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Treatment of domestic wastewater in step-feeding anoxic/oxic activated sludge–biofilm system at low temperature: Performance, removal characteristics, and community

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ABSTRACT

Domestic wastewater was treated with activated sludge system and activated sludge-biofilm system at low temperature (10°C), respectively. In both systems, step-feeding strategy were adopted while the aerobic and anoxic zones were designed. The performances of two different systems were compared. The microbial community in the activated sludge-biofilm system was studied deeply. As a result, the activated sludge-biofilm system achieved higher performance than the activated sludge system. In the activated sludge-biofilm system, the chemical oxygen demand (COD), NH_4^+-N and total nitrogen (TN) removal efficiencies reached 88%, 97% and 65%, respectively. However, in the activated sludge system, the COD, NH_4^+-N and TN removal efficiencies were 86%, 40% and 55%, respectively. Simultaneous nitrification and denitrification (SND) occurred in aerobic zones in activated sludge-biofilm hybrid system. The effluent TN concentration was 8.5 mg/L in average, which was mainly contributed by NO_3^--N. The main microbial communities consisted of *Proteobacteria, Bacteroidetes, Nitrospirae, Firmicutes,* and *Planctomycetes* were at the phylum level, indicating the occurrence of nitrification, denitrification, and anammox.

Keywords: Step-feeding; Activated sludge-biofilm system; Nitrogen removal; Microbial community

1. Introduction

With the development of economy, a large amount of wastewater with high nitrogen concentration was discharged into the receiving waters. Eutrophication occurs when the nitrogen accumulates in water and exceeds the purification capacity of water bodies. Water eutrophication is the main problem of water pollution at present, which seriously affects people's daily production and life [1]. Biological nitrogen removal (BNR) was widely used to remove the nitrogen in both domestic and industrial wastewater treatment. In China, the maximum nutrient concentrations (particularly nitrogen and phosphorus) in the effluent are strictly controlled according to the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant (WWTP) (GB18918-2002). However, biological nitrogen removal technology has some deficiencies, and the most important problem is the relatively low nitrogen removal efficiency. Mainly because it is hard to maintain a good cooperation state of heterotrophic bacteria, nitrification and denitrification bacteria [2–4].

To date, the anaerobic/anoxic/oxic (A/A/O) and pre-denitrification (A/O) methods are widely used in WWTP [5]. Among them, step-feeding process was able to improve the nitrogen removal efficiency by allocate the flow rate of influent, which favored the utilization of organic carbon in

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denitrification process [6]. Under the condition of adequate carbon sources, these processes display relatively suitable nutrient removal efficiencies [7,8]; Due to its promising potential of nitrogen removal, the anoxic/oxic step-feeding activated sludge process has been widely studied and applied [9]. Previous studies were focused on the parameters optimization, such as influent flow rate distribution ratio, sludge recirculation rate and aeration rate. With appropriate control parameters and sufficient organic carbon concentration, high total nitrogen(TN) removal efficiency (over 95%) could be achieved. However, when the influent organic carbon was not sufficient, the nitrogen removal efficiency might be limited [10–12], especially for the wastewater at low temperature. Temperature is an important factor in biological wastewater treatment, which affects the microbial growth and activity significantly [13]. In northern of China, the average wastewater temperature in winter is approximately 10°C. It has been shown that the microbial metabolic rate approximately doubled with every 10°C increase [14]. For the activated sludge process, the ammonia removal efficiency reached 90% at temperature of 15°C, but it dropped to 20% at 10°C and ceased at 5°C [15,16]. At low temperatures, the activated sludge still had certain degrees of capability for pollutant degradation attributable to the existence of cold-adaptive microorganisms. Although psychrophilic bacteria proliferate well and are dominant in activated sludge at low temperatures, the efficiencies on organic removal and denitrification were low [17,18].

Two effective methods could be used to improve efficiency: a change in kinetics can be addressed by increased volume or intensification (e.g., by increasing sludge age) and changes in relative feasibility of reactions or microbial community (e.g., by introducing a biological membrane system). Activated sludge system can achieve high removal performance due to better mass transfer capacity, and can be flexibly operated and controlled. For the biofilm system, it enables a larger biodiversity of the microorganisms owing to its longer SRT, and had shown great potential for partial nitritation as a result of its sufficient oxygen usage and well-stratified distributions of AOB and NOB within the biofilm [19].

Based on the discussion above, the main purpose of this study is to investigate the performance of the activated sludge-biofilm system at 10°C. Besides, the performance of the step-feeding anoxic/oxic activated sludge process and the activated sludge-biofilm system will be compared. Furthermore, the bacteria obtained from the activated sludge–biofilm hybrid system were analyzed with high throughput sequencing technology. It is expected that the knowledge obtained in this study will be critical for the application of step-feeding anoxic/oxic process to the treatment of domestic wastewater in low temperature.

2. Materials and methods

2.1. Description of the step-feeding anoxic/oxic system

Two types of step-feeding anoxic/oxic processes were studied in this study. Fig. 1 shows the configuration scheme of two reactors: A/O step-feeding activated sludge

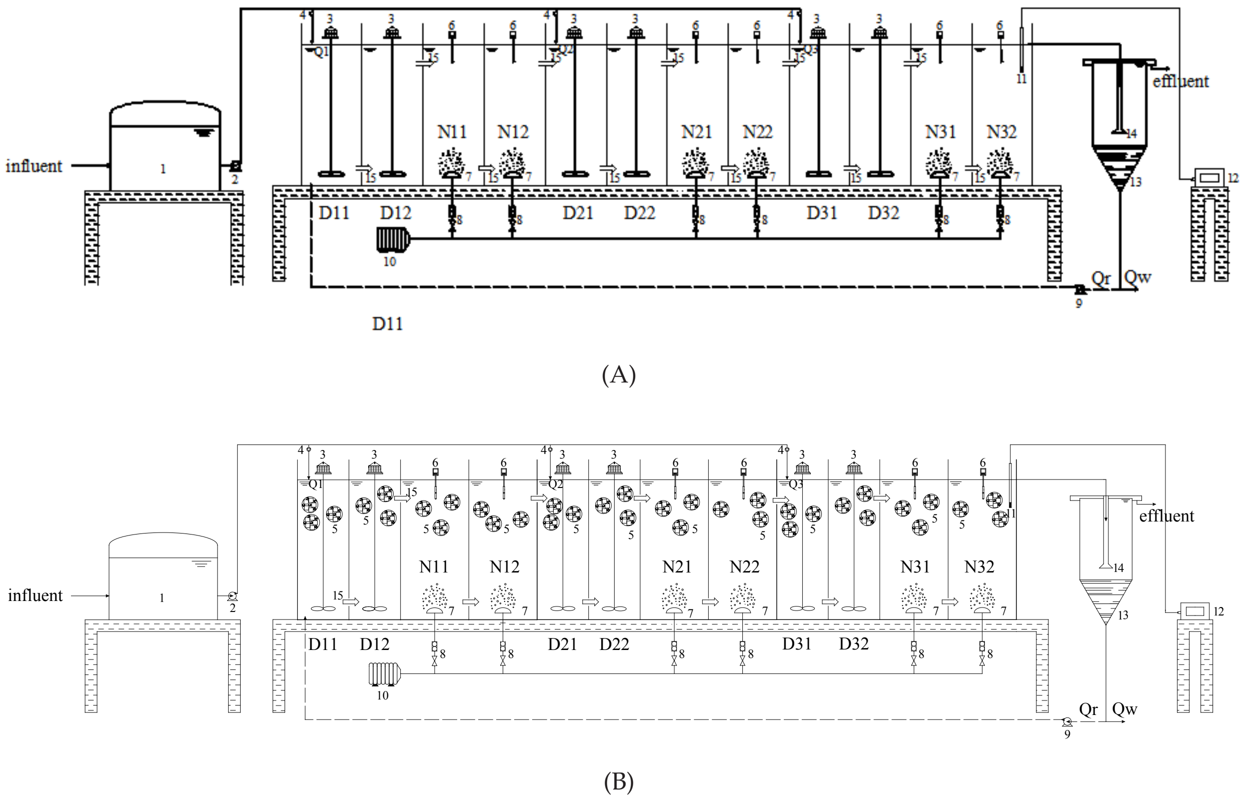

Fig. 1. Schematic diagram of step-feeding anoxic/oxic activated (A) and activated sludge-biofilm process (B).

process named R1 (Fig. 1A) and A/O step-feeding activated sludge-biofilm process named R2 (Fig. 1B). These two reactors were made by acrylic glass material with the effective working volume of 126L, which contained three alternating anoxic/oxic zones. Each anoxic zone or oxic zone was divided into 2 identical compartments. The A/O volume ratios of R1 and R2 were 1:1 and 3:4 (contrast the volume and residence time of aerobic zone), respectively. Six mechanical agitators (JJ-1A 160W, Zhengrong, China) were placed in the anoxic zone to increase mass transfer efficiency. Air was pumped into oxic zone with an air diffuser. The aeration rate was controlled with the flow meters (LZB-DK600-4F, Chengfeng, China). The rotation speed of mechanical agitators and the aeration rate were described in Table 2. In R1, no microbial carriers were added, while sponge and Kaldnes rings were added in R2 as microbial carriers in each compartment. The sponges (square, 3 cm in side length) and Kaldnes rings (cylinder, 2 cm in diameter and 1 cm in height) were filled in the flow-separated ball (8 cm in diameter). The volume of the flow-separated ball was 30% of the reactor volume. A vertical sedimentation tank was placed before every reactor. The effect working volume of the vertical sedimentation tank was 54 L with the height of 116 cm and inner diameter of 30 cm.

2.2. Activated sludge

Activated sludge was collected from the anoxic-oxic tank (Nanbu Wastewater Plant, Changchun) in December. The aerobic and anoxic stages were inoculated into aerobic sludge and anoxic sludge, respectively. Bacteria were cultivated over the course of a week by gradually increasing nitrogen and organic load.

Table 1
Characteristics of the synthetic wastewater

Parameters	Range	Average
COD (mg/L)	204.2–237.8	221.5
TN (mg/L)	18.36–37.72	28.04
NH_4^+-N (mg/L)	14.34–23.38	20.36
NO_3^--N (mg/L)	3.034–8.059	5.54
NO_2^--N (mg/L)	0.015–0.036	0.026
TP (mg/L)	6.89–7.86	7.47
PH	6.84–7.56	7.21

Table 2
Controls parameter of the step-feeding anoxic/oxic hybrid system

Controls parameter	First stage	Second stage	Three stage
Flow rate (ml/min)	100	80	80
Aeration rate (L/min)	2.0	1.6	1.6
Stirring intensity (rad/min)	300	250	200
Sludge recycle flow rate (%)	50		

2.3.Experiment water quality

In this study, synthetic wastewater was used to mimic the characteristics of domestic wastewater in China. Synthetic wastewater was prepared from tap water enriched with beef starch, extract, peptone as the main carbon and nitrogen source, NH_4Cl as a lesser nitrogen source, and K_2HPO_4 as a phosphorus source. Its characteristics are listed in Table 1.

2.4. Operation of the reactors

Two sets of reactors operated in parallel under the condition of the same control. The experiment was operated in the constant temperature room and control the water temperature of 10±0.5°C (two refrigerating machines automatic controlled the air temperature between 9.5°C and 11°C). The synthetic wastewater was pumped into the anoxic zones of the reactor with peristaltic pumps (BT300-2J, Longer Pump, China). The flow distribution ratio of the three anoxic zones was 5:4:4. The operation of R1 and R2 consisted of two main phases. In the first phase, seed sludge was added to the reactors with the sludge concentration of 5000 mg/L. The hydraulic retention time of reactors was set at 24 h, 18 h, 12 h and 8 h respectively. Each hydraulic retention time was operated with three days. The excess sludge was regularly recycled from the vertical sedimentation tank to the first anoxic zone. The recycle ratio was set at 50%. In the second phase, the hydraulic retention time was set at 8 h. The recycle ratio was set at 50%, and 2 L excess sludge was discharged from settling basin every day. Wastewater from the anoxic zone and oxic zone was analyzed to evaluate the nitrogen and COD removal performance.

2.5. Methods

2.5.1. Analysis methods

Influent and effluent samples were taken every 48 h for analysis; pH was first measured with a pH meter (PHS-3C, LeiCi, China). The water samples were filtered through a speed filter paper before conducting other measurements. The levels of COD, NH_4^+-N, TN, and TP were determined using equipment (Lian-Hua, China), include COD rapidly measurement instrument (5B-3C(V8)), NH_4^+-N rapidly measurement instrument (5B-6D(V8)), TN rapidly measurement instrument (LH-3BN), and TP rapidly measurement instrument (5B-6P(V7)). The levels of NO_3^--N and NO_2^--N were determined using ion chromatography (881 Compact IC pro, Metrohm, Switzerland; 863 Compact Autosampler, Switzerland). Lastly, the analytical measurements were conducted according to Standard Methods for the Examination of Water and Waste Water (2005).

2.5.2. DNA extraction and PCR amplification method

Biomass samples from the biofilm in aerobic and anoxic zones were collected and immediately stored at –50°C for subsequent experiments. According to the manufacturer's instructions, E.Z.N.A™ Mag-Bind Soil DNA Kit (OMEGA) was used for total genomic DNA extraction. The integrity of DNA was analyzed through the agarose gel method (gel imaging system from UPV, America).

Qubit3.0 DNA testing kits (Q10212, Life) were used in the first round of PCR amplification to determine if the PCR reaction should join the DNA. Universal primers 341F:CCCTACACGACGCTCTTCCGATCTG (barcode) CCTACGGGNGGCWGCAG and 805R (GACTGGAGTTCCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC) were utilized to amplify V3-V4 (Miseq sequencing platform). PCR primers compatible (Illumina) were introduced for the second round of PCR amplification.

3. Results and discussion

3.1. Performance of pollutant removal in activated sludge system

The pollutant removal in the step-feeding anoxic/oxic activated sludge system is shown in (Fig. 2). By inoculating activated sludge, microbiological culture, microbial growth, and propagation, finally enters the stable operation stage. The removal rate of COD, NH_4^+-N, and TN were 86%, 40%, and 55%, respectively. According to the results of the experiment, higher removal efficiency for organic matter has been obtained, but denitrification efficiency received larger inhibition in the activated sludge system, especially during the nitrification process. Studies have shown that the growth rate of nitrifiers strongly depends on temperature [20]. In our study, under the condition of 10°C, the growth of nitrifying bacteria reproduces slowly and has a lower biological activity. Affected by nitrification, denitrification is insufficient, leading to a low TN removal efficiency. Accordingly, the focus of this study is how to improve the nitrification rate.

3.2. Performance of pollutant removal in hybrid system

The pollutant removal in the step-feeding anoxic/oxic hybrid system is shown in (Fig. 3). As can be observed, the reactor passed through three stages. The first was the culture stage. Through eighteen days of cultivation after the activated sludge was inoculated, the activity of microbial was restored; the concentration of sludge had changed greatly, where the removal rate of COD, NH_4^+-N, and TN were 85%, 60%, and 60%, respectively. According to the value of processing efficiency, it can be determined whether the reactor was successfully inoculated.

The second stage was the adaptive phase. During this period, the microorganisms gradually adapted to the changes of the environment. The number of microorganisms increased until stable. The changing trend regarding the removal rate of COD, NH_4^+-N, and TN displayed in ups and downs; after thirty-eight days operation,the removal rate of COD, NH_4^+-N, and TN was 80%, 95% and 65%, respectively.

The last stage was the stabilization stage. After a period of time, the activity and amount of microorganisms were retained at the same state. In the process of twenty-four days of testing, the mean removal rate of COD, NH_4^+-N, and TN was 88%, 97%, and 65%, respectively; the processing efficiency of contaminant was stable.

From Fig. 3 we can arrive to a conclusion. Under the condition of water temperature at 10°C, higher pollutant

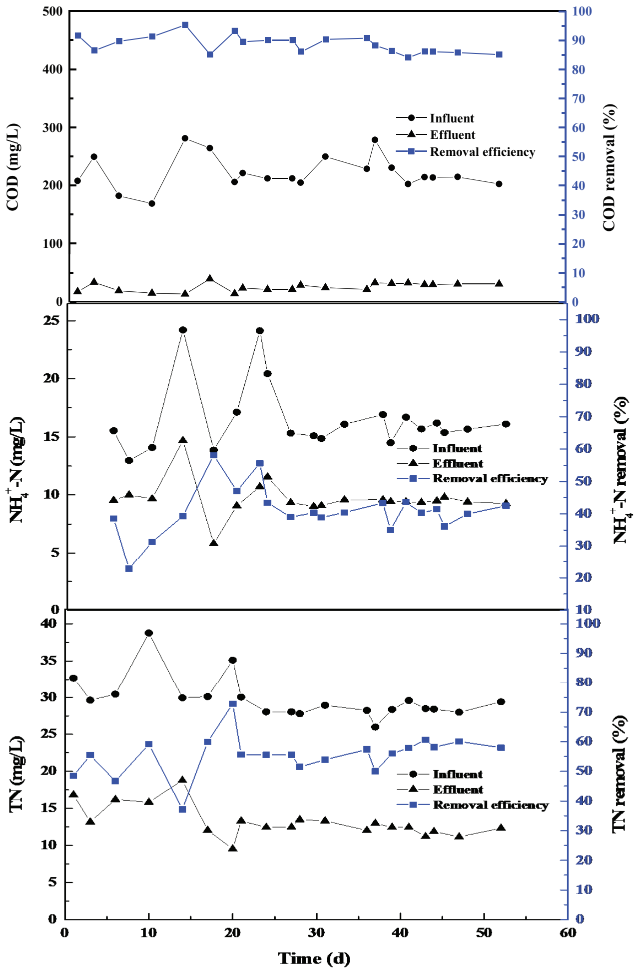

Fig. 2. Performance of a step-feeding anoxic/oxic activated sludge system in terms of COD, NH_4^+-N and TN.

removal efficiency could be obtained for municipal sewage by step-feeding anoxic/oxic hybrid system. The average effluent COD, NH_4^+-N, and TN concentration of step-feeding anoxic/oxic hybrid system at 10ºC could meet the level A of criteria I of Chinese National Discharge Standard of Pollutants for Municipal Wastewater [21] in the last stage.

It is well known that biological reaction rates decreased with decreasing temperature within the physiological range [22]. There was a small influence of COD removal rate under low temperature; the average removal efficiency of COD reached 90.5%, 89.1%, and 90.3% under the temperature of 25°C, 15°C, and 10°C, respectively [23]. The growth rate of nitrifies strongly depends on temperature [20]. So it could be observed that by adding suspended carriers into the reactor and construction of activated sludge – biofilm hybrid system, ammonia removal efficiency was improved, and NH_4^+-N effluent concentrations were lower than 1 mg/L at low temperature, where a high removal rate of ammonia nitrogen was visible. This result could be attributed to the biofilm growth inside the system; the suspended carriers provide sufficient attachment surfaces for microbial bacteria growth and greatly increase the total amount of biomass. This is especially true for the slow growing nitrifying bacte-

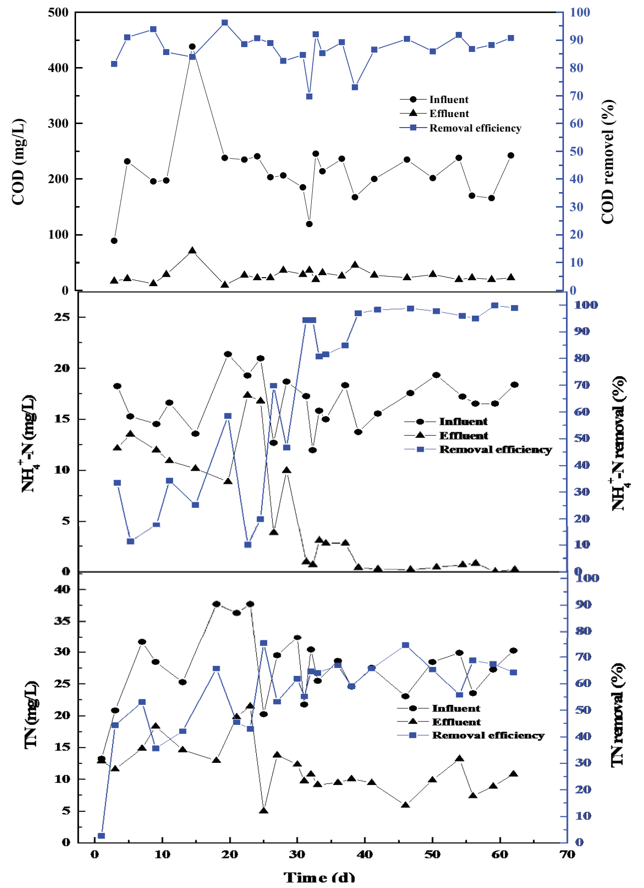

Fig. 3. Performance of a step-feeding anoxic/oxic hybrid system in terms of COD, NH_4^+-N and TN.

ria, which are essential for nitrogen removal in wastewater treatment and, therefore, a good nitrification effect could be obtained under unfavorable conditions.

In the traditional hybrid A/O system, the efficient denitrification was distinctly attributed to the availability of the organic matters in the influent. However, for the step-feeding anoxic/oxic hybrid system, the raw water was put by the anoxic zone to enter. Organic matter was first placed into the anoxic zone to ensure that the denitrification reaction had a sufficient carbon source. Researchers reported that COD/TN ratios (5.3–11) were required for complete denitrification and high TN removal [24,25]. During the entire period, the COD/TN ratio was 7.3 in the influent; using the method of step-feed technology could solve the problem of insufficient carbon source. In addition, it is widely accepted that the hybrid system could enhance microbial concentration [26]. Hence, high organic matter and total nitrogen removal efficiency would be obtained by using a step-feeding anoxic/oxic hybrid system in low temperature and low carbon source level.

3.2.1. Pollutants removal performance in different zone

The pH and DO in the twelve zones are exhibited in Fig. 4. The pH around at 7.0 in the twelve zones. The DO

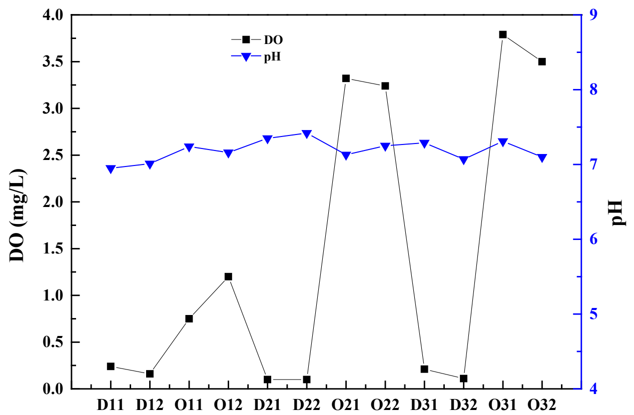

Fig. 4. Variations of DO and pH in each zone under stable state.

concentration in the anoxic zone was controlled between 0.1–0.24 mg/L, which was a typical anoxic environment. The air distribution rule was 5:4:4, which is the same as the water allocation proportion. The DO concentration in aerobic zone was 0.75 mg/L, 1.2 mg/L, 3.32 mg/L, 3.24 mg/L, 3.79 mg/L, and 3.5 mg/L, respectively. From the results it can be determined that the step-feeding anoxic/oxic hybrid system has suitable aerobic and anoxic environments. The variations of total biofilm and sludge concentration in each zone is show in Fig. 5. The mean sludge concentration were 6500 mg/L, 4500 mg/L and 3500 mg/L in first, second and third stage, respectively. The ratio of biofilm amount and suspended sludge is 1/10.

Water samples from twelve zones along the reactor were detected to investigate the growth of biofilm and removal mechanism of pollutant in difference zone. The analysis includes inlet and outlet SCOD (dissolved organic matter), SNH_4^+-N, SNO_3^--N, SNO_2^--N, STN, sludge concentration, biofilm biomass, and dissolved oxygen at aerobic zone. The results are shown in Fig. 6 and Fig. 7.

Concentrations show a trend of gradual decline in each level of the reactor (Fig. 6), the effluent SCOD concentrations in the twelve zones are approximately 30 mg/L. SCOD removal rates were 88.18% in the step-feeding anoxic/oxic hybrid system. The influent SCOD was removed quickly in the anoxic zones, showing that influent organics were successfully involved in the denitrification process, and the change trend was the same as TN degradation. In regards to larger anoxic volumes for SCOD uptake by denitrification bacteria, previous studies reported that less residual SCOD into oxic zones was conductive to nitrification since it greatly weakened the competition between heterotrophic bacteria and nitrifying bacteria for oxygen [8,27]. This conclusion was successfully confirmed in our study.

Fig. 7. shows that the step-feed A/O nitrogen removal process in each tank; ammonia and nitrate nitrogen in the raw wastewater was nearly 62.0% and 23.5%, respectively, of the total amount of the nitrogen. However, nitrate nitrogen in the effluent was more than 95% of the total nitrogen.

NH_4^+-N concentration in last oxic tank was less than 1 mg/L, so in 10°C the last oxic can satisfy complete nitrification. Attributable to the wastewater step feed to each anoxic tank, the NH_4^+-N concentration of the last two anoxic tanks were increased, though the ammonia nitrogen removal

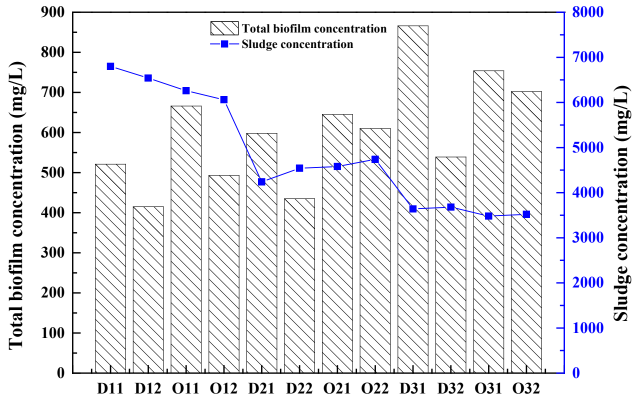

Fig. 5. Variations of total biofilm and sludge concentration in each zone under stable state.

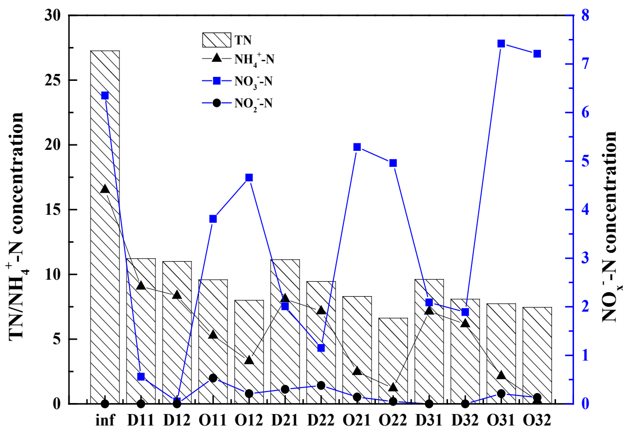

Fig. 7. Variations of ammonia, nitrite, nitrate and TN in each zone under stable state.

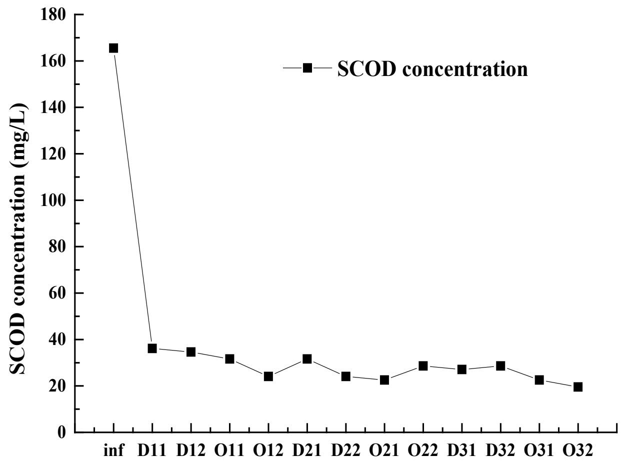

Fig. 6. Variations of SCOD concentration in each zone under stable state.

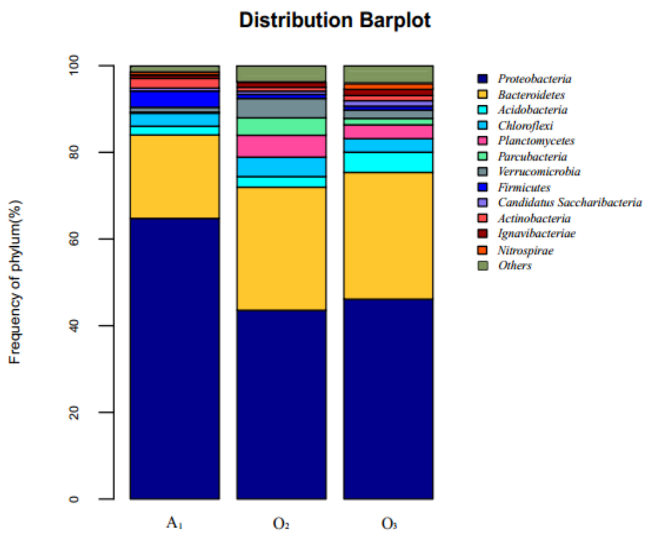

Fig. 8. Bacterial community composition at the Phylum level.

showed a trend of decline at each level and the typical nitrification rate in each oxic tank increased. In our study, there were some different phenomena from the previous research; decreasing concentration of ammonia nitrogen was found in each anoxic tank, the possible reason was the existence of anoxic environment within the suspension condiment.

Ammonia was converted to NO_2^--N and NO_3^--N in the oxic zones. In our study, NO_2^--N concentration in each tank was primarily less than 0.5 mg/L, no accumulation of nitrite was found in our study. Because of the aerobic nitrification, the nitrate nitrogen concentration in each oxic zones were higher than that in the anoxic zones at the same stage. Moreover, in the first stage anoxic zone was close to zero while approaching complete nitrification. The result was the same with previous research [25,28]. Fig. 7 shows little difference in last two stages of NO_3^--N concentration in oxic zones; NO_3^--N concentration decline was also found in anoxic zones. This was mainly attributed to a small amount of denitrifying bacteria attachment in biofilms (Fig. 8).

The variation regularity of TN was the same as NO_3^--N. TN removal in the anoxic zones attribute to denitrification and anoxic ammonia oxidation process. In oxic zones, the TN concentration was reduced because of consumption of microbial cell synthesis and occurrence of SND [29,30]. At the final stage, TN was mainly composed of NO_3^--N, where denitrification was incomplete.

3.2.2. The bacterial community in hybrid system

At the temperature of (10±0.5)°C, high nitrogen removal efficiency was achieved by the step-feeding anoxic/oxic hybrid system. In order to explore the nitrogen removal mechanism, high-throughput sequencing was employed to investigate microbial community changes in operation temperature. The content includes diversity and structure of the microbial community in this study. We selected four biomass samples including the biofilm on the sponge packing in the anoxic zone (A1). This study did not consider the biofilm on the polythene packing for the fact that there was less biomass on the polythene packing; the polythene packing in the anoxic zone only had the effect of support frame.

Table 3
The richness and diversity of 16S rRNA sequences in anaerobic, aerobic biofilm and the mixture of sludge

Sample ID	Seq num	OUT num	Shannon index	ACE index	Chao1 index	Coverage	Simpson
1	43033	1682	5.53	2247.27	2170.49	0.99	0.01
2	47178	2016	5.80	2581.7	2532.79	0.99	0.01
3	48967	2060	5.79	2638.81	2613.59	0.99	0.01
4	46569	1528	5.34	2551.97	2251.61	0.99	0.01

The biofilm on the sponge (O2), polythene (O3) packing in the aerobic zones and the mixture of sludge (S4) from reactor. These samples represented two different oxygen environments. The diversity of the microbial community for each sample is shown in Table 3.

The coverage values of four samples were over 99%, indicating that the sequencing was more than enough to cover the entire microbial community. The total numbers of OTUs estimated by Chao 1 estimator were 1682 (A1), 2016 (O2), 2062 (O3), and 1528 (S4). The results showed that suspended packing in the anoxic zone (A1) and aerobic zones (O2), (O3) exhibited greater microbial richness than the activated sludge system. The Shannon index of suspended packing A1 (5.53), O2 (5.80) and O3 (5.79) were larger than that of S4 (5.34), the Shannon index demonstrations represent the species and evenness of the species [31,32]. Accordingly, it can be inferred that the enriched OTUs of suspended packing in the A1, O2, and O3 communities were more evenly distributed than in activated sludge system S4. The richness and the diversity of the biofilm sample was higher than the sludge sample at 10ºC, this result was consistent with a previous study [23].

To obtain information about the microbial community on the suspended packing, the phylogenetic classification of bacterial sequences from suspended packing (three samples of A1, O2, and O3) at phylum levels is summarized in Fig. 8.

At the phylum level in Fig. 8, 12 identified phyla were observed at ≥1 abundance in at least one sample. *Proteobacteria* was the most abundant (64.75%, 43.56%. and 46.12%) of all the A1, O2, and O3, followed by *bacteroidetes* (19.26% A1, 28.40% O2, and 29.25% O3). A high abundance of *Proteobacteria* and *Bacteroidetes* in suspended packing was consistent with previous studies that investigated microbial diversity in a nitrogen removal system, such as a mixotrophic nitrogen removal system [33]. As such, two kinds of suspended packing were used where the main bioprocess was nitrification. *Nitrospirae* was detected at 0.44% in anoxic suspended packing (A1), which was less than that 0.31% and 1.24% in aerobic suspended packing (O2, O3). *Planctomycetes* was another dominant phylum (5.04%, 3.19%) in O2 and O3. However, it was 0.15% in A1. Many species of *Planctomycetes* possess anoxic ammonium oxidizing (anammox) activity, in which nitrite and ammonium could be converted to nitrogen gas [34]. The possible reason was the existence of anoxic environment within the suspension condiment.

4. Conclusions

The step-feeding anoxic/oxic activated sludge-biofilm system showed excellent removal capacity compared with the pure activated sludge system at 10°C, where the average removal rate of COD, NH_4^+-N, and TN were 88.58±3.20%, 97.66±2.70%, and 65.94±2.79%, respectively. The volume optimization of aerobic zone, anoxic zone, and adding floating ball improved the share of nitrifying bacteria in total bacteria, reduced the loss of the microbes, and improved microbial population diversity. Hybrid system would be effectively suitable for dealing with the domestic wastewater at low temperatures. The main microbial phyla including *Proteobacteria, Bacteroidetes, Nitrospirae, Firmicutes*, and *Planctomycetes* were detected in the systems, indicating the occurrence of nitrification, denitrification, anammox, and COD removal simultaneously existed in a single reactor. The use of a step-feeding anoxic/oxic activated sludge-biofilm system for domestic wastewater treatment at low temperatures appears to be an advantageous solution.

Reference

[1] R.P. Schwarzenbach, T. Egli, T.B. Hofstetter, U. von Gunter, B. Wehrli, Global water pollution and human health, Annu. Rev. Environ. Res., 35 (2010) 109–136.
[2] J.A. Baeza, D. Gabriel, J. Lafuente, Improving the nitrogen removal efficiency of an A2/O based WWTP by using an on-line knowledge based expert system, Water Res., 36 (2002) 2109–2123.
[3] E. Viopoulou, P. Melidis, A. Aivasidis, An activated sludge treatment plant for integrated removal of carbon, nitrogen and phosphorus, Desalination, 211 (2007) 192–199.
[4] E. Viopoulou, A. Aivasidis, A modified UCT method for biological nutrients removal: Configuration and performance, Chemosphere, 72 (2008) 1062–1068.
[5] A.R.M. Nasab, S.M. Soleymani, M. Nosrati, S.M. Mousavi, Performance evaluation of a modified step-feed anaerobic/anoxic/oxic process for organic and nutrient removal, Chinese J. Chem. Eng., (English ed.), 24 (2016) 394–403.
[6] T.Y. Pai, Y.P. Tsai, Y.J. Chou, H.G. Leu, C.F. Ouyang, Microbial kinetic analyses of three different types of EBNR process, Che-mosphere, 55 (2004) 109–118.
[7] K.A. Third, N. Burnett, R. Cord-Ruwisch, Simultaneous nitrification and denitrification using stored substrate (PHB) as the electron donor in an SBR, Biotechnol. Bioeng., 83 (2003) 706–720.
[8] D. Kim, K.Y. Kim, H.D. Ryu, K.K. Min, S.I. Lee, Long term operation of pilot-scale biological nutrients removal process in treating municipal wastewater, Bioresour. Technol., 100 (2009) 3180–3184.
[9] Metcalf & Eddy "Wastewater engineering: treatment and reuse." McGraw-Hill Series in Water Resources and Environmental Engineering, 73 (2003) 50–51.
[10] T. Mino, M.C.M. Van Loosdrecht, J.J. Heijnen, Microbiology and biochemistry of the enhanced biological phosphate removal process, Water Res., 32 (1998) 3193–3207.
[11] J.H. Gau, Y.Z. Peng, S.Y. Wang, Y.N. Zheng, H.J. Huang, Z.W. Wang, Long-term effect of dissolved oxygen on partial nitri-fication performance and microbial community structure, Bioresour. Technol., 100 (2009) 2796–2802.

[12] T. Shoji, H. Satoh, T. Mino, Quantitative estimation of the role of denitrifying accumulating organisms in nutrient removal, Water Sci. Technol., 47 (2003) 23–29.
[13] J. Pietikäinen, M. Pettersson, E. Bååth, Comparison of temperature effects on soil respiration and bacterial and fungal growth rates, Fems Microbiol. Ecol., 52 (2005) 49–58.
[14] K.V. Rajeshwari, M. Balakrishnan, A. Kansal, K. Lata, V.V.N. Kishore, Stateof-the-art of anoxic digestion technology for industrial wastewater treatment, Renew. Sustain. Energy Rev., 4 (2000) 135–156.
[15] N. Sundaresan, L. Philip, Performance evaluation of various aerobic biological systems for the treatment of domestic wastewater at low temperatures, Water Sci. Technol., 58 (2008) 819–830.
[16] L. Sun, L. Wang, X. Yuan, Low temperature influence on package membrane bioreactor (MBR) operation in remote mountain areas, International Conference on Bioinformatics and Biomedical Engineering IEEE., (2009) 1–4.
[17] M. Henze, Wastewater treatment: biological and chemical processes, Progr. Colloid Polym. Sci., 49 (1999) 747–752.
[18] M.K. De Kreuk, M. Pronk, M.C. van Loosdrecht, Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures, Water Res., 39 (2005) 4476–4484.
[19] X. Zhang, Y. Liang, Y. Ma, J. Du, L. Pang, H. Zhang, Ammonia removal and microbial characteristics of partial nitrification in biofilm and activated sludge treating low strength sewage at low temperature, Ecol. Eng., 93 (2016) 104–111.
[20] W. Gujer, M. Henze, T. Mino, M. van Loosdrecht, Activated sludge model no. 3, Water Sci. Technol., 39 (1999) 183–193.
[21] C. GB, Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant. China Environmental Science Press, Beijing, China. 2002.
[22] S. Qiao, N. Matsumoto, T. Shinohara, T. Nishiyama, T. Fujii, Z. Bhatti, K. Furukawa, High-rate partial nitrification performance of high ammonium containing wastewater under low temperatures, Bioresour. Technol., 101 (2010) 111.
[23] H. Zhou, X. Li, Z. Chu, J. Zhang, Effect of temperature downshifts on a bench-scale hybrid A/O system: Process performance and microbial community dynamics, Chemosphere, 153 (2016) 500–507.
[24] Y. Wang, Y. Peng, T. Stephenson, Effect of influent nutrient ratios and hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen removal in a two-sludge sequencing batch reactor process, Bioresour. Technol., 100 (2009) 3506–3512.
[25] Y. Chen, B. Li, L. Ye, Y. Peng, The combined effects of COD/N ratio and nitrate recycling ratio on nitrogen and phosphorus removal in anaerobic/anoxic/aerobic (A2/O)-biological aerated filter (BAF) systems, Biochem. Eng. J., 93 (2015) 235–242.
[26] W. Jianlong, S. Hanchang, Q. Yi, Wastewater treatment in a hybrid biological reactor (HBR): effect of organic loading rates, Process Biochem., 36 (2000) 297–303.
[27] D. Kim, T.S. Kim, H.D. Ryu, S.I. Lee, Treatment of low carbon-to-nitrogen wastewater using two-stage sequencing batch reactor with independent nitrification, Process Biochem., 43 (2008) 406–413.
[28] G. Han, X. Sui, Water quality and control parameters analysis on step-feed anoxic/oxic activated sludge process, International Symposium on Water Resource and Environmental Protection, IEEE, 2011, pp. 1537–1540.
[29] K. Hanaki, C. Wantawin, S. Ohgaki, Nitrification at low level of DO with and without organic loading in a suspended growth reactor, Water Res., 24 (1990) 297–302.
[30] S. Wyffels, K. Pynaert, P. Boeckx, W. Verstraete, O. Van Cleemput, Identification and quantification of nitrogen removal in a rotating biological contactor by 15 N tracer techniques, Water Res., 37 (2003) 1252–1259.
[31] L. Lu, D. Xing, N. Ren, Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis zones involved in enhanced H2 production from waste activated sludge, Water Res., 46 (2012) 2425–2434.
[32] J. Luo, H. Liang, L. Yan, J. Ma, Y. Yang, G. Li, Microbial community structures in a closed raw water distribution system biofilm as revealed by 454- pyrosequencing analysis and the effect of microbial biofilm communities on raw water quality, Bioresour. Technol., 148 (2013) 189–195.
[33] S. Park, J. Yu, I. Byun, S. Cho, T. Park, T. Lee, Microbial community structure and dynamics in a mixotrophic nitrogen removal process using recycled spent caustic under different loading conditions, Bioresour. Technol., 102 (2011) 7265–7271.
[34] S. Kalyuzhnyi, M. Gladchenko, A. Mulder, B. Versprille, DEAMOX--new biological nitrogen removal process based on anoxic ammonia oxidation coupled to sulphide-driven conversion of nitrate into nitrite, Water Res., 40 (2006) 36–37.