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Short Communication

# A micro-aerobic hydrolysis process for sludge in situ reduction: Performance and microbial community structure

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## HIGHLIGHTS

- A micro-aerobic hydrolysis (SPRAS) process was used for sludge in situ reduction.
- SPRAS system achieved good performances of pollutants removal and sludge reduction.
- SPRAS system showed higher relative abundance and stability of microbial communities.
- SPRAS system enriched anaerobic bacteria, slow growers and predatory bacteria.

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## ABSTRACT

A sludge process reduction activated sludge (SPRAS) system by inserting a sludge process reduction (SPR) module, composed of a micro-aerobic tank and a settler, before activated sludge process was operated for sludge in situ reduction. The average removal efficiencies of COD and ammonium nitrogen were 86.6% and 87.9%, respectively. Compared to anoxic/aerobic (AO) process, SPRAS process reduced sludge production by 57.9% with observed sludge yield of 0.076 gVSS/gCOD. Pyrosequencing analyses revealed that the relative abundance and stability of microbial communities in SPRAS system were higher than AO system. Fermentative acidogenic classes *Anaerolineae*, *Actinobacteria*, *Cytophagia* and *Caldilineae* were enriched in the SPR module and responsible for sludge reduction. Specific comparison down to the genus level identified the enrichment of oxyanion-reducing bacteria (*Sulfuritalea*; *Azospira*; *Ramlibacter*), fermentative acidogenic bacteria (*Propionivibrio*; *Opitutus*; *Caldilinea*), slow growers (*Ramlibacter*) and predatory bacteria (*Myxobacteria*) in SPRAS system. Nitrifiers were also more abundant in SPRAS system than AO system.



## 1. Introduction

The generation of a large amount of wastage activated sludge (WAS) in activated sludge (AS) process has become the major problem with the dramatic increase of wastewater treatment plants (WWTP) (Khursheed and Kazmi, 2011). The treatment and disposal of WAS account for 25–65% of the total plant operating costs (Chon et al., 2011; Saby et al., 2003), and will be more challenging and costly because of more stringent regulatory requirements in sludge disposal and reuse. To tackle the problem, sludge in situ reduction (SIR) technology to decrease the amount of sludge production within the AS process has aroused much attention worldwide (Li et al., 2014a; Saby et al., 2003). With the insertion of an anaerobic side-stream reactor (SSR), the aerobic-settling-aerobic (OSA) process caused efficient sludge reduction (Li et al., 2014a; Saby et al., 2003), and was considered as a promising way for full-scale applications (Khursheed and Kazmi, 2011; Wang et al., 2013). Chon et al. (2011) reported another biological SIR process by introducing a portion of returned activated sludge (RAS) or WAS recycled through the anaerobic SSR. In the biological SIR process, uncoupling through the shift between aerobic and anaerobic conditions and sludge decay in the anaerobic tank due to hydrolysis and/or lysis were two major reasons of sludge reduction (Wang et al., 2013).

In our previous studies, a sludge process reduction activated sludge (SPRAS) process was designed for SIR by inserting a sludge process reduction (SPR) module, composed of a micro-aerobic tank and a settler (Fig. 1), before conventional AS process, and showed good performances of pollutant removal and sludge reduction (Zhou et al., 2014). In the SPRAS system, the SPR module creates a micro-aerobic environment with low dissolved oxygen (DO) in favor of facultative anaerobic bacteria by enhancing their growth rate and stimulating their physiological metabolism, and probably

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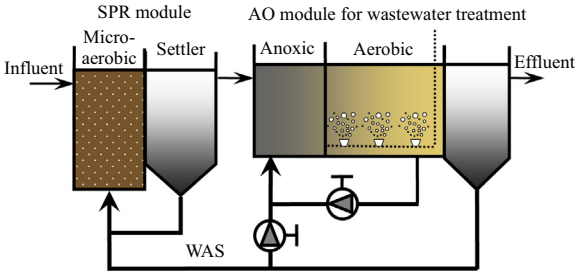


**Fig. 1.** Schematic diagram of the SPRAS sludge in situ reduction process.

has a significant influence on microbial communities. Therefore, information on microbial community structure and composition in SPRAS process is worthy and will deepen the understanding of its sludge reduction mechanisms.

Compared to conventional molecular biology methods (e.g. PCR-DGGE), pyrosequencing can generate huge amount of DNA reads, and identify thousands of operational taxonomic units (OTUs) to capture comprehensive and systematic microbial information of AS system (Hu et al., 2012). In this study, a lab-scale SPRAS system and a conventional anoxic/aerobic (AO) system were operated in parallel for the estimation of sludge reduction and the comparison of microbial community structure. 454-high throughput pyrosequencing technology was applied to investigate the microbial community structure and population composition in both SPRAS and AO.

## 2. Methods

### 2.1. Experimental setup and operating conditions

Two lab-scale bioreactors, a SPRAS and an AO, were both operated for 200 days and fed by wastewater from Dongqu WWTP in Shanghai with flow rate of 50 L/days. Hydraulic retention times (HRTs) of anoxic and aerobic tank in AO were 2.0 and 6.0 h, respectively. In SPRAS system, HRTs of AO module were the same as AO, and HRTs of SPR micro-aerobic tank and settler were 1.5 and 3.0 h (Zhou et al., 2014), respectively. DO values in SPR micro-aerobic tank and aerobic tank were controlled at 0.5–1.0 and 3.0–4.0 mg/L, respectively. Ratios of mixed liquor recirculation and RAS were both maintained at 100% for the two systems. The sludge recycle ratio from SPR settler to micro-aerobic tank was controlled at 50%. The solids retention time (SRT) of AO was controlled at 20 days. SRT of AO module was also kept at 20 days by discharging WAS to SPR module, but no WAS was discharged from the SPRAS.

### 2.2. Microbial community analysis

In this study, three samples for microbial analysis were collected from SPR micro-aerobic tank ($S_{SPR}$), aerobic tank of SPRAS ($S_{SPO}$) and aerobic tank of AO ($S_{AOO}$) on day 109. DNA extraction, PCR amplifications, and amplicons purification and qualification were conducted in accordance with the reported methods (Xie et al., 2014). A mixture of amplification was used for pyrosequencing on a Roche massively parallel 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, USA) according to standard protocols. To obtain database of effective sequences for each sample, random sequencing error and low-quality sequences were detected, trimmed and removed by SEQCLN and MOTHUR software (Lu et al., 2012).

Pyrosequencing reads were clustered into OTUs with an average length of 474 bp by setting a 3% or 5% distance limit ($\alpha$) using the MOTHUR software. Based on cluster information, the following parameters were calculated for each sample: rarefaction curve, Chao1 richness estimator, Shannon diversity index, abundance-based coverage estimator (ACE), Good's coverage for sequencing depth. Representative reads from the clusters were classified with a confidence threshold of 80% at levels of phylum, class and genus based on MOTHUR and SILVA106 database.

### 2.3. Analytical method and sludge reduction calculation

Chemical oxygen demand (COD), ammonium nitrogen ($NH_4$-N), total nitrogen (TN), and total phosphorus (TP) in the influent and effluent were analyzed every two days according to Chinese standard methods. Mixed liquor suspended solids and volatile suspended solids (VSS) were determined twice a week. DO, pH and oxidation–reduction potential (ORP) were monitored using an HQd30 portable meter (HACH Company, USA). The observed sludge yields ($Y_{obs}$) of the two systems were calculated according to the reported method (Zhou et al., 2014).

## 3. Results and discussion

### 3.1. Process performance

Fig. 2 summarizes variations of pollutants concentrations in the influent and effluent of the two systems. Both systems were almost equally effective in COD removal with average efficiency of 86.6%. The SPRAS obtained higher nitrification efficiency (87.9%) than AO (86.1%) owing to the insertion of SPR micro-aerobic tank and the long SRT. The average TN concentrations in the effluent of SPRAS and AO were 22.51 ± 4.05 and 15.12 ± 6.94 mg/L at COD/N ratio of 7.8, and then reduced to 6.84 ± 3.51 and 6.35 ± 3.00 mg/L at COD/N ratio of 11.7, respectively (Fig. 2c). The results indicated that the increase of COD/N ratio improved TN removal performance of the two systems. Compared to SPRAS that removed 28.2% of TP, more sludge discharge of AO led to higher TP removal of 43.9% (Fig. 2d). The SIR process usually deteriorated its phosphorus removal performance (Li et al., 2014a).

According to calculation, $Y_{obs}$ values of SPRAS and AO were 0.076 ($R^2$ = 0.979) and 0.180 ($R^2$ = 0.994) gVSS/gCOD, respectively. The SPRAS reduced sludge production by 57.9% compared to AO, and the result is similar to our previous study (Zhou et al., 2014) and the OSA process with sludge reduction efficiency of 14.0–63.5% (Li et al., 2014a; Saby et al., 2003). The ORP below −200 mV at the bottom of SPR settler indicated the formation of a stable anaerobic environment owing to the accumulation of WAS. Therefore, the SPR module can also be considered as an OSA-like process, and the anaerobic environment at the bottom of SPR settler followed by micro-aerobic tank in a cyclic order reduced the sludge through promotion of catabolism and demotion of anabolism by uncoupling mechanisms (Khursheed and Kazmi, 2011; Zhou et al., 2014).

### 3.2. Microbial community

#### 3.2.1. Richness and diversity of bacteria phylotypes

Three 16s RNA gene libraries were constructed from pyrosequencing of $S_{SPR}$, $S_{SPO}$ and $S_{AOO}$ communities with 10,643, 10,606 and 10,002 high quality sequence tags, which were clustered into different phylogenetic bacterial communities with 2866/2500, 2701/2320 and 2383/2053 OTUs at $\alpha$ of 3%/5% (Table 1), indicating that SPRAS exhibited greater microbial richness than AO. The number of total OTUs estimated by Chao1 estimator (Table 1) also suggested the higher richness of bacterial communities in SPRAS, especially in SPR module. Shannon and ACE indices both demonstrated that microbial communities were more abundant in SPRAS