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Application of biological island grids in wastewater treatment and its microbial mechanisms

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ABSTRACT

Biological island grid system (BIGS) is a new type of biological floating island which allows for larger bacterial population to grow in the system. In this study, the nutrient-removal efficiencies of two different BIGS, constructed with *Oenanthe javanica* and *Iris pseudacorus* were investigated. Molecular technique was used to study the microbial mechanisms of pollutants removal. Results showed that BIGS had an excellent long-term removal performance compared with conventional biological floating island. The NH_4^+-N-removal efficiencies of BIGS were 89.5 and 91.2%, which were 16.3 and 16.9% higher than the control, respectively. Over 75% of chemical oxygen demand (COD) was removed in both BIGS while the COD-removal efficiencies were only 71.4 and 69.1% in the control. Analysis of DGGE pattern showed that the diversity index of the elastic space in BIGS was 1.93–2.65. Furthermore, month played a main role in microbial community structures. About 46.2% of the microorganisms in the system belonged to *Proteobacteria*, followed by uncultured bacteria. There were lots of nitrogen-fixing and nitrate-degradation bacteria among them, which might play an important role in the nitrogen removal from the polluted water. The present study proved that BIGS was an effective approach to improve water quality.

Keywords: Biological island grid system; Removal efficiency; Microbial mechanisms

1. Introduction

Constructed wetlands are engineered systems that have been designed to utilize the natural processes including soils, wetland vegetation, and their associated microbial assemblages to assist in treating wastewater [1]. Compared with conventional constructed wetlands, the biological floating island can be introduced into almost any existing water system, regardless of depth or shape [2]. The effectiveness of the biological floating island has been investigated in several studies [3,4]. One full-strength cattail wetland could remove 534 g/m^2 of N and 79 g/m^2 of P, respectively [5].

The biological floating islands reduce excess aquatic nutrients biologically by a combination of plants

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and microbes. Plants use nitrate as a nutrient source, and anoxic bacteria utilize it as a respiratory electron acceptor [2]. The extent of wastewater treatment in constructed wetlands depends on the wetland design, types of plants involved, and the microbial community [6]. Various studies have investigated the use of floating wetland platforms or the plant selection in structure optimization to enhance wastewater treatment capacity [7]. However, a few studies have focused on improving the removal efficiency by increasing the habitat of micro-organism.

Our group designed a new type of biological floating island named biological island grid system (BIGS). BIGS increases the substrate surface area by adding elastic space packing under the conventional biological floating island, thus forming a new habitat for micro-organism. The operation results of eight months showed that the BIGS performed better than the biological floating island by enhancing its microbial function [8]. The objectives of this study were to investigate the pollutants removal efficiencies of the biological island grid and the microbial mechanism of removing pollutants.

2. Materials and methods

2.1. Experimental design

The present study was conducted in the Baihua Garden of Jinan, Shandong, China, from May to November in 2011. This area was characterized as a temperate monsoon climate, with an annual average air temperature of 14.7°C. The annual precipitation is about 671 mm.

Eight pilot-scale BIGS were constructed in polyvinylchloride plastic tanks with a size of 40 cm × 50 cm (diameter × depth). In the under part of each tank, a water faucet was installed as waterspout in order to take water samples. Rigid polymer resin plates were stuck to the tanks to support the plants. On the plates, there were 2.5 cm diameter holes from which plants were settled with sponge wrapped around their roots.

Two types of hygrophyte, *Oenanthe javanica* and *Iris pseudacorus* were used in BIGS, which were named as OJBIGS and IPBIGS, respectively. The plants were transplanted from Nansi Lake, and planted with the density of 40 and 48 plants/m^2 for *O. javanica* and *I. pseudoacorus*, respectively. The experimental facilities are shown in Fig. 1. Below the resin plate, elastic space packing were hanged about 25 cm under water to increase the microbial adhesion area and improve the pollutants removal efficiency. Systems without elastic space packing were set as control, named as OJBIGS-C and IPBIGS-C, respectively.

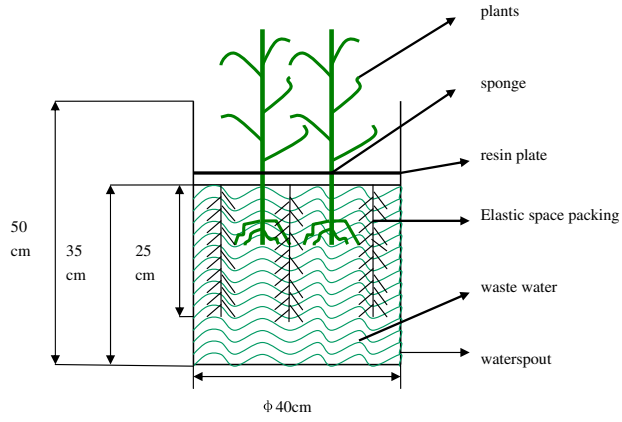

Fig. 1. Schematic diagram of the pilot-scale BIGS.

Synthetic wastewater was used to simulate the effluent of sewage treatment plant, with slight modification. According to the test, it had chemical oxygen demand (COD) 64.54 ± 12.31 mg/L, NH_4^+-N 4.45 ± 0.70 mg/L, pH 7.38 ± 0.57, dissolved oxygen (DO) 8.05 ± 1.95 mg/L, total nitrogen (TN) 7.13 ± 1.01 mg/L, total phosphorus (TP) 1.41 ± 0.38 mg/L.

2.2. Analysis of COD, TN, TP, and NH_4^+-N

Water samples were collected in polyethylene plastic bottles of 100 mL every other day at 8:00–9:00 am for the analysis of water-quality parameters. Once samples were collected, water pH and temperature were tested and DO measured with a HQ30d 53LED-TM dissolved oxygen analyzer immediately (HACH, USA). Standard methods (APHA, 2005) were followed for analysis of COD, NH_4^+-N, TN, and TP [9].

2.3. DNA extraction and PCR-DGGE

Microbial samples were collected from the surface of the elastic space of 15–20 cm under water at the beginning of every month from June to October, which were named Y-6, Y-7, Y-8, Y-9, and Y-10 in IPBIGS and S-6, S-7, S-8, S-9, and S-10 in OJBIGS, respectively.

Soil DNA rapid Isolation kit (Bioteke Ltd, China) was used for the DNA extraction. DNA quality was detected through agarose gel electrophoresis (1%) which was stained with ethidium bromide solution. PCR universal primer pair F338-GC (5′-CGC CCG CCG CGC GCG GCG GGG GCG GGG GCA CGG GGG GAC TCC TAC GGA GGC AGC AG-3′) and R518 (5′-ATT ACC GCG GCT GCT GG-3′) were used to amplify 16S rRNA region of about 200 bp [10].

Each 25 μL PCR reaction included: 0.5 μL of the DNA extract, 0.01 μM each primer, 500 μM dNTP, 2.5 μL of 10-fold PCR buffer, 1 U of Taq DNA polymerase, and sterile deionized water to a final volume of 25 μL. PCR amplification was performed under the following conditions: 95℃ for 4 min; 20 cycles consisting of 95℃ for 1 min, landing annealing at 63–53℃ for 1 min, and 72℃ for 1 min. Then, 20 cycles consisting of 95℃ for 1 min, 53℃ for 1 min, and 72℃ for 1 min, and a final extension step at 72℃ for 7 min. The presence of PCR products was confirmed by electrophoresis on 1.5% agarose gels stained with ethidium bromide. DCode TM475 System (Bio-Rad, USA) was used to perform DGGE analysis. The gels were made with a denaturing gradient ranging from 30 to 50%. Thirty-two microliter PCR products together with 8 μL 6-fold buffer were loaded per well and run at 120 V for 7.5 h. Gels were stained with ethidium bromide solution and images were captured using a Bio-Rad gel documentation system (Bio-Rad, USA). DGGE patterns were analyzed with Quantity One 4.6.

The similarity matrix of the microbial community was examined by the Dice index: CS. Its equation is:

$$C_S = \frac{2j}{a+b} \times 100 \quad (1)$$

where a is the number of bands in one lane, b is the number of bands in another lane, and j refers to the sharing bands in the two lanes. Dendrogram of the DGGE profiles were generated on the base of unweighted pair-group method average.

2.4. Cloning, sequencing, and identifying of DNA fragments

The desired DGGE bands were excised and rinsed in 70% alcohol and TE solution two times separately, then dissolved in 20 μL sterile deionizer water and used as PCR template. PCR was performed as above except with the primer F338 without a GC clamp. The PCR products were purified, cloned, and then screened according to the Dong et al. report [11]. The gene fragment clones were sequenced by SHANGHAI GENESTAR BIotech Co., Ltd. Sequences were compared in GenBank database (www.ncbi.nlm.nih.gov) by using the BLAST. The BioEdit program was used to edit, assemble, and align all gene sequences including the reference sequences obtained from GenBank database. The sequence distance matrix for all pairwise sequence combinations was analyzed by the use of MEGA 4 with the neighbor-joining method of phylogenetic tree construction.

3. Results

3.1. The pollutants removal efficiencies

During the study period, the maximum temperature was 28.3℃ (in July) while the minimum temperature was 15.9℃ (in October). The average pH value of OJBIGS and IPBIGS were 7.17 ± 0.76 and 7.19 ± 0.75, respectively. And the average DO concentrations were 4.53 ± 0.62 mg/L and 4.22 ± 0.84 mg/L, respectively.

The differences in pollutant removal performance between OJBIGS and OJBIGS-C during 6 months were shown in Fig. 2. For NH_4^+-N, the mean removal efficiency of OJBIGS was 89.5%, which was 16.3% higher than that of OJBIGS-C's. For COD, the mean removal efficiency of OJBIGS and OJBIGS-C were 79.2 and 71.4%, respectively, of which the difference was 7.9%.

Differences were found ($p < 0.5$) between the mean removal efficiency of IPBIGS and IPBIGS-C (Fig. 3). In IPBIGS, removal efficiency for NH_4^+-N and COD reached 91.2 and 78.8%, respectively. However, in IPBIGS-C, they were only 74.3 and 69.1%, separately. The difference of the removal efficiency of NH_4^+-N and COD were 16.9 and 9.7%, respectively.

The effluent concentrations of NH_4^+-N and COD in OJBIGS were 0.47 and 13.41 mg/L; and in IPBIGS, the concentrations were 0.39 and 13.67 mg/L, respectively. The water quality of effluent could reach standard III of the groundwater. However, the removal efficiency was lower for TN and TP (about 70 and 56%, respectively) and the concentration of TN and TP in the effluent water was 1.33–3.18 and 0.36–0.85 mg/L, respectively, which were higher than standard III of the groundwater.

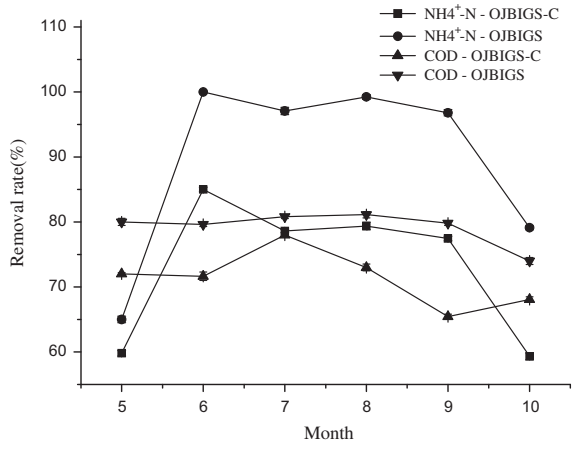

Fig. 2. The comparison of the removal rate of NH_4^+-N, COD treated by OJBIGS, and OJBIGS-C.

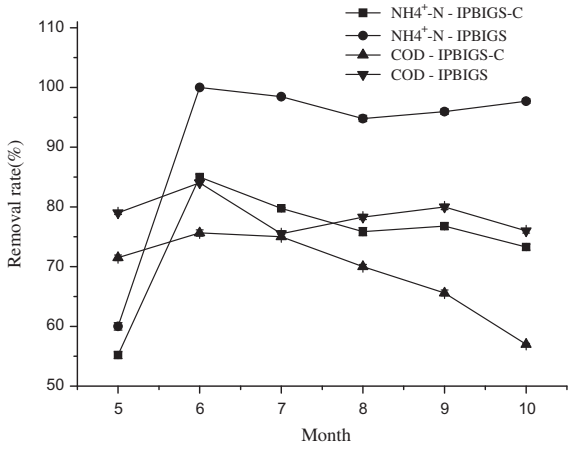

Fig. 3. The comparison of the removal rate of NH_4^+-N, COD treated by IPBIGS, and IPBIGS-C.

3.2. The microbial mechanism of pollutants removal

3.2.1. Bacterial community diversity based on PCR-DGGE and Cluster analysis

The bacterial diversity of all samples was evaluated by DGGE analysis of the amplified partial 16S rRNA genes. The DGGE pattern showed the changing of dominating band in intensity and richness according to different samples and in different months (Fig. 4). Bands 1 and 2 existed in all the systems, indicating that these bacteria existed steadily in all the samples. Band 7, 8, 9, 12, 13, and 14 only appeared in one or two samples, indicating that the microbial community was gradually changed with time in different systems.

Based on the values of the resulting matrix, a cluster analysis was performed and the 10 different samples were visualized in dendrograms. It was demonstrated that the bacterial community profiles were clustered into two groups (Fig. 3), group 1 (S-6, Y-6, S-8, Y-8, and Y-9) and group 2 (S-7, Y-7, S-10, Y-10, and S-9). This indicated that the differences of month had more important impact on the microbial community structure while different species had little effect.

3.2.2. Microbial structure analysis

Shannon-Winner Index was used to indicate the microbial diversity. In IPBIGS and OJBIGS, the H' values of the systems were 1.93 and 2.65, respectively (Fig. 5). There was a similar change pattern in two systems. The H' values were relatively high in June and August but decreased a little in July and September. In October, the *I. pseudacorus* system showed the lowest diversity while the *O. javanica* system showed a slight increase.

The similarity analysis showed that most of the strains had higher similarity to the 16S rDNA sequences deposited in enormous GenBank database, which had a similarity of more than 95% (Table 1). All the best matched bacteria were environmental species. Among them, five unculturable bacterium strains were detected.

A neighbor-joining phylogenetic tree was constructed to visualize the relationship between the sequences from wetland systems and related organisms from the GenBank database (Fig. 6). The detected populations were related to several methanotrophic

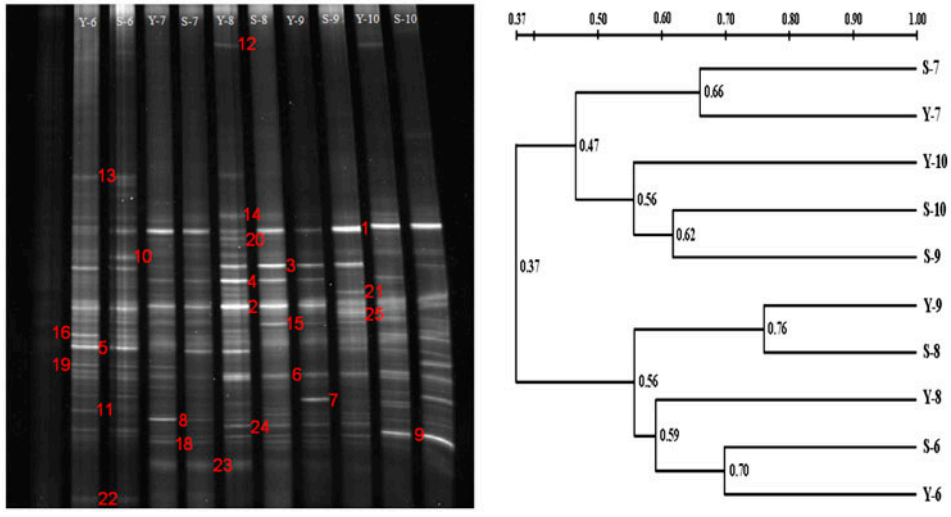

Fig. 4. DGGE analysis of 16S rRNA fragments (Left) and Cluster analysis (Right).

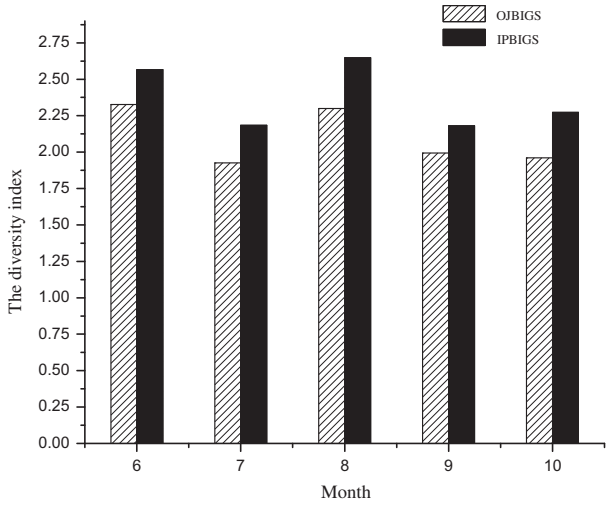

Fig. 5. The diversity index of island grid system.

genera, including *Gamma proteobacteria*, *Bacteroidetes*, *Alpha proteobacteria*, and *Delta proteobacteria*. *Gamma proteobacteria* were the most prevalent, which made up 46.2% of the total samples while there were 30.8% belonging to unculturable bacterium.

4. Discussion

The pollutants removal efficiencies of both OJBIGS and IPBIGS were investigated. Results showed the NH_4^+-N and COD-removal efficiency were high, while it was lower for TN and TP. This was consistent with conventional constructed wetlands in which removal of TN would be a little low because of low nitrification–denitrification [12,13]. Also phosphorus removal was often limited because the only sustainable mechanism for phosphorus removal was plant uptake and subsequent harvesting, also the flooded topsoils in the systems were net sources of P [14, 15].

The bacterial community of the systems has been extensively studied owing to its importance in the function of wastewater treatment [15, 16]. More surface area allows for a larger bacterial population and activity which leads to greater nutrient uptake. Experiments indicated that the biological floating island which differed from conventional constructed wetlands in that the macrophytes extend roots into the water and provide an additional submerged surface area to support the growth of microbes, could remove nitrate about 10 times faster than that of conventional constructed wetlands [2]. In this experiment, the substrate surface area was further increased by adding elastic space packing to increase microbial quantity. The experiments showed that the OJBIGS and IPBIGS removed NH_4^+-N and COD higher than the controls, indicating that biological island grids, which could increase the substrate surface, was an effective method to improve water quality.

In the systems, *I. pseudacorus* and *O. javanica* were used. The cluster analysis showed that the two systems had a similar community structure, particularly in the early period. Furthermore, it indicated that the month had an important impact on the microbial community structure, which was consistent with the results of Rohr et al. that over the course of the year major qualitative and quantitative changes occurred in the structure of the bacteria while different species have little effect [17]. Water temperature in different months were likely to influence the microbial community's structure and then affect the removal efficiency by affecting microbial-mediated reactions, such as mineralization, nitrification, and denitrification [18, 19]. For instance,

Table 1
Bacterial 16S rRNA gene sequences obtained from the systems

Band	Best match database	Similarity (%)
G1	Uncultured bacterium clone F1Q32TO05GI15R(GU501860.1)	99
G2	*Sphingomonas* sp. BAC84(EU131006.1)	99
G3	*Aeromonas veronii* strain w-s-06(JF490065.1)	99
G4	*Thiothrix eikelboomii* strain AP3(NR024758.1)	98
G5	*Pseudomonas fluorescens* strain NFL16(GQ496662.1)	99
G6	Uncultured bacterium clone W_0307_3(GQ379420.1)	99
G7	Uncultured *Bacteroidetes* bacterium clone IRD18H08(AY947982.1)	99
G12	*Bacillus funiculus* strain L2–1(JN941306.1)	98
G14	Uncultured bacterium clone F1Q32TO03C2VWE(GU747550.1)	94
G15	Uncultured bacterium clone:12C-A83(AB205821.1)	95
G16	Uncultured *Bdellovibrio* sp. clone Naukuchia-3(FJ405270.1)	99
G20	Uncultured bacterium clone SBfYyy35(HE574377.1)	100
G25	Uncultured *Rhizobium* sp. clone Cvi50(FJ712870.1)	99

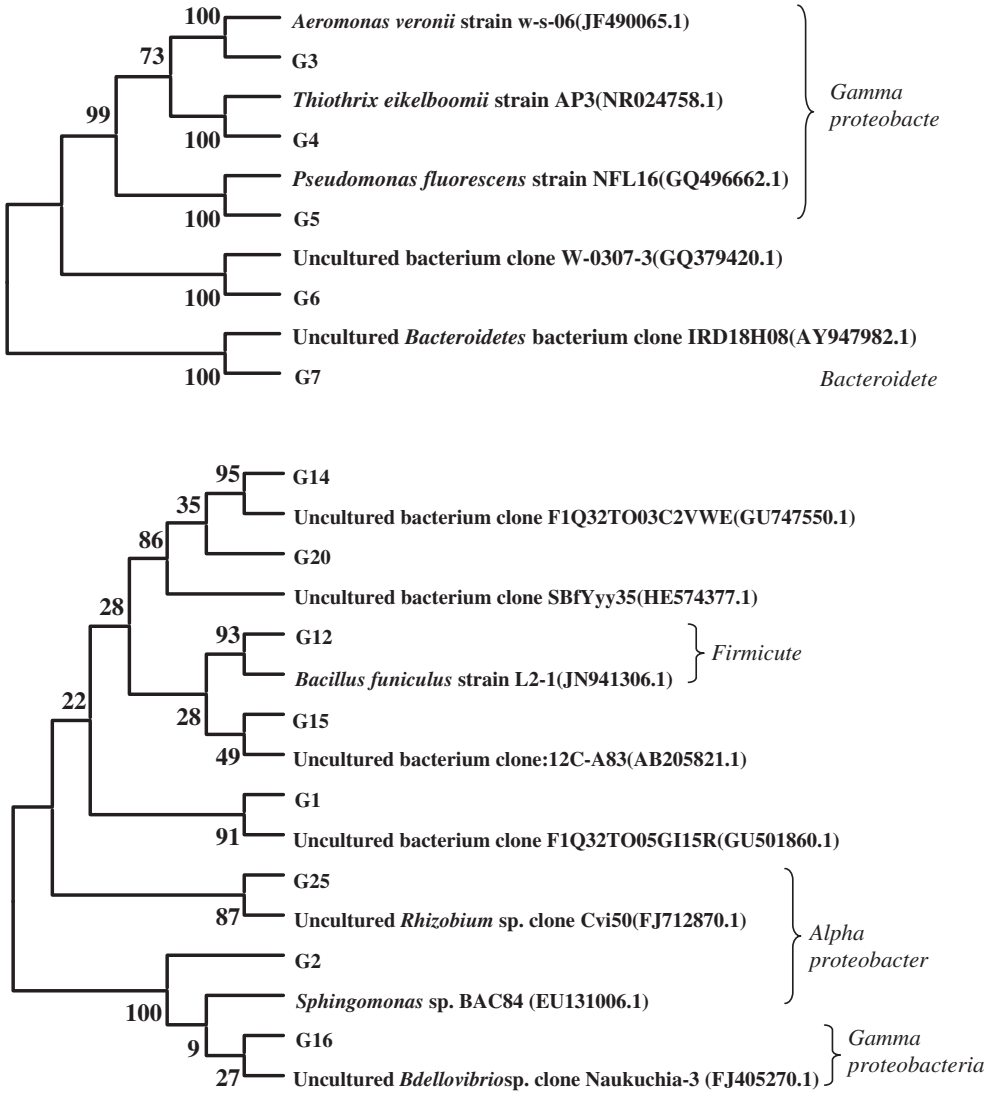

Fig. 6. Phylogenetic relationship between the 16S rRNA gene sequences from wetlands system and related organisms from the GenBank database.

research showed that the denitrification activity increased when there was a rise in the water temperature [20]. The root exudation may also affect the bacterial community [21, 22].

In different systems and in different months, the bacterial community structures were different (Fig. 1). These results suggested that the microbial populations gradually changed during the operation process. Some existed steadily in all the systems indicating that they had an important role in microbial-mediated processes, which perhaps were important to wetlands processes such as denitrification, nitrification, and other enzyme activities [23]. While some other microorganism was sensitive to environmental changes and died off gradually. The band G5 showed 99% similarity with *Pseudomonas fluorescens* strain NFL16, which is popular in soil and water and could use nitrate instead of oxygen as a final electron acceptor during cellular respiration [24]. So the presence of such bacteria in our systems for wastewater treatment could improve the capabilities of N nutrient removal. B and G3, which showed 99% similarity with *Aeromonas veronii* strain; w-s-06 was also detected in membrane biofilm system of microfiltration plant for drinking water treatment as a pathogen [25], which indicated that pathogens also grew in the systems. The phylogenetic tree showed that five different types of unculturable bacteria were detected in the wetland system. This result was consistent with many other researches that detected a lot of the unknown bacteria [26, 27]. Bands G1, G6, G7, G14, G15, G16, G20, and G25 had close relationships with unculturable denitrification bacteria

whose activities might contribute to the nutrient removal [11].

In conclusion, BIGS was an effective approach to improve water quality. Compared to conventional biological floating island, it could increase COD-removal efficiency by 7.9 and 9.7% and NH_4^+-N-removal efficiency by 16.3 and 16.9%. The higher pollutant removal efficiency was caused by the additional submerged surface area which could support the growth of more microbes. And the month was the major factor affecting the microbial community structures.

Acknowledgements

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