



Effects of dissolved oxygen on sludge reduction and microbial community structure in sequencing batch reactors

Yufeng Xu^a, Long Wang^c, Xiaofeng Yin^d, Shengkun Zhang^b, Simin Li^{a,*}, Yanyan Dou^{b,**}

^aCollege of Energy and Environmental Engineering, Hebei University of Engineering, Handan 056038, China, Tel./Fax: +86-310-8578748; email: jackstarfly@126.com (Y. Xu)

^bCollege of Energy and Environmental, Zhongyuan University of technology, Zhengzhou 450000, China

^cChongqing Academy of Metrology and Quality Inspection, Chongqing 401123, China

^dThe 718th research institute of CSIC (PERIC), Handan 056038, China

Received 7 August 2017; Accepted 11 October 2018

ABSTRACT

The effects of dissolved oxygen (DO) on treatment efficiency and sludge reduction in sequencing batch reactors (SBRs) were examined in this study. The results showed that not only was excess sludge reduced but the pollutants were also removed more effectively in the low-DO reactor compared with the control reactor. To further investigate the differences between the sludges produced in the two reactors, the characteristics of each sludge were studied. The average values of MLVSS/MLSS, $SOUR_{EX}$, $SOUR_{EN}$, Y_{H} , and K_d in the low-DO reactor were 0.82, 44 mg O_2 /(g MLSS h), 6.8 mg O_2 /(g MLSS h), 0.61 g cell/g COD, and 0.33 d⁻¹, respectively, and 0.78, 37 mg O_2 /(g MLSS h), 8.2 mg O_2 /(g MLSS h), 0.65 g cell/g COD, and 0.38 d⁻¹, respectively, in the control reactor. In the low-DO reactor, filamentous bacteria, facultative bacteria, and other such bacteria that are able to adapt to the low-DO environment comprising the main bacteria. The low Y_{H} and high total decay of the low-DO reactor sludge contributed to the reduction in sludge quantity by approximately 16% and 84%, respectively.

Keywords: Dissolved oxygen; Low DO; Sequencing batch reactor; Sludge reduction; Microorganisms

1. Introduction

Currently, most urban sewage treatment plants in the world use an activated sludge process because of its ability to effectively remove pollutants with fewer steps, lower cost, and higher treatment capacity [1,2]. However, the activated sludge process generates a large quantity of excess sludge [3], which can pose a threat to the environment and impose a heavy burden on the sewage treatment plant [4,5]. At present, sludge disposal accounts for 50%–60% of worldwide sewage plant operation costs. Thus, the minimization of sludge production during the activated sludge process has become an important goal in the field of wastewater treatment, receiving considerable attention in recent years [6]. Biological, mechanical, thermal, and chemical methods

have been developed to reduce sludge production during the activated sludge process [7,8]. However, these methods require extra energy or chemicals, which prevent the existing sludge reduction technologies from being environmentally sustainable. An advanced sludge reduction strategy needs to be developed to simultaneously reduce excess sludge and enhance nutrient removal during wastewater treatment.

Dissolved oxygen (DO) is one of the key substrates for biological survival. Varying DO concentrations can result in different biochemical characteristics and diverse microbial communities [9]. When O_2 is used as the electron acceptor, more substrates are involved in anabolism compared with when other electron acceptors (NO_3^- , SO_4^{2-} , and CO_2 , etc.) are used [10,11]. This factor accounts for the higher sludge yield

* Corresponding author.

reported for the aerobic activated sludge process compared with other processes such as SHARON, ANAMMOX, and CANON [7,12]. Sadaie et al. [13] reported that sludge production was remarkably reduced when the DO supply decreased.

However, the pollutant removal efficiency remains unchanged in activated sludge treatment systems with low DO.

Guo et al. [14] introduced the limited filamentous bulking sludge under low DO, under the artificial control, the activated sludge treatment system did not lead to filamentous bacteria malignant expansion or serious sludge loss. Tian et al. [15] found that the optimum conditions for integrated nitrogen, phosphorus, and chemical oxygen demand (COD) removal were achieved with a DO of 1.0–1.5 mg/L and a sludge volume index (SVI) level of 170–200. Guo et al. [14] reduced the DO to 0.5 mg/L and successfully maintained an anoxic/oxic (A/O) system at a slightly expanded state. In comparing with the conventional process, the aeration volume was reduced by 57%, and the removal rate of pollutants was unchanged.

It could be inferred from these studies that activated sludge systems maintained in a low-DO state have greater potential for sludge reduction and efficient sewage treatment. Thus, further research on sludge reduction, sewage treatment, and microbiological activities in the low-DO activated sludge process is needed. In this study, the differences in the pollutant treatment efficiency, sludge reduction, and bacterial communities between low-DO-activated sludge systems and Control systems are discussed, in order to provide theoretical support and practical engineering guidance for the development of low-DO-activated sludge treatments.

2. Materials and methods

2.1. Seed sludge

Sludge samples (feed) were collected from the oxic tank of a municipal wastewater treatment plant (WWTP) in Chongqing (P. R. China) and stored at 4°C. The sludge had a pH value of 6.9, volatile solids (VS) concentration of 10,000–11,000 mg/L, and total solids (TS) concentration of 14,500–15,000 mg/L.

2.2. Reactor

Two identical sequencing batch reactors (SBRs) with a 10-L capacity were operated in parallel (Fig. 1). The low-DO reactor L ran an aerobic process with DO concentrations in the range of 0.1–0.8 mg/L (average DO = 0.4 mg/L) while reactor N ran as a control (average DO = 2.5 mg/L). To avoid the deteriorating effects of settling brought on by the overgrowth of filament with long-term operation under low-DO conditions; an appropriate process was introduced to effectively prevent the occurrence of sludge bulking [16]. The experimental reactors were operated through five sequential phases: (1) water inflow (20 min); (2) aerobic (120 min in the control system, 180 min in the low-DO system, stopped by the actual substrate degradation response time); (3) anoxic (60 min); (4) sludge settling (60 min); and (5) effluent discharge (30 min). Operation of the pumps and magnetic stirrer and gas delivery to the SBRs were automated using digital timers. At the end of each cycle, 2,500 mL of supernatant was pumped from the reactor. An equal volume of synthetic wastewater was then allowed

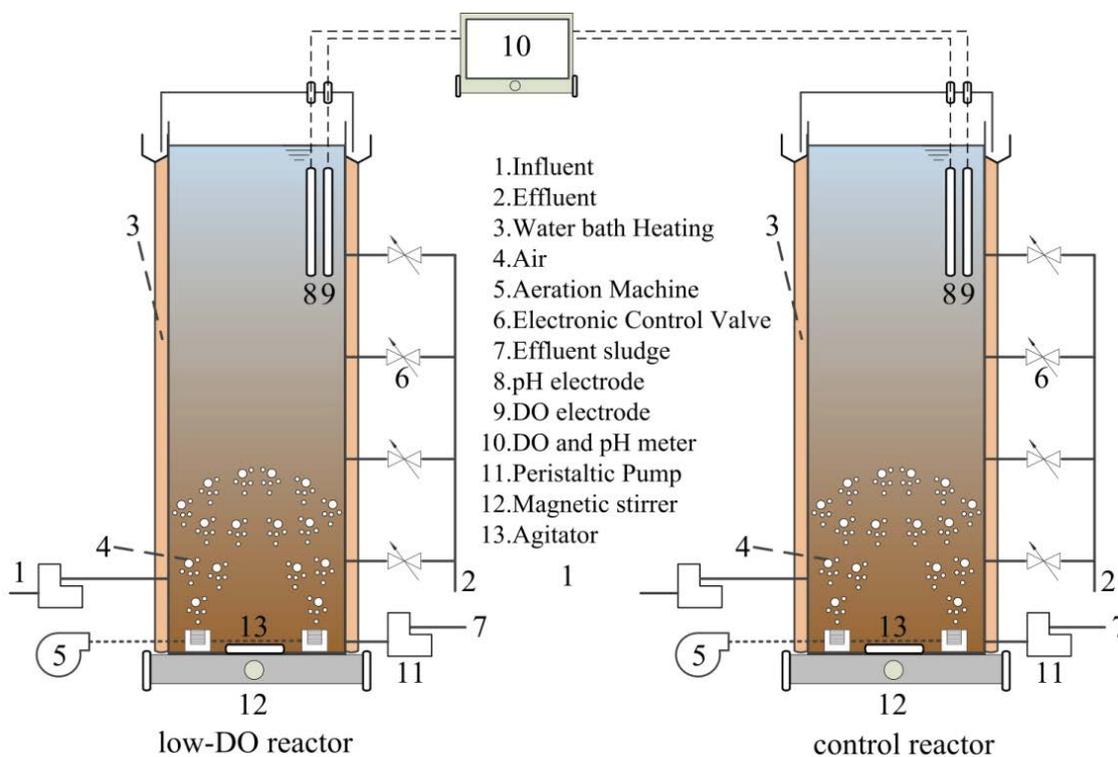


Fig. 1. Low-DO and control reactors.

to flow into the SBRs at the beginning of the next cycle, which corresponded to a hydraulic retention time (HRT) of 24 h. The pH was measured using a PHS-3C pH meter (Sartorius AG, Germany). DO was measured using a DO sensor (YSI55/12FT, USA).

2.3. Synthetic feed

The synthetic feed (influent) was designed to maintain COD, nitrogen, and phosphorus concentrations at 510, 70, and 4 mg/L, respectively. Table 1 summarizes the composition of synthetic feed. The pH was maintained at 7.2 ± 0.5 through the addition of sodium bicarbonate (NaHCO_3 , Jiangsu, China). The composition of the synthetic feed was (per liter) 30 mg glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), 16.7 mg sucrose, 10 mg peptone, 67.5 mg NaHCO_3 , 22.5 mg NH_4Cl , 7.5 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 11.36 mg $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$, 21.94 mg KH_2PO_4 , and 20 mL of trace element solution. The trace nutrient solution was similar to the one used by Goel and Noguera [17].

2.4. Water quality analysis

In this study, samples were collected daily. Standard methods (SEPA [18]) were used to measure nitrate (NO_3N), nitrite (NO_2N), total nitrogen (TN), and COD_{Cr} .

2.5. Sludge analysis

The specific oxygen consumption rate (SOUR) of the activated sludge, which describes the amount of oxygen used by the microorganisms to consume 1 g of COD, was used to evaluate the microbial metabolic activity of the sludge. The SOUR was measured using standard methods (APHA, 1992). The heterotrophic conversion yield (Y_H , kg COD/kg COD) was measured using Strotmann's method [14,19]. A technique adapted from Liu and Wang [20] was used to measure the K_d (endogenous decay coefficient).

2.6. Microbial community analysis

2.6.1. Sample collection and DNA extraction

Sludge samples L (denoting sludge samples from the low-DO system) and N (denoting sludge samples from the control system) were collected from the low-DO and control reactors, respectively. The genomic DNA was extracted from the sludge samples using an OMEGA (Norcross, Georgia, USA) E.Z.N.A Soil DNA kit, and the genomic DNA was detected by 1% agarose gel electrophoresis.

2.6.2. Polymerase chain reaction amplification

Based on the designated sequencing area, the fusion primers with "5'-454A and B joint-specific primer-3'" were

synthesized. The sequencing end of the barcode label was denoted with A to distinguish the different samples, and the B end was connected with the amplification primers. The polymerase chain reaction was carried out using TransGen AP221-02 (Beijing, China): TransStart Fastpfu DNA Polymerase and an ABI GeneAmp@9700 thermocycler.

2.7. Calculation of the observed yield

The sludge observed yield was calculated using the following equation:

$$Y_{\text{obs}} = \frac{((X_0 - X_e) + \Delta X)}{S_0 - S_e} \quad (1)$$

where X_0 represents influent suspended solids (g/L), X_e represents effluent suspended solids (g/L), S_0 represents influent COD (g/L), S_e represents effluent COD (g/L), and ΔX represents solids produced in the SBR (g/L).

The experimental observed yield (Y_{obs} , kg VSS/kg COD) was calculated using Eqs. (2) and (3). The standard error deviation for each Y_{obs} value was calculated based on the VSS (volatile suspended solids) variability for the period considered:

$$X_{B,H} = \frac{\theta_c \times Y_H \times (S_0 - S_e)}{\tau_o \times (1 + K_d \times \theta_c)} \quad (2)$$

$$Y_{\text{obs}} = \frac{\tau_h \times X_{B,H}}{\theta_c \times (S_0 - S_e)} \quad (3)$$

where $X_{B,H}$ represents the sludge concentration (mg/L), τ_o represents the aerobic retention time, τ_h represents the HRT, θ_c represents the sludge retention time (SRT), Y_H represents the heterotrophic yield coefficient (mg/L), K_d represents the endogenous decay coefficient (mg/L), S_0 represents the influent concentration (mg/L), and S_e represents the effluent concentration (mg/L).

3. Results and discussion

3.1. Effect of DO on pollutant removal efficiency

Table 2 summarizes the removal efficiency of each pollutant in the SBRs under different DO concentrations as well as the pollutant concentrations in the influent and effluent. The table shows that the effluent COD removal rate of the low-DO reactor was not significantly different from that of the control reactor. This is because most of the COD in the influent consisted of easily biodegradable substrate, which

Table 1
Composition of synthetic feed (mg/L)

Constituent	Peptone	Glucose	Sucrose	NaHCO_3	CaCl_2	MnCl_2
Concentration	10	30	16.7	67.5	7.5	0.03
Constituent	KH_2PO_4	NH_4Cl	MgSO_4	FeCl_3		
Concentration	21.94	22.5	11.36	4.0		

Table 2
Average effluent quality in each reactor

Project	Low-DO reactor	Control reactor
	Average ± standard deviation	Average ± standard deviation
Influent COD _{Cr} mg/L	482 ± 12	482 ± 12
Effluent COD _{Cr} mg/L	35.6 ± 4.2	37.8 ± 5.5
COD _{Cr} removal rate, %	92.6 ± 1.4	92.2 ± 2.1
Influent TN, mg/L	68.4 ± 4.2	68.4 ± 4.2
Effluent TN, mg/L	16.7 ± 2.1	24.5 ± 3.5
TN removal rate, %	75.58 ± 1.7	64.18 ± 2.1
Influent NH ₄ ⁺ -N, mg/L	63.2 ± 4.7	63.2 ± 4.7
Effluent NH ₄ ⁺ -N, mg/L	3.21 ± 0.52	2.89 ± 0.38
NH ₄ ⁺ -N removal rate, %	94.92 ± 1.1	95.43 ± 1.5
Influent TP, mg/L	4.72 ± 0.51	4.72 ± 0.51
Effluent TP, mg/L	0.35 ± 0.08	0.41 ± 0.12
TP removal rate, %	92.55 ± 1.2	91.28 ± 1.5

would not inhibit COD removal. Furthermore, utilization associated products were adsorbed but difficult to degrade by the sludge, so there was no significant difference in COD degradation. The effluent NH₃-N concentration in the low-DO reactor was 3.21 mg/L, and the removal rate was 95%, much like the control reactor. The average effluent TN concentrations in the low-DO reactor and control reactor were 16.7 and 24.5 mg/L, respectively, and the TN removal rate was 76% and 64%, respectively. Traditional denitrification theory holds that heterotrophic denitrifying bacteria are also facultative anaerobic bacteria. Münch et al. [21] found that when the concentration of DO was 0.5 mg/L, the denitrification and nitrification rates were equal. If the concentration of DO in the system was high, then the in vivo synthesis of denitrifying bacteria with nitrate reductase was inhibited. A lower DO concentration ensured that there was a sufficiently anoxic environment inside the sludge for the activation of denitrifying bacteria. Therefore, simultaneous nitrification and denitrification was more likely to occur in the lower DO system, which improved the TN removal rate.

In the low-DO reactor, the removal rate of total phosphorus reached 92%, which was higher than in the

control reactor. There are two potential reasons for this observation. First, the high phosphorus removal rate obtained in the low-DO reactor may have been based on the enrichment of polyphosphate bacteria; second, gasification that removed phosphorus may have occurred in the low-DO reactor [22,23]. The phosphorus removal mechanism in the low-DO environment requires further study.

3.2. Sludge-observed yield (Y_{obs})

As can be seen from Fig. 2a, under a low-DO environment, the sludge concentration was maintained in the range of 2,150–2,250 mg/L, with an average value of approximately 2,200 mg/L. In the control reactor, the sludge concentration was maintained in the range of 2,540–2,620 mg/L, with an average value of about 2,580 mg/L, and no large-scale sludge loss or growth was observed for the high SVI.

To reduce the influence of daily variation, the growth rate of the sludge was integrated, and the relationship between the accumulation of sludge and the total removal of COD was obtained where the slope of the line was the cumulative sludge observed yield (Y_{obs}). Fig. 2b shows

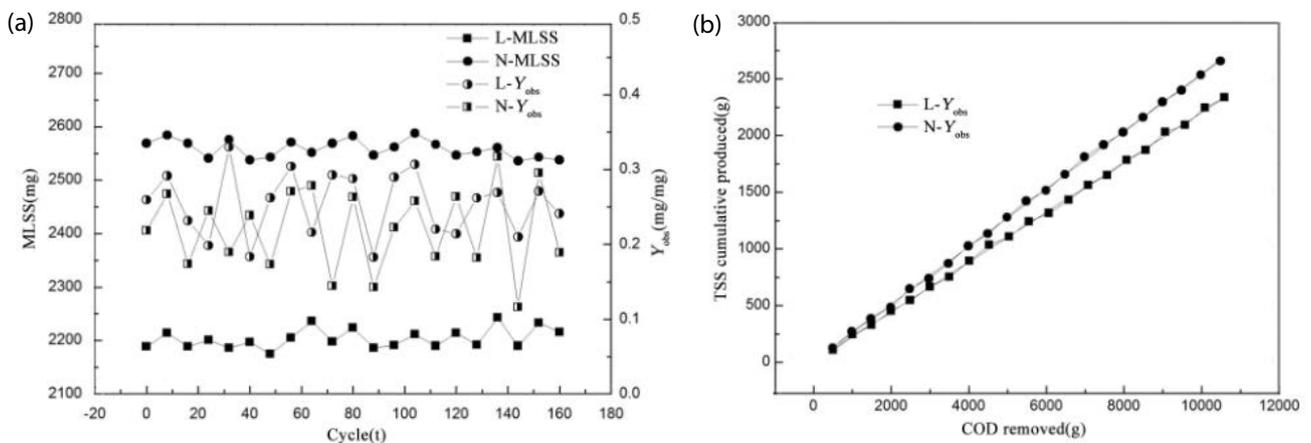


Fig. 2. Sludge observed yield (Y_{obs}) in both the reactors. (a) Daily sludge concentration and sludge observed yield (Y_{obs}) and (b) cumulative sludge observed yield (Y_{obs}).

that the sludge Y_{obs} coefficient of the control reactor was 0.254 g/g COD, while that of the low-DO reactor was only 0.22 g/g COD, 14% lower. This indicates that the DO concentration had an influence on the sludge Y_{obs} coefficient. In order to further investigate the difference in the sludge Y_{obs} coefficient between the reactors, the sludge characteristics were studied.

3.3. Effect of DO on sludge characteristics

3.3.1. Effect of DO on the sludge SVI

As can be seen from Fig. 3a, under a low-DO environment, the SVI was maintained in the range of 173–252 mL/g, with an average value of approximately 236 mL/g. In the control reactor, the SVI was maintained in the range of 46–82 mL/g, with an average value of about 78 mL/g.

The difference in the SVI value between the two reactors was mainly due to the different DO concentrations. In the low-DO environment, the growth of bacteria with a low oxygen affinity was inhibited, while filamentous bacteria with a higher oxygen affinity likely gained a competitive advantage and grew rapidly (Fig. 3b). Therefore, the high SVI was caused by the excessive proliferation of filamentous

bacteria. However, no serious sludge bulking could be caused by the low-DO concentration alone. Guo et al. [24] called this phenomenon stable limited filamentous bulking.

3.3.2. Effect of DO on the sludge MLVSS/MLSS value

The mixed liquor volatile suspended solids (MLVSS) and mixed liquor suspended solids (MLSS) values in the low-DO and control reactors are shown in Fig. 4a. The MLVSS/MLSS ratio of the sludge in the low-DO reactor was approximately 0.82, while that of the sludge in the control reactor was approximately 0.78% and 15% lower. This finding is consistent with the findings of Guo et al. [14] and He et al. [25]. Generally, the MLVSS/MLSS value is related to the SRT. In the long-SRT process, a large amount of degraded sludge results as well as inorganic suspended solids that remain in the residue. Under the same conditions, differences in the MLVSS/MLSS values may be related to differences in the sludge degradation rate, which in turn may be related to differences in the microbial populations. The main strains existing in the low-DO reactor included filamentous and facultative bacteria that can adapt to low-DO environments. In the control reactor, the main strains included floc-forming and aerobic bacteria. A study by

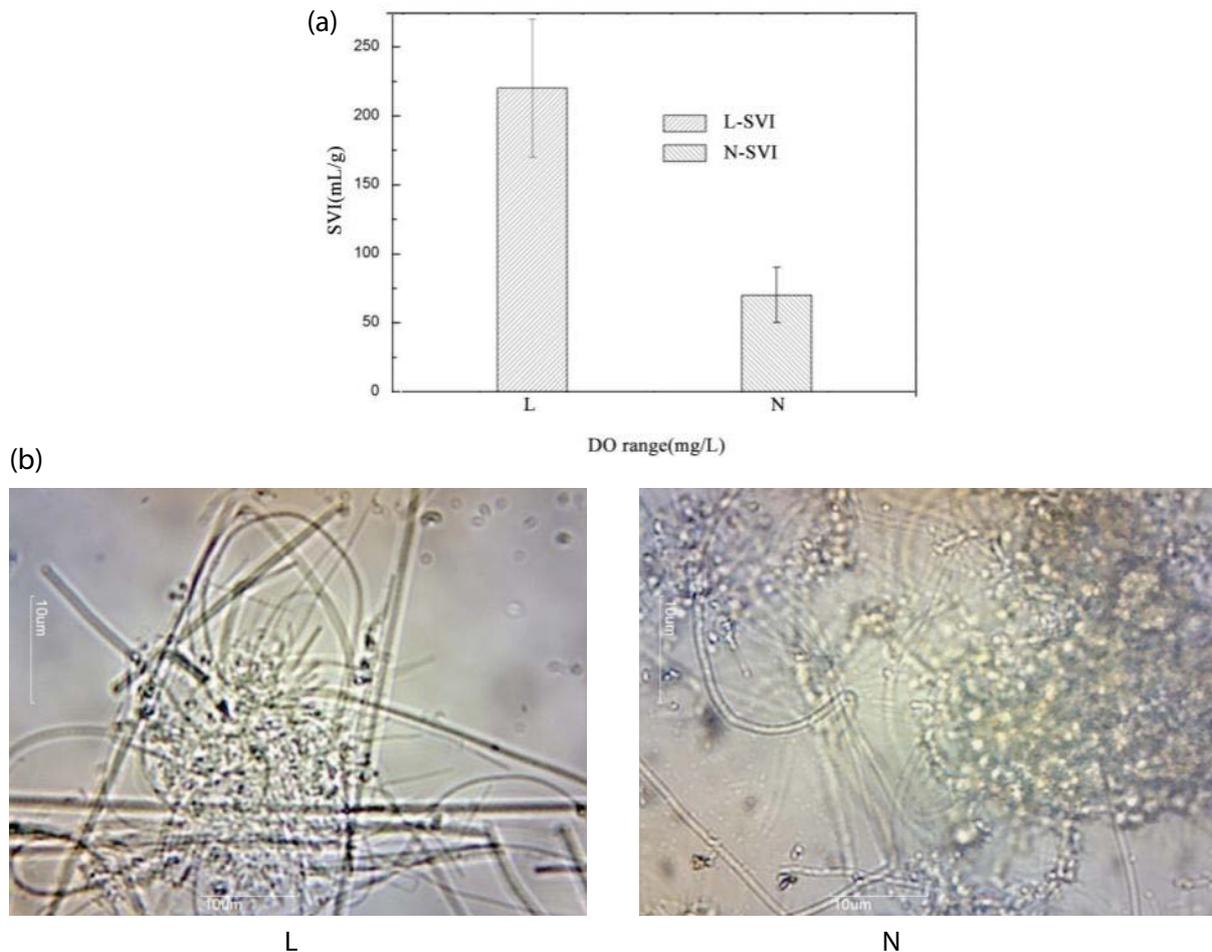


Fig. 3. (a) SVI of the sludge under different DO and (b) optical micrographs of the sludge under different DO. Samples L denotes sludge samples from the low-DO system and N denotes sludge samples from the control system.

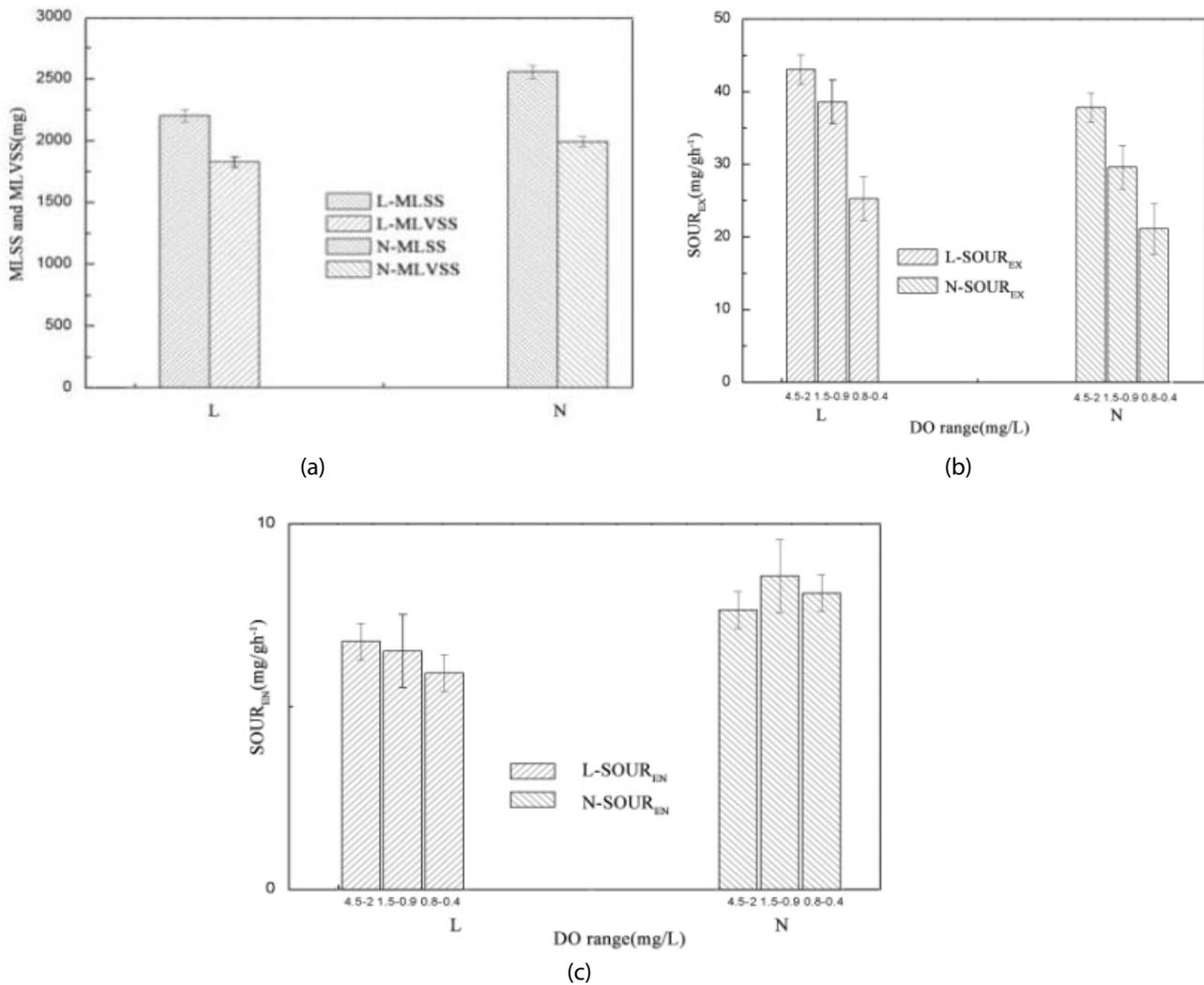


Fig. 4. (a) SS/VSS of the sludge under different DO, (b) $SOUR_{EX}$ of the sludge under different DO, and (c) $SOUR_{EN}$ of the sludge under different DO. Samples L denotes sludge samples from the low-DO system and N denotes sludge samples from the control system.

Contreras et al. [26] determined that the bacterium micelle has a greater self-degradation rate than filamentous bacteria.

3.3.3. Effect of DO on the sludge SOUR value

Generally, $SOUR_{EN}$ (endogenous specific oxygen uptake rate) is used to represent the endogenous respiration rate and $SOUR_{EX}$ (exogenous specific oxygen uptake rate) is used to represent the maximum respiration rate. The $SOUR_{EN}$ and $SOUR_{EX}$ values of the sludge in the low-DO reactor and control reactor under different ranges of DO are shown in Figs. 4b and c. When the DO concentration was higher than 2 mg/L, the $SOUR_{EX}$ values in the low-DO and control reactors were 44 and 37 mg O_2 /(g MLSS h), respectively. When the DO concentration was in the range of 1.5–0.9 mg/L, the $SOUR_{EX}$ values in the low-DO and control reactors were 38 and 31 mg O_2 /(g MLSS h), respectively. When the DO concentration was in the range of 1.8–0.4 mg/L, the $SOUR_{EX}$ values in the low-DO and control reactors were 24 and

21 mg O_2 /(g MLSS h), respectively. These observations indicate that $SOUR_{EX}$ decreases as DO concentration declines.

This was mainly because the microbial degradation of organic matter required an electronic donor and an electron acceptor. Therefore, the utilization rate of electron donors decreased with a decrease in electron acceptor concentrations. While the $SOUR_{EX}$ of the sludge in the low-DO reactor was higher than that in the control reactor, regardless of oxygen concentration. This is mainly attributed to the loose flocs formed in low-DO environments, the large specific surface area of the sludge, and the potential for a high oxygen transfer rate. However, when the oxygen concentration was low, the difference of sludge $SOUR_{EX}$ between the low-DO reactor and control reactor was reduced. This could be explained by the tendency of facultative bacteria in the low-DO reactor to enter anaerobic or anoxic working modes that consume less oxygen.

While $SOUR_{EN}$ and $SOUR_{EX}$ characteristics between reactors differed with oxygen concentration (Figs. 4b and c),

the difference in the $SOUR_{EN}$ value was not obvious due to the small amount of endogenous aerobic respiration. The $SOUR_{EN}$ of the sludge in the low-DO reactor was 20% lower than that of the sludge in the control reactor. This could be explained by the presence of hypoxemic bacteria such as filamentous bacteria, that have a smaller attenuation coefficient and a lower endogenous respiration rate, resulting in a lower $SOUR_{EN}$ for the sludge in the low-DO reactor compared with the control reactor.

3.3.4. Effect of DO on the biomass growth yield coefficient (Y_H)

As shown in Fig. 5, the average Y_H values of the sludge in the low-DO reactor and control reactor were 0.61 and 0.65 g/g, respectively. Thus, the Y_H of the sludge in the low-DO reactor was slightly lower than that of the control (Fig. 5a). Running the reactors in parallel could eliminate the influence of substrate characteristics, heavy metals, and pH on the sludge yield. The observed difference in the Y_H is mainly attributed to the particular varieties of microbial species. Payne [27] studied the Y_H of eight kinds of bacteria grown in a single aerobic glucose culture medium. The results showed that the Y_H of different cells (pure culture) ranged from 0.43 to 0.59 g/g. This test clearly demonstrated the influence of various microorganisms on Y_H . Also, filamentous bacteria presented a low yield coefficient, which might be the reason for the low Y_H of the sludge in low-DO reactor [28,29].

3.3.5. Effect of DO on the endogenous decay coefficient (K_d)

The endogenous decay coefficient (K_d) of the sludge in the low-DO reactor and control reactor were 0.33 and 0.38 d^{-1} , respectively (Fig. 5b). The K_d of the sludge in the low-DO reactor was 12% lower than that of the control.

Microorganism decay is generally affected by heavy metals, toxic metal ions, antibacterial drugs, viruses, microorganism characteristics and starvation. In this study, synthetic wastewater was used and the reactors were run in parallel. Therefore, the difference in the K_d was mainly due to the varying characteristics of the microorganisms. According to a study by Peng and Guo [29], compared

with *Zoogloea*, filamentous bacteria have lower endogenous decay rates. Hence, the K_d of the sludge in the low-DO reactor with a high content of filamentous bacteria was lower.

3.3.6. Effect of DO on the community structure of the sludge

Experimental sludge samples were taken from the low-DO and control reactors. As shown in Table 3, in sludge samples from the low-DO reactor (L), the richness index Chao value was slightly higher than that of sludge from the control reactor (N), but the difference was small. The ACE and Shannon diversity indices showed a similar pattern, while the Simpson index was slightly lower in the L sample, indicating the higher diversity and abundance of microbes in the N sample.

The accumulation percentage of microorganisms above 0.5% in each sludge sample is plotted in Fig. 6. It can be seen that the DO concentration had a great influence on the microbial population. In the L sample, *Acidobacteria*, *BD1-5*, *Bacteroidetes*, *Candidate_division_SR1*, *Candidate_division_TM7*, *Chlorobi*, and *Firmicutes* were the most abundant bacteria, among which *Bacteroidetes*, *Firmicutes*, *Acidophilus*, *Chlorobi*, and *Chloroflexi* were mostly anaerobic or facultatively metabolized. In the N sample, *Chloroflexi*, *Nitrospirae*, and *Proteobacteria* were the most abundant bacteria. The *Proteobacteria* content was high in both types of sludge samples.

In conclusion, the types of microorganisms that were present in the low-DO and control reactors were different. Most of the bacteria in the control reactor were aerobic heterotrophic bacteria, while facultative bacteria, anaerobic bacteria, and filamentous bacteria were dominant in the low-DO reactor.

3.3.7. Analysis of the sludge reduction

From the above analysis, we can see that the Y_{obs} of the low-DO reactor was lower than that of the control reactor, while the previous analysis showed that the $SOUR_{EN}$, Y_H and K_d values of the sludge in the low-DO reactor were lower than those of the control reactor. The lower

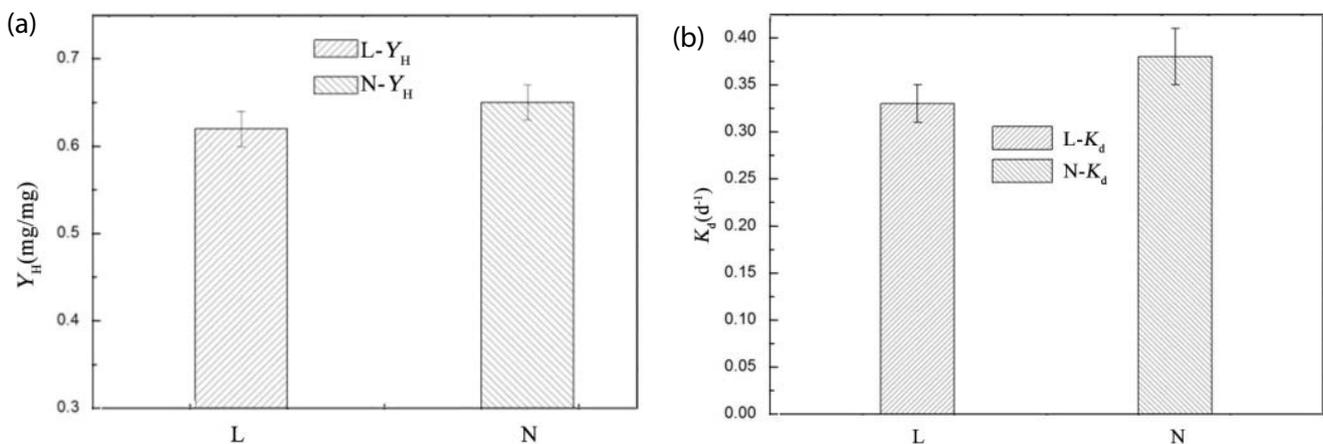


Fig. 5. Y_H of the sludge under different DO and (b) K_d of the sludge under different DO.

Table 3
Diversity index analysis table

	L	N
ACE	5,406	4,169
Chao	4,000	3,081
Coverage	0.926316	0.93655
Shannon	5.35	5.2
Simpson	0.0259	0.0271

Table 4
Contribution rates in sludge reduction

	N	L
Sludge production	0.254	0.22
Relative sludge yield, %	100	86.70
Contribution rate of the lower Y_H	100	16
Contribution rate of total decay amount	100	84

yield and lower decay rate of the sludge in the low-DO reactor made it difficult to determine the contribution rate of each index in the sludge reduction. Thus, it is essential that the growth and decay of the sludge are quantified based on the Lawrence–McCarty model (Fig. 7). The observed yield (Y_{obs} , kg VSS/kg COD) was calculated using Eqs. (2) and (3).

Eqs. (2) and (3) were then combined to obtain Eq. (4).

$$Y_{obs} = \frac{\tau_h \times Y_H}{1 + K_d \theta_c} \tag{4}$$

From Eq. (4), it can be seen that the sludge concentration was related to the Y_H , K_d , SRT, HRT, and aerobic retention

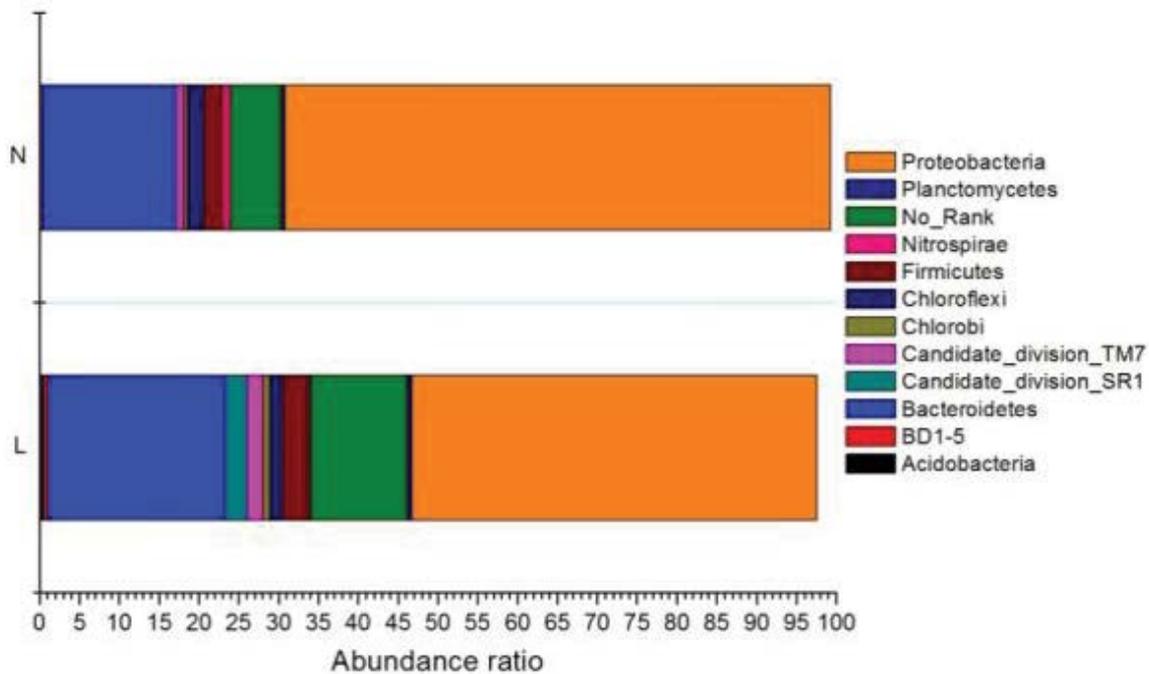


Fig. 6. Distribution of main bacteria in sludge L and N.

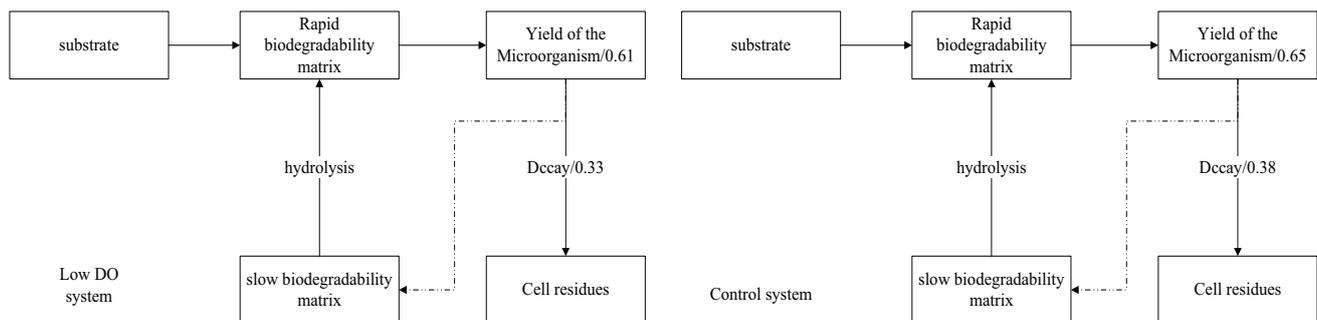


Fig. 7. Analysis of the biomass balance under different DO.

time. Therefore, when the SRT was constant, the decrease in Y_{obs} in the low-DO reactor was attributed to two reasons. The first reason was the lower Y_H of the sludge. The second reason was the higher degree of total decay in the low-DO reactor due to the longer aerobic retention time than in the control reactor. The first and second reasons contributed to the reduction in sludge quantity by approximately 16% and 84%, respectively (Table 4).

4. Conclusions

Compared with the control reactor, the low-DO reactor reduced excess sludge and removed pollutants more effectively. The low-DO reactor exhibited removal efficiencies of over 95% for ammonia, over 70% for TN, over 95% for $PO_4^{3-}-P$, and over 95% for COD. Furthermore, the sludge Y_{obs} coefficient of the low-DO reactor was 14% lower than that of the control reactor.

To further investigate the differences between the sludges produced in each reactor, the sludge characteristics were studied. The MLVSS/MLSS value of the sludge in the low-DO reactor was 15% higher than that in the control reactor. The $SOUR_{EX}$ of the sludge in the low-DO reactor was higher than in the control reactor; however, the $SOUR_{EN}$ of the sludge in the low-DO reactor was 20% lower than in the control reactor. The average Y_H values of the sludge in the low-DO and control reactors were 0.61 and 0.65 g/g, respectively. The average K_d values of the sludge in the low-DO and control reactors were 0.38 and 0.33 d^{-1} , respectively.

The Chao value in the low-DO sludge sample (L) was slightly higher than that in the control sludge (N), but the difference was not significant; the ACE and Shannon indices showed similar trends, while the Simpson index was slightly lower in the low-DO sludge sample, which could explain the high diversity and abundance of microorganisms in samples of low-DO sludge. In the low-DO reactor, filamentous bacteria, facultative bacteria, and other bacteria that were able to adapt to the low-DO environment were the main bacteria present. The microbial yield coefficient of such bacteria was low, which enhances the sludge reduction process.

The mechanism of excess sludge reduction in the low-DO reactor was the combined effect of Y_H , K_d , SRT, HRT, and aerobic retention time. The low Y_H and the high total decay amount of the sludge in the low-DO reactor contributed to the reduction in sludge quantity by approximately 16% and 84%, respectively.

Acknowledgements

This work was supported by the Natural Science Foundation of China (No. 51508149), the Natural Science Foundation of Hebei Province (E2018402172), the Handan science and technology research and development program (No. 1623209044-2), the Key scientific research projects in universities in Henan Province (No. 17B560015), the Project of Scientific and Technological Research Program of Chongqing Municipal Education Commission (KJ160062), and the Natural Science Foundation of Chongqing Science & Technology Commission (cstc2018jcyjAX0734).

References

- [1] J. Tamis, G. van Schouwenburg, R. Kleerebezem, M.C. van Loosdrecht, A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant, *Water Res.*, 45 (2011) 5916–5924.
- [2] S.S. Yang, W.Q. Guo, X.J. Zhou, Z.H. Meng, B. Liu, N.Q. Ren, Optimization of operating parameters for sludge process reduction under alternating aerobic/oxygen-limited conditions by response surface methodology, *Bioresour. Technol.*, 102 (2011) 9843.
- [3] D. H. Chon, M. Rome, Y.M. Kim, K.Y. Park, C. Park, Investigation of the sludge reduction mechanism in the anaerobic side-stream reactor process using several control biological wastewater treatment processes, *Water Res.*, 45 (2011) 6021–6019.
- [4] Y. Tian, Y. Lu, Z. Li, Performance analysis of a combined system of membrane bioreactor and worm reactor: wastewater treatment, sludge reduction and membrane fouling, *Bioresour. Technol.*, 121 (2012) 176–182.
- [5] G. Wang, J. Sui, H. Shen, S. Liang, X. He, M. Zhang, Y. Xie, L. Li, Y. Hu, Reduction of excess sludge production in sequencing batch reactor through incorporation of chlorine dioxide oxidation, *J. Hazard. Mater.*, 192 (2011) 93–98.
- [6] W.Q. Guo, S.S. Yang, W.S. Xiang, X.J. Wang, N.Q. Ren, Minimization of excess sludge production by in-situ activated sludge treatment processes—a comprehensive review, *Biotechnol. Adv.*, 31 (2013) 1386–1396.
- [7] P. Foladori, G. Andreottola, G. Ziglio, *Sludge Reduction Technologies in Wastewater Treatment Plants*, IWA Publishing, London, 2010.
- [8] P. Yan, F. Ji, J. Wang, J. Fan, W. Guan, Q. Chen, Evaluation of sludge reduction and carbon source recovery from excess sludge by the advanced sludge reduction, inorganic solids separation, phosphorus recovery, and enhanced nutrient removal (SIPER) wastewater treatment process, *Bioresour. Technol.*, 150 (2013) 344–351.
- [9] L. Huang, J. Liu, Y. He, S. Sun, J. Chen, J. Sun, K. Chang, J. Kuo, X.A. Ning, Thermodynamics and kinetics parameters of co-combustion between sewage sludge and water hyacinth in CO_2/O_2 atmosphere as biomass to solid biofuel, *Bioresour. Technol.*, 218 (2016) 631–642.
- [10] M. Henze, M.C.M. van Loosdrecht, G.A. Ekama, D. Brdjanovic, *Biological Wastewater Treatment: Principles Modelling and Design*, IWA Publishing, London, 2008.
- [11] B.E. Rittmann, P.L. McCarty, *Environmental Biotechnology: Principles and Applications*, 4th ed. McGraw-Hill Higher Education, New York, 2001.
- [12] L. Daija, A. Selberg, E. Rikmann, I. Zekker, T. Tenno, T. Tenno, The influence of lower temperature, influent fluctuations and long retention time on the performance of an upflow mode laboratory-scale septic tank, *Desal. Wat. Treat.*, 57 (2016) 18679–18687.
- [13] T. Sadaie, A. Sadaie, M. Takada, K. Hamano, J. Ohnishi, N. Ohta, K. Matsumoto, Y. Sadaie, Reducing sludge production and the domination of comamonadaceae by reducing the oxygen supply in the wastewater treatment procedure of a food-processing factory, *Biosci. Biotechnol., Biochem.*, 71 (2007) 791–799.
- [14] J.-H. Guo, Y.-Z. Peng, C.-Y. Peng, S.-Y. Wang, Y. Chen, H.-J. Huang, Z.-R. Sun, Energy saving achieved by limited filamentous bulking sludge under low dissolved oxygen, *Bioresour. Technol.*, 101 (2010) 1120–1126.
- [15] W.D. Tian, W.G. Li, H. Zhang, X.R. Kang, M.C. van Loosdrecht, Limited filamentous bulking in order to enhance integrated nutrient removal and effluent quality, *Water Res.*, 45 (2011) 4877–4884.
- [16] A.M.P. Martins, K. Pagilla, J.J. Heijnen, M.C.M. van Loosdrecht, Filamentous bulking sludge - a critical review, *Water Res.*, 38 (2004) 793–817.
- [17] R.K. Goel, D.R. Noguera, Evaluation of sludge yield and phosphorus removal in a cannibal solids reduction process, *J. Environ. Eng.*, 132 (2006) 1331–1337.

- [18] SEPA (State Environmental Protection Administration of China), Marine Sediment Quality GB18668-2002, Standards Press of China, Beijing, 2002.
- [19] U.J. Strotmann, A. Geldem, A. Kuhn, C. Gendig, S. Klein, Evaluation of a respirometric test method to determine the heterotrophic yield coefficient of activated sludge bacteria, *Chemosphere*, 38 (1999) 3555–3570.
- [20] G. Liu, J. Wang, Modeling effects of DO and SRT on activated sludge decay and production, *Water Res.*, 80 (2015) 169–178.
- [21] E.V. Münch, P. Lant, J. Keller, Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors, *Water Res.*, 30 (1996) 277–284.
- [22] S.C.F. Meijer, M.C.M. Van Loosdrecht, J.J. Heijnen, Metabolic modelling of full-scale biological nitrogen and phosphorus removing WWTP's, *Water Res.*, 35 (2001) 2711–2723.
- [23] Q. Meng, F. Yang, L. Liu, F. Meng, Effects of COD/N ratio and DO concentration on simultaneous nitrification and denitrification in an airlift internal circulation membrane bioreactor, *J. Environ. Sci.*, 20 (2008) 933–939.
- [24] J. Guo, Y. Peng, S. Wang, X. Yang, Z. Wang, A. Zhu, Stable limited filamentous bulking through keeping the competition between floc-formers and filaments in balance, *Bioresour. Technol.*, 103 (2012) 7–15.
- [25] L. He, F.-Y. Ji, X.-L. He, W.-W. Zhou, X. Xu, M.-S. Lai, Validation of accumulation models for inorganic suspended solids of different particle size in an activated sludge system, *Bioresour. Technol.*, 149 (2013) 51–57.
- [26] E.M. Contreras, L. Giannuzzi, N.E. Zaritzky, Growth kinetics of the filamentous microorganism, *Sphaerotilus natans* in a model system of a food industry wastewater, *Water Res.*, 34 (2000) 4455–4463.
- [27] W.J. Payne, Energy yields and growth of heterotrophs, *Annu. Rev. Microbiol.*, 24 (1970) 17–52.
- [28] C.T. Goudar, K.A. Strevett, Estimating growth kinetics of *Penicillium chrysogenum* through the use of respirometry, *J. Chem. Technol. Biotechnol.*, 72 (1998) 207–212.
- [29] Y. Peng, J. Guo, *Water Science and Technology: The Mechanism, Causes and Control of Activated Sludge Bulking*, Science Press, Beijing, 2002.