Supplementary Material

1. The compositions of trace element solution

The synthetic wastewater was prepared with 0.3 mL L^{-1} of trace elements solution prepared according to Smolders et al. (1994) and the detail compositions were $ZnSO_4 \cdot 7H_2O$ 120 mg L^{-1}, H_3BO_3 150 mg L^{-1}, $CuSO_4 \cdot 5H_2O$ 30 mg L^{-1}, $MnCl_2 \cdot 4H_2O$ 120 mg L^{-1}, KI 180 mg L^{-1}, Na_2MoO_4 60 mg L^{-1}, EDTA 10000 mg L^{-1}, $FeSO_4 \cdot 7H_2O$ 1540 mg L^{-1}, $CoCl_2 \cdot 6H_2O$ 150 mg L^{-1}.

2. Experiments on in situ sludge reduction and study of the mechanism

During the sludge decay experiments, biofilm with FSC and SC samples taken from the steady-state $_{FSC}$SBBR and $_{SC}$SBBR were washed to remove any residual substance in the supernatant and biofilm, respectively. The washed biofilm samples were inoculated into two batch reactors, each with an effective volume of 2 L. After running for 24 h, the daily sludge decay yield of SOUR and the concentrations of protein (PN) and polysaccharide (PS) were monitored during the experimental periods.

To further understand the role of energy-uncoupling metabolism in sludge reduction in the FSC-SBBR and SC-SBBR, biofilm samples from FSC and SC were taken from the steady-state FSC-SBBR and SC-SBBR, respectively, and were inoculated into batch reactors, each with an effective volume of 2 L. During the batch experiments, sampling every 1.5 h sampling time coincided with the HRT of SBBR. The changes in Y_{obs} values, sludge-reduction ratios and ATP concentration were examined.

3. Analysis of extracellular polymeric substances

Extraction of biofilm samples on carriers by Ultrasonic Oscillation method: mixed liquid containing carriers was cracked at 40 W, 21 KHz for 2 minutes. Then, the mixture was the suspension containing the biofilm sample. According to Ding et al (2016), extracellular polymer substance extraction method was, firstly, 12000 rpm centrifugation at 4 ℃ for 30 minutes to obtain supernatant. Then, supernatant was heat 80 ℃ water bath for 30 minutes and centrifuged at 4 ℃ 12000 rpm for 20 minutes. The supernatant was filtered through the 0.22 μm cellulose acetate membrane.

PN concentrations were measured by the Lowry method (Bollag et al., 1996) using a protein assay kit (Shanghai LAB-AIDE Co., Ltd., China), with bovine serum albumin (BSA) as the standard. The PS concentrations were measured by the phenol-sulfuric acid method with glucose as the standard.

In this study, the excitation wavelength (λ_{Ex}) scan range and excitation monochromator slit width were 200~450 nm and 5 nm, respectively. The emission wavelength (λ_{Em}) scan range and the excitation monochromator slit width were 220~600 nm and 5 nm, respectively. And the change step length was 5 nm; the scan speed was 12000 nm/ min.

4. Analysis of adenosine triphosphate (ATP) and SOUR

The cellular ATP extract samples were treated by Microbial Cell Viability Assay (Bac Titer-Glo™, USA) and then were measured using a chemiluminescence detector (Shanghai Sp-Max 1800L, China).

A sludge sample of 100 mL was taken from one of the reactors, washed and re-suspended

with distilled water, and then added to a 250 mL conical flask (Yang et al., 2016). An aerated probe was used to aerate the sludge mixed liquid to produce a DO of 5-6 mg L^{-1}. An oxygen electrode was inserted into the flask and immediately sealed with a bottle stopper. During a 15 min period, the DO concentrations were recorded every 30 seconds. The SOUR value was determined based on the bottle volume (V), DO slope (f), and MLSS (Eq. (1)).

$$\mathrm{SOUR} = \frac{3600f}{MLSS \times V} \qquad (1)$$

5. Pretreatment method of SEM

The samples were fixed with 2.5% glutaraldehyde for 2 h at 4 ℃. They were then rinsed in phosphate-buffered saline three times, and placed consecutively in vials containing 30%, 50%, 70%, 90%, and 100% ethanol for 15 min. Following this dehydration, the samples were air-dried, attached onto viewing stages, and then sputter-coated with gold to prevent static build-up during sample viewing. The samples were examined using SEM (ZEISS, Sigma 300).

6. Analysis of microbial community

The 16s rRNA sequences were clustered into operational taxonomic units (OTUs) with an average length of 423 bp by setting a 3% distance limit (equivalent to 97% similarity) for the FSC-SBBR sample (2074 OTUs) and the SC-SBBR sample (2118 OTUs). The Shannon indices for FSC-SBBR and SC-SBBR-samples were 5.97 and 5.87, respectively. Higher richness and diversity in the microbial community can be represented by the higher OTU value and Shannon diversity index indicated. The specific operational steps of DNA extraction, PCR amplification and high-throughput 454 pyrosequencing were performed according to Yang et al. (2016).

7. Performances of biological COD, nitrogen and phosphorus removal

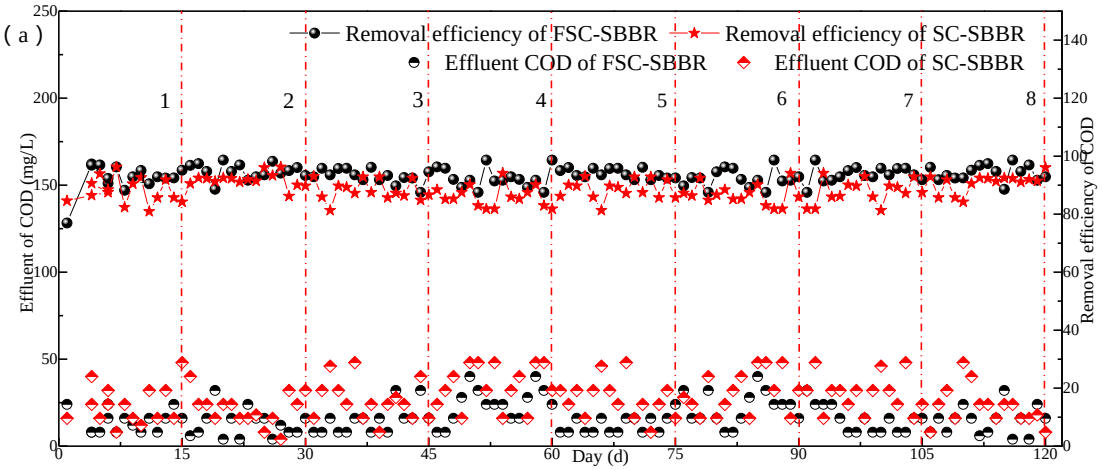
The average removal rates of COD, NH_4^+-N, TN and TP in FSC-SBBR were 93.39%, 96.66%, 82.13% and 60.23%, respectively; the average removal rates of COD, NH_4^+-N, TN and TP in SC-SBBR were 88.52%, 95.28%, 76.73% and 55.73%, respectively (as shown in Fig. 8). This observation shows that the average removal rate of contaminants in FSC-SBBR was higher than that of SC-SBBR. The average removal rates of NH_4^+-N and TN in FSC-SBBR at different DO concentrations were 98.28%, 87.97% (DO= 4~5 mg L^{-1}); 98.06%, 80.73% (DO= 2~3 mg L^{-1}) and 94.14%, 84.30% (DO=6~7 mg L^{-1}), respectively. Biological denitrification was accomplished by nitrification and denitrification (Ni et al., 2013), and there was a positive correlation between denitrification efficiency and DO concentration (Tian et al., 2013). Aerobic autotrophic nitrification bacteria oxidize NH_4^+-N to NO_3^- under aerobic conditions, which is then mixed with the influent synthetic sewage in the next cycle. Heterotrophic denitrifying bacteria utilize the sufficient carbon sources, reducing NO_3^- to N_2 under anoxic/anaerobic conditions at the beginning of the operating cycle. Thus, nitrogen removal is finally complete. Therefore, the NH_4^+ -N removal efficiencies were higher in both systems. In addition, the cell-lysis metabolism could provide additional carbon sources for denitrification.

As discussed in the SOUR experiment, local anoxic/anaerobic environment were formed in the interior of the biofilm of FSC-SBBR. The NO_3^- generated by nitrification in the aerobic phase was transferred to the interior of the biofilm. The NO_3^- was then reduced to N_2 by the oxygen-denitrifying bacteria in an anoxic environment, and simultaneous nitrification and

denitrification was thus completed during the aerobic phase. This phenomenon enhanced the efficiency of denitrification.

In addition to increasing the nitrogen removal efficiency, a higher COD removal rate was observed in FSC-SBBR (Fig.8 (a)). The average removal rate of COD was 93.39% in FSC-SBBR, while the average removal rate of COD was only 88.52% in SC-SBBR. The microbial communities of the biofilm attached to FSC was more complex than those attached to SC, and the heterotrophic bacteria continued to consume organic matter for their own metabolism during the cycle. Additionally, simultaneous nitrification and denitrification occurred, which also consumed organic matter in an aerobic environment in the FSC-SBBR. Therefore, the removal rate of COD was higher in FSC-SBBR.

The removal rates of TP fluctuated downward in both FSC-SBBR and SC-SBBR over a long period of operation (Fig. 8 (d)). The average removal rates of TP were 48.49% and 36.73%, respectively. However, the average concentrations of TP in the effluent were 4.57 mg/L and 5.00 mg L^{-1}, respectively, both of which were very close to the concentration of the influent TP (10 mg L^{-1}). This phenomenon was similar to the studies of Wang et al. (2012), and biological phosphorus removal mainly relied on the exclusion of phosphorus-rich sludge. The Y_{obs} values of FSC-SBBR and SC-SBBR was only 0.16 g MLSS g^{-1} COD^{-1} and 0.22 g MLSS g^{-1} COD^{-1}, respectively, and the low yield of excess sludge resulted in low TP removal efficiency. However, the efficiency of phosphorus removal in $_{FSC}$SBBR reached 60.01% during initial operation, indicting the adsorption by the biofilm induced by EPS secreted by microbes during the formation of the biofilm (Pan & Han, 2016).



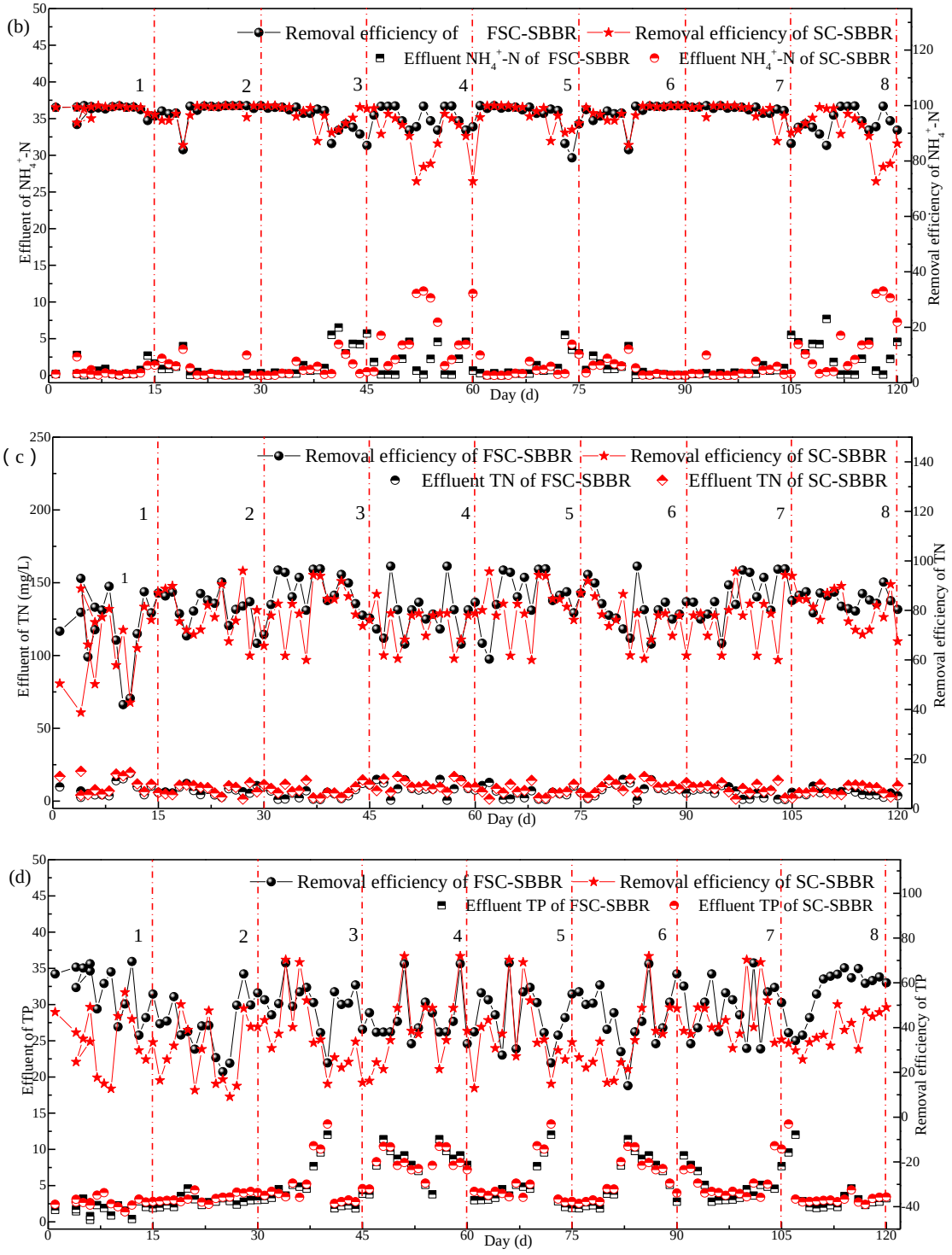


Fig. 1 Average effluent of (a) COD, (b) NH_4^+-N, (c) TN and (d) TP, and removal efficiencies in FSC-SBBR and SC-SBBR systems during 120 days of operation.

8. The impact of different operation conditions

Comparing reaction conditions 1~4, the temperature has a positive correlation with sludge reduction effect in the in situ sludge reduction under suitable condition, and the sludge reduction efficiency increases with increasing temperature. The optimum operating temperature of the two

in situ sludge reduction systems was 25℃ and Y_{obs} were 0.22±0.10 (FSC-SBBR) and 0.28±0.10 (SC-SBBR) g MLSS·（g COD）$^{-1}$, respectively. The temperature will influence the in-situ sludge reduction by affecting the microbial activity and the microbial population richness on the biofilm. At the optimal operating temperature, earthworm and decomposed sludge indicative microbiology were observed on the biofilm of FSC-SBBR. The suitable temperatures are conducive to the formation of a rich microbial community structure and a stable food chain, promoting the in situ sludge reduction induced by microbial predation.

Comparing reaction conditions 3, 5 and 6, prolonging the HRT time can increase the effect of in-situ sludge reduction. The optimal HRT of the two in situ sludge reduction systems was 12 h. The suitable HRT could make the biofilm thoroughly hydrolyzed under anaerobic stage, promoting lysis-cryptic growth metabolism, and making the in sit sludge reduction more effective. According to the previous stud of Sun et al. (2016) also showed that HRT will affect sludge reduction by affecting the microbial lysis phenomenon, and with the prolonged HRT, the soluble organic matter in the supernatant will show the trend of increasing first and then decreasing.

Comparing reaction conditions 3, 7 and 8, the DO concentration was positively correlated with the sludge reduction effect of the two in situ sludge reduction systems. The optimal DO concentration for the two systems is 4~5 mg/L. DO could influence the sludge in-situ reduction performance by affecting the liquid ORP value and changing the living environment of the microorganism. The ORP value can reflect the system liquid microbial activity (Gong et al., 2015), and lower ORP value can promote sludge in situ reduction (Eusebi et al., 2015). In addition, the microbial activity is enhanced and the substrate is consumed in large quantities for its own metabolism with the ORP value rising rapidly. However, the limited substrate concentration may constrain the anabolism of microorganisms under high ORP environment (Chen et al., 2003), inducing the in situ sludge reduction.

References

Bollag D M, Rozycki M D, Edelstein S J. 1996　Protein Methods - 2nd edition.

Chen G H, An KJ, Saby S, Brois E，Djafer M (2003). Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process), Water Research, 37(16):3855.

Eusebi A L, Battistoni P (2015). Reduction of the excess sludge production by biological alternating process: real application results and metabolic uncoupling mechanism. Environmental Technology, 36(1-4):137.

Gong B, Wang Y, Wang J, Dou Y, Zhou J (2017). In situ excess sludge reduction in SBBR through uncoupling of metabolism induced by novel aeration modes. Rsc Advances, 7(46): 29058-29064.

Ni B J, Ruscalleda M，Pellicern àcher C, Smets B F (2013). Modeling Nitrous Oxide Production during Biological Nitrogen Removal via Nitrification and Denitrification: Extensions to the General ASM Models. Environmental Science & Technology, 47(20): 11908-9.

Pan L T, Han Y (2016). A novel anoxic-aerobic biofilter process using new composite packing material for the treatment of rural domestic wastewater. Water Science & Technology, A Journal of the International Association on Water Pollution Research, 73(10): 2486.

Sun L, Chen L, Cai S, Guo W Z, Ye T J, Cui Y H (2016). Effect of sludge reduction in oxic-settling-anaerobic (OSA) systems with different anaerobic hydraulic retention times (HRTs). Desalination & Water Treatment, 57(23):1-5.

Tian Y, Li Z, Ding Y, Lu Y (2013). Identification of the change in fouling potential of soluble microbial products

(SMP) in membrane bioreactor coupled with worm reactor. Water Research, 47(6): 2015-24.

Wang J, Li X, Fu W, Wu S, Li C (2012). Treatment of artificial soybean wastewater anaerobic effluent in a continuous aerobic anaerobic coupled (CAAC) process with excess sludge reduction. Bioresource Technology, 126(126C)：142.

Yang, S, Guo W, Chen Y, Peng S, Du J, Zheng H, Feng X, Ren N (2016). Simultaneous in-situ sludge reduction and nutrient removal in an A_2MO-M system: Performances, mechanisms, and modeling with an extended ASM2d model. Water Research, 88(4): 524-537.