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# The role of microbial diversity and composition in minimizing sludge production in the oxic-settling-anoxic process

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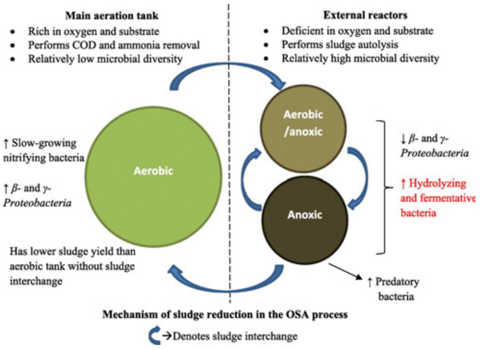
## HIGHLIGHTS

- The mechanistic process of OSA from a microbiological perspective was elucidated
- Redox conditions were the key factor affecting microbial community structure
- $SBR_{OSA}$ contained more slow-growing nitrifiers than $SBR_{control}$
- Denitrifiers and predators enhanced sludge reduction in external reactors
- Both oxygen and substrate deficiency were critical for sludge reduction

## GRAPHICAL ABSTRACT



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## ABSTRACT

The oxic-settling-anoxic (OSA) process, which involves an aerobic tank attached to oxygen- and substrate-deficient external anoxic reactors, minimizes sludge production in biological wastewater treatment. In this study, the microbial community structure of OSA was determined. Principal coordinate analysis showed that among the three operational factors, *i.e.*, (i) redox condition, (ii) external reactor sludge retention time ($SRT_{ext}$), and (iii) sludge interchange between aerobic and anoxic reactors, redox condition had the greatest impact on microbial diversity. Generally, reactors with lower oxidation-reduction potential had higher microbial diversity. The main aerobic sequencing batch reactor of OSA ($SBR_{OSA}$) that interchanged sludge with an external anoxic reactor had greater microbial diversity than $SBR_{control}$ which did not have sludge interchange. $SBR_{OSA}$ sustained high abundance of the slow-growing nitrifying bacteria (*e.g.*, *Nitrospirales* and *Nitrosomondales*) and consequently exhibited reduced sludge yield. Specific groups of bacteria facilitated sludge autolysis in the external reactors. Hydrolyzing (*e.g.*, *Bacteroidetes* and *Chloroflexi*) and fermentative (*e.g.*, *Firmicutes*) bacteria, which can break down cellular matter, proliferated in both the external aerobic/anoxic and anoxic reactors. Sludge autolysis in the anoxic reactor was enhanced with the increase of predatory bacteria (*e.g.*, order *Myxobacteriales* and genus *Bdellovibrio*) that can contribute to biomass decay. Furthermore, β- and γ-*Proteobacteria* were identified as the bacterial phyla that primarily underwent decay in the external reactors.

## 1. Introduction

The management of excess sludge constitutes a significant fraction of the total operation cost of biological wastewater treatment. Because

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sludge contains active biomass and biodegradable materials, treatment is required prior to disposal to prevent negative impact on public sanitation and environment. Sludge treatment, which mainly involves the removal of water, volatile solids, and pathogens, is a challenging process due to the strong binding of water molecules to sludge flocs and the slow biodegradation of the volatile fraction under ambient conditions (Mowla et al., 2013; Tchobanoglus et al., 2003). Furthermore, there are only a few options for ultimate sludge disposal. Ocean-dumping and land-filling were the traditional means of disposing of sludge; however, the former has been banned to protect marine life and while the latter has been restricted due to the high cost of landfill operation. Current practices, such as sludge incineration and re-use of sludge as land-applicable biosolids, have some inherent disadvantages. For instance, incinerating sludge is highly effective in removing volatile solids, but has high energy requirements. Re-using sludge enables the recovery of organic matter and nutrients, but the conversion of sludge into high-quality biosolids that can be safely used in agricultural applications and the transport of biosolids from metropolitan facilities to farmlands are expensive (Semblante et al., 2014; Tchobanoglus et al., 2003). These concerns emphasize the need to minimize sludge production. Reducing sludge will decrease costs for dewatering, stabilization, transportation, and other aspects of sludge management (Foladori et al., 2010; Semblante et al., 2014). A number of approaches have been devised to minimize sludge, such as controlling dissolved oxygen (DO) and sludge retention time (SRT) of the aeration tank, adding chemicals to decrease sludge growth, and destroying sludge using advanced oxidation processes. However, the full-scale implementation of these approaches are hindered because they either require significant capital and operating cost or only result in a marginal sludge reduction (Foladori et al., 2010).

The oxic-settling-anoxic (OSA) process is a potentially economical alternative to sludge reduction. It involves the addition of external anoxic reactor/s in the return sludge loop of the conventional activated sludge (CAS) process. Due to its simple design, OSA can be set up using readily available equipment (*e.g.*, tanks, pipes, and pumps) and requires minimal maintenance (Semblante et al., 2014). The sludge interchange between the external anoxic reactor/s and the main aerobic tank results in net sludge reduction. Recent research demonstrated that manipulating parameters such as oxidation-reduction potential (ORP), sludge interchange rate and external reactor SRT influences the autolysis of sludge in the oxygen- and substrate-deficient external anoxic reactor/s (Khursheed et al., 2015; Saby et al., 2003; Semblante et al., 2016b). Sludge can be reduced by >35% depending on the aforementioned parameters and wastewater characteristics (Saby et al., 2003; Semblante et al., 2015).

Previous studies hypothesized that sludge reduction in the OSA process is driven by the selection of a distinct microbial community brought about by the interchange of sludge between different redox regimes (Goel and Noguera, 2006; Kim et al., 2012; Semblante et al., 2014). Conventional techniques such as polymerase chain reaction-denaturing gradient gel electrophoresis have shown that the microbial community in an oxic-settling-anaerobic system was similar to that of anaerobic digesters, therefore reactions such as sulfate reduction and methane production took place in the external anaerobic reactor (Kim et al., 2012; Saby et al., 2003). High-throughput sequencing methods (*i.e.,* pyrosequencing and Illumina sequencing) produce higher resolution than conventional techniques and hence achieve better characterization of microbial communities. Application of pyrosequencing analysis showed that an aerobic/anoxic system with external anaerobic reactor has greater microbial diversity than a control aerobic/anoxic system (Ning et al., 2014; Zhou et al., 2015) probably because slow-growing fermentative (*Azospira*, *Propionivibrio* and *Sulfuritalea*) (Zhou et al., 2015) and hydrolyzing (*Sphingobacteria*) (Ning et al., 2014) bacteria were enriched under anaerobic conditions. Meanwhile, Illumina sequencing analysis in the study by Cheng et al. (2017) showed enrichment of different types of bacteria in an aerobic membrane bioreactor (MBR), *e.g., Nitrospirae*, and the attached external anaerobic reactor, *e.g., Chloroflexi* and *Armatimonadetes*. It was further observed that microbial groups that facilitate sludge autolysis, such as those that perform degradation of extracellular polymeric substances (EPS), sulfate reduction, and fermentation, survive in the external anaerobic reactor attached to an anoxic/aerobic sequencing batch reactor (SBR) (Cheng et al., 2017; Ferrentino et al., 2016). These studies imply that aerobic-anaerobic interchange enriched sludge biomass and that microbial composition yielded useful information regarding potential biological reactions relevant to sludge reduction (Cheng et al., 2017; Ferrentino et al., 2016; Ning et al., 2014; Zhou et al., 2015). Therefore, it is necessary to characterize the microbial community structure of OSA, which is expected to differ from that of other configurations available in literature due to the sensitivity of bacteria to redox and other operating conditions, to understand its effects on sludge reduction. Addressing this crucial knowledge gap will be useful in designing bioreactors and selecting operating conditions that will facilitate sludge reduction.

The aim of this study was to determine the microbial community structure in OSA to provide insight in its role in sludge reduction mechanisms. A laboratory-scale OSA (SBR with external aerobic/anoxic and anoxic reactors) with real wastewater was operated alongside a control (SBR with single-pass aerobic digester). To systematically determine the effects of microbial community on sludge reduction, Illumina sequencing analysis was performed when SRT of the SBRs (henceforth called $\text{SRT}_{\text{SBR}}$) was kept constant (10 days) and the SRT of the external reactors (henceforth called $\text{SRT}_{\text{ext}}$) were varied (10, 20, and 40 days). The potential linkage between operating parameters (*e.g.*, redox condition, $\text{SRT}_{\text{ext}}$, and sludge interchange between aerobic and anoxic reactors) and microbial community was determined. Variation in microbial diversity and taxonomic classifications were also systematically investigated.

## 2. Materials and methods

### 2.1. Wastewater characteristics

Wastewater was obtained fortnightly from the beginning of the primary sedimentation channel of Wollongong wastewater treatment plant (WWTP), New South Wales, Australia. It was stored at 4 °C in plastic containers prior to use. Wastewater characteristics are summarized in Supplementary Table S1.

### 2.2. Reactor configuration and operation

The detailed description of the configuration and operation of the OSA and control systems is discussed elsewhere (Semblante et al., 2016b). Briefly, the OSA system consisted of a $\text{SBR}_{\text{OSA}}$ (5 L) attached to external aerobic/anoxic (2 L) and anoxic reactors (2 L) (Fig. 1a). The control system consisted of $\text{SBR}_{\text{control}}$ (5 L) attached to a single-pass aerobic digester (2 L) (Fig. 1b).

$\text{SBR}_{\text{control}}$ and $\text{SBR}_{\text{OSA}}$ were fed with domestic sewage (Section 2.1) and operated at 4 cycles/day and HRT of 12 h. Each cycle comprised of 15 min of filling, 4.5 h of aeration, 1 h of settling, and 15 min of decanting. The SRT of $\text{SBR}_{\text{control}}$ and $\text{SBR}_{\text{OSA}}$ were maintained at 10 days by regular sludge wastage (W) (Fig. 1).

The aerobic digester of the control system (Fig. 1a) was continuously aerated. The SRT of this digester was adjusted to 40, 20, and 10 days at Phase I, II, and III of the study, respectively, through sludge wastage ($Q_{\text{out}}$). The aerobic digester was fed from sludge obtained from $\text{SBR}_{\text{control}}$ thickened to 5–10 g/L ($q_1$) by centrifugation for 10 min at 3300 × *g*. Sludge was obtained from the $\text{SBR}_{\text{control}}$ near the end of its aeration phase (*e.g.*, at 4.5 h) to ensure that the majority of the total chemical oxygen demand (tCOD) had been consumed.

The aerobic/anoxic reactor of the OSA system was intermittently aerated (*i.e.*, 8/16 h aeration on/off). The aerobic/anoxic reactor was fed with sludge from $\text{SBR}_{\text{OSA}}$ thickened to 5–10 g/L ($q_1$) by