# Effect of aeration rates on the performance of an OSA-based sludge reduction process: limitations and implications

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Received 14 July 2016; Accepted 9 November 2016

# ABSTRACT

The oxic-settling-anaerobic (OSA) process can decrease the amount of sludge generated during wastewater treatment. In this study, the effect of aeration rates on the performance of an OSA-based sludge reduction process was investigated. Aeration rates did not significantly impact on sludge reduction in the main reactor (44.5% reduction at the lower aeration *versus* 45.4% reduction at the higher aeration rate). Integrating a side reactor for hydrolysis/acidification did not affect effluent quality, but resulted in slightly greater dominance of *Proteobacteria* and *Dechloromona* in the main wastewater treatment reactor, especially under the low aeration. Recycling anaerobic hydrolysate improved denitrification, while nitrification was unaffected. However, recycling the anaerobic hydrolysate decreased sludge settle ability under the low aeration. These results have implications for operating and optimizing an OSA process for sludge reduction.

Keywords: Sludge reduction; Aeration rates; Oxic-settling-anaerobic; Sludge settle ability

#### 1. Introduction

Activated sludge is widely used for biological wastewater treatment due to its low operational cost and high pollutant removal efficiencies. However, it generates a large amount of biomass, commonly known as waste activated sludge. A combination of stricter discharge limits and a greater volume of wastewater treatment required will inevitably lead to increased waste activated sludge production, if the current treatment methods are not improved. Efficient treatment and disposal of this excess biomass can advance sustainable wastewater treatment. Therefore, the development of highly efficient sludge

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treatment and disposal process is an important environmental issue.

There are two widely-adopted approaches practiced today aimed at decreasing sludge generation during wastewater treatment or during post-treatment [1,2]. One of these, sludge recycling from a side reactor into the main reactor during wastewater treatment, can lower biomass generation. In the main reactor, applying an energy uncoupling strategy (i.e., favoring catabolic over anabolic metabolism), adding enzymes and enriching specific micro organisms can lower sludge produced by 50–89% [3]. A side reactor, using thermal/thermo-chemical treatment, ultrasound treatment, ozone oxidation, chlorine oxidation and anaerobic hydroly-sis/acidification can lower sludge by 39–78% [4–6]. However, these side processes have high operational costs, and

may produce toxic by-products. Hydrolysis and acidification can be beneficial as this does not produce hazardous by-products and may improve organic matter removal and sludge settle ability. These benefits, combined with cost-efficiency, make them particularly attractive as side processes for sludge reduction [7].

The hydrolysis/acidification sludge reduction process was first developed by Westgarth et al. [8]. They incorporated an anaerobic tank in the return sludge line of conventional activated sludge (CAS) process and reduced sludge by 50%. Subsequently, Chudoba et al. [9,10] modified the process and developed the "oxic-settling-anaerobic"(OSA) process. Compared with CAS, OSA achieved sludge reduction of 20-65% [9,10]. Recently, Quan et al. [11] obtained 64% sludge reduction by incorporating a hydrolysis/acidification side reactor. Nowadays, continuous improvement and optimization have enabled the development of different OSA-based sludge reduction processes such as SBR-SSR (sequencing batch reactor-side stream reactor), BIMINEX and Cannibal. Sludge reduction in the anaerobic side reactor is predominantly affected by the ORP (oxidation/ reduction potential) and sludge retention time (SRT). A low ORP can improve sludge reduction. Sludge yield decreased from 0.22 to 0.19 mg MLSS/mg COD removed when the ORP decreased from -100 mV to -250 mV, where MLSS is mixed liquor suspended solids and COD is chemical oxygen demand [12]. Generally, the longer the SRT, the lower the sludge yield [13]. Ye et al. [14] examined SRTs of 5.5, 7.6 and 11.5 h in the OSA process. They obtained the lowest sludge yield at a SRT of 7.6 h, and observed a non-linear relationship between the SRT and sludge production. The sludge recycling ratio and the proportion of sludge recycled to a side reactor both affect sludge reduction. Coma et al. [15] obtained the highest sludge reduction when recycling 100% of the sludge in the BIMINEX process. Sun et al. [16] found that, at the same sludge recycling ratio, improved sludge reduction with the increased sludge recycling to a side reactor. Zhou et al. [17] found that the anaerobic-OSA process improved both process performance and microbial community stability, with a notably lower sludge production rate (0.179 g MLSS/g COD) compared to the anoxic/ aerobic main reactor (0.257 g MLSS/g COD). Goel and Noguera [18] found that coupling the enhanced biological phosphorus removal in a SBR with a side anaerobic reactor, the sludge reduction was not evident. The potential for the OSA process to improve removal efficiencies and decrease sludge production clearly warrants further research as it may have significant financial ramifications for wastewater treatment.

Optimizing aeration is important to reduce energy consumption by wastewater treatment facilities, as aeration accounts for more than 50% of the total energy consumption [19]. Wastewater treatment at a low dissolved oxygen (DO) concentration can lower the energy cost and has been widely applied in wastewater treatment. In addition, low DO (or low aeration) may benefit simultaneous nitrification and denitrification, short-term nitrification and denitrification as well as phosphorus removal, that is, improve both nitrogen and phosphorus removal efficiencies [20,21].

To date, the effect of aeration on sludge reduction in the OSA-based process has not been investigated. Therefore, the objectives of this study were to assess the effect of aeration rate in the OSA-based SBRs on: (1) sludge reduction; (2) nutrient removal during wastewater treatment; (3) sludge properties and (4) microbial community. This would establish the potential to improve sludge reduction in the OSA-based process by changing aeration.

#### 2. Materials and methods

### 2.1. Experimental setup and operation

Four parallel 12-L SBRs were operated at 25°C. Reactor 1 (R1) was operated at a higher aeration rate of 3.34 L/min during the aerobic phase in an anaerobic/aerobic SBR (SBR<sub>H</sub>). Reactor 2 (R2) was operated by incorporating a side hydrolysis and acidification reactor (SHAR) to the  $SBR_{H}$  type reactor (SBR<sub>H</sub>-SHAR). Reactor 3 (R3) was operated at a lower aeration rate of 1.67 L/min during the aerobic phase in ananaerobic/aerobic SBR (SBR<sub>1</sub>). Reactor 4 (R4) was operated by incorporating a SHAR to the SBR type reactor (SBR, -SHAR). All SBRs performed six reaction cycles per day, with each reaction cycle consisting of a 60 min of an anaerobic phase (including 10 min filling), 120 min of an aeration phase, and 60 min of a settling, decanting and idle phase. The hydraulic retention time (HRT) in each reactor was 8 h, and half the reactor volume (mixed liquor after settling) was discharged. The reactors were mixed by mechanical mixers during the anaerobic and aerobic phases. Aeration was provided at the reactor base through airstones. Reactor filling, withdrawal, aeration and mixing was controlled by timers. All SBRs were seeded with activated sludge taken from the 7th wastewater treatment plant in Kunming, China.

In the side reactor of SBR<sub>H</sub>-SHAR and SBR<sub>L</sub>-SHAR, 500 mL of mixed liquor from the main reactor were anaerobically digested at 35°C with a SRT of 6 d. After anaerobic digestion, 80 mL mixed liquor was pumped to SBR<sub>H</sub>-SHAR and SBR<sub>L</sub>-SHAR during each filling phase, respectively.

The influent to the main reactor was the effluent from a grit sedimentation tank in a wastewater treatment plant in Kunming, China. It contained approximately 250 mg/L of COD, 17.75 mg/L of ammonium nitrogen (NH<sub>4</sub><sup>+</sup>–N), 28.68 mg/L of total nitrogen (TN), 1.32 mg/L of orthophosphate ( $PO_4^{3-}$ –P) and 2.09 mg/L of total phosphorus (TP).

#### 2.2. Batch experiments

Experiments were performed for all SBRs under steady state. The removal of pollutants within a typical SBR cycle and microbial activities of nitrification and denitrification were examined. In addition, the effect of aeration rate on sludge reduction was evaluated in terms of the sludge MLSS and mixed liquor volatile suspended solids (MLVSS) in the main reactor and the side reactor.

During the typical SBR cycle, samples were taken at intervals of 10 or 15 min, with simultaneous pH and DO measurements. The samples were filtered through 0.45  $\mu$ m membranes, and the NH<sub>4</sub><sup>+</sup>–N, nitrite nitrogen (NO<sub>2</sub><sup>-</sup>–N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>–N) and PO<sub>4</sub><sup>3–</sup>–P concentrations in the liquid were determined.

For the nitrification experiment, 500 mL of mixed liquor was removed from each reactor at the end of the aerobic phase. The mixed liquor was centrifuged, and the solids were re-suspended to 500 mL in glass-flasks containing synthetic wastewater without organic carbon or  $NH_4^+-N$ .  $NH_4Cl$  and  $NaNO_2$  were added so that the initial  $NH_4^+-N$  and  $NO_2^--N$  concentrations were both 20 mg/L, and then aerated to initiate nitrification. Samples were removed every 10 min for 90 min. Concentrations of  $NH_4^+-N$ ,  $NO_2^--N$ ,  $NO_3^--N$ , MLSS and MLVSS were determined to calculate activities of the aerobic ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB).

For the denitrification experiment, the mixed liquor was pre-treated in the same manner as for the nitrification experiment. MLSS were re-suspended in 500 mL glass-flasks in the synthetic wastewater containing no organic carbon. Sodium acetate (CH<sub>3</sub>COONa), KNO<sub>3</sub> and NaNO<sub>2</sub> were added with the initial COD, NO<sub>3</sub><sup>-</sup>–N and NO<sub>2</sub><sup>-–</sup>N concentrations of 500, 30 and 20 mg/L, respectively. Flasks were sealed using rubber stoppers and there was a single port for sampling. The mixed liquor inside the flasks was agitated using magnetic stirrers. Samples were removed every 10 min over a 90 min period. Concentrations of NO<sub>2</sub><sup>-–</sup>N, NO<sub>3</sub><sup>-–</sup>N, MLSS and MLVSS were determined to calculate the specific denitrifying activity.

## 2.3. Analytical methods

COD, NH<sup>+</sup><sub>4</sub>–N, NO<sup>-</sup><sub>2</sub>–N, NO<sup>-</sup><sub>3</sub>–N, TN, PO<sup>+</sup><sub>4</sub>–P, TP, MLSS and MLVSS were measured according to standard methods [22]. DO and pH were measured with a portable DO meter (flexi, HACH, USA) and a pH meter (HQ11d, HACH, USA), respectively. DNA was extracted from activated sludge using a Fast DNA Spin Kit (PowerSoil DNA Isolation Kit, Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. After extraction,DNA was amplified by the V4 region of the bacterial 16-s rRNA gene and the microbial community was analyzed using high-throughput 16S rRNA gene sequencing [23].

Volatile fatty acids (VFAs) were quantified using a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector and a capillary Inert Cap WAX-HT column (30 m by 0.25 mm by 0.25  $\mu$ m). The inert carrier gas was N<sub>2</sub> at a flow rate of 50 mL/min, a split ratio of 15 at a flow rate of 1.1 mL/min in the column, and a purge flow rate of 3 mL/min. The oven temperature was increased linearly from 70 to 200°C for more than 10 min, then held at 200°C for 2 min. The temperatures of injector and detector were both 240°C. The samples were acidified by adding formic acid to adjust the pH to below 3, and the injection volume was 1  $\mu$ L.

Eq. (1) shows the relationship between the observed yield  $(Y_{obs})$  and the SRT, which was revised from the equation proposed by Rittmann and McCarty [24] to better describe the condition applicable to the current study:

$$Y_{obs} = \frac{(\alpha Y_a + \lambda Y_{an}) \left[ 1 + \left( 1 - f_d \right) * SRT * b \right]}{1 + b * SRT}$$
(1)

where  $\alpha$  is the ratio of the aerobic time period to the total time period: 0.67 in this study;  $Y_a$  is the theoretically aerobic yield coefficient, 0.45 g MLVSS/g COD;  $\lambda$  is the ratio of

the anaerobic time period to the total time period: 0.33 in this study;  $Y_{an}$  is the theoretical anoxic yield coefficient, 0.3 g MLVSS/g COD;  $f_d$  is fraction of the biodegradable active biomass, 0.8; and *b* is the decay coefficient, 1/d.

#### 3. Results and discussion

#### 3.1. Sludge reduction under different aeration rates

Fig. 1 shows the cumulative MLVSS discharged from four reactors under long-term operation. The sludge production rates in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR were 2.86, 1.57, 2.91 and 1.61 g MLVSS/d, respectively, calculated by linear least squares regression analysis. Under steady state, based on mass balance and data presented in Table 1, the corresponding COD removal rates were 9.03, 9.09, 9.13 and 9.10 g COD/day. Therefore, the corresponding sludge yield coefficients ( $Y_{obs}$ ) calculated from the above data were 0.319, 0.177, 0.317 and 0.173 g MLVSS/g COD, respectively. The sludge yield decreased by 45.4% when incorporating the side reactor in the sludge recycling line under high aeration, and decreased by 44.5% under low aeration. The data verifies an effective reduction in sludge



Fig. 1. Accumulative MLVSS discharged from all reactors during the long-term acclimation period.

 Table 1

 The influent and effluent water quality under steady state

	Influent (mg/L)	Effluent (mg/L)				
		SBR <sub>H</sub>	SBR <sub>H</sub> -SHAR	SBR	SBR <sub>L</sub> -SHAR	
NH <sub>4</sub> <sup>+</sup> -N	17.75	0.24	0.23	0.25	0.24	
NO <sub>3</sub> -N	_	8.23	7.96	7.17	7.38	
NO <sub>2</sub> -N	-	0.07	0.02	0.07	0.04	
TN	28.68	12.33	11	10.76	10.58	
PO <sub>4</sub> <sup>3–</sup> –P	1.32	0.02	0.01	0.04	0.02	
TP	2.09	0.17	0.07	0.31	0.3	
COD	250	33	32	30	31	

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by incorporating a hydrolysis and acidification process, but no difference was observed due to either aeration rate used in the main reactor.

The SRT in the side reactor has a strong impact on sludge reduction. Novak et al. [25] achieved a sludge reduction of up to 60% with the SRT of 10 days in the side reactor. Zhou et al. [17] obtained a lower sludge reduction (32%) using a shorter SRT (6 h) in their side reactor (in an A-OSA process with the conventional aerobic process in the main reactor, under similar aeration to SBR<sub>1</sub>-SHAR). Sun et al. [26] lowered sludge reduction by 48% in biological denitrification and phosphorus removal process. Their sludge yield was 0.151 mg MLSS/mg COD in the UNITANK-OSA process using aside reactor with a 5 day SRT, while the sludge yield in the UNITANK process without a side reactor was 0.288 mg MLSS/mg COD. In the current study, enhanced biological nitrogen and phosphorus removal occurred in the main reactor and similar sludge reduction was achieved. However, it is evident that the operational parameters regarding side hydrolysis and acidification will have a significant influence on sludge reduction. The mechanisms involved in sludge reduction in the side reactor of the OSA-based process include: (1) endogenous metabolism, (2) uncoupling of catabolic and anabolic metabolism, (3) selective enrichment of slow-growing microorganisms, and (4) destruction of extracellular polymeric substances (EPS) [10,17,18].

The SRTs of  $SBR_{H}$  and  $SBR_{I}$  were both 10 days. In the SBR<sub>H</sub>-SHAR and SBR<sub>1</sub>-SHAR, the theoretical SRT increased to 17 days because mixed liquor from the side reactor was recycled to the main reactor. Under high aeration, the decay coefficient (b) of 0.035 day<sup>-1</sup> was calculated according to Eq. (1) with  $Y_{obs}$  in SBR<sub>H</sub> of 0.317 g MLVSS/g COD. Assuming that the decay coefficient was identical, the  $Y_{obs}$  predicted in SBR<sub>H</sub>-SHAR was 0.281 g MLVSS/g COD, which was higher than the observed coefficient of 0.173 g MLVSS/g COD. Similarly, the theoretical  $Y_{obs}$  in SBR<sub>L</sub>-SHAR was 0.283 g MLVSS/g COD, while the observed  $Y_{abs}$  was 0.177 g MLVSS/g COD - also much lower than the predicted value.This indicated greater sludge reduction due to the longer SRTs. Sludge reduction attributable to the longer SRT was similar for both high (38.4%) and low aeration (37.5%). The extended SRT would mainly enhance the endogenous microbial metabolism, resulting in a decreased sludge yield. Datta et al. [27] reported a sludge reduction efficiency of up to 63% with a SRT of 100 days in a SBR-OSA process. However, extended SRTs need to be balanced by gains in sludge reduction in relation to the spatial requirements of the side reactor.

Under steady state conditions, MLVSS in the SBR<sub>H</sub>-SHAR main reactor and the side hydrolysis and acidification reactor were 2.96 g/L and 2.75 g/L, respectively. Sludge was reduced by 12.2% in the side reactor. For the SBR<sub>L</sub>-SHAR reactors, MLVSS was 2.77 g/L in the main reactor and 2.63 g/L in the side reactor – a sludge reduction of 11.2%. The volume of the main reactor was 12 L, and only 480 mL of recycled mixed liquor was fed daily to the side reactors. Consequently, the hydrolysis and acidification side reactors contributed little (0.45 ± 0.05%) with regard to total sludge reduction.

Under a high aeration, the total sludge reduction of 45.4%, with 38.4% attributable to the longer SRT, 0.5% to the

side reactor, and 6.5% to the energy uncoupling metabolism and other processes. Under lower aeration, the total sludge reduction was about 44.5%, with 37.5% attributable to the longer SRT, 0.4% to the side reactor, and 6.6% to the energy uncoupling metabolism and other processes. The similarity in sludge reduction indicates that the aeration rate in the main reactor did not affect the sludge reduction mechanism in the OSA-based process.

# 3.2. Effect of sludge reduction on nutrient removal under different aeration rates

The effluent water quality under steady state is shown in Table 1. The effluent COD concentrations were all approximately 30 mg/L, with removal efficiencies of approximately 87%. This was consistent with previous studies, where a side hydrolysis and acidification process had little effect on COD removal [10,14]. Concentrations of NH<sup>+</sup><sub>4</sub>–N in the effluent were all below 0.25 mg/L, and concentrations of NO<sub>2</sub>-N were all below 0.07 mg/L. Nitrate removal efficiencies were similar. The effluent NO<sub>2</sub>-N concentration was approximately 8 mg/L for the high aeration rate and 7 mg/L for the low aeration rate. The TN removal percentage of SBR<sub>H</sub>-SHAR was 5% greater than for SBR<sub>H</sub>, indicating that the recycle of side reactor effluent enhanced TN removal. However, no such effect was observed at a low aeration rate. Datta et al. [27] observed no obvious improvement in nitrogen removal (NH<sub>4</sub><sup>+</sup>–N, NO<sub>3</sub><sup>-</sup>–N and  $NO_2$ –N) when using a side reactor in a SBR process. The effluent TN concentration under a high aeration condition was less than that under a low aeration condition.

The use of a side reactor made no obvious difference to effluent phosphate (PO<sub>4</sub><sup>3-</sup>–P) concentrations, which ranged from 0.07 to 0.31 mg/L. Phosphorus removal correlated with the ORP in the OSA reactor - the lower the ORP, the more phosphorus released. Consequently, the more phosphorus was recycled to the main reactor. Although the influent organic carbon increased due to recycling the soluble COD from the side reactor, so did the phosphorus loading rate, confounding any benefit to phosphorus removal. Chudoba et al. [10] and Ye et al. [14] combined an anaerobic side reactor with an aerobic primary reactor, which improved phosphorus removal by enriching polyphosphate accumulating microorganisms (PAOs). In the current study, the main reactor alternated between anaerobic and aerobic phases, which benefited both nitrogen and phosphorus removal. Saby et al. [28] observed similar phosphorus removal efficiencies for a membrane bioreactor (MBR)-OSA process and a MBR.

The dynamics of typical parameters in a SBR cycle under steady state are shown in Fig. 2. All reactors performed well regarding nitrification, denitrification, phosphorus release and uptake. The recycling of anaerobic hydrolysate had little effect on nitrification. NO<sub>2</sub>–N accumulated in SBR<sub>H</sub> and SBR<sub>H</sub>-SHAR after 60 min, with peak concentrations of 2.05 and 3.17 mg/L, respectively. The correspondingNO<sub>2</sub>–N concentrations in SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR were 2.05 and 3.17 mg/L after 60 min and 70 min, respectively. Under low aeration, it took longer for NO<sub>3</sub>–N and NH<sub>4</sub><sup>+</sup>–N in SBR<sub>L</sub> to reach steady state than for SBR<sub>L</sub>-SHAR. The maximum PO<sub>4</sub><sup>3</sup>–P concentration after anaerobic release in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>I</sub> and SBR<sub>I</sub>-SHAR was 3.78, 5.87, 6.23 and 6.24 mg/L,



Fig. 2. Dynamics of typical parameters in the SBR cycle under steady state.

respectively. This indicated that the recycle of an anaerobic hydrolysate could improve  $PO_4^{3-}-P$  release under high aeration. All effluent  $PO_4^{3-}-P$  concentrations were less than 0.17 mg/L, in part due to low  $PO_4^{3-}-P$  concentrations in the influent. The results indicate that aeration had an insignificant effect on the effluent quality with the incorporation of a side reactor, but it did affect metabolic processes.

Recycling the anaerobic hydrolysate increased loading rates to the main reactor, but this had a minor effect on system performance. Under high aeration, recycling the anaerobic hydrolysate increased nutrient loading by 3.7% for NH<sub>4</sub><sup>+</sup>–N, 4.6% for PO<sub>4</sub><sup>3–</sup>–P and 2.7% for COD. Correspondingly, under the low aeration, the values increased by 3.7%, 4.8% and 2.7%, respectively. Propionic acid and acetic acid were the dominant VFAs produced during hydrolysis and acidification in SBR<sub>H</sub>-SHAR (35.4% and 31.0%, respectively) and SBR<sub>L</sub>-SHAR (48.5% and 18.6%, respectively). VFAs contained in soluble COD are known to benefit nitrogen and phosphorus removal. Although the increased COD loading in the main reactor was low, VFAs are easily utilized and are generally beneficial to nitrogen and phosphorus removal after acclimation.

3.3. Microbial structure and activities of functional microbial communities

The microbial community structure in the OSA-based sludge process was analyzed using high-throughput 16S rRNA sequencing (Fig.3). Generally, the incorporation of the side reactor increased the microbial diversity in the main reactor, which was consistent with previous studies [7].

The microbial community phyla in the main reactors included *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*. Among them, *Proteobacteria* and *Bacteroidetes* were dominant, also consistent with previous studies [29]. The proportion of *Proteobacteria* was 37.0%, 42.4%, 41.4% and 45.0% in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR, respectively. The proportion of *Proteobacteria* increased slightly with the incorporation of the side reactor. In addition, the proportion of *Proteobacteria* at the low aeration was higher than that at the high aeration. The proportion of *Bacteroidetes* in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR was 40.4%, 33.0%, 33.8% and 33.8%, respectively. Under high aeration, the use of a side reactor affected the proportion of *Bacteroidetes*, while it had no effect at a low aeration. Though

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*Proteobacteria* and *Bacteroidetes* were also dominant in the side reactor, the proportion of *Firmicutes* (about 5%) in the side reactor was marginally higher than in the main reactor. There was no difference under different aeration rates. *Firmicutes* are known to produce extracellular enzymes capable of degrading macromolecules into small molecules. By contributing to sludge degradation and VFA synthesis, the *Firmicutes* are an important component of the microcosm. *Spirochaetes*, gram-negative photoheterotrophs, were



Fig. 3. Taxonomic analysis of microbial communities based on 16-s rRNA gene sequencing for all reactors under steady state.

not detected in the main reactors, but a small population (1%) was detected in the side reactor of the low aeration (SBR<sub>L</sub>-SHAR) reactor. Servin et al. [30] detected *Spirochaetes* in activated sludge and concluded that they might be correlated to the reduction of organic carbon. The side reactors contained *Chloroflexi* (3%), which were not detected in the main reactors. According to some studies, *Chloroflexi* belong to photosynthetic microorganisms, and the long SRT due to the side reactor may have promoted its growth [31].

*Thauera, Terrimonas, Lewinella* and *Dechloromonas* were the dominant genera detected in the primary reactors. The proportion of *Dechloromonas* in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>1</sub> and SBR<sub>L</sub>-SHAR main reactors was 0.8%, 0.8%, 1.2% and 5.2%, respectively. With a low aeration rate, the proportion of *Dechloromonas* was significantly higher when using a side reactor. Its presence coincided with the lowest TN concentration observed in the effluent of the SBR<sub>L</sub>-SHAR reactor. *Dechloromonas* is capable of denitrification, and can completely reduce nitrite to N<sub>2</sub> [32,33]. The *Thauera* in SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR contents were 6.0%, 7.4%, 4.3% and 6.1%, respectively. *Thauera* and *Dechloromonas* also played key roles in producing intracellular organic carbon by activated sludge, which can contribute to enhanced nitrogen removal by better utilization of organic carbon [34].

Nitrification and denitrification activity at steady state are displayed in Table 2. Generally, the recycling of anaerobic hydrolysate had no obvious influence on the activities of AOB or NOB. However, it did affect denitrification. The first step (reduction of  $NO_3^--N$ ) and second (reduction of  $NO_2^--N$ ) step of denitrification in SBR<sub>H</sub>-SHAR were higher than those in SBR<sub>H</sub> by a factor of 1.62 and 1.66 times, respectively. Recycling the anaerobic hydrolysate could be used to improve denitrification under the high aeration. The activity of denitrifying  $NO_3^--N$  in SBR<sub>L</sub>-SHAR was similar to that in SBR<sub>H</sub>-SHAR, while the activity of denitrifying  $NO_2^--N$  in SBR<sub>L</sub>-SHAR was slightly less than that in SBR<sub>H</sub>-SHAR.

Under steady state, the average sludge volume index (SVI) for SBR<sub>H</sub>, SBR<sub>H</sub>-SHAR, SBR<sub>L</sub> and SBR<sub>L</sub>-SHAR were 160, 153, 142 and 177 mL/g, respectively. When the primary reactor had a high aeration rate, the recycling of anaerobic hydrolysate had no impact on sludge settle ability. Ye et al. [14] found that SVI for a CAS-OSA process was less than that of the CAS process, that is, for an aerobic primary reactor a side treatment improved sludge settle ability. However, when the primary reactor uses anoxic/aerobic processes, the influence of a side-anaerobic/anoxic reactor on the sludge settle ability may be negligible. In the current study, aeration rate affected sludge settle ability. Sludge settle ability was better with the

Table 2

Microbial activities of nitrification and denitrification for acclimated activated sludge

	SBR <sub>H</sub>	SBR <sub>H</sub> -SHAR	SBR	SBR <sub>L</sub> -SHAR
AOB (mg NH <sub>4</sub> <sup>+</sup> –N/g MLVSS/h)	3.23	3.57	3.62	3.72
NOB (mg NO <sub>2</sub> -N/g MLVSS/h)	2.64	2.65	2.25	1.94
DNO <sub>3</sub> (mg NO <sub>3</sub> -N/g MLVSS/h) <sup>a</sup>	2.16	3.78	3.84	4.54
DNO <sub>2</sub> (mg NO <sub>2</sub> -N/g MLVSS/h) <sup>a</sup>	2.46	4.12	5.96	4.87

 $^{a}$ DNO<sub>3</sub> and DNO<sub>2</sub> represent the first and second denitrification steps, that is, reduction of NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N.

higher aeration rate. Further research would be required to establish how side hydrolysis and acidification affected settle ability. In addition, sludge settle ability might be also affected by SRT [7]. The effects of aeration rates with increasing SRTs when employing side hydrolysis and acidification should be investigated.

#### 4. Conclusions

The aeration rate in a primary reactor had little impact on the sludge reduction when incorporating an anaerobic side reactor for hydrolysis and acidification. Total sludge reduction was mainly attributable to an extended SRT. The inclusion of a side reactor had no significant impact on the effluent water quality, but it did affect microbial metabolism. Microbial diversity and dominant populations in the main reactor were enhanced due to the integration of the side reactor. Proteobacteria and Dechloromona increased slightly with the incorporation of a side reactor, especially under low aeration. The recycling of anaerobic hydrolysate enhanced nitrate reduction, but had no obvious influence on activities of AOB or NOB. Pilot-scale studies are necessary to confirm these findings and optimize the OSA process.

#### Acknowledgments

The research was supported by the Major Science and Technology Program for Water Pollution Control and Treatment of China (No. 2011ZX07317-001) and Kunming Science and Technology Plan (2014-04-A-S-01-3065).

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