CHEMICAL ENGINEERING TRANSACTIONS
VOL. 49, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216
DOI: 10.3303/CET1649079

Preliminary Evaluation of Sludge Minimization by a Lab-Scale OSA (Oxic - Settling - Anaerobic) System

Rosa Vitanza[a*], Tommaso Diociaiuti[b], Maria E. De Arana-Sarabia[a], Iginio Colussi[a], Angelo Cortesi[a], Vittorino Gallo[a].

[a]Department of Engineering and Architecture, University of Trieste, via A. Valerio 6, 34127 Trieste, Italy
[b]Department of Life Sciences, University of Trieste, Via E. Weiss 2, 34128 Trieste, Italy
rosa.vitanza@di3.units.it

The biological wastewater treatment process involves a significant production of sludge that must be treated before its ultimate disposal. The treatment and disposal of the excess sludge are expensive processes and the minimization of sludge production is a topic of great interest. The oxic – settling – anaerobic (OSA) process is an adaptation of the conventional activated sludge (CAS) treatment, set up by inserting a sludge holding tank (SHT) in the sludge return line. The OSA technology demonstrated to reduce the excess sludge production and several studies have confirmed that the enhanced sludge decay is the decisive cause in OSA process.
The present paper reports the preliminary results obtained by an OSA lab-scale plant working with real influent wastewater. The plant operations were monitored by calculations of removal efficiencies and by microfauna composition analysis. Two runs of one month were performed: the average removal efficiencies were 76% for soluble COD and 82% for ammonia nitrogen. Biological denitrification was also observed with an efficiency of 34% for the first run and of 19% for the second one. The microfauna observations showed that the activated sludge remained efficient during the two runs with a high density of microfauna (always greater than 10^6 organisms per liter) and a highly diversified community.

1. Introduction

In the last decades, the growth of the households connected to the wastewater treatment plants (WWTPs) and the major environmental limitations in water disposal have increased the amount of generated sewage sludge in Europe (De Filippis et al., 2013). The EU-28 sewage sludge production during the year 2010 was about 10 million tons of dry solids (http://epp.eurostat.ec.europa.eu) and it is expected that, up to 2020, the previous value will increase exceeding 13 million tons of dry solids (Kelessidis and Stasinakis, 2012). The treatment and disposal of the excess sludge are expensive processes accounting for 25% - 60% of the total plant operation costs (Etienne, 2012), corresponding to 25-35 €/(person x year) (Braguglia et al., 2012). For this reason, the production of excess sludge from biological treatment is one of the most vexing problems for WWTPs necessitating effective management strategies (Semblante et al., 2014). Three main approaches may be followed for sludge minimization in WWTPs (Perez-Elvira et al., 2006): (i) processes in the water line, directed to lower the yield coefficient; (ii) processes in the sludge line, aiming to reduce the final stream of sludge to be disposed of; (iii) processes in the final waste line (that do not represent a minimization strategy, but a post-treatment for the final disposal of the sewage solids).
The oxic-settling-anaerobic (OSA) process, first hypothesized by Westgarth et al. (1964), belongs to the first process type and is an adaptation of the conventional activated sludge (CAS) treatment, set up by inserting an anaerobic sludge holding tank (SHT) in the sludge return line (Chudoba et al., 1992). This anaerobic tank is different from the anaerobic reactors generally operating in biological nutrient removal processes: indeed, in the SHT no external substrate is supplied and the feed is represented by the few organic substances left from the previous oxidation tank (Chen et al., 2003). In addition, since neither physical or chemical treatment are needed (Chen et al., 2003), the OSA process seems to be an economical option to reduce excess sludge

Please cite this article as: Vitanza R., Diociaiuti T., De Arana-Sarabia M.E., Colussi I., Cortesi A., Gallo V., 2016, Preliminary evaluation of sludge minimization by a lab-scale osa (oxic – settling - anaerobic) system, Chemical Engineering Transactions, 49, 469-474
DOI: 10.3303/CET1649079

production and can be readily retrofitted to existing plants as well as implemented in new designs (Semblante et al., 2014).
The OSA technology demonstrated to reduce the excess sludge production by 23% to 58% (Wang et al., 2008), but the reason by which this occurs is unclear. Semblante et al. (2014) summarized the sludge reduction mechanisms reported in literature - *i.e.* enhanced endogenous decay, EPS destruction, biomass feasting/fasting, energy uncoupling/spilling, slow-growing bacteria selection and predation on bacteria by higher organisms – pointing out that it is difficult to isolate a single cause because it is possible that these mechanisms are overlapping. Wang et al. (2008) investigated the effects of sludge decay, energy uncoupling and low sludge yield of anaerobic oxidation on the excess sludge minimization concluding that the sludge decay is the decisive cause in the OSA process, accounting for 66.7% of sludge production reduction.
The oxidation-reduction potential (ORP) is a crucial operating parameter affecting the OSA process: it was proved that when the ORP level is controlled at -250 mV, the excess sludge can be reduced by 58% compared to a CAS process (Saby et al., 2003). Also the sludge retention time (SRT) plays a role, being in general inversely proportional to the sludge yield (Semblante et al., 2014).
In this paper, the preliminary results obtained from an OSA lab-scale plant working with real influent wastewater are reported. The process was studied by correlating the treatment efficiency with the species structure of the microfauna. Thus, the plant operations were monitored by routine physical-chemical analysis and by microfauna composition observations.

2. Materials and Methods

2.1 Experimental set-up and operation

The lab-scale OSA system is reported in Figure 1. It is composed by an oxidation reactor of 3.0 L, a settling tank of 0.45 L and an anaerobic sludge holding tank of 4.5 L. Influent and recirculation flowrates (of 9 L/d and 13 L/d, respectively) are fed by the control system BioKontrol_Mark2 that provides also an air flow of 2.0-2.3 L/min. The effluent is discarded from the top of settling tank by a peristaltic pump. A gasometer is connected to the SHT in order to collect the produced biogas (if any). Dissolved oxygen into the aeration tank is measured by the Hanna Instruments probe HI76401 and ORP values are detected by the Hanna Instrument electrode HI3230.
Two runs of operations were performed: run #1 (during summer season) and run #2 (end summer-autumn).
Influent and activated sludge came from a municipal WWTP located in the Friuli Venezia Giulia region operating in the CAS configuration. The wastewater in the influent barrel was replaced every 3-4 days with 30-40 L of fresh influent: the feed occurred only when the barrel was totally empty, in order do not mix different samples. At the same time, the effluent barrel (not visible in Figure 1a) was emptied in order to analyze it and to perform the mass balances.
Total and volatile solids into the influent, the effluent and the sludge were analyzed according to Standard Methods (APHA, 2005). COD, nitrogen (ammonium and nitrate) and phosphate concentrations were detected by the Hach-Lange test cuvettes.
In Table 1 the characteristics of the influent and the initial conditions of the activated sludge for the two runs are reported.

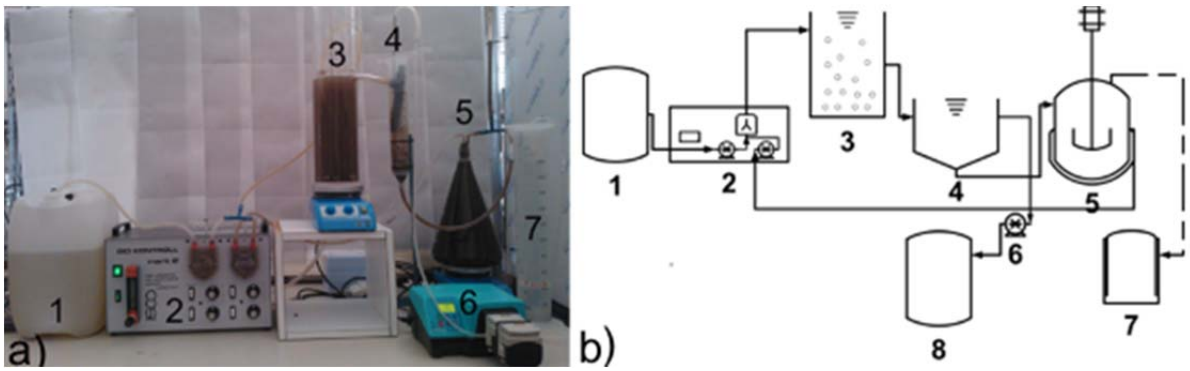

Figure 1: a) Experimental set-up, b) PFD: 1) Influent barrel, 2) Control system, 3) Oxidation reactor, 4) Settling tank, 5) Anaerobic sludge holding tank, 6) Peristaltic pump, 7) Gasometer, 8) Effluent barrel

Table 1: Influent characteristics and initial conditions of activated sludge

	SS [mg·L^{-1}]	VSS [mg·L^{-1}]	tCOD [mg·L^{-1}]	sCOD [mg·L^{-1}]	N-NH_4 [mgN·L^{-1}]	N-NO_3 [mgN·L^{-1}]	PO_4 [mg·L^{-1}]
Influent characteristics							
Run #1	168±150	61±50	490±135	188±71	49.0±20.3	2.7±1.0	15.7±6.2
Run #2	183±150	137±113	440±134	198±78	53.3±21.8	3.5±1.4	14.3±7.9
AS initial conditions							
Run #1	5990	4030	-	20	2.5	23.5	14.8
Run #2	4900	3840	-	37	5.8	10.8	8.8

2.2 Microfauna observation

To monitor the microfauna, volumes of activated sludge at regular time intervals were sampled and stained with Rose Bengal. After staining, each sample was divided in two portions: one part was analyzed in vivo to identify the contained species and the other one was formalin-fixed and utilized to characterize and quantify the microfauna. Protozoa and small metazoan were identified using an optical microscope at 100 X following the method proposed by Madoni (1994). Most protozoa were identified to the species level according to morphology and movements. Organisms not able to be identified to the species level were recorded as units. Small metazoa were classified into 3 units: gastrotricha, oligochaete and rotifers. The count was performed by observing the formalin-fixed sample with an inverted microscope following the Utermohl protocol (1958). The average number of organisms was standardized per liter of sample.

3. Results

3.1 Process performance and physico-chemical analysis

As stated before, the effluent barrel was emptied every time the influent changed, resulting in 8 samples for run #1 and 13 samples for run #2. The removal efficiencies for sCOD, ammonia and phosphate, showed in Figure 2, were calculated from the mass balances done at each sampling. The N-NO_3 removal was obtained by comparing the actual effluent nitrate concentration with the amount of nitrified ammonia.

As regards the first run, the average daily removal were: 1.42 g·d^{-1} for the soluble COD, 0.33 g·d^{-1} for ammonia, 0.12 g·d^{-1} for N-NO_3 and 0.02 g·d^{-1} for PO_4. In the second run similar values were obtained: 1.32 g·d^{-1} for sCOD, 0.38 g·d^{-1} for N-NH_4, 0.09 g·d^{-1} for nitrate and 0.01 g·d^{-1} for phosphate. The average removal efficiency for nitrate was of 33.5% for run #1 and 18.8% for run #2. Since the DO concentration into the aeration tank ranged from 2.2 mg·L^{-1} to 3.5 mg·L^{-1}, it is reasonable to suppose that denitrification took place into the SHT, where anaerobic/anoxic conditions stood. Furthermore, as no external substrate was supplied to the sludge holding tank, the biological denitrification occurred using hydrolysed decayed biomass as electrons donor. The average values of ORP measured into the anaerobic sludge holding tank during the two runs were -160 mV for run #1 and -55 mV for run #2.

3.2 Observed sludge yield

To evaluate the sludge production, the observed sludge yield Y_{obs} was calculated. Starting from the fundamental definition of yield, “the amount of sludge formed per the amount of substrate removed” (Grady et al., 1999), in literature several examples of yield calculation for OSA and side-stream reactors exist, differing by the expression of the substrate removed (as soluble COD or as total COD) (Wang et al., 2008; Chon et al., 2011; Torregrossa et al., 2012; Coma et al., 2013).

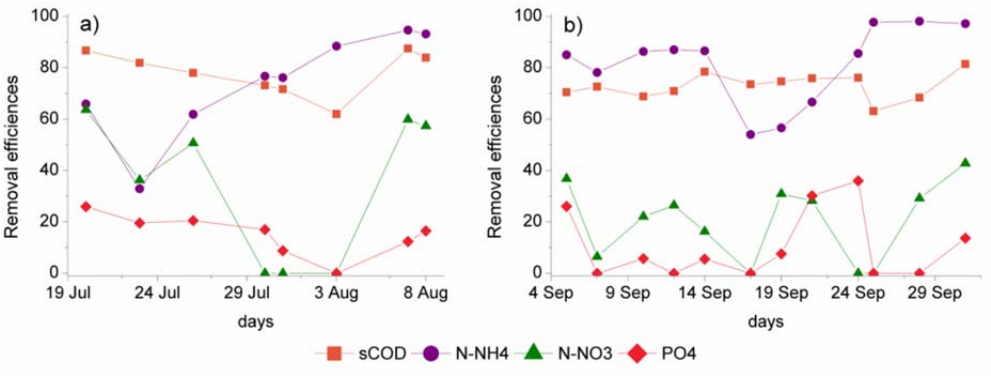

Figure 2: Removal efficiencies: a) run #1, b) run #2

In this work, the approach of Chon et al. (2011) was followed, calculating the observed yield by equation (1):

$$Y_{obs} = \frac{\Delta X_{AS} V_{AS} + \Delta X_{SHT} V_{SHT} + X_{eff} Q_{eff} \Delta t}{\Sigma (S_{in} Q_{in} - S_{eff} Q_{eff}) \Delta t} \quad (1)$$

where:

- $\Delta X_{AS} V_{AS}$ and $\Delta X_{SHT} V_{SHT}$ are the changes of sludge amounts into the activated sludge tank and in the sludge holding tank, respectively;
- Q_{eff} is the effluent flowrate having the solid concentration X_{eff} ($X_{eff} Q_{eff} \Delta t$ represents the sludge wastage from the effluent in the given time - there was no sludge wastage from the return activated sludge);
- S_{in} and S_{eff} represent the soluble COD concentrations in the influent and in the effluent, respectively.

Cumulative terms were used to quantify changes in both solids and substrate because solids concentrations in both oxidation reactor and sludge holding tank changed during OSA process operations. The Y_{obs} was obtained as the slope of the regression line of the cumulative generated sludge versus the cumulative consumed substrate. As showed in Figure 3, the observed yields were 0.335 gVSS·g^{-1}sCOD for the run #1 and 0.408 gVSS·g^{-1}sCOD for the run #2, values similar than those found by Wang et al. (2008) in their lab-scale plant.

3.3 Analysis of microfauna

The biological community of the activated sludge consists of various populations competing with each other for food: decomposers (bacteria and fungi) utilize the dissolved organic matter in the wastewater and consumers (protozoa and small metazoan) feed on dispersed bacteria and other organisms (Madoni, 1994; Signorile et al., 2010). Within the aeration tank of the activated sludge system, the protozoa acquire special significance: they not only consume most of the dispersed bacteria in the mixed liquor (contributing in reduction of suspended solids), but also are sensitive to changes in environmental conditions, allowing the evaluation of the system performance (Araújo dos Santos et al., 2014). According to Madoni (1994), an efficient activated sludge should have the following characteristics: (i) high number of microfauna cells (≥10^6 organisms per liter); (ii) microfauna composed chiefly by crawling and attached ciliates; (iii) a highly diversified community.

A summary of the results of microfauna analysis is presented in Table 2 in which the main species abundances (expressed as total individual number and in percent) are reported for the beginning of the experiment (*i.e.* in the real WWTP conditions) and for the end of the OSA configuration period. As it can be seen, the initial conditions of the two samples agree with the characteristics reported by Madoni (1994), therefore the original activated sludge (from the WWTP) can be classified as efficient. On Figure 4, the four most abundant taxa observed during the experiments are reported. As it can be observed for run #1, the testate amoebae Euglypha *sp*. and Arcella *sp*. were found in all the four periodic samples, indicating an excellent quality of effluent and a high performance of the treatment (Madoni, 1994). From the figure, it is possible to notice that their relative abundances decreased in the sample collected in the 30 of July, when the abundance of Opercularia *sp.* increased. This could be a symptom of a deterioration of the activated sludge, because these ciliates are observed to survive in stressed environments better than other protozoans (Madoni, 1994). Transient conditions in the sludge structure, probably due to the adaptation from CAS configuration to OSA system, were proved by the increase, noticed on the 23 of July, of the attached ciliate Thuricola sp., (Madoni, 1994). Another interesting item was the progressive disappearance of the crawling ciliates that decreased from the 6% of the total microfauna in the first sample, to 0% in the last one (passing through the 4% in the second sample and 1% in the third one).

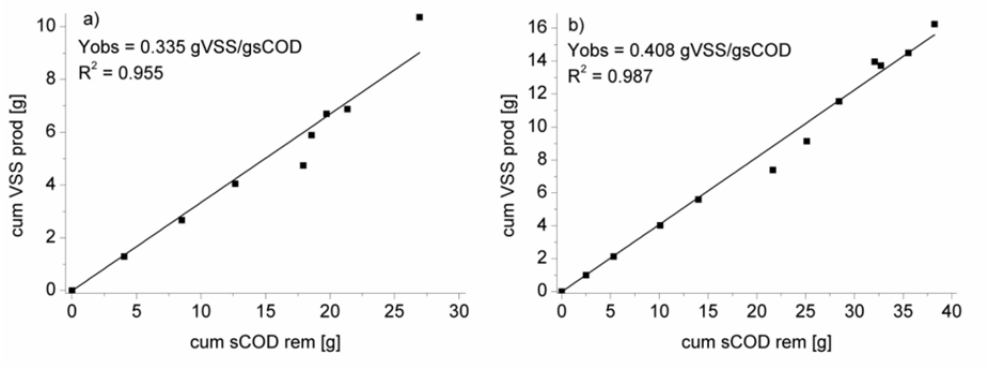

Figure 3: Observed yield from cumulative biomass production and organic matter removal: a) run #1, b) run #2

Table 2: Main species revealed during the run #1 and the run #2

	Run #1				Run #2			
	Initial Abudance		Final Abudance		Initial Abudance		Final Abudance	
	No/L	%	No/L	%	No/L	%	No/L	%
Swimming ciliates	360,000	10.7	140,000	7.1	360,000	5.8	100,000	5.3
Crawling ciliates	200,000	5.9	0.0	0.0	280,000	4.5	40,000	2.1
Attached ciliates	700,000	20.7	480,000	24.2	3,920,000	62.8	900,000	47.4
Carnivorous ciliates	60,000	1.8	20,000	1.0	100,000	1.6	0.0	0.0
Amoebae	140,000	4.1	80,000	4.0	0,0	0.0	20,000	1.1
Testate amoebae	1,780,000	52.7	1,180,000	59.6	480,000	7.7	660,000	34.7
Oligochaeta	120,000	3.6	40,000	2.0	120,000	1.9	60,000	3.2
Rotifers	20,000	0.6	0.0	0.0	100,000	1.6	100,000	5.3
Gastrotricha	-	-	-	-	0,0	0.0	20,000	1.1
Total microfauna	3,380,000		1,980,000		6,240,000		1,900,000	
Total number of taxa	19		12		14		12	

Simultaneously to the disappearance of the crawling ciliates, a worsening in the settling was observed, probably explained with the inverse relationship of the crawling ciliates with the SVI (Madoni, 1994). In spite of the previous negative symptoms, the treatment efficiency was high during the whole experiment, except for the days near the 30 of July when there was no denitrification. It is worthy to note that, during the same time, the conditions of the activated sludge changed also in the full scale CAS plant. Indeed, the initial sludge sampling of the second run revealed a high abundance of Opercularia *sp.* and a decrease in the number of crawling ciliates (among which only Aspidiscia costata was identified). However, despite being the activated sludge 'stressed', good removal performances for COD and ammonia were obtained also during the run #2, as proved by the constant presence of Euglypha *sp*. and Arcella *sp.* (frequency of 100%).
A microscopic analysis done for the sludge contained in the SHT at the end of run #2 revealed a prevalence of the Opercularia *sp.* (43% of abundance), the unique species of attached ciliates found, followed by the swimming ciliate Cinetochilum *sp.* (19%) and by the testate amoeba Arcella *sp*. (11%).

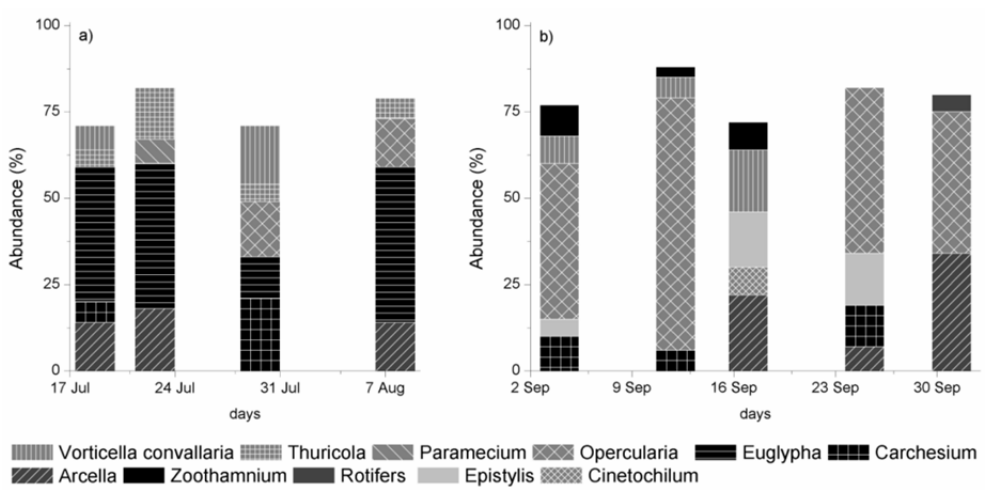

Figure 4: The most abundant taxa trend: a) run #1, b) run #2

4. Conclusions

The performances of a lab-scale OSA system were evaluated by coupling removal efficiencies with microfauna variation analysis. Two experimentation runs were performed.
The results showed that the removal of COD and ammonia remained high during the acclimatization of the activated sludge to the OSA conditions. The occurred denitrification (with the average efficiencies of 33.5% for run #1 and 18.8% for run #2) proved the hydrolysis of decayed biomass into the anaerobic sludge holding tank, suggesting that the sludge reduction was due to the enhanced endogenous decay. As regards the microfauna dynamics, despite the increase of the *Opercularia sp*., on the whole it can be stated that there was

not a worsening in the conditions of the activated sludge, that remained efficient with a number of cells higher than 10^6 and a diversified number of taxa.
The sludge production during the experiments was low: the calculated observed yield were 0.335 $gVSS \cdot g^{-1}sCOD$ for the run #1 and 0.408 $gVSS \cdot g^{-1}sCOD$ for the run #2.

Acknowledgments

The Authors thank CAFC S.p.A. for the financial support.

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