



# Influence of an oxic settling anoxic system on biomass yield, protozoa and filamentous bacteria



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## HIGHLIGHTS

- The oxic settling anoxic system reduced the biomass yield and active biomass.
- The oxic settling anoxic system most likely will improve settling properties.
- The oxic settling anoxic system showed a higher metabolic activity than control.
- The oxic settling anoxic system decrease both protozoa density and diversity.
- Diversity and crawling ciliates have been shown as promising bioindicators.

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## ABSTRACT

An oxic settling anoxic system coupled with an activated sludge process has been studied to reduce sewage sludge production. The reduction of sludge yield, excess sludge production and active biomass yield were 51.7%, 52.9% and 67.1%, respectively, compared with the control system. The oxic reactor of the oxic settling anoxic system, even with a lower active biomass concentration than the oxic reactor of control system, showed a higher metabolic activity in their active biomass. Diversity and crawling ciliates group have been shown as promising bioindicators of active biomass yield reduction. The identification of floc-forming bacteria in the control system suggested that oxic settling anoxic system will improve settling properties compared to a Conventional Activated Sludge process.

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## 1. Introduction

The Conventional Activated Sludge (CAS) process has been extensively applied to wastewater treatment (Zhou et al., 2015). The large amount of sewage sludge produced from CAS process is

a waste management problem. Handling, treatment and disposal costs of the sewage sludge are important concerns for most countries. The increase of the annual sewage sludge worldwide production is expecting to exceed 13 million tons Dried Solids (DS) in 2020 (Kelessidis and Stasinakis, 2012). In view of this, the reduction of sewage sludge production has become one of the first priorities in waste management hierarchy (CEU, 2008).

Many technologies are currently developed to reduce and/or treat sewage sludge in large installations (Cieřlik et al., 2015) and small-scale installations (Wu et al., 2013). However, medium size installations, i.e., around 50,000 equivalent population are still a challenge in order to efficiently reduce sludge production. Several technologies are currently developed to reduce sludge production in medium size installations such as the sludge drying bed, worm treatment, thermal processes, drying systems, incineration or co-incineration (Cieřlik et al., 2015).

**Abbreviations:** OSA, oxic settling anoxic; CAS, Conventional Activated Sludge; DS, Dried Solids; MBR, Membrane Bioreactor; MBBR, Moving Bed Biofilm Reactor; SSR, Side-Stream Reactor; SBR, Sequencing Batch Reactor; TSS, Total Suspended Solids; VSS, Volatile Suspended Solids; SLP, Suspended Lipid Phosphate; COD, Chemical Organic Demand; HRT, Hydraulic Retention Time; DO, Dissolved Oxygen; MLSS, Mixed Liquor Suspended Solids; ORP, Oxidation–Reduction Potential;  $Y_s$ , sludge yield;  $Y_{ab}$ , active biomass yield; SBI, Sludge Biotic Index;  $H'$ , Shannon diversity index;  $\alpha$ , statistical significance of Pearson correlation test; EPS, extracellular polymeric substances; TCS, 3,3',4',5-tetrachlorosalicylanilide.

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One promising option as sewage sludge reduction method is the application of the oxic settling anoxic (OSA) system, which consist in the implementation of an anoxic reactor in the sludge recirculation line of a CAS process. The OSA system shows many advantages such as easy application, avoids undesirable products and seems sustainable in the long term (Coma et al., 2013; Guo et al., 2013; Zhou et al., 2014, 2015). The OSA system is easy to implement into existing wastewater treatment processes and its operation should not be more complex than the previously existing wastewater treatment process. The OSA system has been shown as a feasible solution of sewage sludge reduction in medium size installations (Coma et al., 2013).

The alternating of the oxic/anoxic/anaerobic environments in wastewater treatment processes has been studied to reduce sewage sludge production in several configurations. The OSA system implementation in a CAS process has been studied by the inclusion of an anoxic reactor in the sludge recirculation line (An and Chen, 2008; Chudoba et al., 1992; Ye et al., 2008). The inclusion of a settler after the Membrane Bioreactor (MBR) and an anaerobic reactor in the sludge recirculation line has been studied as well (Chen et al., 2003; Saby et al., 2003). Also, the inclusion of an anoxic, anaerobic and aerobic reactor after the Moving Bed Biofilm Reactor (MBBR) has been studied (Li et al., 2014). Furthermore, in an anaerobic, anoxic and aerobic reactor has been studied by the inclusion of an anaerobic Side-Stream Reactor (SSR) in the sludge recirculation line (Coma et al., 2013). The inclusion of an anaerobic SSR in the sludge recirculation line of an aerobic Sequencing Batch Reactor (SBR) has been studied as well (Yagci et al., 2015). Also, inclusion of an anaerobic reactor in the sludge recirculation line in an anoxic, oxic reactor has been studied (Zhou et al., 2014, 2015). All of these authors has been reported that the alternating of the oxic/anoxic/anaerobic environments is a highly promising option for sewage sludge reduction.

Up to our knowledge, few studies have focused on microbiological dynamics in the OSA system. The anoxic environment have been shown to impact in the cellular metabolism, sludge ecology, protozoa and filamentous bacteria (Rodríguez-Pérez and Fermoso, 2015), but it has not been far investigated in a continuous lab scale process. The study of protozoa and filamentous bacteria could be important for the further understanding of the process, due to its role as potential bioindicator as was suggested by Rodríguez-Pérez and Fermoso (2015).

The aim of this study was the implementation of the OSA system in a continuous CAS process, focusing on the biological sludge minimization. The impact of the OSA system application in the cellular metabolism, protozoa population and filamentous bacteria were investigated. Protozoa population was analyzed to determine its role as a potential bioindicators.

## 2. Methods

### 2.1. Inoculum and influent

The inoculum was taken from the secondary sludge recirculation line of a municipal wastewater treatment plant in Seville, Spain. The characteristics of the inoculum used were: 3.4 g L<sup>-1</sup> of Total Suspended Solids (TSS) concentration, 2.3 g L<sup>-1</sup> of Volatile Suspended Solids (VSS) concentration and 85 ± 10 nmol mL<sup>-1</sup> of the Suspended Lipid Phosphate (SLP) concentration.

The influent of the continuous systems was prepared with a mixture of glucose, as sole added organic matter, in a mineral medium described by Rodríguez-Pérez and Fermoso (2015). The pH was adjusted to an initial value of 7.25 ± 0.25 and was kept with phosphate buffer solution. The mean concentration of the influent Chemical Organic Demand (COD) was 282 ± 7 mg COD L<sup>-1</sup>.

### 2.2. Experimental procedure

#### 2.2.1. Lab scale reactor

Two identical lab scale reactors were designed, built and operated in parallel (Fig. 1). One of them was used as a control system and operated as a CAS process (Fig. 1). The other one was a modified CAS process, which included an anoxic reactor in the sludge recirculation line, i.e., the OSA system (Fig. 1). The work volumes of oxic reactors and settlers were 2.5 L and 3.7 L, respectively, in both lab scale reactors. Bubble diffusers located at the bottom of the oxic reactors provided the aeration at 1.5 L min<sup>-1</sup>. The OSA system was designed with a Hydraulic Retention Time anoxic/oxic ratio equal to 0.27. The anoxic reactor was designed with a volume of 1.35 L.

#### 2.2.2. Experimental conditions

The lab scale reactors were fed with the same feeding flow rate, 6.4 L d<sup>-1</sup>. The returned sludge ratio was set to 200% in both lab scale reactors. Hydraulic Retention Time (HRT), Dissolved Oxygen (DO) concentration and Mixed Liquor Suspended Solids (MLSS) concentration in both oxic reactors were set at 9 h, 6 mg L<sup>-1</sup> and 2.5 g L<sup>-1</sup>, respectively. HRT, DO concentration and MLSS concentration, in the anoxic reactor included in the OSA system, were set at 2.5 h, 0.23 mg L<sup>-1</sup>, 5 g L<sup>-1</sup> and, respectively. HRT in the control system was 23 h, while HRT in the OSA system was 25.5 h. The sludge age for the activated sludge in the control system and OSA system were 4 days, and 14 days, respectively. The reaction temperature was the ambient temperature, c.a. 25 ± 5 °C. The lab scale reactors operation lasted 100 days.

### 2.3. Chemical analysis

Soluble COD, TSS and VSS concentrations were determined according to Standard Methods of analysis (Eaton et al., 2005). Active biomass was measured by SLP concentration following the procedure by Arnaiz et al. (2006). Phospholipids are the major lipids in bacteria, being 90–98% of the bacterial membrane. Phospholipids are relatively constant membrane compound and are not forming part of cell reserves, which make it reasonable to use the SLP concentration as active biomass (Arnaiz et al., 2006). The DO concentration was measured using a DO meter (HQ30D Flexi, Hach-Lange), the Oxidation–Reduction Potential (ORP) and pH were measured by digital pH meter (GLP22, Crison). All measurements were made in triplicate.

### 2.4. Sludge production

The sludge yield ( $Y_s$ ) was calculated to evaluate the sludge production. Since solids concentrations in the systems changed, cumulative terms during the period of study were used (Eq. (1)). For this reason, COD and VSS were quantified daily.

$$Y_s = \frac{\sum \text{VSS produced}}{\sum \text{COD removed}} = \frac{\sum (F_w * \text{VSS}_w + F_e * \text{VSS}_e + \Delta \text{VSS}_{\text{sys}})}{\sum (F_i * (\text{COD}_i - \text{COD}_e))} \quad (1)$$

where  $F_w$ ,  $F_i$  and  $F_e$  correspond to the waste, influent and effluent flows in m<sup>3</sup> d<sup>-1</sup> (Fig. 1), respectively;  $\text{COD}_i$  and  $\text{COD}_e$  correspond to the soluble organic matter in the influent and effluent (g COD m<sup>-3</sup>), respectively;  $\text{VSS}_w$  and  $\text{VSS}_e$  correspond to the Volatile Suspended Solids in the waste and effluent (g VSS m<sup>-3</sup>), respectively; and  $\Delta \text{VSS}_{\text{sys}}$  corresponds to the biomass accumulated in the system (g VSS d<sup>-1</sup>).

$Y_s$  was determined by regression of the cumulative sludge production versus the cumulative organic matter removal as describes Coma et al. (2013). The slope of the regression line is considered the mean  $Y_s$  (Fig. 2A).

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