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REDUCTION OF EXCESS SLUDGE PRODUCTION IN AN ACTIVATED SLUDGE SYSTEM BASED ON LYSIS-CRYPTIC GROWTH, UNCOUPLING METABOLISM AND FOLIC ACID ADDITION

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Abstract - The following sludge reduction alternatives were tested in wastewater biological reactors: oxic-settling-anaerobic (OSA-process); ultrasonic disintegration (UD); chlorination (CH); 3,3',4',5-tetrachlorosalicylanilide (TCS); and folic acid (FA). Compared to the control system, UD reduced 55% of the sludge production, and greater substrate and nutrient removal efficiency was achieved. CH worsened the sludge settleability and increased the SVI values; the system achieved 25% of sludge reduction. OSA showed 50% and 60% of sludge reduction after 16 and 10 hours under anaerobic conditions, respectively. The observed sludge yield during TCS addition was decreased by 40%, and the sludge settleability worsened. FA presented the highest sludge reduction (75%), and the system improved the nutrient removal efficiency by 30% compared to the control system and maintained the sludge properties. Acute toxicity conducted with *Daphnia magna* classified the effluent from the sludge reduction systems as non-toxic for discharge into water sources.
Keywords: Wastewater; Activated sludge; Sludge reduction; Lysis-cryptic growth; Uncoupling metabolism; Folic acid.

INTRODUCTION

Activated sludge is an efficient and reliable process for the treatment of wastewater; however, the process produces a large amount of excess sludge that must be treated and disposed of (Pérez-Elvira *et al*., 2006). This amount is set to rise, considering the preservation of water resources and the more stringent effluent regulations that increase the population connected to the sewage treatment network. Sludge management has become one of the most critical challenges in the field of sewage treatment (Foladori *et al*., 2010). Sludge management is usually limited to its stabilization and dewatering, and the typical final disposal of this waste is in landfills or via composting or incineration. Operating costs associated with the more stringent disposal regulations may contribute to make the current conventional alternatives for sludge treatment and disposal limited or unviable in the near future (Liu & Tay, 2001).

An ideal approach to solving the waste sludge problem would be the reduction of excess sludge production in the water line of the wastewater treatment plant (WWTP). The treatment should be cost-effective and would not affect the effluent quality and settling properties (Easwaran, 2006). The most

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widely adopted techniques in sludge reduction are based on mechanisms such as cell lysis-cryptic growth and uncoupling metabolism. Moreover, the addition of folic acid (B vitamin) as a nutrient source to improve the biological activity and sludge flocculation has also been shown to be an effective alternative to reduce the production of excess sludge (Akerboom *et al*., 1994), as subsequently described.

The cryptic growth mechanism is based on the reutilization of intracellular compounds that are released during cell lysis. This organic autochthonous substrate is reused in microbial metabolism, and a portion of the carbon is liberated as products of respiration, which results in an overall reduction of the biomass production (Mason and Hamer, 1987). Cell lysis and consequential cryptic growth could be promoted using physical, chemical and combined methods to reduce sludge production. Conventional inorganic oxidation such as chlorination can provide sludge solubilization, cell lysis and thus sludge reduction by 65% (Saby *et al*., 2002). The reduction of sludge production can achieve 20-50% using ultrasonic disintegration, which enhances the disaggregation of biological flocs and increases the biodegradability of the sludge (Pilli *et al*., 2011).

Uncoupling metabolism is a mechanism that increases the discrepancy in the energy level between catabolism and anabolism, thus limiting the energy available for anabolism. The energy is first used to satisfy the maintenance requirements before it is used for cell growth, i.e., the energy supply is limited to convert metabolites into new cells. The biomass yield is thus reduced, and the sludge production decreases (Russel and Cook, 1995). Uncoupling metabolism may be carried out under abnormal conditions such as the presence of inhibitory compounds, an excess energy source, non-optimal temperatures, nutrient limitations, chemical uncouplers (2,4-dinitrophenol: 2,4-DNP; or 3,3',4',5-tetrachlorosalicylanilide: TCS; or heavy metals) and oxic-anoxic (or oxic-anaerobic) cycling conditions (Ye and Li, 2010; Foladori *et al*., 2010; Coma *et al*., 2013). In this manner, the OSA (oxic-settling-anaerobic) process has been confirmed to reduce excess sludge production by 23% to 58% due to the physiological shock created by a lack of oxygen and of substrate (Saby *et al*., 2003). The effectiveness of the chemical uncoupler TCS has been demonstrated by a sludge reduction of 40% at concentrations in the range of 0.4-1.0 mg/L.

The addition of folic acid in the WWTP serves the particular function to regulate 1-carbon metabolism (Anderl, 1987). In systems that are deficient in folic acid, metabolic processes would occur at a significantly lower rate. The addition of folic acid promotes fast metabolic activity in the activated sludge process, which results in a decrease in the daily sludge growth (Strunkheide, 2004). Operational data evaluated in more than 60 municipal and industrial WWTPs in North and South America have confirmed that the addition of folic acid reduced the production of excess sludge by approximately 50% (Senörer and Barlas, 2004). Initial operational testing with this product in Germany has supported the possibility of a reduction of excess sludge on the order of 30 - 60% (Strunkheide, 2004).

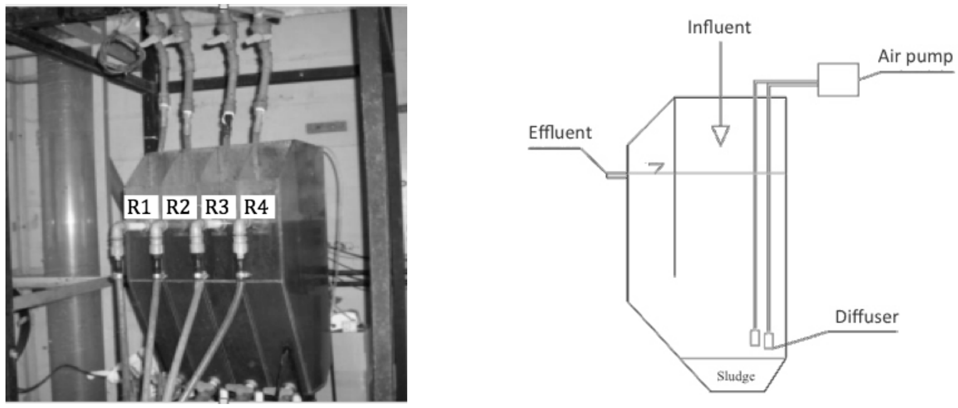
The overall aim of this study was to investigate and compare the ability of different sludge reduction alternatives that were applied in a bench-scale activated sludge system. The purpose was to investigate the reduction of sludge production and the wastewater treatment performance in the system using different configurations such as conventional activated sludge and modified activated sludge based on cryptic growth, uncoupling metabolism and folic acid addition.

MATERIALS AND METHODS

Bench-Scale Treatment System

The biological reactors used in this research consisted of four acrylic tanks with a working volume of 10 L each (Figure 1). These reactors were fed with domestic wastewater taken from the municipal sewage collecting system by a submerged pump. A microbial seed, which was obtained from a sewage treatment plant (Florianópolis-Brazil), was inoculated into the biological reactors. The hydraulic retention time (HRT) was controlled at 10 h; the dissolved oxygen (DO) concentration and the effective mixing in the aerobic tanks were provided by a bead air diffuser at a volumetric airflow rate of 60 L/h, which maintained a dissolved oxygen concentration in the four reactors above 5 mg O_2/L. The systems were operated in continuous flow as an activated sludge system. After 1 month of sludge cultivation, the sludge in the system was acclimatized. Sludge reduction alternatives were applied during the stable operational period of the system. The entire experimental period consisted of two distinct phases, which differed in the sludge reduction alternatives applied simultaneously. The systems were monitored twice a week prior to the application of the sludge reduction alternative.

The characteristics of the influent and the operational conditions throughout the experiment are presented in Table 1.



(A) (B)

Figure 1: (A) Picture of the reactors; (B) Scheme of the effluent stream.

Table 1: The influent characteristics and the operational conditions of the system.

Parameters	Phase 1	Phase 2
Bench-scale system		
Duration (days)	70	90
Total COD (mg/L)	393 ± 104	491 ± 146
Organic loading rate (Kg COD/m^3·d)	0.94	1.18
NH_4^+-N (mg/L)	62 ± 17	80 ± 16
Ammonium loading rate (kg NH_4^+-N/m^3·d)	0.15	0.19
Total BOD_5 (mg/L)	252 ± 82	325 ± 136
Total suspended solids (mg/L)	41 ± 27	150 ± 89
Hydraulic retention time (hours)	10	10
Input flow (m^3/d)	0.024	0.024
Microbial seed		
Total Suspended Solids (g/L)	3.1	2.4
Sludge Volume Index (mL/g)	210	150

Sludge Reduction Alternatives

In the study, three different alternatives for sludge reduction were tested during each phase, resulting in a total of six alternatives during the entire experiment. The performance of each reactor was compared to the others to determine which reactor promoted greater efficiency in reducing sludge production without significant interference in the effluent quality.

Sludge reduction alternatives were tested twice a week for the following treatments: oxic-settling-anaerobic processes, chlorination and ultrasonic disintegration. To achieve this analysis, the aeration of well-mixed sludge was switched off; the sludge was then thickened by 30 minutes of settling; 500 mL of sludge was collected from the sample points located at the bottom of the reactors to perform the sludge reduction alternatives. After the application of the sludge reduction treatments, the sludge samples were returned to the activated sludge systems.

The tested alternatives were:

1) Oxic-settling-anaerobic process (OSA_{16h}.R) – The excess sludge produced was maintained under anaerobic conditions for 16 hours (Ye *et al*., 2008; Foladori *et al*., 2010) and then returned to the aerobic reactor (twice a week). A fraction of approximately 15% of settled sludge (500 mL) was maintained under anaerobic conditions for 16 hours in a hermetically sealed container.

2) Chlorination combined with activated sludge process (CH.R) – Chlorination treatment of excess sludge was applied according to Saby *et al*. (2002) at dosages of 0.066 g Cl_2/g TSS and 1 min of contact time followed by the return of the treated sludge to the aerobic reactor (twice a week). A volume of 500 mL of sludge that had settled for 30 minutes was combined with sodium hypochlorite solution (2.5%

of active chlorine – Anhembi SA, Brazilian Industry), at a chlorine dose of 0.066 g Cl_2 / g SST.

3) Ultrasonic disintegration (UD.R) – The returned sludge was treated with sonotrodes (twice a week). A volume of 500 mL of sludge that had settled for 30 minutes was sonicated in an ultrasonic cell disruptor (DES500 – 100 W, Unique) at a frequency of 35 kHz for 90 seconds according to Wang *et al*. (2005) and Foladori *et al*. (2010).

4) Oxic-settling-anaerobic process (OSA_{10h}.R) – The excess sludge produced was maintained under anaerobic conditions for 10 hours (Ye *et al*., 2008; Foladori *et al*., 2010) and then returned to the aerobic reactor (twice a week). A fraction of approximately 15% of the settled sludge (500 mL) was maintained under anaerobic conditions for 10 hours in a hermetically sealed container.

5) TCS as chemical uncoupler (TCS.R) – The 3,3',4',5-tetrachlorosalicylanilide was tested at a concentration of 0.4 mg/L according to Wei *et al*. (2003). The reagent feed was delivered continuously via a peristaltic pump (Watson Marlow 505S) directly to the aeration tank.

6) Folic acid addition (FA.R) – Folic acid (folic acid 98% - Vetec, Sigma-Aldrich) addition was tested at a concentration of 0.2 mg/L according to Dubé *et al*. (2002) and Bertacchi (2005). The reagent feed was delivered continuously via a peristaltic pump (Cole Parmer Masterflex 7519-20) directly to the aeration tank.

In each phase, a biological reactor (C.R) served as the control system without any sludge reduction alternative applied, thus operating as conventional activated sludge.

Analytical Methods

The samples were stored in appropriate vials and transported to the laboratory for both physical and chemical analyses. The samples were analyzed to determine the pH, temperature, dissolved oxygen (DO), total chemical and total biochemical oxygen demand (COD and BOD_5, respectively), ammonium nitrogen (NH_4^+-N), and total suspended solids (TSS). The dissolved oxygen concentration, pH, and temperature were measured online with a multiparameter probe (YSI 6600). All of the analyses were conducted according to Standard Methods (2005).

The COD concentration was determined by the closed reflux colorimetric method (Standard Method 5220 D). The biochemical oxygen demand (BOD_5) was determined using the manometric method (Standard Method 5210 D), in which the sample was digested over 5 days of incubation on a shaker base at 20 ± 1 °C. The total suspended solids (TSS) were determined using Standard Methods (SM 2540 D), in which the samples were dried to a constant weight at 105 °C. The amounts of NH_4^+-N were determined by distillation followed by back titration of the boric acid distillates using 0.2 N sulfuric acid (SM 4500-NH_3 BC).

Respirometry tests were performed online, i.e., inside the biological reactors, to determine the oxygen uptake rate (OUR) of the mixed liquor during the operation of the sludge reduction alternatives. These tests were conducted according to the adapted method described by Wolff *et al*. (2003) based on Ochoa *et al*. (2002).

The possibility of biomass changes within the biological tanks was verified by optical microscopy (Olympus BX-40).

To verify the possibility of effluent toxicity after the application of the sludge reduction alternatives to the systems, a toxicity analysis was performed according to Brazilian Technical Standards (NBR 12713, ABNT 2003). The acute toxicity tests were conducted with *Daphnia magna*, a freshwater microcrustacean. The results are normally expressed as the effective concentration (EC_{50}), which corresponds to the sample concentration that causes an acute effect (immobility/death) in 50% of the tested organisms exposed to the test solution for 48 hours. The degree of sludge sample toxicity was established according to Marsalek *et al*. (1999): non-toxic $EC_{50} > 100$; potentially toxic $100 > EC_{50} > 40$; toxic $40 > EC_{50} > 10$; very toxic $EC_{50} < 9$.

Observed Sludge Yield (Yobs) Calculation

The observed sludge yield (Y_{obs}) was determined for each monitoring phase using a regression method applied to the masses of TSS produced and organic matter removed (Chon *et al*., 2011; Coma *et al*., 2013).

The mass of TSS produced ($\Delta TSS_{produced}$) was calculated as follows including all the TSS variations that occurred in the system:

$$\Delta TSS_{produced} = \Delta X_{AS} \cdot V_{AS} + ((Q_W \cdot X_W + Q_{ef} \cdot X_{ef}) \cdot \Delta t)$$

where the terms ΔX_{AS}, X_W and X_{ef} are the variations of solids in the activated sludge tank, in the wastage sludge, and in the effluent, respectively (kgTSS).

The mass of COD removed ($\Delta COD_{removed}$) was calculated twice a week as follows:

$$\Delta COD_{removed} = \left(Q_{in} \cdot \left(COD_{in} - COD_{ef}\right)\right) \cdot \Delta t$$

where Q_{in} is the influent flow ($L \cdot d^{-1}$), which is considered to be equal to Q_{ef}. COD_{in} and COD_{ef} correspond to the COD concentration (mg/L) in the influent and effluent wastewater, respectively. Δt is the elapsed time in days between each sludge reduction alternative application.

The mean value of Y_{obs} for each monitoring phase was calculated as the slope of the linear regression curve obtained from the data for the cumulative TSS produced versus the cumulative COD removed:

$$Y_{obs} = \frac{TSS_{produced}}{COD_{removed}}$$

Sludge Retention Time (SRT) Calculation

The sludge retention time (SRT) was calculated according to Chon *et al*. (2011) as the ratio between the total mass of solids in the reactor and the mass of solids extracted daily from the system.

$$SRT = \frac{X_{AS} . V_{AS} + X_{Ri} . V_{Ri}}{X_{EF} . Q_{EF} + X_{AM} \cdot \frac{V_{AM}}{\Delta t}}$$

where X_{AS}, X_{Ri}, X_{EF} and X_{AM} are the concentrations of total suspended solids (TSS mg/L) in the activated sludge tanks, in the settled sludge, in the effluent, and in the activated sludge samples, respectively. V_{AS}, V_{Ri} and V_{AM} are the volumes (L) of the activated sludge system, the settled sludge, and the activated sludge samples, respectively. Q_{EF} is the effluent flow (L/d). Δt is the elapsed time in days between each sampling.

The systems were operated as no sludge wastage systems, and therefore, there was no intentional sludge wasting, excluding the sampling for measurements and the sludge washout in the effluent.

Statistical Analysis

To verify the possibility of interference in the removal efficiencies and to check the difference in sludge production for each sludge reduction alternative, analysis of variance (ANOVA) was conducted using STATISTICAW 7.0 software. Tukey's test at a significance level of 5% was used to compare the average values of the variables obtained between the four biological reactors.

RESULTS AND DISCUSSION

Effluent Quality

Table 2 summarizes the removal efficiency and the effluent concentration of the main parameters during the experiment. It can be observed that the sludge reduction alternatives did not influence the treatment performance of the system, otherwise the results were consistent with the control system, or even slightly better.

Despite the variability in the activated sludge operational configuration, the average COD and BOD_5 concentrations in the effluent were below 100 mg/L and 60 mg/L, respectively, in all of the biological tanks, excluding the system that used chlorination as a sludge reduction alternative (CH.R). The treatment systems also provided values below the discharge limit imposed by Brazilian (120 mg/L or 60% of efficiency for BOD_5) and Santa Catarina (60 mg/L or 80% of efficiency for BOD_5) regulations (Conama resolution 430/2011; Santa Catarina law 14.675/2009). The average COD and BOD_5 removal efficiencies remained over 80%, even with the possibility of the considerable generation of soluble compounds in the systems due to sludge lysis and the increase in SRT, typical of most of the applied sludge reduction alternatives in this study. It can be assumed that the soluble compounds released during the sludge reduction were immediately consumed in the system simultaneously with the influent in the aerobic tank and that they did not interfere with the COD and BOD_5 removal efficiency.

The chlorination system (CH.R) showed a low treatment performance, and the results for efficiency were statistically comparable to the control system. However, the effluent concentration revealed a decline in effluent quality, with average values that nearly exceeded the state discharge limit. According to Foladori *et al*. (2010), at high dosages, chlorination may worsen the efficiency of the biological process, in particular the efficiency of nitrification due to the disinfection effect.

The OSA_{16h}.R system presented results for COD, BOD_5 and NH_4^+-N that were slightly better compared to the control system, exhibiting an improvement of the effluent quality. These results are in agreement with those of Saby *et al*. (2003); these authors showed that the aerobic-anaerobic cycle system could improve the effluent quality due to the increased rate of substrate uptake imposed by stress conditions (low availability of substrate and high concentration of biomass). OSA_{10h}.R basically differed in the number of hours that the thickened sludge

Table 2: Effluent concentration and removal efficiency of COD, BOD_5, NH_4^+-N and TSS during the experiment.

Treatment Unit	COD (mg/L)		BOD_5 (mg/L)		NH_4^+-N (mg/L)		TSS (mg/L)	
	Out (n=20)	R (%)	Out (n=20)	R (%)	Out (n=20)	R (%)	Out (n=20)	R (%)
C.R	72±56 **a**	82 **a**	30±19 **a**	88 **a**	37±17 **a**	40 **a**	51±37 **a**	-
OSA_{16h}.R	51±33 **b**	87 **a**	14±10 **a**	94 **b**	24±15 **a**	61 **b**	38±23 **a**	7 **a**
CH.R	92±20 **a**	77 **a**	59±31 **b**	77 **a**	37±20 **a**	40 **a**	39±14 **a**	5 **a**
UD.R	51±22 **b**	87 **a**	18±15 **a**	93 **b**	25±17 **a**	60 **b**	38±26 **a**	7 **a**
C.R	80±29 **a**	84 **a**	18±13 **a**	93 **a**	27±6 **a**	65 **a**	29±22 **a**	81 **a**
OSA_{10h}.R	78±34 **a**	84 **a**	25±9 **a**	91 **a**	28±10 **a**	65 **a**	30±19 **a**	80 **a**
TCS.R	62±29 **a**	87 **a**	13±11 **b**	96 **a**	11±9 **b**	85 **b**	28±18 **a**	81 **a**
FA.R	78±46 **a**	84 **a**	11±7 **b**	95 **a**	13±7 **b**	83 **b**	29±19 **a**	81 **a**

C.R – control reactor; OSA_{16h}.R - oxic-settling-anaerobic process (16 h of anaerobic conditions); CH.R – chlorination treatment; UD.R – ultrasonic disintegration; OSA_{10h}.R – oxic-settling-anaerobic process (10 h of anaerobic conditions); TCS.R - 3,3',4',5-tetrachlorosalicylanilide addition; FA.R – folic acid addition.
Column values followed by the same letter do not differ (5% of significance level using Tukey's test). Out = effluent; R = removal efficiency.

was maintained under anaerobic conditions. A decrease in the time that the sludge was maintained under anaerobic conditions did not affect the effluent quality, and the results were statistically the same as those obtained for the control system.

The results for the ultrasonic disintegration system (UD.R) revealed an improvement of the substrate and nutrient removal efficiencies, and the effluent concentrations were lower compared to the control system. These findings are consistent with those of Zhang *et al*. (2008), who observed an increase in the COD removal efficiency by 5-12%, and an increase of 28% in the oxygen uptake rate (OUR) by treating sludge with ultrasound disintegration at 25 kHz and 30s. The improvement of the removal efficiency could be explained by the increase in the specific surface of sludge flocs and the dispersion of dense flocs that was observed during the sonication. These characteristics favour contact between bacteria, substrates and enzymes, resulting in an enhancement of the overall sludge biodegradability (Foladori *et al*., 2010).

Similarly, the NH_4^+-N removal efficiency was not affected by the sludge reduction alternatives, and most of the systems presented greater efficiency than the control system, excluding the chlorination system. The folic acid addition (FA.R) and TCS.R systems showed efficiencies of more than 80%, and an increase 30% of the nutrient removal efficiencies compared to the control system. Folic acid addition accelerates metabolism activity, which could result in greater substrate and nutrient consumption, thus providing additional efficiency compared to conventional activated sludge. Nevertheless, several studies investigating folic acid addition described no significant interference in the substrate and nutrient removal efficiencies (Dubé *et al*., 2002; Bertacchi, 2005). Using TCS and TCS combined with the OSA process, Ye and Li (2005) demonstrated a decline in the nutrient removal rate, with effluent concentrations that were higher compared to the control system. However, as demonstrated in this study, the authors found that the substrate removal efficiency was not affected, and the system could also stimulate an increase in the rate of specific substrate uptake (Ye and Li, 2005; Low and Chase, 1998).

The TSS effluent concentrations were in the same range for all of the systems in both phases of the experiment; however, the phase 2 influent concentration was greater than the concentration in phase 1. Therefore, the efficiency results were higher for phase 2 and statistically equivalent for all of the biological reactors.

Sludge Production and Settleability

Table 3 summarizes the results obtained for the variables used to evaluate the reduction of excess sludge production and settleability.

The TSS concentrations in the mixed liquor during the experiment were maintained at approximately 2 g/L in phase 1 and at about 1 g/L in phase 2 and they remained stable during all of the periods.

The oxic-settling-anaerobic process was the sludge reduction alternative, which presented the second largest reduction of excess sludge production during both phases, showing 50% and 60% of reduction (Table 3). The effluent treatment was maintained in the same range or improved after applying the OSA process, confirming that this system performed as efficiently in reducing excess sludge production at a low cost and with good stability.

Table 3: Total suspended solids, sludge volume index, sludge retention time, observed growth yield and reduction of excess sludge production in the treatment unit during both distinct phases.

Treatment Unit	MLSS (mg/L)	SVI (mL/g)	SRT (days)	Yobs	
				Value (kg/kg)	Reduction (%)
C.R	1.160 ± 423	92 ± 34	12.5	0.20	-
OSA_{16h}.R	2.136 ± 555	76 ± 19	26.7	0.10	50 **a**
CH.R	2.151 ± 798	328 ± 239	26.2	0.15	25 **b**
UD.R	2.127 ± 607	139 ± 96	26.6	0.09	55 **a**
C.R	1.230 ± 539	71 ± 29	17.9	0.20	-
OSA_{10h}.R	916 ± 459	64 ± 32	22.7	0.08	60 **a**
TCS.R	1.034 ± 450	169 ± 64	20.8	0.12	40 **b**
FA.R	1.180 ± 578	99 ± 48	22.0	0.05	75 **c**

Column values followed by the same letter do not differ (5% of significance level using Tukey's test).

Previously reported experiments (Chen *et al*., 2001; Saby *et al*., 2003) have shown that the recirculation of sludge between the aerobic reactor and anaerobic process induces a favorable sludge reduction mechanism and leads to effective flocculation. This phenomenon may be attributed to the release of intracellular polymers under anoxic/anaerobic condition, which can act as floc-bridging agents to improve the settling properties of the sludge (Ye and Li, 2005), as observed for the average values obtained for SVI in OSA.R (76 mL/g and 64 mL/g, for OSA_{16h}.R and OSA_{10h}.R, respectively).

During chlorination treatment, sludge production decreased by 25% in terms of the growth yield. The system presented the highest SVI values, and the settleability worsened. These results were comparable with those reported by Saby *et al*. (2002), who also identified worsening sludge settleability. However, these authors obtained a greater sludge reduction of approximately 65%. In contrast, Wang *et al*. (2011) treated sludge with a ClO_2 dose of 10 mg ClO_2/ g TSS for 40 min and observed a sludge reduction of 58% without deterioration of the sludge settleability.

Ultrasonic disintegration was the sludge reduction alternative that demonstrated the largest reduction of excess sludge production during phase 1 (Table 3), with a verified reduction of 55% of excess sludge production. The system showed stable sludge settling, as indicated by the SVI value (average of 139 mL/g).

The observed sludge yield during TCS addition was decreased by 40%, and the sludge settleability worsened and the SVI values increased 2.4-fold compared to the control system. The negative side effects, such as the potential worsening sludge settleability, may have been due to the dosage of the chemical uncoupler; the optimal conditions for this process are not completely understood (Wei *et al*., 2003).

The FA.R system presented the lowest observed sludge yield, with a reduction of 75% compared to the control system. The SVI values remained at less than 100 mL/g. The sludge reduction results presented in this study were higher than those reported in the literature, which range from 30% to 60% (Sen̈örer and Barlas, 2004; Strunkheide, 2004). Ostrander *et al*. (1992) and Dubé *et al*. (2002) verified the improvement of sludge settleability after folic acid addition in an active sludge system, reporting SVI data reductions of 75% and 63% with average values of 250 mL/g and 147 mL/g, respectively.

In the control systems, the average SRT was 12.5 d and 17.9 d (Table 3). The SRT increased to approximately 26 d in phase 1 and 22 d in phase 2 in the systems that were operated with a sludge reduction alternative. A long SRT may often cause a worsening of the settling properties and effluent quality; however, the sludge reduction systems assessed herein showed good stability, excluding the chlorination system, which showed high SVI values, removal efficiencies that were slightly lower than the control system, and the lowest sludge reduction among all of the experiments.

Respirometry, Microscopy and Toxicity Results

The specific oxygen uptake rate (SOUR) of the systems was monitored approximately every 10 days (phase 1) and every 15 days (phase 2) during the experiment to investigate the effect of the sludge reduction alternatives on the sludge activity.

Figure 2A shows that there was a large decrease in SOUR in the sludge reduction operating systems. This could be attributed to the biomass inactivation that mainly occurred in the lysis-cryptic growth systems (CH.R and UD.R); however, the effluent quality did not change, as previously demonstrated (Table 1). Adverse environmental conditions, such as physical-chemical interventions, could lead to cell death and/or activity decay, which cause a decrease in bacterial activity in the activated sludge (Mason *et al*., 1986; van Loosdrecht and Henze, 1999). Moreover, under stressful conditions, bacteria might adjust their metabolic processes and decelerate their requirements for maintenance energy. This phenomenon is observed as a decrease in the specific activity (Arbrige and Chesbro, 1982).

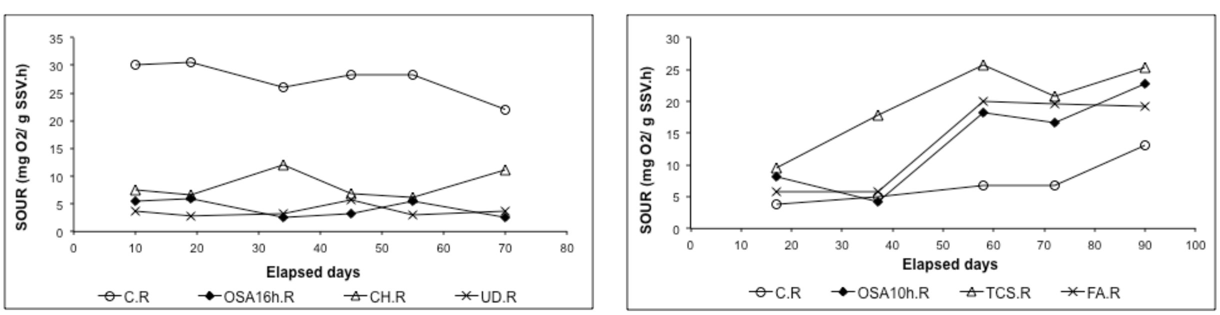
Takdastan *et al*. (2009) also found low SOUR values (average of 3 mg O_2/g VSS·h) at chlorine dosages of 20 mg/g MLSS. The authors affirmed that the SOUR values were reduced because a significant portion of microorganisms were killed due to the inhibitory role of chlorine.

The TCS.R and FA.R systems (Figure 2B) showed an increase at the beginning of the monitoring period and then stabilized in the range of 20 – 25 mg O_2/ g VSS·h. The microbial activity in these systems was high compared to the control system. Chen *et al*. (2004) tested different doses of TCS and verified that a higher TCS concentration (up to 3.6 mg/L) resulted in a more rapid oxygen uptake rate, thus providing higher levels of metabolic uncoupling. They concluded that the over-consumption of oxygen suggested the occurrence of a high level of energy dissipation for metabolic regulation, which could be the cause of the reduction in sludge growth.

The OSA systems presented an inverse behaviour. During phase 1, OSA_{16h}.R demonstrated that the exposure of bacteria to anaerobic conditions reduces their activity compared to the control system. According to Saby *et al*. (2003), the reduced bacterial activity might be due to the low ORP conditions to which they are submitted under anaerobic conditions (in this case, 16 hours). Otherwise, the cell activities in the OSA_{10h}.R system during phase 2 might not have been affected at the beginning of this phase, showing SOUR values in the same range as those observed in the control system. However, after approximately 35 days of system operation, the SOUR values increased and stabilized at approximately 20 mg O_2/g VSS·h. The difference between these two systems in terms of the SOUR values could be explained by the duration for which the sludge was maintained under anaerobic conditions. Phase 1 had a longer duration under anaerobic conditions, resulting in lower ORP values that influenced the bacterial activity.

The microscopic examination revealed qualitative similarities; microbes agglomerated in dense flocs of predominantly cocci and short bacilli. Samples of the mixed liquor showed a constant presence of protozoa such as crawling ciliates (*Aspidisca* sp.), amoebae (*Arcella* sp.) and rotifers. Ciliated species that were adhered to the floc (*Vorticellas* sp.) were also frequently observed in the microscopic analyses. Representatives of free ciliates (*Paramecium* sp., *Litonotus* sp.) and the rotifer gender *Rotatoria* sp. were also verified. Fernandes *et al*. (2013) used a full-scale SBR to treat domestic wastewater and showed a highly concentrated sludge that was formed by compact flocs. In that study, the zooplankton comprised ciliated protozoa (attached and crawling ciliates), naked amoebae and rotifers. According to Zhou *et al*. (2008), these organisms are very important for the system because they feed mainly on bacteria. Consequently, they maintain the bacterial densities and rejuvenate the population via predation. They also contribute to improving the effluent quality by functioning as effective flocculation agents.

Overall, the sludge reduction alternatives did not promote great differences in the biomass characteristics; the systems maintained good sludge settleability and a stable efficiency. However, filamentous bacteria were detected in the chlorination system. This finding suggested that a shift in the predominant microbial population occurred after the addition of sodium hypochlorite solution, resulting in the formation of flocs with a reduced density that contributed to the worsening sludge settleability, as demonstrated by the high SVI values, and a decline in the treatment performance.



(A) (B)

Figure 2: Variations of the specific oxygen uptake rate (SOUR) in the control system and in three sludge reduction systems. (A) Phase 1. (B) Phase 2.

Some of sludge reduction alternatives tested in the present study introduced chemical compounds into the biological systems, which could produce toxic effects in the effluent by interacting with organic matter and forming new compounds. Thus, the effluents in these systems were tested for acute toxicity. The results obtained for the acute toxicity tests showed that the effluent samples from all of the systems, including the control system, were non-toxic (EC_{50}> 100).

CONCLUSIONS

The results of the present study show that the tested sludge reduction alternatives can effectively reduce sludge growth, with sludge reduction efficiencies ranging from 25 to 75%.

The excess sludge production observed in the OSA_{16h}.R and UD.R systems was reduced by 50% and 55%, respectively. These systems showed a decrease in biomass activity that may have been due to cellular inactivation due to lysis via ultrasonic disintegration, as well as to the low ORP values observed under anaerobic conditions. Nevertheless, the systems maintained their performance in the effluent treatment. The removal efficiencies were consistent with, or even better than, the control system.

It can be assumed that the role of low ORP values in biomass activity is greatly dependent on the duration of exposure to anaerobic conditions; longer durations are required to achieve low ORP values. This effect was noticeable when comparing the two OSA systems, which basically differed in terms of the time that the thickened sludge was maintained under anaerobic conditions. The OSA_{10h}.R presented an enhancement of bacterial activity compared to the control system. This increase could explain the increased sludge reduction that was verified in this system in comparison to OSA_{16h}.R.

The chlorination treatment resulted in negative side effects, demonstrating that this alternative is not an ideal option for sludge reduction. The removal efficiencies decreased by 12% and 6%, for COD and BOD_5, respectively. The sludge settleability was affected concomitantly with a change in the microbial population that occurred after the 50-day operation. Filamentous bacteria were observed that resulted in flocs with a reduced density and in high SVI values. In addition, the sludge yield was not significantly decreased (25%), as verified in the other systems.

The sludge growth could be reduced by approximately 40% and 75% with the TCS system and FA system, respectively. No negative effects were observed for the treatment efficiency in terms of the COD and BOD_5 removal rate. Furthermore, the systems showed improvements in NH_4^+-N removal efficiencies by 30% compared to the control system. In addition, the microbial activity was enhanced in these systems.

The acute toxicity tests of the effluent in all of the systems were performed with *Daphnia magna*, and all of the samples were classified as non-toxic. Thus, there was no toxicity risk of discharge into water sources.

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