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Effect of adding exogenous nutrients on refractory wastewater treatment and bacterial community in activated sludge

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ABSTRACT

Conventional biochemical processes have unsatisfactory effects on refractory wastewater treatment. Thus, a technically feasible and economical treatment should be established. In the conventional culture of microorganisms, adding exogenous nutrients to a medium can effectively promote microorganism growth. Therefore, using exogenous nutrients in wastewater treatment may enhance microbial activity, subsequently speeding up the removal of contaminants. In this study, nutrients with different carbon and nitrogen (C/N) ratios were added to activated sludge and a chemical oxygen demand (COD) of 1000 mg/L was considered the benchmark for carbon source in the activated sludge process. Results showed that wastewater COD was reduced below 130 mg/L, and the removal rate reached 90% when the C/N ratio was 5:1. Along with the results from the study on F/M, dehydrogenase activity, content of extracellular polymeric substances, and microbial community in the wastewater treatment process, our findings indicated that adding exogenous nutrients increase microbial community diversity and promote the activity of microorganisms, thereby wastewater COD was reduced to 130 mg/L after 12 h and the removal rate reached approximately 90%. Simultaneously, analysis results on microbial community structure indicated that *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were the dominant species of bacteria. The addition of exogenous nutrients may increase the abundances of *Pseudomonas* and *Bacillus* species, which were essential in COD removal.

Keywords: Refractory wastewater; Wastewater treatment; Chemical oxygen demand; Microbial community; Bioaugmentation technology

1. Introduction

Toxic and harmful refractory organics, such as phenols, chlorophenols, pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, nitroaromatic compounds, and dyes, which are inevitably released from the processes of chemical industry production, can cause significant environmental pollution because of the rapid development of economic production. Industrial wastewater constantly contains many refractory organics that possess complex composition, high chromaticity, high salinity, and refractory characteristics (specifically reflected in its biological oxygen demand (BOD)/chemical oxygen demand (COD) that is below 0.3) [1]. Many organic pollutants exist in wastewater, and their presence indicates physiological toxicity that results in great inhibitory effects on the growth and metabolism of microorganisms; thus, effective biological treatment in industrial wastewater treatment remains a challenge [2]. Conventional biological treatment technology negatively affects COD removal in refractory wastewater for its high concentrations and low biodegrading characteristics. The present treatment for this type of wastewater constantly uses several combined biochemical processes along with complicated processes and high energy consumption [3]. Bioaugmentation is a technically feasible and economical

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approach and has strong practical significance for refractory wastewater treatment [4].

In the conventional culture of microorganisms, adding certain exogenous nutrients in a microbial culture medium can provide these microorganisms an appropriate nutritional environment and can make them grow efficiently. Similarly, using bioaugmentation technology in adding exogenous nutrients in activated sludge can improve the original nutritional environment in wastewater treatment and promotes the co-metabolism of microorganisms in sludge. Thus, in organic degradation, refractory organics can be easily degraded when certain organic substances; that is, glucose, is present. The effect of wastewater treatment may be enhanced by efficiently promoting the growth of certain microorganisms and changing the microbial community structure of activated sludge [5,6]. Several papers have reported on bioaugmentation in activated sludge systems. In the treatment of dyeing wastewater anaerobic baffled reactor (ABR) process, adding a certain proportion of nutrients in the wastewater and changing the nutritional environment in which the sludge is located can play different roles in the growth of activated sludge; hence, wastewater treatment can be conducted in different directions [7]. Ahmad et al. [8] used two laboratory-scale biological reactors (one reactor under aerobic conditions and the other under anaerobic conditions) for pesticide wastewater treatment, which used glucose as a supplemental carbon substrate. More than 96% pesticide was removed when the optimum ratio of substrate (Vydine) to co-substrate (glucose) was 1:100. Sen et al. [9] used an anaerobic fluidized bed in real textile wastewater treatment when additional glucose concentration was 2 g/L. The corresponding maximum COD, BOD_5 and color removals were found to be approximately 82%, 94%, and 59%, respectively. Given the aforementioned conditions, adding different proportions of nutrients in activated sludge in wastewater treatment can change the activity and community structure of microorganisms and thus improve wastewater treatment.

The refractory wastewater involved in this paper was a type of fur dyeing wastewater. The COD of such wastewater after conventional activated sludge treatment can only reach approximately 200 mg/L, which cannot easily satisfy emission standards. Thus, we must consider other methods for wastewater COD reduction. On the basis of the conventional activated sludge process, the varied effects of different exogenous nutrients with different COD and ammonia nitrogen (C/N) ratios on wastewater treatment were studied and analyzed in the present study. The optimum C/N ratio of added exogenous nutrients was determined to find the lowest COD that could be reached in refractory wastewater treatment. Thus, the difference of microbial ecology caused by adding different exogenous nutrients was analyzed, and the dominant bacteria in the sludge during the treatment were revealed based on the effects of wastewater treatment and the changes of microbial community structure.

2. Materials and methods

2.1 Materials

The wastewater used in the experiment was obtained from the fur dyeing section of a fur factory in Zhejiang Province, China (Table 1). The concentration of NH_4^+–N was relatively low and could be ignored. Thus, only COD removal was considered in subsequent experiments. A 5 L biological treatment device made of polyethylene material was used in the experiments (Fig. 1). The experimental device contained a foam plastic box with a certain amount of water and a thermostatic stick to ensure a constant temperature. A5 L polyethylene plastic bucket was placed in the foam box. An external aeration device was attached, and the aeration head was located at the bottom of the plastic bucket but was not in contact with the bottom of the bucket. The activated sludge, which was starved for 1 week, was placed in the reactor for aerobic aeration, and the mixed liquid suspended solid (MLSS) was 5000 mg/L. The sludge was cultivated in the operating mode of sequencing batch reactor (SBR). The cycle time was 24 h, as follows: influent for 0.5 h, aeration for 20 h, setting aside for 1 h, outflow for 0.5 h, and the rest for 2 h. The concentration of dissolved oxygen was kept at approximately 2 mg/L, and the temperature was maintained at 25°C. In the initial stage of cultivation, simulated artificial wastewater was introduced in the reactor rather than wastewater, and the concentration of wastewater was gradually increased until the wastewater replaced the simulated wastewater completely. Concurrently, the

Table 1
Chemical characteristics of refractory wastewater influent used in this study

Parameter *	Concentration
COD_{Cr}	1000–1500
BOD_5	250–400
BOD/COD	<0.25
NH_4^+–N	10–15
SS	<100
Chromaticity (times)	125–150
pH	10.5–11.5

*All values are expressed in (mg/L), except for pH and chromaticity.

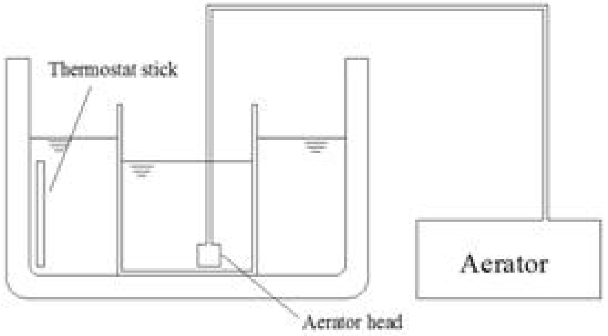

Fig. 1. Experimental device. The device was first used for sludge culture. The cycle was 24 h. After the successful cultivation of the sludge (after 45 d), the bioaugmentation in activated sludge process for the refractory wastewater treatment was conducted in this device, and the experimental time was set to 72 h.

microorganisms adapted to the wastewater environment (this phase lasted approximately 45 d). The effluent COD concentration was determined. The sludge cultivation was considered successful when the effluent COD concentration remained the same. Then, the experiment on the effects of adding exogenous nutrients to wastewater treatment could be conducted.

2.2. Design and sampling of experiment

Exogenous nutrients (C and N sources) were added to the reactor. The C source was glucose (1.067 g glucose = 1,000 mg/L COD), and the N source was chlorine ammonium chloride. To determine whether adding a C source promotes sludge activity and induces complete degradation, we added at 1000 mg/L COD to the C source according to the methods described in several references and previous studies. The carbon/nitrogen ratio (C/N ratio) of the added exogenous nutrients was defined as its COD and total nitrogen ratio (COD/TN). Only ammonium chloride was added as exogenous nutrients. Thus, the C/N ratio can be regarded as the ratio between COD and NH_4^+–N. The values of C/N ratios should be above 1 [10], and C content should not be lower than the N content, which ensured that the microorganisms could grow in the activated sludge with a good nutrition environment. The growth of microorganisms was inhibited when the C/N ratio of exogenous nutrients was extremely high, thereby specifically reflecting that the nitrifying bacteria could not grow because of the lack of NH_4^+–N and affected the entire nitrification and denitrification processes [11]. In the experiment, six groups were set according to the added amount of nutrients to the sludge at different C/N ratios. The activated sludge which was successfully cultivated was added to each experimental device. At the same time, the COD of the wastewater at 0h was determined. After this was done, the wastewater and nutrients were added to the sludge system at the same time. Aeration time lasted for 72 h, which is the time required for the complete consumption of the added exogenous nutrients. In this process, the supernatant of each group was used to measure the change in water quality, and the muddy water mixture was used as the sample for molecular biology and was stored at −20°C.

Table 2
Grouping of experimental design and the amount of applied reagents

Experimental grouping	C/N ratio of nutrients	Glucose*	Ammonium chloride*
#1	Without C or N source	–	–
#2	Only C source	1.067	–
#3	Only N source	–	3.0
#4	C/N=5:1	1.067	0.6
#5	C/N=2.5:1	1.067	1.2
#6	C/N=1:1	1.067	3.0

*All values are expressed in (g/L)

2.3. Water quality determination method

In the above experiment, COD was determined by the fast digestion spectrophotometric method. Nitrate nitrogen was determined by spectrophotometric method with phenol disulfonic acid. BOD_5 was determined by dilution and inoculation methods. MLSS and solid suspension were measured by gravimetric method. Chromaticity was determined by dilution factor method. Sludge loading (F/M) was determined using Eq. (1), as follows:

$$F / M = QS / (VX) \tag{1}$$

where F/M is the sludge loading, kg COD/kg MLSS·d; Q is the daily water volume of inflow, m^3/d; S is the COD concentration, mg/L; V is the effective volume of reactor, m^3; and X is the MLSS, mg/L.

2.4. Determination of dehydrogenase activity (DHA) and extracellular polymeric substance (EPS) in activated sludge

The dehydrogenase concentration in activated sludge was measured by modified 2,3,5-triphenyl tetrazolium chloride (TTC) method [12]. Absorbance was measured at a wavelength of 492 nm, and the amount of μg TF produced per hour was one unit of enzyme activity.

EPS extraction was measured by the method mentioned in [13]. The main components of EPS are polysaccharides and proteins; the content of polysaccharides was determined by anthrone-H_2SO_4 colorimetric method, and the content of proteins was determined by UV absorption method [14].

2.5. Analysis of microbial communities

Deoxyribonucleic acid (DNA) extraction: The total DNA was extracted from the initial activated sludge, and the activated sludge was extracted after different wastewater treatments. The DNA quality and fragment size were checked by 0.5% agarose gel electrophoresis, and the samples were stored at −20°C before use.

Polymerase chain reaction (PCR) amplification: The 27f/1492r target was amplified by PCR by using bacterial universal primers, and genomic DNA was used as a template. The obtained products were separated by electrophoresis in a 2% agarose gel and were purified by a rapid agarose gel DNA recovery kit for recovery.

Terminal restriction fragment length polymorphism (T-RFLP) analysis: The method in [15] was used for detecting changes in microbial community structure by T-RFLP analysis.

2.6. Microbial diversity and similarity analysis

Each terminal restriction fragment (T-RF) in the T-RFLP analysis can be considered an operational taxonomic unit. We used the α-Diversity index to analyze the diversity of microbial communities in the activated sludge, including Shannon–Wiener (H), Simpson (J), Brillouin, and McIntosh (Dmc) indexes [16–18]. Species evenness index based on Gini index for calculating inter specific encounter probability index was also analyzed [16]. Simultaneously, the cluster analyses of microbial community structures under different treatment processes were conducted based on Euclidean distance by using SPSS 20.0 software. The similarity of community composition was compared.

3. Results and discussion

3.1. Treatment of wastewater

3.1.1. COD removal

In the experiments, #1 can be regarded as the conventional activated sludge treatment (Fig. 2a), and its COD can be reduced to approximately 250 mg/L. In contrast to the CODs of the other groups, the COD in #1 continued to decline after 72 h. This condition indicated that the hydraulic retention time in the conventional activated sludge process should be at least 72 h for the complete removal of COD. This time requirement is obviously unrealistic in the actual project. Furthermore, COD in #1 can be reduced to 600 mg/L after 6 h because of the addition of exogenous nutrients. However, the trend of COD indicated differences after 6 h. In #2 and #4, COD was still effectively removed after 6 h, and the effect of COD in #4 was superior to those in the other groups. The COD of wastewater in #4 was reduced to 130 mg/L after 12 h, and the removal rate reached approximately 90%. COD removal rates in #3, #5, and #6 were considerably poor and were lower than the conventional activated sludge process after 48 h. The result showed that the COD removal rate of SBR could reach approximately 85% in the treatment of similar refractory wastewater and that the COD removal rate of hydrolytic acidification-SBR could reach approximately 90% [19]. However, the process used in this study reached a COD removal rate of 90% and attained the effects of several biochemical combination processes in wastewater treatment.

The sludge loading (F/M) in the reactor was also analyzed. After 12 h, the removal rates of COD were greatly reduced and stabilized in all experimental groups. The F/M changes were shown in Fig. 2b. The F/M values did not change much in all experimental groups. After 12 h of reaction in #4, the COD rapidly decreased to 130 mg/L, and the F/M reached 0.38 kg COD/(kg MLSS·d).

3.1.2. Dehydrogenase concentration changes

Changes in DHA in the activated sludge during different processes were investigated (Fig. 3). In #1, which can be regarded as the conventional activated sludge process, DHA concentration was the lowest among the six groups. This result also showed that this refractory wastewater significantly inhibited the growth of microorganisms in the activated sludge. When the experiments proceeded to the later stage, the microorganisms in #1 possessed insufficient nutrients, indicating ineffective wastewater treatment. Simultaneously, the DHA concentration was directly related to the COD removal efficiency of wastewater. DHA concentration in #4 was constantly at a high level (>200 μgTF/L). At this concentration, microorganisms have high dehydrogenase activity and can effectively degrade organic compounds in wastewater, which also obtained a good effect on wastewater treatment. The DHA concentrations in #2, #3, #5, and #6 were consistently at a medium level (in the range of 100 μgTF/L to 200 μgTF/L). Notably, the DHA concentrations in #5 and #6 were higher, although

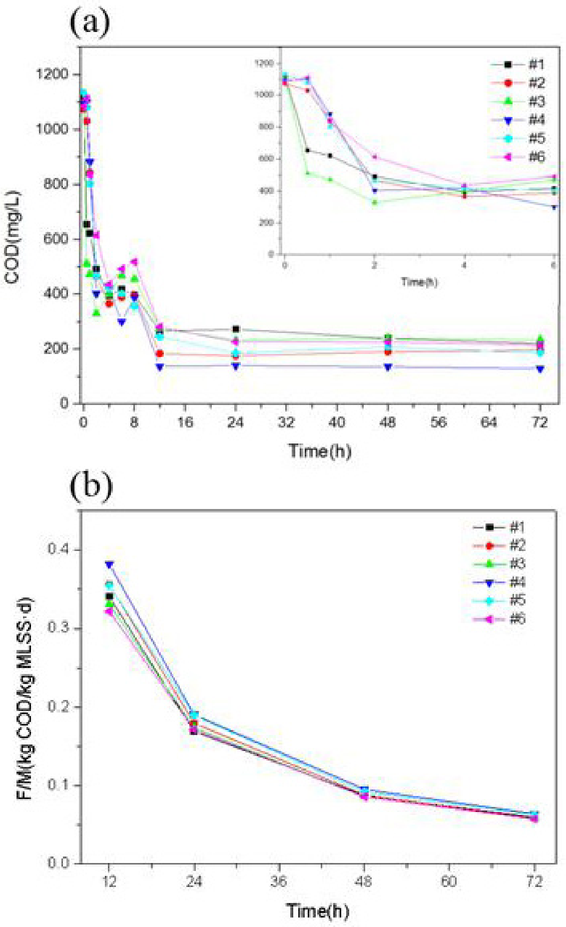

Fig. 2. Change curves of COD and F/M in wastewater treatment. (a) COD change curve; (b) F/M change curve.

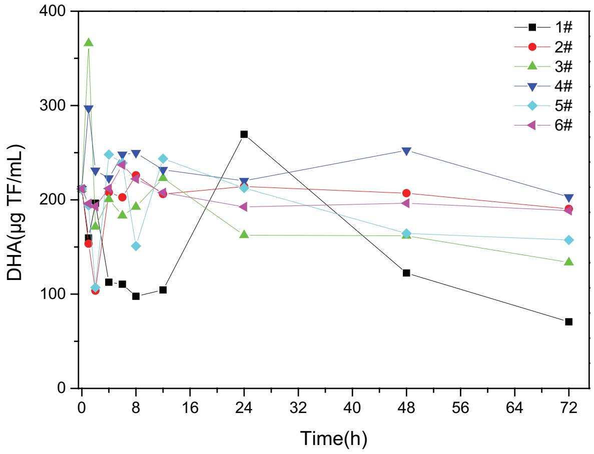

Fig. 3. DHA concentration of activated sludge in wastewater treatment.

the COD removal rates of these two groups were lower than #1. This finding indicated that adding exogenous nutrients could promote the activity of microorganisms in the sludge. However, its effect on wastewater treatment was uncertain. Moreover, only adding C source in the sludge (#2) indicated a positive effect in wastewater treatment, and adding only an N source (#3) was ineffective in promoting the final effect of treatment. According to previous studies on COD removal and DHA, adding exogenous C source in the sludge effectively enhanced the effect on wastewater treatment. In cases #4, #5, and #6, in which the addition of C source was the same (1.067g/L glucose) and N source was different, N sources were added and considerable inhibition of wastewater treatment occurred. On the basis of adding C source in the sludge, adding N source effectively promoted the results of wastewater treatment. However, the addition of exogenous nutrients in the sludge had an optimal C/N ratio, in which a significant effect on wastewater treatment was achieved [6]. In this experiment, the optimum C/N ratio was 5:1, which was also the optimum condition for the growth of microorganisms.

3.1.3. Extracellular polymeric substances (EPS) changes

EPSs in activated sludge are types of high polymers secreted in vitro under certain environmental conditions by microorganisms, which are mainly bacteria. The main components of EPS are proteins and polysaccharides [20]. EPS can accumulate microorganisms for a long period; this characteristic can be beneficial in forming stable symbiotic colonies of microorganisms such that microorganisms can continuously grow in a stable mixed community with high cell concentration. Therefore, activated sludge can maintain strong microbial activity. The contents of EPS secreted by the bacteria will be affected when the sludge is in a different nutrition environment [21]. The quality of living environment and activity of microorganisms in activated sludge can be analyzed by studying EPS content. The chemical compositions of EPS in different environments vary, and EPS plays an important role in activity recovery. Under starvation conditions, microorganisms can use EPS to maintain their viability and microbial activity.

Fig. 4 shows the contents of protein and polysaccharide in the activated sludge in different wastewater treatments. The protein contents in different groups at different times were measured to reflect the characteristics and indirectly explain the biomass in the activated sludge. As shown in Fig. 4a, the protein contents in #4 were constantly at a high state and reached 8.4879 mg/g sludge. Thus, the wastewater treatment in #4 was effective. The protein contents in #1, #3, and #6 were extremely low, and the microorganisms did not grow well under the condition of low protein contents, thereby resulting in unsatisfactory experimental results. However, the protein content in sludge EPS was less than in the domestic sludge because of the special nature of refractory wastewater. This result was caused by the easily degradable and considerably energetic substances in the domestic sewage, which the microorganisms could easily use. Moreover, the domestic sewage sludge secreted more proteins than did refractory wastewater.

As shown in Fig. 4b, the polysaccharide content was opposite to that of the protein content. The polysaccharide contents in #1, #3, and #6 were higher than those in the other groups. The polysaccharide content in #1 reached 12.8546 mg/g sludge, and that in #4 was the lowest at 8.5934 mg/g sludge. Microbial cells actively secrete polysaccharides in adverse external environment to protect themselves against external pressure [22]. The polysaccharide contents in #1, #3, and #6 were high because the existing external conditions of the activated sludge in the three groups were unfavorable. Specifically, the polysaccharide content in #1 was increasingly difficult for microorganisms to extract sufficient nutrients during the process of degrading organics in wastewater. In #4, given that the microorganisms were constantly in acceptable biomasses and biological activities and that the polysaccharides could be rapidly degraded, this group exhibited low levels of polysaccharide content.

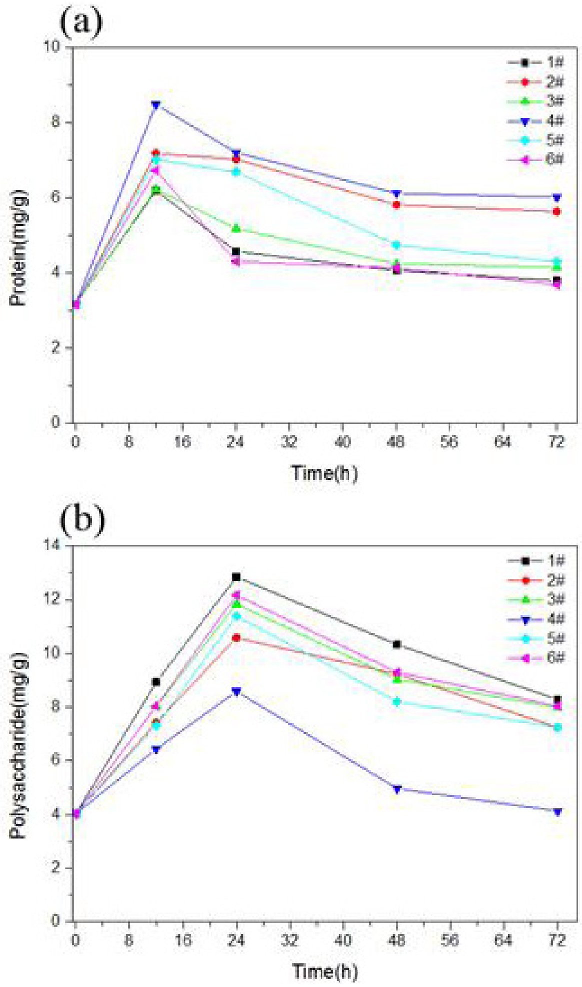

Fig. 4. Protein and polysaccharide content changes in wastewater treatment. (a) protein content curve; (b) polysaccharide content curve.

The above experimental results indicate that DHA and protein content in #4 were at high states. Thus, the microorganisms in #4 possessed high biomass and biological activity. Therefore, the COD removal of refractory wastewater was effective under the conditions in #4.

3.2. Changes of microbial community in activated sludge

3.2.1. Microbial diversity of activated sludge

The Shannon–Wiener index of bacterial species diversity shows the heterogeneity of measured communities (a higher heterogeneity indicates a more dispersed profile of an organism). The Simpson concordance and evenness indexes show that among various individuals, the more the individuals are evenly distributed, the higher the *H* value is. If each individual belongs to different species, then the diversity index will be high. Conversely, if each individual belongs to the same species, then the diversity index is minimal. Table 3 shows the results of the microbial diversity index of the activated sludge in different experimental groups. All diversity indexes in #3 were lower than the remainder of the groups. Together with the results of COD removal in wastewater, the microorganisms could only use organic pollutants in wastewater as C source because only N source was added in #3. The microorganisms in #3 indicated a negative removal effect on these refractory pollutants. Therefore, adding appropriate proportions of C source could enhance the diversity of microorganisms in the activated sludge (the microbial diversity index is higher in the experimental group than the initial sludge (IS)), and only adding N source was not effective in COD removal. Simultaneously, the diversity indexes in #4, #5, and #6 were in a relatively high state compared with the previous groups. This result showed that the diversity of microorganisms in the activated sludge can be promoted by adding C and N sources and different C/N ratios may result in various amplitudes. Among the seven samples, #4 had the highest diversity indexes and the best COD removal effect. Therefore, a high diversity of the indexes of microorganisms in the activated sludge indicates an improved removal effect.

3.2.2. Analysis of microbial similarity clustering

Fig. 5 shows the cluster analysis of the similarity in activated sludge samples with different treatment processes using the average connection (between groups) by using SPSS 20.0 software. The similarity between the IS and the remaining six groups varied significantly. Most of the microorganisms in the IS were neither able to adopt in the wastewater and die nor mutate, thereby leading to changes in their microbial community structure. Groups in #4, #5, and #6 had the highest similarity because C and N sources were added in the sludge during the wastewater treatment process and formed a similar ecological environment. Together with the analysis of microbial diversity index in the activated sludge, the bacterial populations in #4 were the largest, the species was the most abundant, and the effect of wastewater treatment was the best. In #3, the microorganisms were only able to use the organics in the wastewater because only the N source was added. These organic compounds inhibited the growth of microorganisms and caused their diversity to be low. The similarity analysis showed that the microbial community in #3 was easily destroyed and led to the decrease of community diversity. All these phenomena caused the species of microorganisms to become considerably simple, and the wastewater treatment indicated a negative effect [23].

Table 3
Test results of microbial diversity index in the activated sludge at different treatments

Sample	Simpson (*J*)	Shannon (*H*)	Evenness	Brillouin	McIntosh (*Dmc*)
IS	0.6875	2.3171	0.6463	2.0876	0.483
#1	0.7684	3.1656	0.6904	2.7585	0.5671
#2	0.801	3.2496	0.6998	2.8336	0.6053
#3	0.4456	1.8611	0.4381	1.5666	0.2807
#4	0.9053	3.7987	0.8649	3.3782	0.7531
#5	0.8917	4.1608	0.8112	3.579	0.7301
#6	0.8641	3.7235	0.7665	3.2269	0.6892

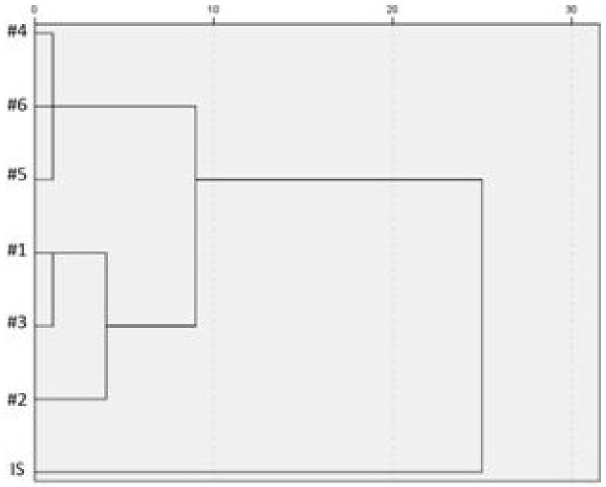

Fig. 5. Unweighted pair group method with arithmetic mean cluster analysis of different samples.

3.2.3. Analysis of microbial community structures and their potential functions

The activated sludge bacterial community of different experimental groups at the compositions of phyla was investigated (Fig. 6). The relative abundances of uncultured bacteria were 81.59% of total reads in the IS. When the uncultured bacteria were ignored, the relative abundance of *Proteobacteria* (4.17% of total reads) in the IS was less than all experimental groups. As shown in all the experimental groups, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were the dominant species, and *Actinobacteria* were present but their abundances were lower than that in the IS. In #4, the abundances of *Proteobacteria* (11.41% of total reads) and *Firmicutes* (15.76% of total reads) were high, and other species, such as *Cyanobacteria*, *Chloroflexi*, *Acidobacteria*, and *Verrucomicrobia* were also found and occupied a certain proportion. Increasing the types of microorganisms with high microbial diversity in the activated sludge could diversify metabolic pathways, enhance metabolic compensation capability, and improve the effect of wastewater treatment. However, the analysis on the level of phyla could not effectively indicate the shifts of specific microorganism species in wastewater treatment.

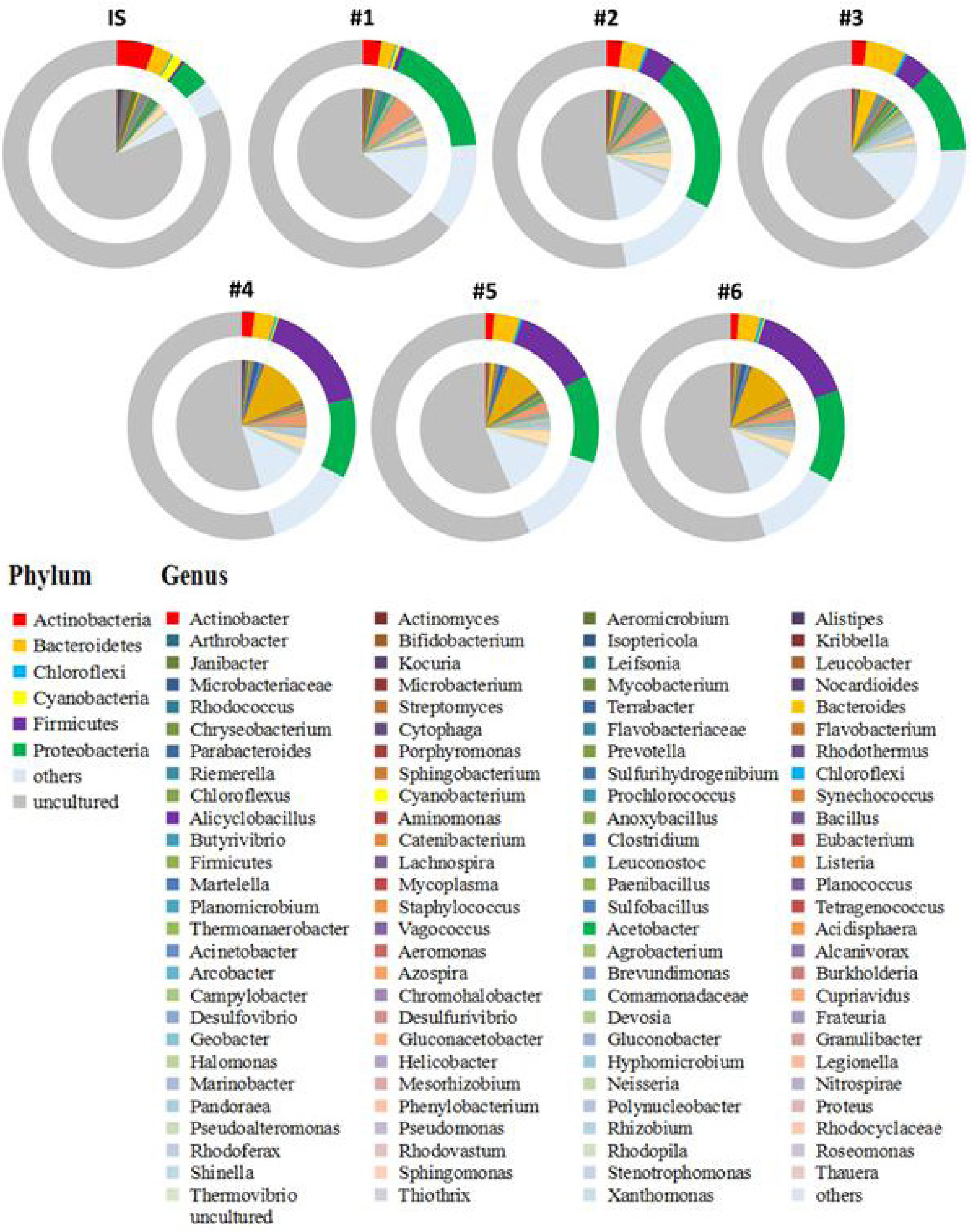

Fig. 6. Double pie charts of microbial communities in different wastewater treatments. The inner and outer pies represent the compositions of phyla and genera, respectively.

Community shifts were also investigated at the genus level (Fig. 7). Given the inability to obtain considerable information on the phyla level, several representative bacteria from more than 10,000 bacteria eventually detected by the sludge used in the experiment were selected for analysis. *Mycobacterium* and *Prevotella* were constantly the dominant species at the beginning of the experiment. However, the abundances were declining as the experiment progressed not only because this refractory wastewater inhibited the growth of the bacteria but also because the added nutrients were not used efficiently. Thus, the two types of bacteria were dominant in the IS and decreased in all experimental groups. However, these bacteria did not disappear completely, thereby indicating that they could finally adapt to the environment. By contrast, *Burkholderia* and *Leuconostoc*, which did not exist in the IS, could gradually grow and be

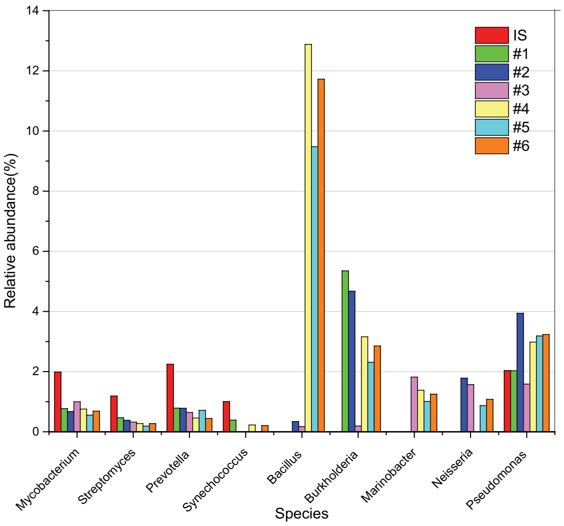

Fig. 7. Comparative analysis of dominant species in different experimental groups. Dominant species that represent more than 1% of total reads from each group are selected for analysis.

dominant. At the same time, *Pseudomonas*, which was one of the several bacteria commonly found in wastewater treatment, did not occur in significant proportions in the IS. In each experimental group, *Pseudomonas* was clearly a dominant species and played a key role in COD removal of wastewater. In addition, adding exogenous nutrients during wastewater treatment could effectively increase the abundance of *Bacillus* in the sludge, and the abundances of *Bacillus* in #4, #5, and #6 could all reach approximately 10% of the total reads, which were significantly higher than those of #2 and #3. Thus, *Bacillus* could play a positive role in refractory wastewater treatment.

Actinomycetes could be adopted in extreme environmental conditions in many wastewater treatments [24]. However, it did not effectively use the refractory wastewater and its abundance was gradually reduced. By contrast, *Firmicutes* could effectively grow in wastewater, thereby significantly increasing its abundance. In the activated sludge process, a certain number of denitrifying bacteria, such as *Pseudomonas* and *Bacillus*, existed, which possessed the potential to remove toxic or refractory contaminants in wastewater treatment and thus contributed to COD removal in wastewater [25]. *Burkholderia* grew well and became the dominant species in the high-salinity, refractory wastewater and it was an excellent bioflocculant that could effectively remove COD [26].

4. Conclusions

Adding exogenous nutrients can effectively promote the growth of microorganisms in activated sludge. Compared with conventional methods for activating sludge, adding exogenous nutrients in activated sludge with a suitable ratio (C/N ratio of 5:1 in the experiment) can change the microbial community structure of the activated sludge and increase the abundances of microorganisms, such as *Pseudomonas* and *Bacillus*, which played a positive role in wastewater treatment. The mechanism of the promotion of COD removal by the addition of exogenous nutrients in refractory wastewater was revealed, through combined analysis of dehydrogenase activity, EPS contents, and microbial community structure. Thus, the effects of exogeneous nutrients on wastewater treatment were higher. When the added exogenous nutrients had an unreasonable C/N ratio, the growth of microorganisms was inhibited in the activated sludge, indicating the hindered growth of the activated sludge and resulting in poor removal of wastewater. Adding appropriate proportion of exogenous nutrients in the activated sludge was feasible for biochemical treatment of refractory wastewater. The result showed that the COD removal rate of Sequencing Batch Reactor (SBR) can reach approximately 85% and the COD removal rate of hydrolytic acidification-SBR can reach approximately 90%. However, the process used in this study reached a COD removal rate of 90% and achieved the effect of several biochemical combination processes on wastewater treatment. In contrast to the combined process, the process used in this study exhibited characteristics of simple facilities and low energy consumption and thus can be applied to refractory wastewater treatment. Therefore, we can try to add other exogenous nutrients in the practical application, such as some sugary industrial by-products, to replace the glucose used in the experiment.

Acknowledgments

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