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Suspended membrane bioreactor with extracellular polymeric substances as reserve carbon source for low carbon to nitrogen ratio wastewater: Performance and microbial community composition

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Abstract−A suspended membrane bioreactor (SMBR) was employed to treat low carbon to nitrogen (C/N) ratio wastewater. The suspended membranes amplified by *Comamonas* sp. YSF15 were collected to develop the SMBR, which showed excellent performance for nitrate (NO_3^-) removal. The maximum nitrate removal efficiency of 95.22% was obtained at an initial nitrate concentration of 20 mg L^{-1}, HRT of 6 h, and C/N=2.5 : 1 (molar ratio). In addition, the polysaccharide (PS) and protein (PN) compositions of the soluble microbial products (SMP), extracellular polymeric substances (EPS) and the Fourier transform infrared (FTIR) spectra indicated that the suspended membrane utilized its SMP and EPS as reserve C source to achieve higher denitrification performance under the low C/N ratio. With the increase of pH, the generated SMP and EPS in SMBR continued to increase. The increase in PS content was significantly greater than that of PN, thus forming a suspended membrane with a certain mechanical strength. High-throughput sequencing data indicated that *Comamonas* sp. YSF15 played a key role in effective nitrate removal by SMBR. It can adapt to a nutrient-deficient environment, especially low (C/N) ratio, and greatly increase in the late operation of the reactor.

Keywords: Exopolysaccharide, High-throughput Sequencing, Low C/N Ratio, Nitrate Removal, Suspended Membrane Bioreactor

INTRODUCTION

Nitrate nitrogen (NO_3-N) pollution has received great attention in wastewater treatment due to eutrophication that would be aggravated by higher levels of nitrogen compounds released into the aquatic ecosystem [1,2]. Nitrate becomes a potential source of pollution in the wastewater and poses a threat to human health and aquatic micro-flora and fauna [3,4]. However, the nitrate nitrogen in wastewater is hard to completely remove under the low carbon-nitrogen (C/N) ratio due to insufficient carbon for complete denitrification; thus, it requires an external carbon source for effieicient performance of the bioreactor [5-8].

The deficient carbon in domestic wastewater leads to excessive nitrate in the effluent released from biological reaction tanks, which is already being a challenge for wastewater treatment plants to achieve efficient nutrient removal [9,10]. As the carbon acts as electron donor for denitrification, the traditional solution to this problem is an external addition of carbon source (ethanol, methanol, glucose or sodium acetate) for denitrification [11]. Carbon addition is costly and not applicable on large scale [12]. Therefore, the development of new technologies to treat low C/N ratio wastewater is a research hotspot.

Previous studies have shown that soluble microbial products (SMP) and extracellular polymeric substances (EPS) could be employed as carbon sources to improve the treatment effect of low C/N wastewater [13,14]. Under the oligotrophic conditions, microorganisms metabolize SMP and EPS to maintain their normal growth and development [4]. The macromolecular carbonitrides produced by EPS hydrolysis can be used for the synthesis of active biomass by microorganisms [15]. In addition, researchers have demonstrated that SMP is biodegradable [16]. Additionally, Hiebner et al. found that polysaccharides (PS) are the main components of SMP and EPS under anaerobic conditions and used as carbon sources by microorganisms [17]. SMP and EPS are produced by microorganisms and form biofilms [17]. Ozturk et al. have reported that biofilms have a greater potential to withstand environmental pressures [18], such as changes in pH and salt content, as microbes are protected in the matrix [19]. In fact, biofilms showed about 1,000-fold higher resistance to antibiotics or disinfectants compared to planktonic cells [20].

Due to the strong adaptability and stability of biofilm to the environment, some researchers have been exploring its application technology in various fields for decades. In electrochemistry field, UV-curable glyceryl methacrylate (GMA) based solid polymer electrolyte (SPE) [21], PEO/LAGP mixed solid polymer electrolyte (HSPE) [22], UV crosslinked composite polymer electrolyte (CPE) [23], PEO/LiTFSi-based polymer electrolyte [24], porous carbon film (LCM) [25] and other film composites have opened up a broad prospect for the development of all solid-state energy storage equipment. In the field of optical materials, thin film coating has been

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widely used in national economy and national defense construction [26]. In the field of water treatment, MBR technology has potential in energy production, wastewater treatment and environmental protection, and is considered to be an excellent wastewater treatment and energy production process [27]. In addition, some achievements have been made in the exploration of biofilm reactors for the treatment of low carbon and nitrogen wastewater. For instance, Iannacone et al. prepared a microaerobic moving bed biofilm reactor for treating low C/N ratio wastewater [28]. The biomass cultivation at low C/N (2.7 and 4.2) resulted in the highest nitrifying and denitrifying activities. Zou et al. and Gou et al. established a novel sequential batch biofilm reactor (NSBBR) and suspended growth bioreactor to improve the nitrogen removal efficiency of low C/N wastewater [29,30]. NSBBR operated at a lower C/N ratio (2.7) resulted in 2.6-fold higher denitrification activity than that of traditional SBBR [29]. In case of suspended growth bioreactor, the increasing the C/N strengthened the removal efficiency total phosphorus and chemical oxygen demand. The C/N ratio impacts the growth and reproduction of microorganisms in the reactor [30]. However, at present, the biofilm treatment for wastewater with low C/N is always inefficient.

In this study, a suspended membrane bioreactor (SMBR), produced by an effective denitrifying strain (*Comamonas* sp. YSF15) under low C/N, is proposed as a novel effective nitrate removal membrane in the bioreactor. The objectives of this research were to (i) evaluate the influence of different C/N ratios, hydraulic retention time (HRT) and initial nitrate concentration to affect nitrate removal in SMBR and optimize the operating conditions of the reactor, (ii) analyze the composition and characteristics of SMP and EPS by Fourier transform infrared spectroscopy (FTIR) and further understand the mechanism of high-efficiency denitrification in low C/N reactor, and (iii) analyze the influence of variable C/N ratios on the microbial community diversity in SMBR.

MATERIALS AND METHODS

1. Formation of the Suspended Membrane

In the previous study we isolated a strain YSF15 that was able to efficiently denitrify under low C/N conditions [31]. When the pH of the medium was set to 9.0, it was unexpectedly found that YSF15 produced some membranous substances with a certain mechanical strength in the culture apparatus (Fig. S1). This membranous substance floats in a suspended state in the liquid and should be a special complex membrane produced by bacteria in order to adapt to the infertile environment.

To test the application of suspended membrane in biological nitrate removal, strain YSF15 was inoculated into the medium for cultivation at pH=7.0 [31]. The medium was cultured constantly at 30 °C for one week and the formation of the membrane was observed. When the membrane was stirred at 30 °C and 120 rpm min^{-1} without breaking, the well-preserved membrane was selected, separated and collected in a storage glass bottle, and stored at 4 °C.

2. Reactor Operating Conditions

A 500 mL bottle was employed as a reactor to study the denitrification performance of the SMBR in the anaerobic condition. At the same time, the reactor was optimized by different parameters (C/N, HRT, etc.) to obtain the optimal operating conditions of the SMBR. The optimized parameters were C/N ratio (2.0, 2.5 and 3.0), hydraulic retention time (2, 4 and 6 h) and initial concentration of nitrate (15.0, 20.0 and 25.0 mg L^{-1}). The specific operating parameters of the SMBR reactor are shown in Table S1. Each stage in the table runs for seven days, which can well illustrate the reproducibility of the SMBR reactor. The nitrate and nitrite nitrogen of the influent and effluent for each phase were measured and recorded separately.

3. Mechanism Exploration

For understanding the mechanism of nitrate removal by SMBR, the SMP and EPS changes in the system were extracted according to Kunacheva and Stuckey [32]. The rubber plug was inserted for gas sampling into the sealed syringe to extract the gas produced in the reactor. The gas composition in the reactor was detected by gas chromatography instruments (Agilent 6890, Japan; PerkinElmer clarus 600, USA) during the stable period to verify the nitrate removal pathway during reactor operation. The specific measurement methods are referred to previous studies [3]. Fourier transform infrared (FTIR; Nicolet iS50, Thermo, USA) spectrometer was used to detect in the composition of the suspended membrane composition when the reactor was running for 0 and 6 h. To explore the membrane-forming principle of the suspended membrane, different pH conditions were set (6.0, 7.0 and 8.0) to observe the SMP and EPS changes under each condition.

4. High-throughput Sequencing Analyses for Microbial Diversity

The sequencing analyses were carried out by using the Majorbio Cloud Platform to study the change in species composition and dominant flora during reactor operation. Based on the different C/N ratios, we selected three representative cycle samples during the operating period, namely, YSF1 (C/N=3.0), YSF2 (C/N=2.5) and YSF3 (C/N=2.0) at an initial nitrate concentration of 25.0 mg L^{-1} and HRT of 6 h. The 16S rRNA gene product of DNA was amplified using a specific primer, purified and Illumina (Solexa) sequencing and analyzed according to Hao et al. [33].

5. Analytical Methods

The standard N-(1-naphthalene)-diaminoethane spectrophotometry and UV spectrophotometric methods (UV-2802, Unico, China) were used for the nitrite and nitrate concentrations, respectively. The pH was determined with a pH meter (MM110, HACH, USA). The measurement of PS and protein (PN) adopted standard procedures [34,35].

RESULTS AND DISCUSSION

1. Denitrification Performance of SMBR

The removal characteristics of nitrate in the reactor are shown in Fig. 1. The operation process of the SMBR is segmented into stage 1 (C/N=3.0), stage 2 (C/N=2.5) and stage 3 (C/N=2.0) based on the different C/N ratios. Each stage, depending on the initial nitrate concentration and HRT, was divided into 27 phases (NO_3^- =15.0, 20.0, 25.0 mg L^{-1}, HRT=2, 4 and 6 h) as presented in phase 1 to 27 (Table S1). The SMBR reactor achieved stable and efficient removal of NO_3^--N in each operation cycle. It is speculated that due to the accumulation of more biomass in the reactor, the

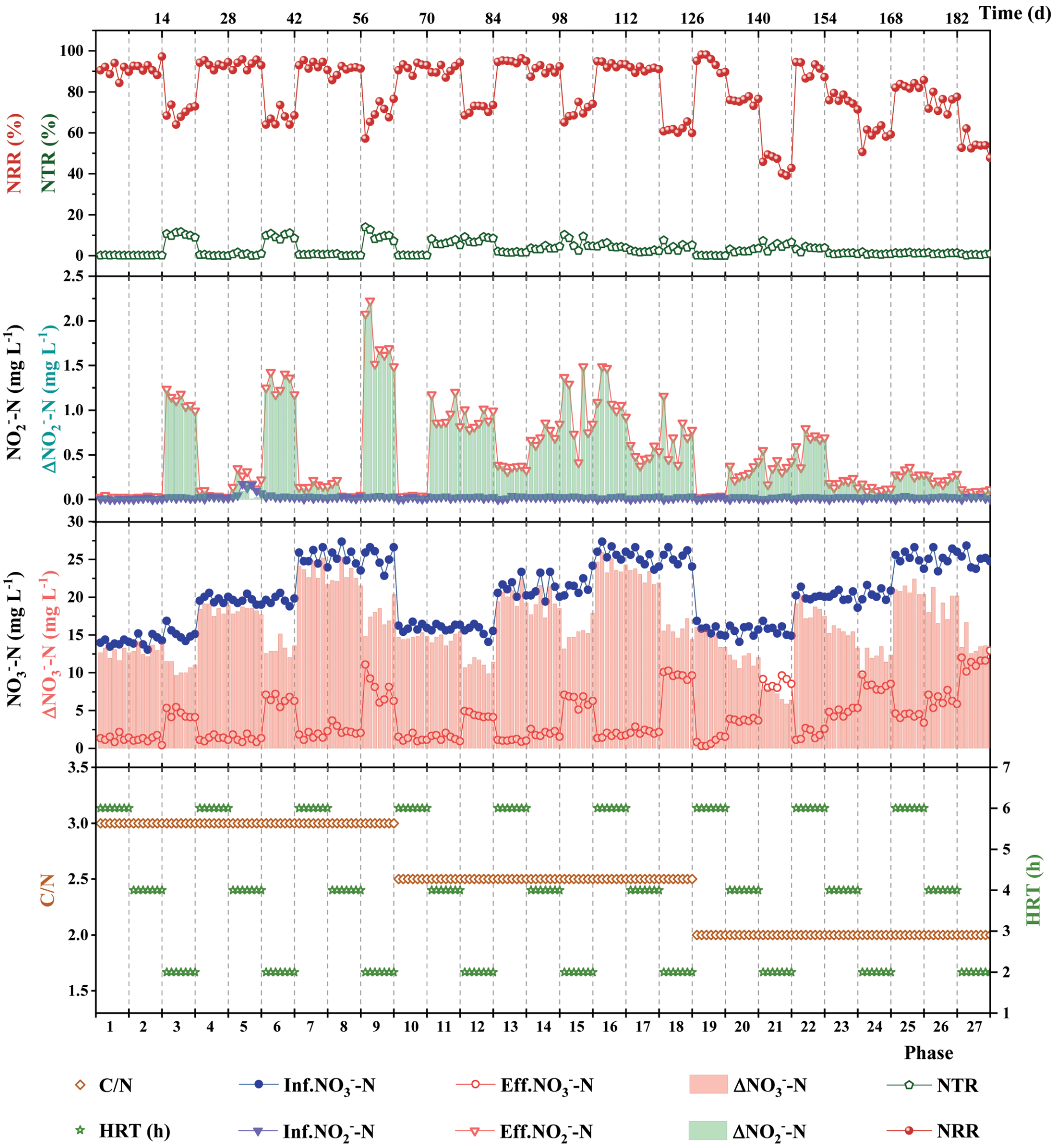

Fig. 1. Nitrate removal performance of the SMBR reactor.

impact resistance capacity is higher and the system operation is relatively more stable.

1-1. Effect of C/N Ratios on Denitrification Performance

The average removal ratio of nitrate (NRR) values at the C/N of 3.0, 2.5 and 2.0 were 84.21, 84.02, and 72.36%, respectively (Fig. 1). The experimental results revealed an interesting phenomenon of no difference in the NRR between C/N=3.0 and 2.5; both of them had a certain amount of accumulation of nitrite, indicating that *Comamonas* sp. YSF15 adapted to the new environment during operation. It seemed that the SMBR reactor can achieve a good denitrification effect at the C/N of 2.5. A significant decrease in the NRR was observed at C/N of 2.0 compared to the C/N of 3.0 and 2.5. These results showed clearly, with the same initial nitrate concentration, the continuous decrease of C/N would lead to further decrease the NRR and led to the deterioration of the reactor denitrification effect, which was in lined with the previous findings [5,30]. Morover, Machat et al. also reported that higher C/N ratio promoted the carbon and nitrate removal in a hybrid biological reactor [6].

When the HRT was adjusted at 6 h and the concentration of influent nitrate was 20 mg L^{-1}, the highest NRR values at C/N ratio of 3, 2.5 and 2 were 95.38, 96.34 and 94.42% (3.18, 3.74 and 3.18 mg L^{-1} h^{-1}), respectively. It can be concluded that the NRR is higher when the C/N ratio is 2.5 rather than 3. However, Machat et al. indicated that the efficiency of the nitrate removal falls with a reduced content of C/N [6] and Tian et al. reported that the higher the carbon source concentration, the higher is the nitrate removal efficiency [5]. So, it might be because YSF15 uses EPS and SMP as a reserve C source when the C/N ratio is 2.5.

1-2. Effect of HRT on Denitrification Performance

As displayed in Fig. 1, the HRT was set at 2, 4 and 6 h. At C/N of 2.5 and the initial nitrate concentration of 20.0 mg L^{-1}, the maximum nitrate removal efficiency was 96.34% (3.74 mg L^{-1} h^{-1}) at HRT of 6 h. The removal ratio of nitrate declined remarkably at the same time from 96.34 to 69.87% (3.74 to 7.70 mg L^{-1} h^{-1}) when the HRT was reduced from 6 to 2 h. The nitrate concentration decreased significantly, which confirmed that the *Comamonas* sp. YSF15 had sufficient time to denitrify efficiently. This indicated that longer HRT provides sufficient time to the suspended membrane to adapt to the new environment for denitrification. These findings are in line with the previous work of Yang et al. [36]. Gadow and Li also showed that the nitrate removal efficiency would be lower at short HRT in the denitrification bioreactor [37]. A similar tendency was even more pronounced at the C/N ratios of 2 and 3.

A gradual decrease in the concentration of nitrite was observed with increasing the HRT from 2 to 6 h. When the initial nitrate concentration was adjusted at 20.0 mg L^{-1} and the C/N at 2.5, the effluent nitrite concentration reached its highest point (1.47 mg L^{-1}) at an HRT of 2 h after 4 days. This demonstrated that, as the HRT increased, the removal efficiency of nitrate and nitrite also greatly increased. As reported by Zhang et al., HRT influences the denitrification efficiency of the reactor [38]. The results indicated that a longer HRT was essential for a higher denitrification performance [39].

1-3. Effect of Initial Nitrate Concentration on Denitrification Performance

At C/N of 2.5 and the HRT of 6 h (Fig. 1), the initial content of nitrate dropped from 25.0 to 20.0 and 15.0 mg L^{-1} (Phase 1.5), respectively. The nitrate removal efficiency was 93.42, 94.98 and 91.90% (4.04, 3.37, 2.46 mg L^{-1} h^{-1}), respectively. Meanwhile, the nitrite accumulation was only 0.19, 0.06 and 0.01 mg L^{-1} h^{-1}, respectively, at the time of the initial nitrate content of 25, 20 and 15 mg L^{-1}. The increase in nitrate concentration hardly decreased the removal rate, so the reactor had a certain impact load resistance capability. As reported by Su et al., the change in initial nitrate concentration had little influence on the biological nitrate removal capacity by *Comamonas* YSF15 [31]. Finally, it was obvious that no impact of initial nitrate concentration on the removal ratios of nitrate at SMBR reactor was demonstrated in the present experiment. At the same time, we propose that the SMBR has great environmental adaptability, the nitrate concentration range of wastewater can be widely treated, and all of them can meet the sanitary standards for drinking water in China (GB5749-2006).

It can be observed by comparing the experimental results of the three stages that HRT and C/N revealed significant effects on the nitrate removal performance of the SMBR. The increase in HRT had positive feedback to the nitrate removal ratio in the reactor, and the C/N was always below 3 : 1 during the whole operation of the SMBR. Therefore, the SMBR reactor could run stably at a low C/N ratio, while the average nitrate removal ratio was 80.20% and the accumulation of nitrite was only 0.49 mg L^{-1}. Taken together, the SMBR reactor based on the efficient denitrification characteristics of strain YSF15 was able to conduct complete denitrification under the poor nutrition condition of a C/N less than 3.0. Overall, the SMBR reactor achieved the best efficiency of denitrification as the C/N was 2.5, HRT was 6 h and the concentration of the initial nitrate was 20.0 mg L^{-1}, which was set as the optimum conditions for the subsequent stable operation.

2. Denitrification Performance of Stable Period

The SMBR reactor was optimized by different operating parameters to obtain the optimal operating conditions. The removal effect was further investigated by taking the C/N of 2.5, HRT of 6 h and the nitrate concentration of 20 mg L^{-1} as the optimum condition for the subsequent denitrification operation. The results of the stable operation are presented in Fig. 2. After seven days of stable operation of the SMBR, the average NRR in the reactor was 95.22% (3.33 mg L^{-1} h^{-1}), whereas the highest was 95.97% (3.42 mg L^{-1} h^{-1}) on the fourth day. For nitrite, the average accumulation of nitrite in the whole period was only 0.32 mg L^{-1}, no significant accumulation of nitrites occurred. The average nitrate was 1 mg L^{-1}, which is lower than the sanitary standards for drinking water in China (GB5749-2006). Moreover, it corroborates that SMBR has a good effect on removing nitrate, which could achieve the removal of over 95% nitrate at the C/N of 2.5, HRT of 6 h and the nitrate concentration of 20.0 mg L^{-1}. These findings are in agreement with the previous finding of Gou et al., who reported an enhancement in the denitrification with an increasing C/N ratio in a suspended growth bioreactors [30].

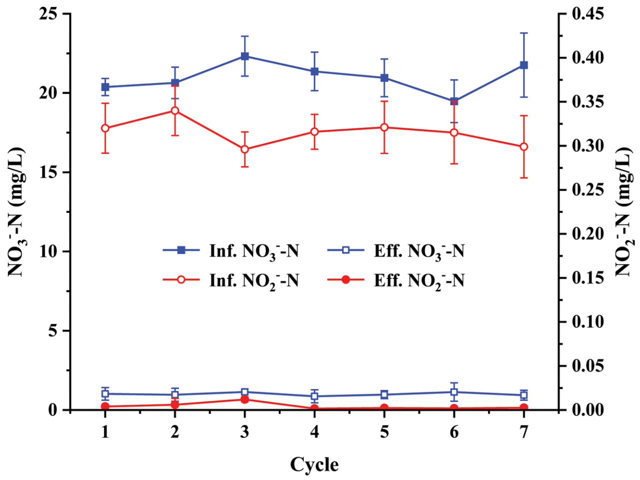

Fig. 2. Stable operation of the SMBR reactor (Inf. stands for influent concentration of contaminants; Eff. stands for effluent concentration of contaminants).

3. Gas Composition Analysis

To verify the nitrate removal pathway during the operation of the SMBR, the gas composition was detected by gas chromatography in the stable operation period and the results are presented in Fig. 3. The results reveal that 99.82% of N_2 was detected in the gas production process. N_2O, which is a serious threat to the ozone layer, has the potential to cause global warming [40]. However, most of the nitrate removed was converted to a gaseous product N_2, which demonstrated that complete denitrification occurred in the SMBR and no intermediate compound (N_2O) was accumulated. Therefore, it was concluded that the denitrification efficiency is high in the SMBR and has little effect on the gas environment. Furthermore, Chen et al. also proposed that the denitrification process created an alkaline environment and part of CO_2 might be converted into HCO_3 and CO_3^- to achieve lower carbon emissions [41].

4. Analysis of SMP and EPS Content

Generally, the EPS released by bacteria are divided into loosely

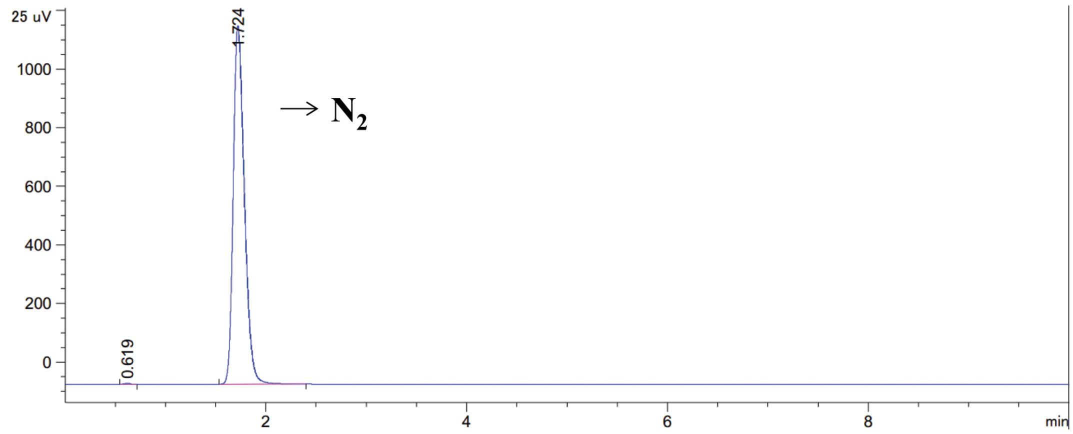

Fig. 3. The gas composition in the SMBR.

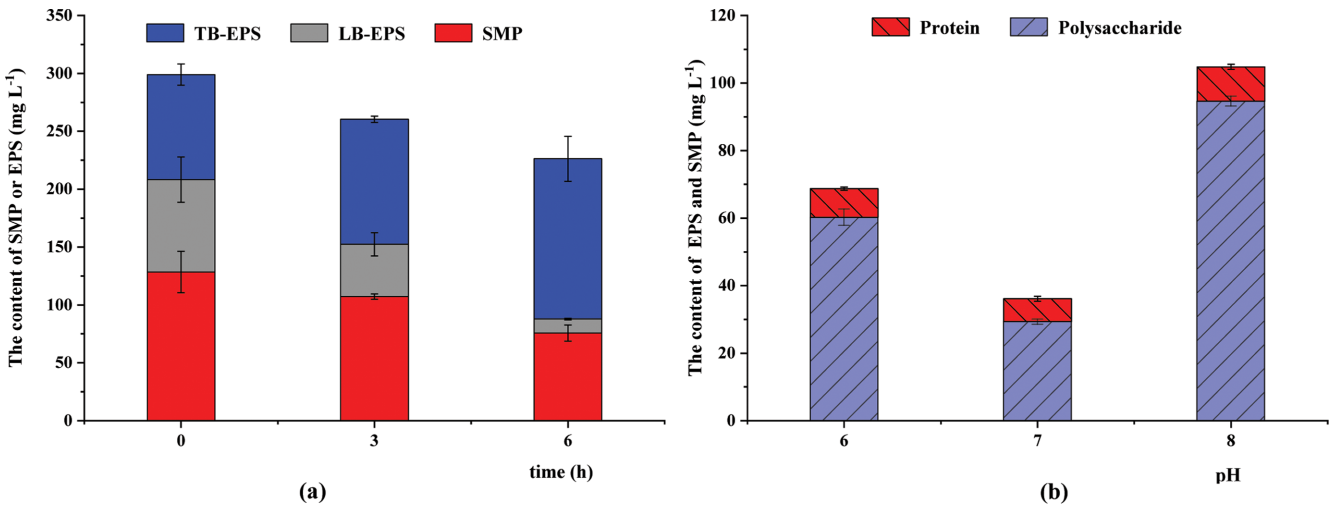

Fig. 4. EPS content in different stages of reactor (a) and the changes in SMP and EPS contents in the system under different pH in the SMBR (b).

bound extracellular polymer (LB-EPS) and tightly bound extracellular polymer (TB-EPS) [42]. The variation in SMP and different types of EPS in the reactor over time are shown Fig. 4. Overall, the total content of SMP and EPS showed a decreasing trend. It was proved that the SMBR used SMP and EPS as the reserve carbon sources when the C/N was 2.5. The content of SMP and EPS at 0, 3 and 6 h was 271.94, 235.35 and 199.42 mg L^{-1}, respectively. The SMP is the first one to be consumed, gradually decreasing from 128.53 (0 h) to 75.73 mg L^{-1} (6 h). In addition, different types of EPS have different trends. On the one hand, LB-EPS gradually decreased from 80.22 (0 h) to 12.15 mg L^{-1} (6 h), while on the other hand TB-EPS increased from 90.71 to 138.34 mg L^{-1}. These results hav that TB-EPS, as a part of the close combination with the bacterial cell, has poor availability [43] and needs to be converted into LB-EPS to be easily used by YSF15. The production rate is greater than the utilization rate, TB-EPS showing an increasing trend. The LB-EPS were used by YSF15 before the TB-EPS and showed a decreasing trend. SMP dissolved in sewage and were easily used [13], so there is a reducing trend. This also verified that SMBR used SMP and EPS as supplementary carbon source to treat wastewater. The changes in SMP and EPS contents in the system under different pH conditions are shown in Fig. 4. An interesting phenomenon can be seen. The content of PS in SMBR was significantly higher than PN. The specific reason of this special case needs further investigation and analysis. It can be seen from the figure that the release of SMP and EPS is the least at pH=7.0. Among them, the PN and PS content was 4.35 and 29.67 mg L^{-1}, respectively. In addition, the release amount of SMP and EPS under acidic and alkaline conditions was higher than neutral conditions. When pH=6.0, the PN and PS content was 5.88 and 58.10 mg L^{-1}, respectively. When pH=8.0, the PN and PS concentration was 6.32 and 93.76 mg L^{-1}, respectively. The data illustrated that YSF15 had different ability to release EPS and SMP under different pH conditions.

YSF15 would release more PS and PN to adapt to the barren environment in alkaline conditions. These extra EPS and SMP would serve as the reserve electron donor for the denitrification and promote the nitrate removal [20]. In addition, we can observe that with the increase in pH, the increase in PS content is more obvious than that of PN. Exo-polysaccharide itself is a polymer viscous material, which could be used as a matrix for intercellular

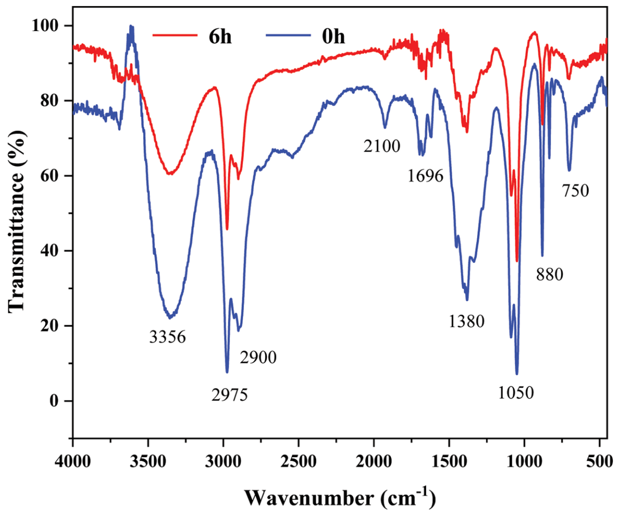

Fig. 5. FTIR observations of SMBR reactor.

connection and adhesion [44], as well as promotes the aggregation of YSF15 to form stable membrane-like substances.

5. Fourier Transform Infrared Analysis

Fig. 5 shows the FTIR spectra of the reactor at 0 h and 6 h. The peaks vary greatly at 3,356, 2,900, 1,380 and 1,050 cm^{-1}. The peaks at 3,356 and 1,380 cm^{-1} belong to the absorption peak of PN substances, corresponding to the O-H group or C-H group stretching vibration [45,46]. The stretching vibration of the O-H group occurred in a wide frequency range, indicating the existence of free hydroxyl groups (OH) and bonded O-H bands in carboxylic acids [47]. The sample shows peaks at 1,696 cm^{-1}, corresponding to a secondary amine [48]. Peaks at 2,975 and 2,900 cm^{-1} both refer to the symmetric and asymmetric stretching vibration of C-H in PS [45]. At 1,050 cm^{-1}, the peak corresponds to C-O stretching vibration of PS [48]. This indicates that the main substances in the sample are SMP and EPS containing PN and PS. Compared with the 0 h sample, the absorption peak transmittance of the sample at 6 h significantly increased. It indicates that EPS and SMP are continuously reduced as the reaction proceeds, further illustrating that YSF15 used EPS and SMP as its reserve carbon source under a low C/N ratio condition [49].

6. Biological Community Diversity Analysis

6-1. Species Diversity Analysis

The species diversity of the samples are presented by Alpha diversity, whose common metrics includes the Sobs, Chao, Shannon, Ace, Simpson and Coverage indices [50]. The coverage index of the three samples is greater than 0.999 (Table 1), suggesting that the sequences can well represent the bacterial OTUs in each sample. The Sobs index indicates that YSF2 has the highest species richness compared to the other two samples. A large number of microorganisms could not adapt to the harsh environment and died at the end of reactor operation. The main cause of the minimum YSF3 species richness was a reduced C/N, which is similar to the reported results of Jia et al. [51]. As is shown in Table 1, the Simpson index of YSF1 is the largest and the Shannon index is the smallest. The results exhibit that the community diversity of the YSF1 was the lowest among the three samples and the dominant species in YSF1 had the highest proportion.

6-2. Species Composition Analysis

A Venn diagram was used to describe the similarity and uniqueness of the three samples in different environments for further analysis of the microbial community [52]. We can see that the number of common OTUs was 29 in three samples (Fig. 6(a)). Besides, the common OTUs between YSF2 and YSF3 was 53, which was higher than the number of common OTUs between YSF1 and YSF2 (44), or YSF1 and YSF3 (32). This suggests that the species compositions of YSF2 and YSF3 are more similar than those of YSF1 and YSF2 or YSF1 and YSF3.

We used the Venn pie chart to show the common species among the three samples. The core genus OTU179 (*Comamonas*) in the SMBR occupied a certain proportion in each species sample. OTU179 accounts for 10.57% in YSF1, YSF2 and YSF3 on OUT level (Fig. 6(b)), 4.47% in YSF1 and YSF2 (Fig. 6(c)), 9.98% in YSF1 and YSF3 (Fig. 6(d)), 15.65% in YSF2 and YSF3 (Fig. 6(e)). The increasing proportion of OTU179 indicated that, with the gradual deterioration of the reaction environment and the gradual decrease in C/N, the advantages of *Comamonas* sp. represented by core strain YSF15 are further reflected. Similarly, Zhan et al. reached the same conclusions [53].

6-3. Bacterial Community Analysis

The bacterial community composition varied with different C/N ratio in the SMBR. The species abundance of each sample was categorized at the phylum and genus level and the community composition is shown in Fig. 7. The bacterial abundance at the phylum level is shown in Fig. 7(a). The *Proteobacteria* accounted for the highest proportion of 96.74, 76.92 and 85.74% in YSF1, YSF2 and YSF3, respectively. By comparing the relative abundance of *Proteobacteria* in the three samples, it can be concluded that the proportion of *Proteobacteria* was higher at the beginning and late stage of reactor operation. This indicates that *Proteobacteria* has become a dominant specie because of its ability to adapt to harsh environment. Ma et al. also reported that *Proteobacteria* was a dominant phylum for efficient nitrogen removal of biofilms [54]. *Proteobacteria* is an abundant phyla in wastewater reactor and play a critical role in carbon degradation and nitrogen removal in wastewater treatment reactors [8].

Actinobacteria (common probiotic) degrade the complex organ-

Table 1. Alpha diversity index

Sample	S_{obs}	Shannon	Simpson	ace	Chao	coverage
YSF1	61	0.545639	0.8237	70.3031	68.8	0.99959
YSF2	163	3.276111	0.0632	191.0361	182.8	0.99906
YSF3	62	1.975169	0.1924	70.0133	66.1	0.99969

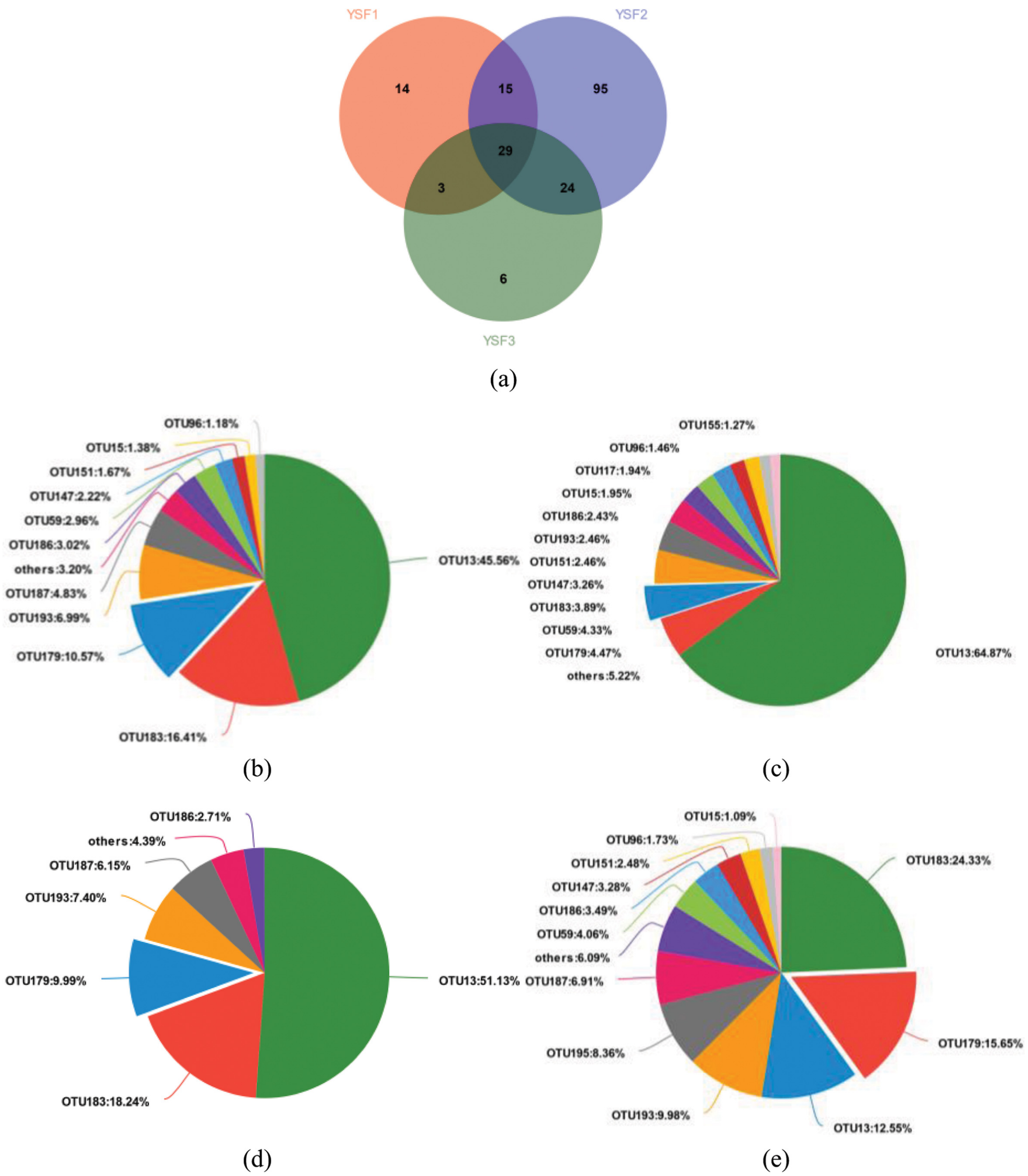

Fig. 6. Venn diagram based on bacterial OTUs at 97% similarity in three samples (a), Venn pie diagrams of YSF1, YSF2 and YSF3 (b), YSF1 and YSF2 (c), YSF1 and YSF3 (d), YSF2 and YSF3 (e) on OUT level.

ics in bioreactors and cellulose and are capable to produce beneficial substances in specific environments [8]. Besides, *Actinobacteria* decompose the organic matter of dead organisms and are found abundant in soil and aquatic sediments [30].

The SMBR abundance at the genus level is shown in Fig. 7(b). In YSF1, *Pseudomonas* accounted for the highest proportion (90.79%), while in YSF2 and YSF3, *Pseudomonas* accounted for a decreasing proportion of 16.97 and 3.71%, respectively. *Comamonas* was only detected as 0.24% in YSF1, while it accounted for an increasing proportion of 7.37% and 19.11% in YSF2 and YSF3, respectively. YSF15 belongs to the *Comamonas* genus. The strong evidence proved that common denitrifying bacteria such as *Pseudomonas* cannot maintain their growth in the SMBR at a reduced C/N, causing a decrease in their growth and development due to unsuitable conditions. Huang et al. and Li et al. also confirmed that the denitrification effect of *Pseudomonas* was lower at reduced C/N ratio [55,56]. However, *Comamomas* can adapt to the oligotrophic environment and increase in abundance at the end of reactor operation [57]. The *Comamomas* contributed significantly to nitrate removal in the water treatment process [53].

Circos is a visualization that describes the correspondence between samples and species. The proportion of *Comamonas* was 0.93, 27 and 72% in YSF1, YSF2 and YSF3, respectively (Fig. 7(c)). *Comamonas* genus showed good adaptability during the opera-

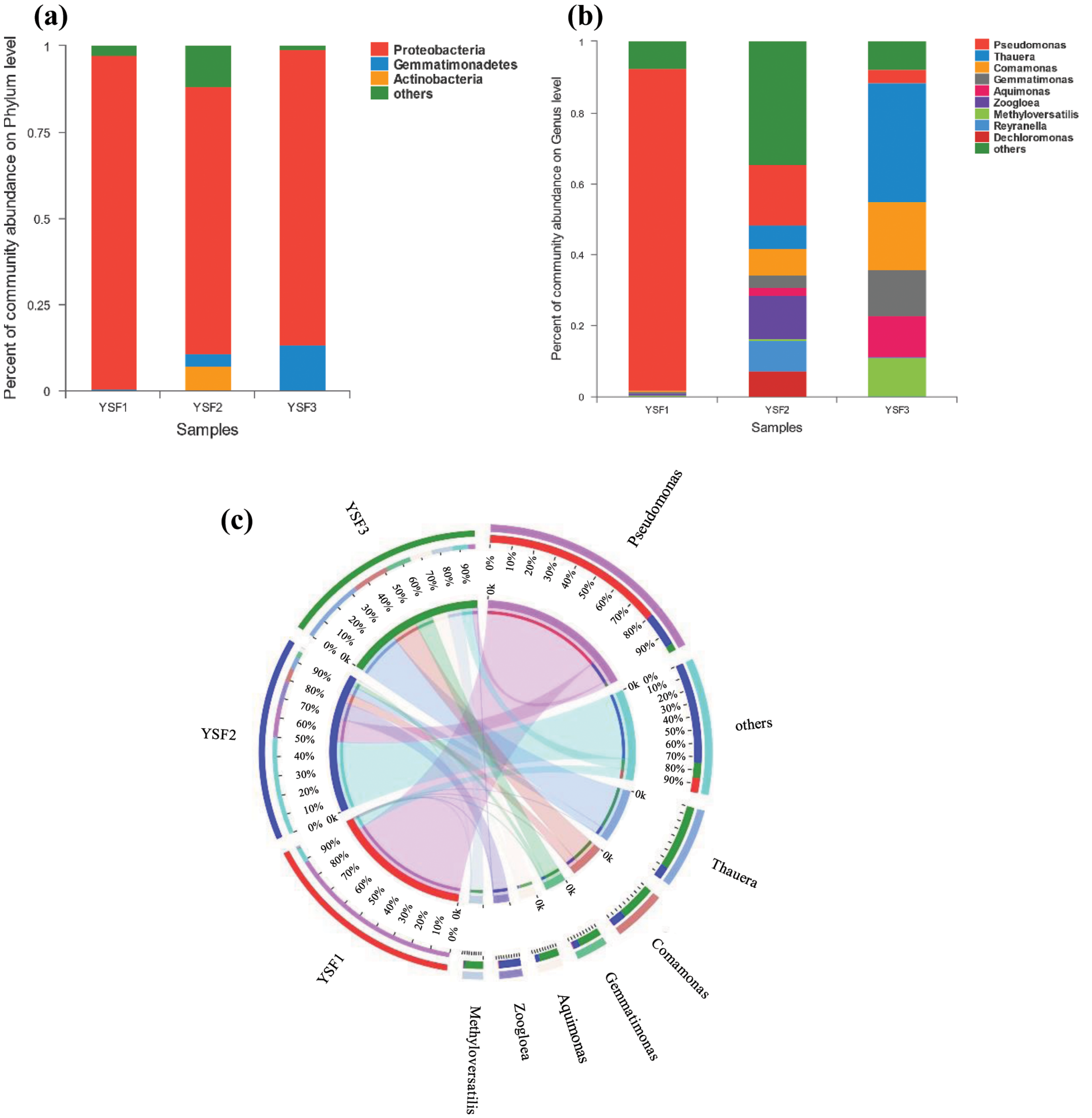

Fig. 7. The community composition at the level of phylum (a) and genus (b) classification. The circos of the relationship between sample and species (c).

tion; the corresponding increase in abundance and content enabled the SMBR to effectively remove nitrate under the low C/N conditions.

CONCLUSION

It was concluded that SMBR exhibited excellent performance for the nitrate removal in the low C/N ratio wastewater. At an initial nitrate concentration of 20.0 mg L^{-1}, HRT of 6 h and C/N=2.5, the average nitrate efficiency in the SMBR reached to 95.22%. It was found that, with the increment of pH, the EPS produced by strain YSF15 continued to increase, which resulted in the formation of the suspended membrane with a certain mechanical strength. Also, the compositon and FTIR observation indicated that SMBR could utilize SMP and EPS produced by itself as the reserve carbon sources to promote denitrification at low C/N ratio. High-throughput sequencing indicated the existence of *Comamonas* genus was the leading contributor for effective nitrate removal in the denitrification. It can adapt to an oligotrophic environment and grealty increase in abundance with the operation of the reactor.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

Suspended membrane bioreactor with extracellular polymeric substances as reserve carbon source for low carbon to nitrogen ratio wastewater: Performance and microbial community composition

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1. Operating Characteristics of the SMBRR in the Reactor

As shown in Fig. S1, the SMBR is suspended in the liquid and is in a floating state. These membranous materials are white, flocculated and have a good sedimentation rate of 10 mL g^{-1}. In addition, these suspended membranes also have a certain mechanical strength. Whenever the reactor is shaken by an external force, they move back and forth in the water and do not break.

2. Operating Conditions of the SMBR Reactor

A 500 ml anaerobic bottle was employed as a reactor to study the denitrification performance of the SMBR. The three C/N ratios (2.0, 2.5 and 3.0), hydraulic retention time (2.0 h, 4.0 h and 6.0 h) and initial concentrations of nitrate (15.0, 20.0 and 25.0 mg·L^{-1}) were set for the experiment. The specific operating parameters are presented in Table S1.

Fig. S1. Biofilm aggregated by strain YSF15.

Table S1. Operating conditions

Phase	C/N	HRT (h)	NO_3^--N (mg L^{-1})
1	3.0	6.0	15.0
2	3.0	4.0	15.0
3	3.0	2.0	15.0
4	3.0	6.0	20.0
5	3.0	4.0	20.0
6	3.0	2.0	20.0
7	3.0	6.0	25.0
8	3.0	4.0	25.0
9	3.0	2.0	25.0
10	2.5	6.0	15.0
11	2.5	4.0	15.0
12	2.5	2.0	15.0
13	2.5	6.0	20.0
14	2.5	4.0	20.0
15	2.5	2.0	20.0
16	2.5	6.0	25.0
17	2.5	4.0	25.0
18	2.5	2.0	25.0
19	2.0	6.0	15.0
20	2.0	4.0	15.0
21	2.0	2.0	15.0
22	2.0	6.0	20.0
23	2.0	4.0	20.0
24	2.0	2.0	20.0
25	2.0	6.0	25.0
26	2.0	4.0	25.0
27	2.0	2.0	25.0