis in the anaerobic reactor. These authors measured in fact a sludge decay rate in the anaerobic environment ($ka = 0.13\ d^{-1}$) much higher than those reported in the literature in the anaerobic reactor without cyclic changes ($ka = 0.04\ d^{-1}$). Furthermore, it is reasonably assured that the reduction of excess sludge is related to the increase of the sludge decay, which leads to cell lysis, releasing protein compounds that are rapidly assimilated by the biomass (Novak *et al.* 2007; Wang *et al.* 2008). In a previous study, Foladori *et al.* (2015) observed that hydrolysis and solubilization of non-bacterial material occurred under anaerobic conditions in a full-scale ASSR plant, and a large increase in soluble biodegradable chemical oxygen demand (COD) and NH_4^+-N in the anaerobically treated sludge was noticed; conversely, bacterial cell decay and lysis occurred mainly under aerobic conditions.

To understand what happens to the microorganisms when they are subjected to cyclical alternation between aerobic and anaerobic conditions, in the last few years, several studies with the OSA process and ASSR system have been applied. The results obtained by different authors have always shown the validity of the OSA process from the point of view of both the efficiency in the wastewater treatment and the reduction of excess sludge production compared to a conventional activated sludge system (Foladori *et al.* 2010; Salehiziri *et al.* 2018; Ferrentino *et al.* 2019). However, many of these studies have been applied at laboratory scale using synthetic wastewater, which enables the control of the typical operating parameters and simplifies the data acquisition of sludge production, generally related to the observed sludge yield values for the assessment of sludge reduction, but does not exactly represent a real picture of wastewater complexity.

To the best of our knowledge, only a few studies have focused on the ASSR system fed with real raw wastewater; nevertheless, these studies were conducted at laboratory scale (Coma *et al.* 2013; Zhou *et al.* 2015; Martins *et al.* 2016; Semblante *et al.* 2017; Ferrentino *et al.* 2019). This study depicts the monitoring of a full-scale plant performed with real wastewater for sludge reduction. Therefore, the aim of this study was to investigate the excess sludge production in a full-scale WWTP modified for sludge reduction (Levico WWTP, North Italy) by applying a solid mass balance from the start-up of the ASSR through an overall period of 270 days.

To understand the effects of the alternate cycles of anaerobic and aerobic conditions on the heterotrophic and autotrophic biomass activity, respirometric tests were applied to mixed liquor samples taken from the ASSR and the aerobic reactors. Furthermore, the role of AS + ASSR configuration on sludge decay, in terms of COD, NH_4^+-N and phosphorous solubilization were also discussed. The results of this study are expected to verify the role of ASSR on sludge decay in terms of hydrolysis and biomass activity, and provide a useful route for sludge reduction determination.

MATERIALS AND METHODS

Full-scale WWTP and ASSR configuration

The study was conducted in a WWTP in Levico (North Italy) that treats a mean population equivalent of 48,000. The WWTP consists of pre-treatments (screening, degritting), biological pre-denitrification (two reactors, total volume of 2,000 m^3), oxidation/nitrification basins (three reactors, total volume of 5,000 m^3) and final settlers (three tanks, total volume of 4,763 m^3). The sludge line is composed of an aerobic digester for the stabilization of secondary waste sludge. The digested sludge is thickened and subsequently dewatered by centrifugation.

The CAS configuration (conventional activated sludge) was modified by introducing an ASSR tank (Cannibal® process, volume of 2,293 m^3) in the return activated sludge (RAS) circuit, obtaining a configuration called hereafter AS + ASSR.

The characterization of the influent wastewater and the operational conditions in the WWTP are summarised in Table 1.

Prior to the ASSR tank, the RAS was screened for inert coarse solids separation, which jointly with the thickened sludge comprises the excess waste sludge. The hydrocyclones, based on centrifugal forces, were included in the original implementation for separating heavy organic material, grit and dense inorganic particles from RAS. However, the mass separated was negligible during the operation and thus the use of hydrocyclones was stopped and not included in this monitoring.

The ASSR tank treats a part of the sludge (330 m^3/d) separated from the return flow before being returned to the activated sludge reactors, resulting in an amount of 8.0% of the activated sludge mass recirculated daily in the ASSR. The ASSR tank was equipped with an intermittent mixer in order to ensure a homogenous condition in the sludge. The theoretical hydraulic retention time (HRT) in the ASSR tank was about 7 d, coinciding in practice with the sludge retention time (SRT) in the anaerobic tank. The

Table 1 | Operational conditions in the WWTP and characterization of the influent wastewater

Parameter	Unit	CAS	AS + ASSR
Operational conditions			
Duration of monitoring	Days	30	240
Number of samples	No.	4	35
Organic loading rate	$g\ COD{\cdot}m^{-3}{\cdot}d^{-1}$	738	815
Nitrogen loading rate	$g\ TN{\cdot}m^{-3}{\cdot}d^{-1}$	84	86
Hydraulic retention time in the anaerobic tank	Days	–	6.9
Sludge retention time	Days	17	32
Influent wastewater characteristics			
Flow rate	m^3/d	14,998 ± 4,988	15,043 ± 5,703
Chemical oxygen demand (COD)	mg/L	342 ± 116	379 ± 107
Biochemical oxygen demand at 5 days (BOD_5)	mg/L	125 ± 73	181 ± 43
Total nitrogen (TN)	mg/L	40 ± 14	40 ± 11
Ammonium-nitrogen (NH_4^+-N)	mg/L	31 ± 14	28 ± 12
Total phosphorus (TP)	mg/L	4.7 ± 3.1	3.5 ± 1.5
Total suspended solids (TSS)	mg/L	144 ± 55	188 ± 33
Temperature	°C	16.8 ± 4.1	12.2 ± 3.6

CAS phase refers to conventional activated sludge; AS + ASSR phase refers to activated sludge implemented with ASSR system.

applied sludge loading rate (ASLR) was calculated according to Coma *et al.* (2013). It considers the flow rate and the VSS concentration entering the ASSR divided by the ASSR volume. The ASLR value was 1.4 kg VSS m^{-3} d^{-1}, which indicates the quantity of sludge treated daily in the anaerobic holding tank.

Low oxidation-reduction potential (ORP) values (below −250 mV) were maintained inside the ASSR tank and values of pH were 6–6.5. The average TSS concentration maintained within the aerobic reactor was 4.0 g/L in the CAS configuration and around 5.0 g/L in the AS + ASSR configuration; the average TSS concentration within the ASSR was 9.1 g/L.

Sampling

The AS + ASSR configuration of Levico WWTP was operated for five straight years (January 2008 – March 2013) until the ASSR tank was emptied for structural maintenance. The present study was conducted before and after the reactivation of the AS + ASSR system, for a total period of 270 days (October 2013–June 2014). The system was operating as a conventional activated sludge system (CAS) at the beginning of the monitored period (30 days), and then the AS + ASSR system was followed up in the remaining period (240 days). The new start-up of the system allowed observation of the evolution of sludge production in the modified configuration of the WWTP.

Sampling was carried out throughout the sewage and sludge treatment process (Figure 1). All samples were taken at the outlet of each treatment step including pre-denitrification tanks (DN_1 and DN_2), oxidation/nitrification tanks (OX_1, OX_2 and OX_3), final settlers (S_1, S_2 and S_3), sludge aerobic stabilization tank (STA) and ASSR. All the samples were analyzed for total suspended solids (TSS), except for thickened and screened sludge, which were analyzed for dry solids (DS).

The common parameters of wastewater treatment were monitored weekly during all the monitoring phases, ensuring the steady state of the WWTP. Specific routines such as respirometric tests, flow cytometry and solid mass balance were established from October 2013.

Sludge samples from the oxidation/nitrification tanks and anaerobic-side-stream reactor were also taken for the biomass activity characterization by respirometric tests.

Analytical methods

Biochemical oxygen demand at 5 days (BOD_5), COD, total nitrogen (TN), NH_4-N, total phosphorus (TP), orthophosphate (PO_4^{3-}P) and TSS were analyzed in the influent and effluent wastewater according to *Standard Methods* (APHA 2012).

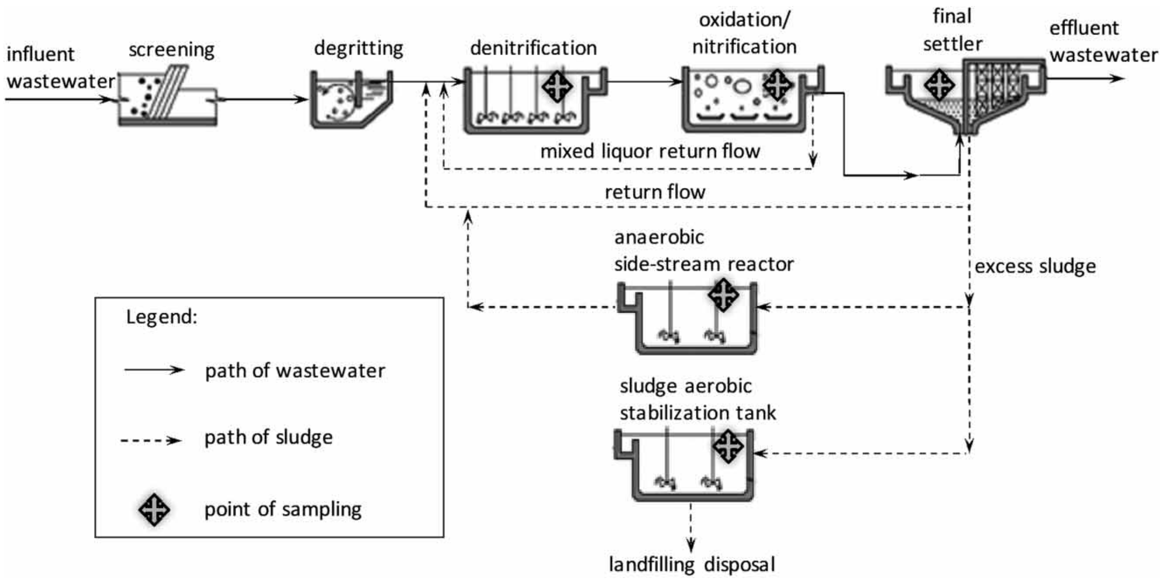

Figure 1 | Scheme of the full-scale WWTP modified with the implementation of the ASSR.

Analyses of TSS and volatile suspended solids (VSS) were carried out on sludge samples taken from the biological reactors and from the ASSR tank (APHA 2012). DS was analyzed on sludge (APHA 2012) to calculate excess sludge production.

Analysis of variance (ANOVA) was conducted using STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA). Tukey's test at a significance level of 5% was used to compare the average values of the parameters obtained between the two operated configurations of the system.

Specific oxygen uptake rate

Respirometry tests were performed to measure the specific oxygen uptake rate (SOUR) of the biomass during the conventional (CAS) and modified (AS + ASSR) configuration of the activated sludge system. It enables investigation of the activity of the biomass, which had undergone alternate aerobic and anaerobic conditions. The maximum substrate consumption rate (v_{max}) was determined according to Andreottola *et al.* (2002), by assuming Michaelis-Menten kinetics to describe the experimental SOUR data.

Mass balance calculation

Mass balance was calculated to estimate the sludge production of the AS + ASSR system, and to estimate the sludge reduction in comparison with the CAS configuration. The balance was done considering the following solids contributions: (i) quantity of sludge removed (dewatered and screened sludge); (ii) quantity of sludge leakage (sludge leaving the effluent); and (iii) variation of internal mass (sludge inside the system units). Therefore, the balance of solids was given as:

$$X_{total} = X_{dewatered} + X_{effluent} + \Delta X_{system} \quad [ton\ \ TSS] \tag{1}$$

Equation (1) can be clarified separately as:

$$X_{dewatered} = X_{mech\_dew} + X_{screened} = \{[P_{mech\_dew}] .[\%\ \ of\ \ dry\ \ matter] + [P_{screened}] .[\%\ \ of\ \ dry\ \ matter]\} \tag{2}$$

$$X_{effluent} = C_{effluent} \cdot Q_{effluent} \tag{3}$$

where $X_{dewatered}$ – mass of sludge dewatered (kg/d); $P_{mech-dew}$ – amount of sludge daily mechanically dewatered (kg/d); $P_{screened}$ – amount of coarse sludge separated daily before the ASSR system (kg/d); $X_{effluent}$ – mass of sludge leakage in the effluent (kg/d); $C_{effluent}$ – TSS concentration in the effluent (g/m^3); $Q_{effluent}$ – effluent flow rate (m^3/d).

Variation of internal mass (ΔX_{system}) is the difference between total mass in the system from the initial ASSR operational day ($X_{November/13}$) to the last day of the sampling period ($X_{June/14}$) and it can be calculated as:

$$\Delta X_{system} = X_{June/14} - X_{November/13} \quad [ton\ \ TSS] \tag{4}$$

$$X_{November/13} = \sum (C_i.\ V_i)_{November/13} \quad [ton\ \ TSS] \tag{5}$$

$$X_{June/14} = \sum (C_i.V_i)_{June/14} \quad [ton\ \ TSS] \tag{6}$$

where $(C_i. V_i)_{November/13}$ and $(C_i.V_i)_{June/14}$ – quantity of sludge inside the treatment units on the initial ASSR operational day and the last day of the sampling period, respectively. It can be achieved by multiplying the volume and TSS concentration of each treatment unit of the system: pre-denitrification tanks (DN_1 and DN_2), oxidation/nitrification tanks (OX_1, OX_2 and OX_3), final settlers (S_1, S_2 and S_3), sludge aerobic stabilization tank (STA) and anaerobic side-stream reactor (ASSR).

The observed sludge yield (Y_{obs}) is defined as the ratio of the amount of sludge produced and the amount of COD removed from the system. This can be expressed as:

$$Y_{obs} = \frac{X_{total}}{(COD_{in} - COD_{out}).\ Q_{influent}} \quad [ton\ \ TSS/ton\ \ COD] \tag{7}$$

where X_{total} – mass of sludge production in terms of TSS (ton); COD_{in} and COD_{out} – influent and effluent COD concentration (mg/L), respectively; $Q_{influent}$ – influent flow rate (m^3/d).

RESULTS AND DISCUSSION

Performance of AS + ASSR system on wastewater treatment and sludge reduction

A summary of the effluent characteristics is shown in Table 2. The results showed that almost all measured parameters were not impaired after inserting ASSR. Substrate removal in terms of COD and BOD_5 presented up to 94% of removal efficiency. The removal efficiencies were in the common range for OSA and ASSR process (84–91%) treating real wastewater (Zhou *et al.* 2015; Martins *et al.* 2016; Ferrentino *et al.* 2019).

Table 2 | Effluent characteristics of CAS and AS + ASSR and removal efficiencies

	CAS		AS + ASSR	
Parameter	Value	Removal (%)	Value	Removal (%)
COD (mg/L)	19 ± 3	94	20 ± 3	95
BOD_5 (mg/L)	5.0 ± 0.1	96	5.0 ± 0.3	97
TN (mg/L)	9 ± 2	78	9 ± 3	78
NH_4^+-N (mg/L)	0.55 ± 0.11	98	0.49 ± 0.14	98
TP (mg/L)	1.6 ± 1.0	66	1.4 ± 0.7	60
TSS (mg/L)	5.3 ± 0.3	96	6.7 ± 3.2	96

CAS phase refers to conventional activated sludge; AS + ASSR phase refers to activated sludge implemented with ASSR system.

ANOVA and Tukey's test results demonstrated that phosphorus removal efficiency was slightly decreased after inserting ASSR. The fate of total phosphorus and orthophosphate in the biological systems aimed at sludge reduction are not yet fully clarified. Although phosphorus removal efficiencies of 60–70% may be reached, exceeding up to 90% (Ferrentino *et al.* 2019), the ASSR can affect the mechanism of biological phosphorus removal. In OSA-like processes an increase of orthophosphate concentration in the effluent could be observed (Foladori *et al.* 2010; Zhou *et al.* 2015).

The performance of the AS + ASSR system in terms of sludge reduction was evaluated considering the net mass balance of the system. For ASSR reactivation (November 2013) the excess sludge produced in the WWTP was used for filling the ASSR, which took approximately 10 days; then the recirculation of the anaerobically treated sludge began.

Table 3 summarizes the solids balance results achieved in the AS + ASSR system during the monitored period. It can be noticed a great difference of solids amount and distribution through the WWTP. The units of the CAS system (pre-denitrification, oxidation tanks and final settlers) presented values higher than those measured in the AS + ASSR configuration.

After the start-up of the AS + ASSR system was possible to observe the variation on sludge mass distribution. A significant mass of sludge was included in the ASSR tank, and a considerable higher mass was observed in the STA unit. In the other tanks, a general reduction of sludge mass was observed. In particular, the AS + ASSR system mainly influenced the final settlers' operation. A reduction of 64% of total solids mass was measured in the final settlers in comparison with the CAS configuration, which should be attributed to the rise of the total system volume.

Figure 2 presents the sludge production and the amount of COD removed during the operation of AS + ASSR configuration (November 2013–June 2014).

The cumulative sludge production during the AS + ASSR operation (from Nov/13 to Jun/14) in terms of mechanically dewatered and screened sludge were 346 and 23 ton TSS, respectively. The cumulative quantity of sludge leakage in the effluent during this period was 20 ton TSS, and the cumulative amount of COD removed in the system was 1,261 ton COD.

The excess sludge production was determined by the observed sludge yield as described in Equation (7) (Y_{obs}, expressed as ton TSS/ton COD removed). The reduction

Table 3 | Solids mass balance of the AS + ASSR system during the monitored period

Unit	Abbreviation	CAS $X_{November/13}$ (ton TSS)	AS + ASSR $X_{June/14}$ (ton TSS)	Difference $X_{June/14} - X_{November/13}$ (ton TSS)
Sludge aerobic stabilization tank	STA	12.75 ± 1.15	20.92 ± 2.31	8.17
Pre-denitrification tanks	DN_1	4.05 ± 0.33	3.58 ± 0.23	−0.47
	DN_2	4.46 ± 0.24	3.51 ± 0.28	−0.95
Oxidation/nitrification tanks	OX_1	10.02 ± 1.52	8.00 ± 1.17	−2.02
	OX_2	5.06 ± 0.65	4.05 ± 0.57	−1.01
	OX_3	10.6 ± 1.06	8.94 ± 1.17	−1.66
Final settlers	S_1	2.34 ± 0.19	1.50 ± 0.10	−0.84
	S_2	2.84 ± 0.27	1.22 ± 0.07	−1.62
	S_3	9.81 ± 0.36	1.21 ± 0.04	−8.60
Anaerobic side-stream reactor	ASSR	0	16.65 ± 1.25	16.65
Total mass in the system (ton TSS)		**61.93**	**69.58**	–
ΔX_{system} (ton TSS)		–	–	**7.65**

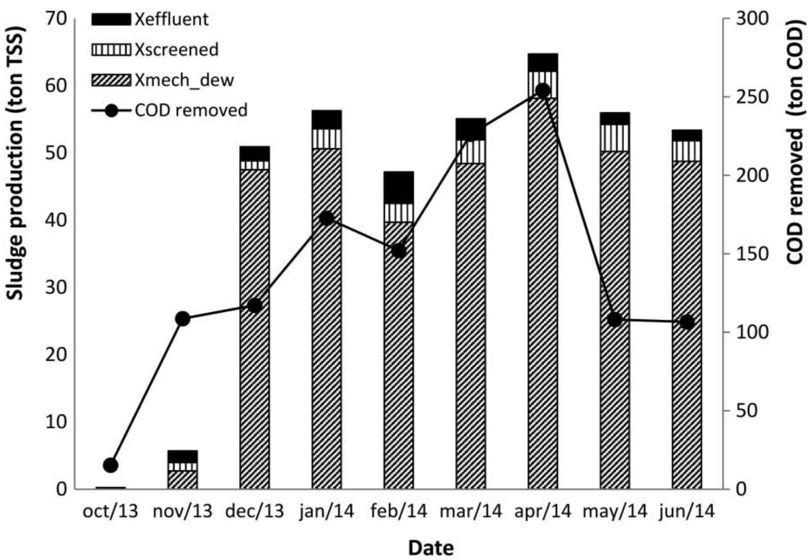

Figure 2 | The sludge production and the amount of COD removed during the AS + ASSR operation.

of excess sludge production was calculated by comparing the specific sludge yield values achieved during CAS and AS + ASSR configurations. The results obtained for the variables used to evaluate the reduction of excess sludge production are summarized in Table 4. In the AS + ASSR configuration, Y_{obs} was 0.315, leading to a mean sludge reduction of 28% compared to the CAS configuration. The sludge reduction of 28% observed in this monitored period was slightly higher than that estimated in a previous study (Velho *et al.* 2016b) for the same operational configuration, where the AS + ASSR configuration monitored for a long period (five years) showed a mean sludge reduction of 20%.

The introduction of the ASSR promoted an increase of 88% in the system SRT. According to Salehiziri *et al.* (2018), the reduction of sludge production can be attributed to the endogenous mechanisms due to the long SRT owing to the addition of the ASSR in the system (SRT > 30 days). However, this mechanism could not be considered as being only responsible for sludge reduction, once compared to the systems with high sludge age (sequencing batch reactor systems), the AS + ASSR system presents an additional reduction of 14–16% (Goel & Noguera 2006). Habermacher *et al.* (2015) verified that significant sludge reduction can be achieved in AS + ASSR by increasing the overall SRT. The authors highlighted that increasing the overall SRT from

Table 4 | Observed sludge yield (Y_{obs}) and percentage of sludge reduction in AS + ASSR configuration

Configuration	Operational period	X_{total} (ton TSS)	$COD_{removed}$ (ton COD)	Observed sludge yield (Y_{obs}, ton TSS/ton COD)	Reduction of Y_{obs}
CAS	Apr 13–Oct 13	392.43	913.37	0.438	–
AS + ASSR	Oct 13–Jun 14	397.30	1,261.14	0.315	−28%

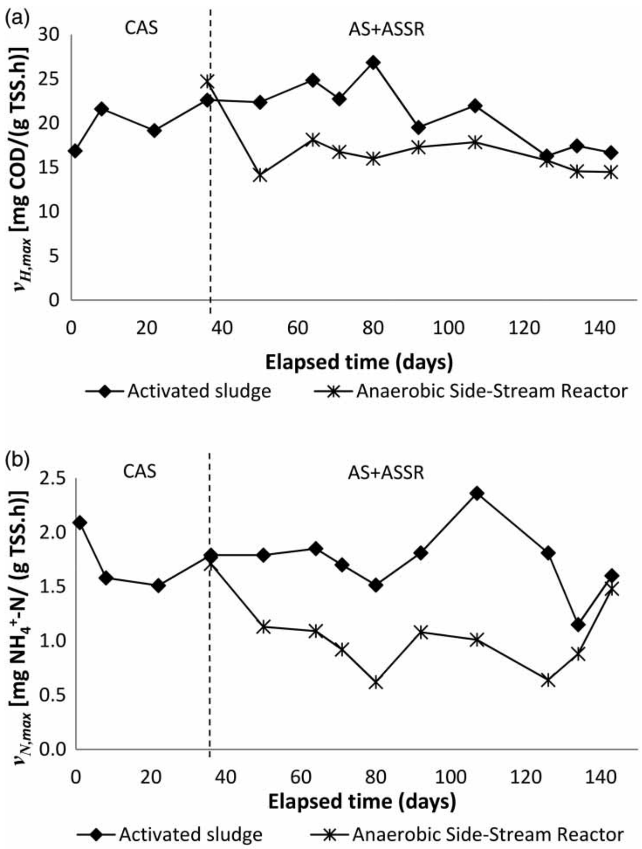

Figure 3 | Time-profiles of maximum specific oxidation rate for heterotrophic (a) and nitrifying (b) biomass under the two operational conditions.

high (25 d) to very high (80 d) values helped to further decrease the Y_{obs} by up to 36%.

The alternation between aerobic and anaerobic conditions is one of the most important mechanisms in sludge reduction. It enhances solubilization, decay rate (Foladori et al. 2015; Jiang et al. 2018) and degradation of iron and/or aluminum associated particles (Yagci et al. 2015). However, at the full-scale plant, the biomass could adapt to this mechanism during long periods and thus become less susceptible to reduction. Our previous experience has already demonstrated that the implementation of the ASSR led to the immediate reduction of sludge production, but the hypothesized biomass adaptation could be noticed as from 12 months of the monitored period; i.e., a progressive decrease of the sludge reduction performance was observed over the monitored period. The reduction of sludge production was 20% with 16 months of AS + ASSR system operation and decreased to 10% with 47 months (Velho et al. 2016b). The observed sludge reduction in this study could be representative of the immediate sludge reduction observed at the full-scale plant. However, the maintenance of these values should be further investigated once the biomass adaptation has already been verified at the system.

Influence of ASSR on biomass activity

SOUR tests were conducted on mixed liquor samples taken from both the ASSR and the aerobic reactors. It was possible to evaluate the effect of the AS-ASSR system on the performance of the active biomass. Figure 3(a) and 3(b) shows the time profiles of the maximum specific substrate removal rate, measured for the heterotrophic ($v_{H,max}$) and nitrifying ($v_{N,max}$) biomass, respectively.

From Figure 3(a), the difference in the biomass behavior between the activated sludge (aerobic reactor) and the anaerobic sludge (ASSR) appears immediately during AS + ASSR operation. The maximum specific substrate removal rate ($v_{H,max}$) in the ASSR was always lower than in the activated sludge. During the first 90 days, a decrease of 15% of the heterotrophic biomass activity from the anaerobic reactor was observed compared to the biomass activity in the CAS configuration. After that period, a similar decay effect on biomass activity from the aerobic reactor was observed (36% of reduction). It could be stated that approximately three months was required to notice the effects of cyclical transition between aerobic and anaerobic conditions on the biomass activity from the activated sludge.

Similar results were found by Velho et al. (2016a) who observed a decrease on biomass activity in a bench-scale OSA-like process, where a fraction of approximately 15% of returned activated sludge (500 mL) was maintained under anaerobic conditions for 16 hours before being recycled to the aerobic reactor. Saby et al. (2003) and Chen et al. (2003) observed that in an OSA system, the proportion of active bacteria was lower than in the reference system (CAS configuration), which suggests that the bacteria in the OSA sludge may have lower activity. Conversely, Wang *et al.* (2008) and Velho *et al.* (2016a) demonstrated an increase in biomass activity of activated sludge held under short anaerobic periods (8 and 10 hours, respectively). According to Khursheed *et al.* (2015) the rise of biomass activity, measured based on the SOUR test, may be induced by increased energy requirement stimulated by the prolonged fasting in the anaerobic reactor (HRT around 1–2 days). These results suggest that a longer SRT under anaerobic/anoxic conditions may lead to a greater tendency to reduce the biomass activity. Oliveira *et al.* (2018) stated that in plants operated with very high SRT or complete sludge retention, the biomass activity decrease can be associated to biomass aging, which also involves a decrease in the heterotrophic active fraction.

The relatively long HRT (7 days) in the anaerobic tank resulted in stable and low ORP values that were always below −250 mV. According to Torregrossa *et al.* (2012) and Oliveira *et al.* (2018) under low ORP condition biomass underwent to metabolic stress which affects the cell activity and induces the increase of sludge decay coefficient, which explains a low production rate of the excess sludge in the system. In addition, Rodriguez-Perez & Fermoso (2016) have also stated that the anoxic environment has been shown to impact in the cellular metabolism and sludge ecology.

For nitrifying biomass (Figure 3(b)), the maximum specific substrate removal rate ($v_{N,max}$) decreased throughout the AS + ASSR operation. Conversely, activated sludge (biomass taken from the aerobic reactor) maintained the $v_{N,max}$ values in the same range of CAS configuration. It could be assumed that the alternation between anaerobic and aerobic conditions has not promoted a pronounced interference on the nitrifying activity. A reduction of $v_{N,max}$ in the anaerobic sludge was observed; however, the microbial activity was reestablished when the aerobic condition was recovered, and remained as high as in the CAS configuration. The results were consistent with those presented by Capodici *et al.* (2016) which observed that the autotrophic metabolic activity is generally higher in alternating aerobic/anoxic conditions. The authors stated that such conditions allow maintenance of a good balance between heterotrophic and autotrophic microorganisms, reduction in the competition for oxygen, and promotion of higher autotrophic growth rates.

Solubilization of COD, nitrogen and phosphorus in the ASSR

Figure 4 summarizes the concentrations of soluble COD and ammonium in the anaerobic and aerobic sludge taken from the ASSR and the aerobic reactor, respectively.

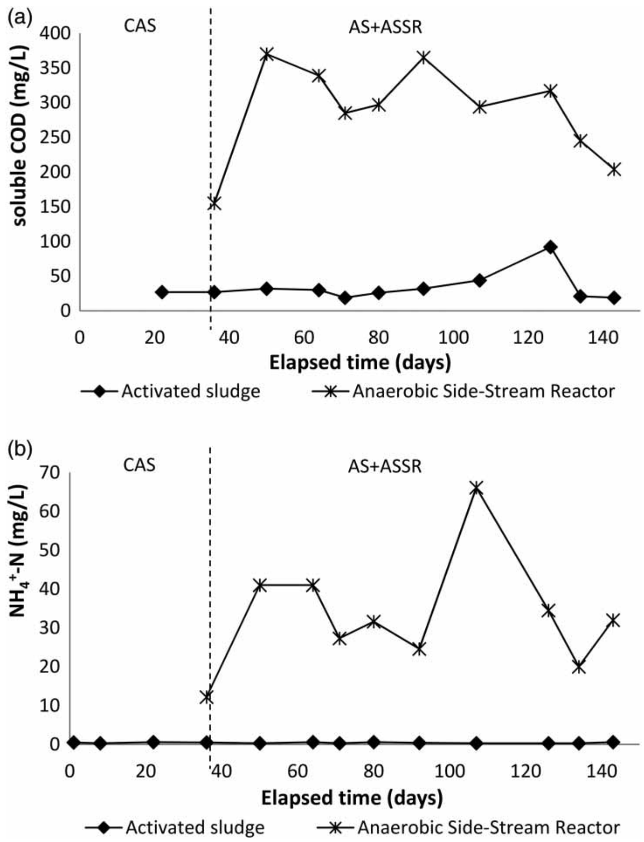

Figure 4 | Time profiles of (a) COD and (b) NH_4^+-N in the sludge samples over the monitored periods.

The mean value of soluble COD was lower than 50 mg/L in the activated sludge during both monitored periods, while it was 308 mg/L in the ASSR sludge, evidencing the substrate solubilization. The release of COD and NH_4^+-N in the anaerobic tank, associated with rapid consumption of the solubilized compounds under recovered aerobic conditions, was already demonstrated in the literature (Novak *et al.* 2007; Wang *et al.* 2008). It means that most of the solubilized compounds generated in the anaerobic reactor are biodegradable, and a negligible interference with removal efficiency was normally observed (Semblante *et al.* 2017). In line, Oliveira *et al.* (2018) indicated that the release of COD in the anaerobic tank can be triggered by the stressful environment (low ORP level and starvation condition in the ASSR).

For ammonium-nitrogen, the difference in the sludge samples was very pronounced: the mean value of NH_4^+-N under anaerobic conditions was 36 mg/L, while it was consistently low (0.43 mg/L on average) in the activated sludge and thus in the effluent. Zhou *et al.* (2015) showed that the increase of NH_4^+-N and total organic nitrogen (TON) concentrations in the sludge holding tank (SHT) further demonstrated the sludge decay under anaerobic conditions. The authors claimed that the release of soluble microbial products (SMPs) was associated with hydrolysis of particulate organic matters and cell lysis in the SHT. A previous laboratory study with the sludge samples of this full-scale plant (Foladori *et al.* 2015) has already demonstrated a large amount of soluble and biodegradable organic substrates and nitrogen available in the anaerobically digested sludge. However, the authors verified at the single-cell level by flow cytometry that the number of dead cells increased under anaerobic conditions, but the bacteria did not undergo cell lysis. Similarly, the flow cytometry tests applied through the overall monitored period (data not shown) revealed that the number of total cells increased under anaerobic conditions (from 9.5×10^8 cells/mgTSS to 1.3×10^9 cells/mgTSS), which according to Foladori *et al.* (2015) indicates that cell lysis did not occur. Similarly, Jiang *et al.* (2018) have also verified, by DNA sequencing and a model of sludge reduction, that cell lysis occurred under aerobic condition and anaerobic hydrolysis dominated in sludge decay.

Although literature findings have confirmed that anaerobic conditions in the ASSR caused cell lysis (Zhou *et al.* 2015; Cheng *et al.* 2017; Ferrentino *et al.* 2019), the differences in operating conditions as well as the differences between fundamental principles of the methods used to investigate cell lysis could likely explain these antagonistic results. The cell lysis can be estimated on the basis of organic matter releases; and, associated with molecular methods, a more accurate view of bacteria populations and dynamics can be provided.

The concentrations of total phosphorus (Figure 5(a)) in the activated sludge samples were always below those in the anaerobic sludge, amounting to 119 and 242 mg/L on average, respectively.

The average values of orthophosphate were 3 mg/L for activated sludge and 44 mg/L for ASSR sludge. Expressive partial hydrolysis was verified under anaerobic conditions, complex phosphorous compounds were transformed to its readily assimilable form. Approximately 2% and 22% of total P in terms of orthophosphate were measured in the activated sludge and in the anaerobic sludge, respectively. According to Oliveira *et al.* (2018), the rise of orthophosphate released under anaerobic conditions could be related to the increase of poli-P organisms. The alternation between aerobic and anaerobic conditions may favor the growth of Phosphate Accumulating Organisms (PAOs), which are able to accomplish the biological phosphorous removal (Goel & Noguera 2006; Ferrentino *et al.* 2019).

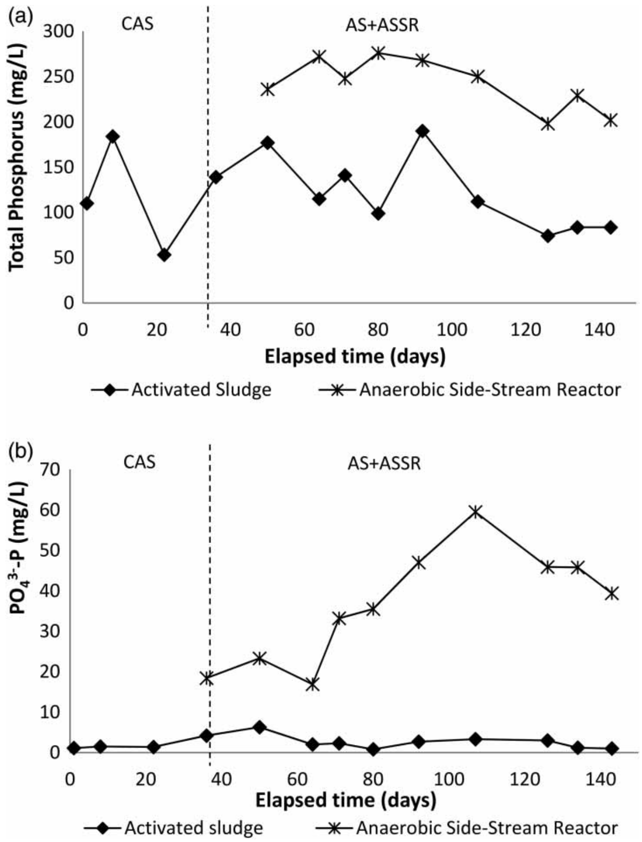

Figure 5 | Time profiles of (a) Total Phosphorus and (b) PO_4^{3-}-P in the sludge samples over the monitored periods.

An advantage of ASSR in sludge reduction is the biomass selection that also contributes to nutrient removal, once its establishment also occurs inside the main reactor (activated sludge) due to the sludge recirculation (Goel & Noguera 2006). A biomass taxonomic characterization was not realized; however, the enrichment of PAOs in the AS + ASSR system could be assumed considering the great difference between TP and orthophosphate concentrations in comparison to the CAS configuration. The OSA-like process reduces sludge by selecting certain enriched bacteria that readily consume the biodegradable products of solubilization (Semblante *et al.* 2016, 2017).

CONCLUSIONS

A full-scale WWTP implemented with an ASSR was monitored to investigate the sludge reduction and the effect of the ASSR on sludge decay and biomass activity. The system achieved efficient substrate and nutrient removal. The cyclic aerobic and anaerobic conditions promoted a decrease in heterotrophic biomass activity, the maximum specific substrate removal rate on activated sludge was 36% smaller compared to the CAS configuration, while the autotrophic biomass activity was not impaired.

The solubilization of COD, NH_4^+-N and orthophosphate confirmed the hydrolysis of organic matter under anaerobic conditions. The net solids balance exhibited the excess sludge reduction. The results suggest that the possible mechanisms of sludge reduction were the increase in the system SRT by the addition of ASSR, and the reduction in heterotrophic biomass activity added to the organic compounds' hydrolysis.

ACKNOWLEDGEMENTS

V.F. Velho held a PhD scholar internship of CAPES Foundation, Ministry of Education of Brazil, as a part of the International Mobility Program 'Ciência sem Fronteiras', Federal University of Santa Catarina/BR and University of Trento/IT. The authors thank 'Agenzia per la Depurazione' of Autonomous Province of Trento for the analyses and access to the plant.

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First received 1 August 2018; accepted in revised form 11 March 2019. Available online 20 March 2019