



Treatment of domestic sewage by biological contact oxidation of different packings under hypoxia

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ABSTRACT

Treating low C/N domestic sewage is one of the difficult tasks in wastewater treatment. This study investigated the treatment of low C/N domestic sewage under low dissolved oxygen condition via self-built biological contact oxidation reactor with composite packing and polyurethane suspension packing. Meanwhile, MiSeq high-throughput sequencing was performed on the microbial communities on the surface of the packing to analyze the changes in microbial community structure. The results showed that polyurethane suspension packing reactor had better removal efficacy on $\text{NH}_4\text{-N}$, TN and COD than the combined packing reactor, and only the effluent indexes of polyurethane suspension packing reactor could reach the first-class A emission standard of Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002). The removal of biological phosphorus of the single-contact oxidation process was not ideal and could be combined with anaerobic reactor. The richness and diversity of the bacterial community in polyurethane suspension packing reactor was higher than those of composite packing reactor. Polyurethane suspension packing reactor contained relatively richer nitrification-related flora *Nitrospira*, which provide better growth and reproduction conditions for the denitrification-related flora *Betaproteobacteria* and *Denitratisoma*. The large amount of facultative heterotrophic bacteria in *Proteobacteria* had a significant effect on the removal of organic pollutants. This study provides a theoretical basis for subsequent in-depth research and practical application.

Keywords: Low dissolved oxygen; Low C/N domestic sewage; Packing; Biological contact oxidation; Microbial community structure

1. Introduction

Water pollution is a primary problem that plagues social development and survival [1–3]. It is difficult to treat urban sewage with low concentration and low C/N ratio in southern China. There are many problems with the traditional biological method used to treat low C/N domestic sewage, such as excessive aeration, insufficient organic carbon source and low nitrogen removal efficiency [4]. If aeration is used to increase the dissolved oxygen (DO), it is necessary to add

more carbon sources, which increases the cost of the wastewater treatment [5]. The biological contact oxidation method is an efficient sewage treatment process, which combines the characteristics of activated sludge method and biofilm method, and has the advantages of simple management, less residual sludge, impact load resistance and stable treatment effect. It is widely used in the treatment of industrial wastewater and decentralized domestic sewage in China [6,7]. Yin et al. [8] used anaerobic baffled reactor and two-step biological contact oxidation to treat dyeing wastewater. The COD,

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BOD and color of the effluent met the requirements of industrial emission standards [8].

Packings can be used to remove contaminants in a variety of ways, such as adsorption [9], biodegradation, chemical reactions, etc. El-Khateeb et al. [10] used non-woven sheets of polyethylene terephthalate (that could be made from waste plastic bottles) as packings for UASB/DHFW combined system to treat domestic sewage, effectively removing COD, BOD, TSS and fecal coliforms [10]. The choice of packings in biological contact oxidation processes is critical, requiring packings with high mechanical strength, hydraulic stability and specific surface area to allow more biofilms to adhere to the packing. Especially under the condition of DO less than 2 mg/L, it is still able to attach, grow and maintain the high activity of microorganisms [11,12].

In recent years, molecular biology methods such as gradient gel electrophoresis, clone library and high-throughput sequencing have been widely used to study the microbial community structure and diversity in wastewater treatment. MiSeq high-throughput sequencing is based on Illumina's sequencing technology. Simultaneous large-scale parallel sequencing of millions of gene fragments by reversible termination reagent method enables accurate and rapid analysis of results and is therefore widely used in microbial community structure and diversity in wastewater treatment [13–15]. In this paper, a biological contact oxidation reactor experimental device was designed. Under the condition of low DO (1.5–2.0 mg/L), the treatment effect of two different packing biological contact oxidation processes on low C/N domestic sewage was investigated. MiSeq high-throughput sequencing was used to analyze the microbial community structure in different packings and to provide basic data and theoretical guidance for the development of a low C/N domestic wastewater process with good denitrification effect and low energy consumption.

2. Materials and methods

2.1. Experimental device and process

The experimental device and process of the biological contact oxidation reactor are shown in Fig. 1. The main body

of the reactor is a plexiglass cylinder of $H500\text{ mm}\times\Phi150\text{ mm}$ (i.d.) with an effective volume of 8 L, and can be filled with contact packing. One side of the reactor has a water inlet near the bottom, and the other side has a water outlet or sampling ports every 90 mm from top to bottom, five in total. The bottom is equipped with a drainpipe. A high-temperature-sintered bubble stone aerator is installed under the inlet nozzle near the bottom of the reactor, which can be connected to an external air pump to realize oxygenation and aeration function.

Two reactors was pre-packed and labeled as No. 1 and No. 2. No. 1 reactor was filled with the composite packing, and No. 2 reactor was added with polyurethane suspended packing (recorded as 1# packing, 2# packing, respectively). The fill ratio of reactors No. 1 and No. 2 was 50% (packing volume/reactor effective volume). Both packings (Fig. 2) were purchased from a packing plant in Beijing, and their physical and chemical properties are shown in Table 1. Fig. 3 is the SEM for experimental selected packings. Polyurethane suspension packing has a mesh structure inside with many voids and a smooth surface. The interior of composite packing is branched and dense.

2.2. Experimental operating parameters

In the experiment, the inlet water of the reactor was manually distributed. The simulated domestic sewage was mixed with NH_4Cl , NaNO_2 , K_2HPO_4 , potassium hydrogen phthalate and glucose. The inoculated sludge was taken from the Gaobeidian sewage treatment plant. After the preparation work was completed, the peristaltic pump and the outlet valve of the tower top were opened, and the simulated domestic sewage was taken out from the inlet tank and pumped into the reactor to realize the continuous water inlet and outlet. The air pump was turned on, and the DO content in the reactor was controlled by the rotor regulating flow meter.

The biofilm was cultured from activated sludge inoculation in the start-up phase of the bioreactor. We first used a high-concentration sewage to grow the biofilm, and then used a low-concentration sewage to acclimate the biofilm. After the reactor was operated for 30 d, the

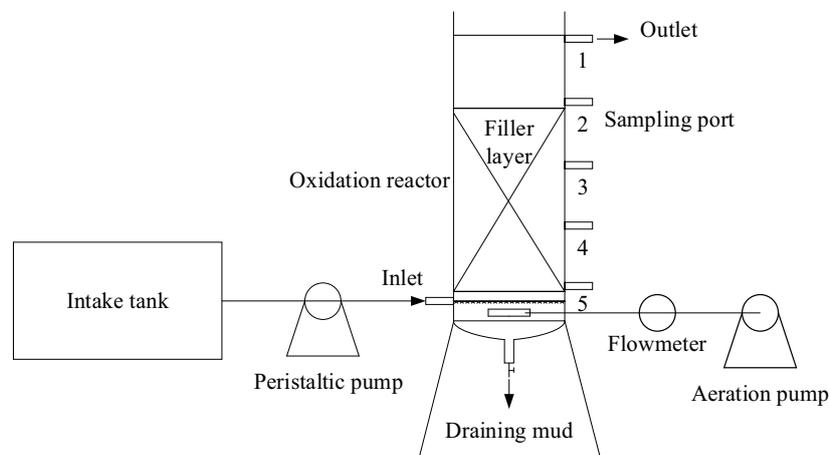


Fig. 1. Experimental device and process of the biological contact oxidation reactor.

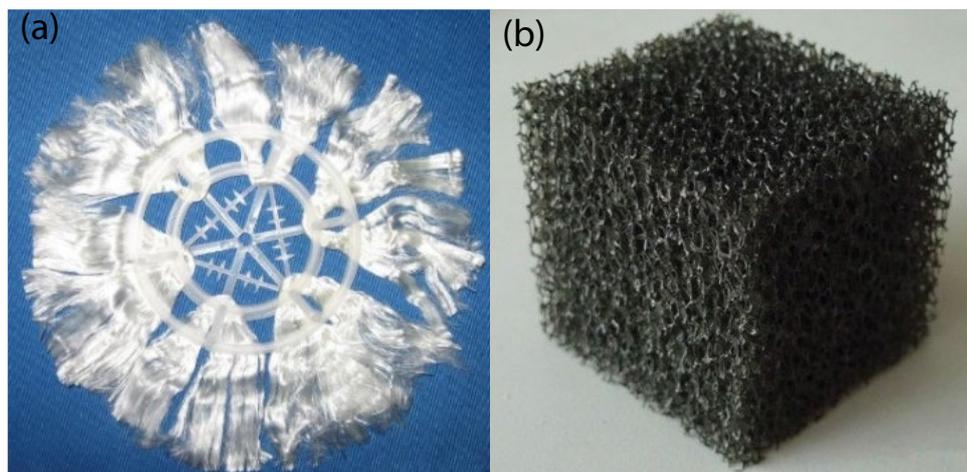


Fig. 2. Experimental selected packings ((a) composite packing and (b) polyurethane suspension packing).

Table 1
Physical and chemical properties of packings

Sort	Size (mm)	Specific surface area (m ² ·m ⁻³)	Porosity (%)
Composite packing	Φ150	290–310	96
Polyurethane suspension packing	D20	1,000–1,200	>99

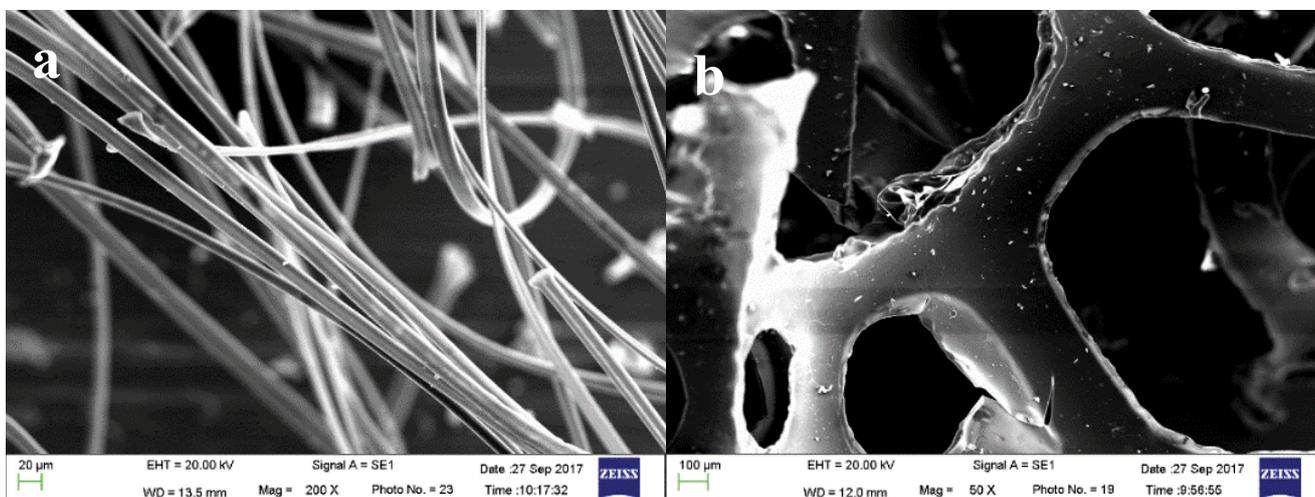


Fig. 3. SEM for experimental selected packings ((a) composite packing and (b) polyurethane suspension packing).

adhesion of the biofilm was enhanced, and the biochemical function became more efficient. At this time, the effluent water became gradually clearer. When the removal rate of COD and NH₄⁺-N in the effluent was greater than 60%, the biofilm cultivation is successful [16]. After the reactor was stably operated, biological samples of the two packing surface biofilms were collected and analyzed for microbial composition. The operating parameters and influent water quality indicators of the biological contact oxidation reactor are shown in Tables 2 and 3.

2.3. Analysis methods

2.3.1. Determination and analysis of water quality indicators

The concentration of COD in the influent and effluent water was determined by potassium dichromate rapid digestion spectrophotometry. The concentration of NH₄⁺-N was determined by Nessler's reagent spectrophotometry. The concentration of TN was determined by alkaline potassium persulfate digestion ultraviolet spectrophotometry.

Table 2
Process operating parameters

HRT (h)	Influent flow (L/h)	DO (mg/L)	Temperature (°C)
6	1.34	1.5–2.0	24 ± 2

Table 3
Influent water quality indexes

Indexes	Range	Average
pH	7.08–7.91	7.47
NH ₄ ⁺ -N/(mg/L)	10.52–56.74	27.35
TN/(mg/L)	17.01–72.51	32.16
COD/(mg/L)	66.41–225.87	124.26
TP/(mg/L)	3.24–6.15	4.89

The concentration of TP was determined by potassium persulfate digestion molybdenum antimony anti-spectrophotometry. The absorbance was measured using HACH DR5000 UV-visible spectrophotometer [17], and the DO concentration was measured with a HI9146 portable DO meter (the measurement position was in the middle of the packed bed).

3.2.2. Determination and analysis of microbial composition

Two different packing surface biofilms were taken as samples for biodiversity measurement. After sampling, the samples were stored in a –80°C refrigerator and tested in time.

DNA extraction and PCR amplification: The DNA was extracted using the procedure provided in the EZNA Soil DNA Kit (Omega, USA) instructions, and the extracted DNA was detected by 1% agarose gel electrophoresis [18,19]. The amplified region of PCR was the V3-V4 region of 16S rRNA, and the primer for bacterial 16S rRNA amplification was universal primer (338F/806R). The PCR amplification system contained 30 ng DNA sample, 1 μL forward primer (5 uM), 1 μL reverse primer (5 uM), 3 μL BSA (2 ng/μL), 12.5 μL 2xTaq PCR reagent, and 7.5-X μL sterile double distilled water. Reaction procedure: the system was preheated at 95°C for 5 min, and then carried out 28 cycles (95°C deformation 30 s, 56°C annealing 30 s, 72°C extension 40 s), and finally extended at 72°C for 10 min. The PCR products were then recovered using an AxyPrep DNA Gel Recovery Kit (Axygen), eluted with Tris-HCl, and detected by 2% agarose gel electrophoresis. Based on the results of electrophoresis, the PCR products were quantified by QuantiFluor TM-ST (Promega (Beijing) Biotechnology Co., Ltd., No. 907-909, Block B, Global Trade Center, 36 North Third Ring Road East, Dongcheng District, Beijing), and the corresponding proportions were mixed according to the sequencing amount of each sample [15]. The Illumina platform library was constructed after fluorescence quantification.

3. Results and discussion

3.1. Influence of packing type on treatment effect

3.1.1. Influence of NH₄⁺-N removal effect

The effect of the two packings on NH₄⁺-N removal is shown in Fig. 4. The removal rate of NH₄⁺-N fluctuated

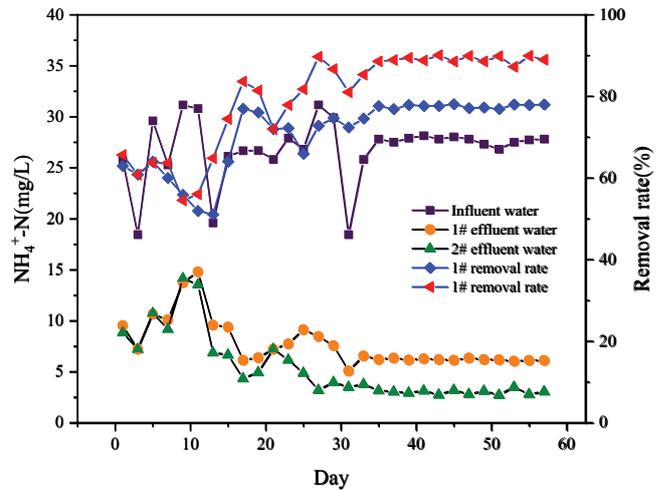


Fig. 4. Effect of two kinds of packings on the removal of NH₄⁺-N from wastewater.

greatly, but Fig. 4 shows a trend of decreasing first, then increasing and finally stabilizing. In general, 2# packing was better than 1# packing for NH₄⁺-N removal. In the early stage of the reaction, the removal rate of NH₄⁺-N of 1# packing and 2# packing was low because the biofilm had not yet fully formed, and the growth cycle of nitrifying bacteria was slower, and the adaptation period was long. Therefore, nitrifying bacteria were at an inferior stage in competition with heterotrophic bacteria.

During the whole experiment, the lowest removal rate of 1# packing was 51.52%; the highest removal rate was 78.11% and the final removal rate was stabilized at 77.61%. The lowest removal rate of 2# packing was 51.21%; and the highest removal rate was 90.11% and the final removal rate was stabilized at around 89.22%. The reason may be that 2# packing has a special mesh structure and a higher void ratio than that of 1# packing, which is more conducive to the retention of NH₄⁺-N. As the reaction proceeds, the pores of the packing began to become smaller due to the adsorption and clogging of the sludge and microorganisms, and the removal rate began to decrease. However, when the reaction proceeded for about 13 d, the complete yellow-brown biofilm was observed on the surface of the 1# and 2# packings, which played a major role in the removal of NH₄⁺-N. Therefore, the removal rates of NH₄⁺-N began to gradually increase. Studies have shown that the removal of ammonia nitrogen at the beginning of the experiment relies mainly on packing adsorption and physical retention [20]. The surface of the packing in the early stage of the reaction is rough, having many voids, large specific surface area, irregular particles, and can fully contact and react with the organic matter in the sewage to increase the retention of pollutants [21].

The NH₄⁺-N effluent concentration of the two packings generally decreased gradually, but only that of the 2# packing could reach and stabilize below 5 mg/L, which satisfied the first-grade A discharge standard. This indicates that the 2# packing is feasible to treat low-concentration ammonia-nitrogen wastewater. The reason may be that low DO inhibits the activity and proliferation rate of nitrifying bacteria.

2# packing can form the ideal state of coexistence of various microorganisms due to its reticulated three-dimensional structure. Its surface and center can also be biologically denitrified by aerobic microorganisms and anaerobic microorganisms, respectively [22], which is more suitable for the reproduction of nitrifying bacteria.

3.1.2. Influence of TN removal effect

The effect of the two packings on TN removal is shown in Fig. 5. The removal rate of TN of the two packings generally shows a trend of decreasing first, then increasing and finally stabilizing. Moreover, the removal rate of TN of 2# packing was higher than that of 1# packing, and the results are similar to those of Fig. 4. The lowest removal rate of 1# packing was 47.92%; the highest removal rate was 55.32%, and the final removal rate was stabilized at 54.74%. The lowest removal rate of 2# packing was 48.85%; the highest removal rate was 63.51%, and the final removal rate was stabilized at 61.21%.

The TN removal rates of the two packings were not high, which is similar to the related research results. Wang et al. [23] found that when the DO is less than 2 mg/L, the TN removal rate of the biological contact oxidation method is low. The reason may be that even under low DO conditions, the anoxic environment required for denitrification cannot be satisfied. It is difficult for oxygen molecules to replace nitrate nitrogen as an electron acceptor, thereby inhibiting denitrification of denitrifying bacteria. In addition, the lack of organic carbon sources in low C/N domestic wastewater is also one of the reasons for the low TN removal rate [20].

It can be seen from Fig. 5 that the TN removal rate of the 2# packing was higher than that of the 1# packing. Only the TN effluent concentration of the 2# packing reactor could be lower than 15 mg/L, which reached the first-grade A discharge standard. On the one hand, because the porosity and specific surface area of the 2# packing were higher than those of the 1# packing, the 2# packing could enhance the diffusion and mass transfer of DO in the biofilm under low oxygen conditions, thereby increasing the nitrification ability. A larger specific surface area means more biofilm, thereby

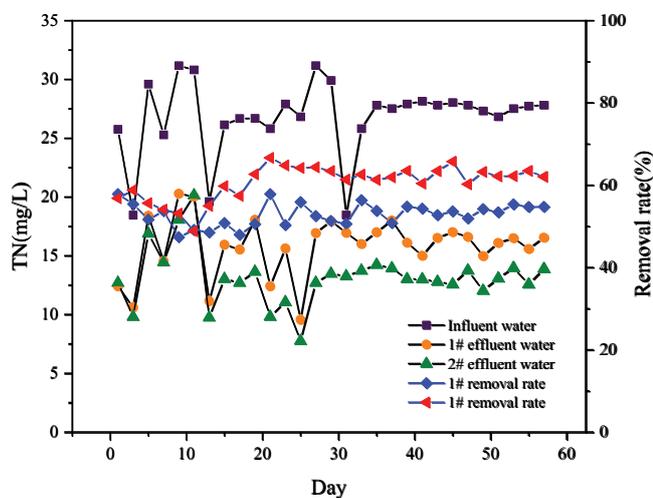


Fig. 5. Effect of two kinds of packings on the removal of TN from wastewater.

expanding the range of anaerobic layers in the biofilm micro-environment of the 2# packing. A large number of heterotrophic nitrifying bacteria suitable for growth under low DO were grown on the surface of the packing, and the anoxic microenvironment formed under low DO conditions makes the denitrification process easier [24]. On the other hand, in the biological contact oxidation reactor, the anoxic tank was not disposed, so the removal of TN mainly depended on the simultaneous nitrification and denitrification reaction occurring on the biofilm. Ammonia oxidizing bacteria have higher affinity for oxygen than nitrite oxidizing bacteria, so low DO conditions are beneficial to the short-range nitrification reaction [25].

3.1.3. Influence of COD removal effect

The effect of the two packings on COD removal is shown in Fig. 6. Both packings have high COD removal rates. The lowest removal rate of 1# packing was 80.41%; the highest removal rate was 89.14%, and the final removal rate was stabilized at 87.41%. The lowest removal rate of 2# packing was 78.32%; the highest removal rate was 92.14%, and the final removal rate was stabilized at 91.23%.

The DO concentration limits the microbial activity and various biochemical reactions, and therefore has an important effect on the removal of organic matter. Both sets of reactors had a high removal rate of COD. On the one hand, under the condition of low DO, the area of the anoxic layer in the membrane expands, which is more suitable for the growth of facultative heterotrophic bacteria. In the reactor system, the removal of organic matter was originally mainly caused by the decomposition of aerobic heterotrophic bacteria, and then mainly by the facultative heterotrophic bacteria. This is consistent with the findings of Wang et al. [26]. On the other hand, COD removal relies mainly on the physical adsorption and retention of the packing, as well as the synergistic effect of heterotrophic microbial growth and reproduction [27]. In this study, the special mesh structure of 2# packing and the dense branch structure of 1# packing were beneficial to retain organic matter and increase the mass transfer rate of

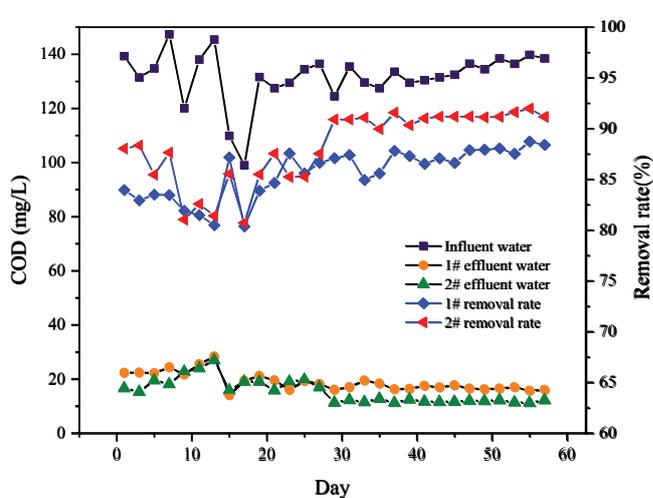


Fig. 6. Effect of two kinds of packings on the removal of COD from wastewater.

oxygen. It could provide sufficient nutrients for the growth and attachment of microorganisms and meet the metabolic activities of heterotrophic bacteria.

3.1.4. Influence of TP removal effect

The effect of the two packings on the removal of TP is shown in Fig. 7. As can be seen from Fig. 7, the removal rates of TP by the two packings fluctuated greatly, showing a trend of decreasing first and then stabilizing overall. The final removal rate of TP of 1# and 2# packings was similar, and the removal rate of TP of 2# packing was more stable. Among them, the lowest removal rate of 1# packing was 19.13%; the highest removal rate was 53.17%, and the final removal rate was about 27.96%. The lowest removal rate of 2# packing was 20.97%; the highest removal rate was 37.72%, and the final removal rate was about 27.89%.

The removal of phosphorus by biofilm process is usually achieved by combined treatment process. Phosphorus removal by the combined process of attachment growth and suspension growth is usually chemical rather than biological. This is because most biological phosphorus removal processes require an anaerobic reactor at the front end. For most combined processes, this requires the backflow of mixed liquor in the biofilm reactor, which is usually difficult to achieve. Some researchers have also explored the phosphorus removal effect of denitrifying phosphorus accumulating bacteria in the combined treatment process. For example, in Zhang et al.'s [28] A_2/O -BCO double sludge system, by refluxing the nitrate from the contact oxidation stage into the anoxic tank, a large amount of NO_x-N is provided as an electron acceptor for denitrifying phosphorus accumulating bacteria to achieve the phosphorus accumulating effect.

The experimental device in this study was a single-contact oxidation process. The process did not have the conditions of anaerobic/aerobic alternate operation, and there was no sludge system, so the effect of biological phosphorus removal was not ideal. According to the analysis, there are three main ways to remove phosphorus in the system

adopted by this research. (1) The adsorption and retention of fillers have certain effect on the removal of insoluble phosphorus in sewage. (2) The metabolism of microorganisms needs to absorb phosphorus in the process of growth, that is, assimilation. (3) Alkalinity will be produced in denitrification process. The precipitation effect of some phosphorus occurs in the process of pH change. Among them, microbial assimilation played the most important role in the removal process of this system [29].

Based on the results of Figs. 4–7, 2# packing was generally better than 1# packing for the removal of NH_4^+-N , TN, COD and TP, and the removal of pollutants from wastewater disposal after aeration. The main reason is that the surface of the 2# packing has some hydrophilic hydroxyl, cation and other reactive groups, which can be combined with negatively charged microorganisms to produce a stable chemical bond and valence combination. This allows the biofilm to adhere firmly to the surface of the packing, which facilitates the growth of the biofilm and enhances the removal of contaminants. Table 4 shows the comparison between effluent water quality indexes of the wastewater treated by the two packings and some water quality indexes of the first-class A emission standard of Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002). The removal efficiency of NH_4^+-N , TN and COD in No. 2 reactor was better than that of No. 1 reactor, and only the effluent indexes of No. 2 reactor could reach the discharge standard. The biological phosphorus removal effect of the single-contact oxidation process was not ideal and could be combined with anaerobic reactor.

3.2. Analytical results of microbial composition

In order to investigate the effects of different packings on biomes in low C/N domestic sewage, the biofilm on the surface of the stationary phase was sampled. The high-throughput sequencing of the surface biofilms of the two packings (labeled 1# packing and 2# packing) was performed using the MiSeq platform to obtain 36,512 and 35,789 optimized sequences, respectively. The results are shown in Table 5. The two sets of optimized sequences were intercepted and aligned with the SILVA106 library, and then clustered. Under the condition of 97% similarity, 927 and 1,035 operational taxonomic units (OTUs) were obtained in the optimized sequences. The Chao index indicates that the larger the richness index of the microbial community in the sample, the greater the abundance of the microbial flora. The Shannon index indicates that the larger the diversity index of the microbial community in the sample, the more abundant the microbial diversity contained in the sample. And the size of the coverage value reflects the proximity of the test results to the sample.

It can be seen from Table 5 that the coverage rates of the two groups of experimental samples were 99.44% and 99.49%, respectively, both greater than 95%, indicating that the sequence library constructed in this study could cover the diversity of bacterial communities. In addition, the experimental results of the two packings showed that: OTUs were 2# packing > 1# packing, Chao index was 2# packing > 1# packing, Shannon index was 2# packing > 1# packing. Therefore, under the condition of low DO (DO = 1.5–2.0 mg/L), the 2# packing had higher microbial richness and diversity index,

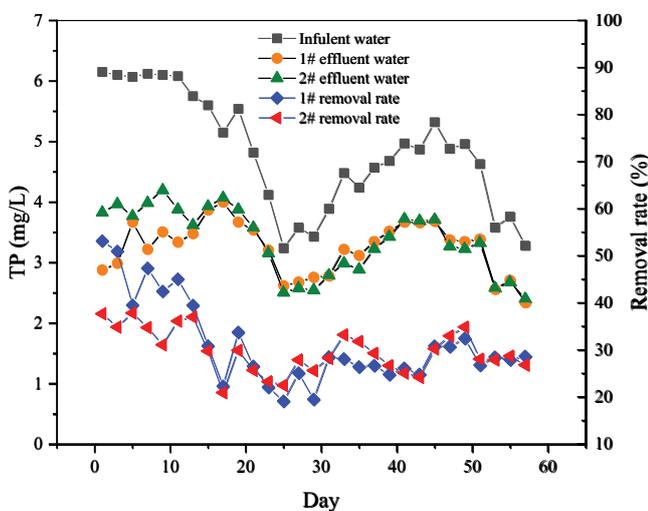


Fig. 7. Effect of two kinds of packings on the removal of TP from wastewater.

Table 4

Comparison between effluent water quality indexes and some water quality indexes in the first-class A emission standard of Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002)

Indexes	1# average (mg/L)	2# average (mg/L)	First-class A emission standard of Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002)
NH ₄ ⁺ -N	5.15	3.11	5
TN	16.36	13.28	15
COD	16.99	11.88	50
TP	3.19	3.14	0.5

Table 5

Microbial richness and polymorphism index analysis table

Sample	1	2
Coverage rate, %	99.44	99.49
OTUs	927	1,035
Chao index	999.11	1,089.87
Shannon index	7.27	8.36

which indicates that 2# packing is more suitable for the growth and reproduction of microorganisms, while increasing the removal rate of COD, TN and NH₄⁺-N.

3.3. Analysis results of community structure

Based on the classification information of SILVA database, the high-throughput sequencing data of the attached colonies on the two kinds of packings were classified at the phylum, the class and the genus level, and the community structure maps were analyzed which are shown in Figs. 8–10.

At the phylum category level shown in Fig. 8, there were 12 large groups of biofilm samples on the surface of the two packings (relative abundance greater than 1%), including Proteobacteria (61.68%, 52.91%), Bacteroidetes (7.96%, 11.43%), Chloroflexi (5.06%, 7.13%), Acidobacteria (5.44%, 4.81%), Nitrospirae (3.46%, 5.55%) and Planctomycetes (3.12%, 4.51%).

The dominant phylum was Proteobacteria, which is the largest species of bacteria in the phylum level and contains a wide variety of bacterial species, most of which are facultative or obligate anaerobic heterotrophic bacteria. 67.15% of Proteobacteria are Betaproteobacteria, and most of them tend to acquire nutrients by decomposing organic matter under anaerobic conditions. Some Betaproteobacteria can utilize hydrogen, ammonia, methane and volatile fatty acids, which are thought to be closely related to sludge denitrification [14,30].

Under the low DO conditions of the experimental study, the microorganisms on the packing were mainly facultative heterotrophic bacteria. The heterotrophic bacteria had a significant effect on organic pollutants removal in wastewater, which is consistent with the results of the above two experiments. It is worth noting that the relative richness of Nitrospirae of the 2# packing was greater than that of the 1# packing. Nitrospirae is the main nitrous acid oxidizing bacteria, which oxidizes NO₂⁻ to NO₃⁻. This also explains the

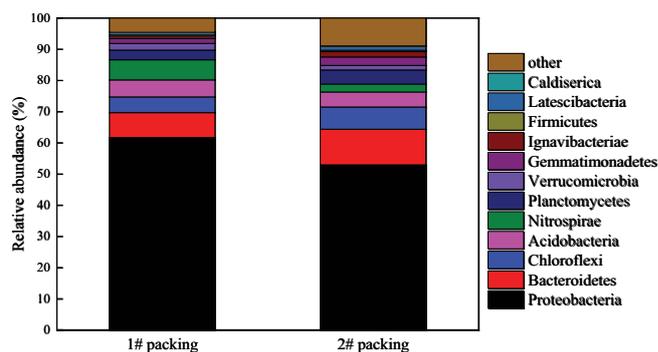


Fig. 8. Community structure diagram at the phylum level.

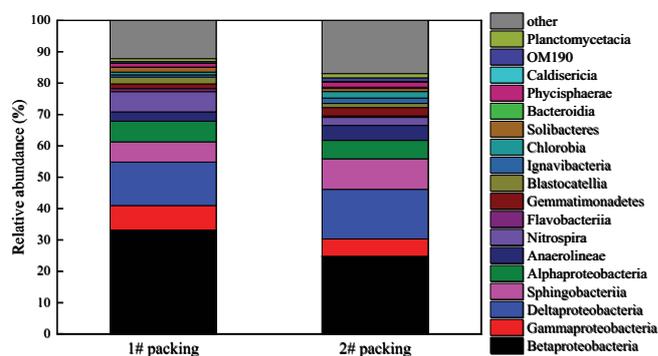


Fig. 9. Community structure diagram at the class level.

phenomenon that the effluent concentration of NH₄⁺-N and TN of the 2# packing was lower than that of the 1# packing.

At the class category level shown in Fig. 9, the surface biofilm samples of the two packings belonged to 18 groups (relative abundance greater than 1%), including Beta proteobacteria (33.08%, 24.72%), Gammaproteobacteria (7.81%, 5.50%), Deltaproteobacteria (13.89%, 15.85%), Sphingobacteria (6.45%, 9.73%), Alphaproteobacteria (6.60%, 5.89%) and Anaerolineae (2.95%, 4.77%). The dominant class of microorganisms on both packings were Betaproteobacteria, and some species of Betaproteobacteria are related to the denitrification process [31,32]. Although the Betaproteobacteria richness of the 1# packing was greater than that of the 2# packing, the premise of denitrification is to have a good nitrification reaction, and the experimental results of the 2# packing (shown in Fig. 4) met this requirement. The nitrification of 2# packing was better than that of 1# packing, and the removal rate of TN was also higher.

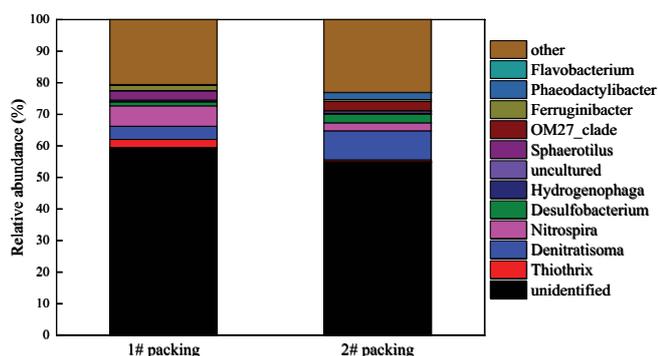


Fig. 10. Community structure diagram at the genus level.

At the genus category level shown in Fig. 10, there were 10 large groups (relative abundance greater than 1%) in the biofilm samples attached to the two packings, including *Thiiothrix* (2.52%, 0.54%), *Denitratisoma* (4.20%, 9.20%), *Nitrospira* (3.44%, 5.51%) and *Desulfobacterium* (1.25%, 2.79%). The dominant genus of both experimental packings was *Denitratisoma*, which is similar to the related research results. Zhao et al. [14] found *Denitratisoma* (3.71%) associated with denitrification in the study of denitrification and microbial community characteristics of composite carbon source packings.

The results of Fahrback et al. [33] showed that *Denitratisoma* isolated from activated sludge from municipal sewage treatment plants is a newly discovered genus belonging to Rhodocyclaceae. *Denitratisoma* has the ability of aerobic denitrification and short-range nitrification, which can directly convert NO_2^- into gaseous nitrogen. The relative richness of *Denitratisoma* in 2# packing was greater than that in 1# packing, which indicates that 2# packing can provide a good environment for microbial growth, strengthen the bioenrichment of *Denitratisoma* and make TN removal better than that of 1# packing (shown in Fig. 5). In addition, *Nitrospira* is a genus of nitrifying bacteria, and the contents of *Nitrospira* in the #1 and 2# packings were 3.44% and 5.51%, respectively. This also creates the possibility of *Nitrospira*'s enrichment. Liu and Wang [34] also confirmed that long-term operation under low DO conditions can enrich *Nitrospira* [35].

As can be seen from Figs. 8–10, the dominant flora in the two groups of reactors was Proteobacteria, Betaproteobacteria and *Denitratisoma*. Polyurethane suspension packing reactor contained relatively richer nitrification-related flora *Nitrospira*, which provide better growth conditions for the denitrification-related flora Betaproteobacteria and *Denitratisoma*. This is consistent with the better removal of NH_4^+-N and TN in polyurethane suspension reactor (Figs. 4 and 5). The large amount of facultative heterotrophic bacteria in Proteobacteria had a significant effect on organic pollutants removal, which is consistent with the COD removal result in Fig. 6. In the future, Proteobacteria, Betaproteobacteria, *Denitratisoma* and *Nitrospira* flora can be cultivated in a targeted manner to improve the treatment of low C/N domestic sewage under low DO conditions.

4. Conclusion

- The polyurethane suspension packing reactor had better removal efficacy on NH_4^+-N , TN and COD than the

combined packing reactor, and only the effluent indexes of polyurethane suspension packing reactor could reach the first-class A emission standard of Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002). The removal of biological phosphorus of the single-contact oxidation process was not ideal and could be combined with anaerobic reactor. Under low DO, the use of polyurethane suspension packing by biological contact oxidation method was feasible in removing domestic sewage NH_4^+-N , TN and COD.

- The richness and diversity of bacterial communities in polyurethane suspension packing reactor were higher than those of composite packing reactor.
- The dominant flora in the two groups was Proteobacteria, Betaproteobacteria and *Denitratisoma*. Polyurethane suspension packing reactor contained relatively richer nitrification-related flora *Nitrospira*, which provide better growth and reproduction conditions for the denitrification-related flora Betaproteobacteria and *Denitratisoma*. The large amount of facultative heterotrophic bacteria in the Proteobacteria had a significant effect on the removal of organic pollutants.

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