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Microbial mechanisms of using enhanced ecological floating beds for eutrophic water improvement

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HIGHLIGHTS

- EEFBs had significant removal efficiencies of organics and nitrogen.
- Microbial community structure had obvious differences between devices.
- Bacteria were mainly attached on the fiber filling.
- The majority of bacteria on plant roots were *β-Proteobacteria* and *α-Proteobacteria*.

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ABSTRACT

Enhanced ecological floating beds were implemented to reduce nutrient quantity and improve the water quality of a eutrophic lake. The results showed that average removal efficiencies of COD_{Cr}, total nitrogen, NH_3-N and total phosphorus for *Canna indica* L. set-up were 23.1%, 15.3%, 18.1% and 19.4% higher, respectively, than that of the setup with only substrate, and 14.2%, 12.8%, 7.9% and 11.9% higher than *Iris pseudacorus* L. ecological floating bed. The microbial community structure had obvious differences between devices and low similarity; bacteria were mainly attached on the fiber filling. The microbial population was abundant at the start and end of the experiment. Shannon index of samples selected ranged from 0.85 to 1.05. The sequencing results showed that fiber filling collected most uncultured bacteria species and the majority of bacteria on the plant roots were *β-Proteobacteria* and *α-Proteobacteria*. The co-dominant species attaching to the filling and plant was *Nitrosomonadaceae*.

1. Introduction

From the latter half of the last century, there has been increasing concern over the elevated nutrient status and eutrophication of rivers and lakes (Sierp et al., 2009; Zhao et al., 2012b). Over the past 20 years, eutrophication has played a major role in a number of serious disasters (Zhao et al., 2012a,b). One of the most widespread examples of pollution is eutrophication due to inputs of large quantities of inorganic nutrients, particularly nitrogen and phosphorus, to freshwater rivers, lakes, streams and reservoirs (Yang et al., 2008b; Zhu et al., 2011). Excessive nitrogen discharged into water can cause serious ecological problems such as eutrophication, algae blooms and habitat degradation in lakes and rivers. It was reported that eutrophication due to nitrogen pollution was serious in some lakes and reservoirs that are nationally protected basins in China (Chen et al., 2014; Yang et al., 2008a). China's water pollution situation is not optimistic, and the shortage of water resources makes the situation even worse. The data in the report of "2014 environment quality bulletin" showed that in 62 lakes (reservoirs) monitored in China, the water quality of 20 lakes (reservoirs) met the category III water quality standards, 15 lakes (reservoirs) met the category IV water quality standards, four lakes (reservoirs) met the category V water quality standards, and five lakes (reservoirs) were worse than category V water quality standards (China's environmental bulletin in 2014, 2015).

Eutrophication has become a matter of concern in aquatic ecological research. It has therefore become necessary to reduce nitrogen (N) levels in river water, and an increasing number of researchers are focusing their efforts on limiting eutrophication. Conventional methods of removing nitrogen from wastewater and natural lakes include physical, chemical and biological approaches. Biological aerobic nitrification and anaerobic denitrification have been found to be reliable and should be encouraged due to their easy implementation and efficiency (Borges et al.,

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2003). Macrophytes have been widely applied in ecological engineering for the treatment of surface water and wastewater due mainly to their efficacy in assimilating nutrients and in creating favorable conditions for the microbial decomposition of organic matter (Bustamante et al., 2011; Li et al., 2010). Ecological restoration technology has been widely used in landscape water treatment. Ecological floating beds (EFBs) have the unique advantage of occupying no land area when compared with conventional macrophyte-based constructed wetlands in the hydrophytic ecosystem (Huang et al., 2013; Iamchaturapatr et al., 2007; Li et al., 2010; Sun et al., 2009;) Microbes in enhanced ecological floating beds are fixed mainly on the fiber fillers and plant roots. Microbes can effectively decompose or mineralize organic matter and gain energy and material. At the same time, microbes can provide food for aquatic animals and provide nutrients or substances. They are an important part of the food chain of the water ecosystem. Plant absorption and microbial decomposition result in water quality purification and governance.

An enhanced ecological floating bed (EEFB) is a type of ecological restoration strengthening device, composed mainly of microbial carrier packing and aquatic plants. The device uses the synergy of aquatic plants and microbes to complete the function of ecological restoration. A larger biomass can be enriched within the limited space of the device to achieve fast and efficient treatment. Plants and microorganisms are important ecological factors of the enhanced ecological floating bed, as plants can absorb N, P and some inorganic salt in water. Assimilation is fixed in plants, allowing the harvest of plants to remove nitrogen and phosphorus from the aquatic environment. At the same time, the roots can attract microorganisms, enriching the microbial community, increasing the biomass and secreting oxygen to benefit the growth of aerobic microorganisms, completing the degradation function.

Biological grid microbes are mainly fixed on packing and plant roots. Microbes will effectively mineralize organic matter decomposition, to grow and gain energy and material while providing food for aquatic animals. Plants can absorb nutrients or substances that are mineralized by the microbes, making microorganisms an important part of the food chain in biological grid ecological systems. Plant absorption of the mineralized nutrient products of microbial decomposition result in water purification and governance.

Two types of phytoplankton (*Iris pseudacorus* L. and *Canna indica* L.) were selected for the study, each species placed on a biological grid. Monitoring the improvement of eutrophic landscape water quality would support remediation and was done through a comparison of the microbial populations between the plant root and fiber filling of the four pilot devices to study the effects of pollutant removal efficiency and the number of microbes. PCR–DGGE biological research was used in the biological grid system to identify the dominant bacteria, and the Shannon-Wiener diversity index was used to characterize the microbial community diversity and variation, laying a foundation for understanding microbial population dynamics and characteristics.

2. Material and methods

2.1. Laboratory-scale biological grid systems

Four parallel laboratory-scale EEFBs (Fig. 1) were developed. The size of the devices were 25 cm × 25 cm × 60 cm, the effective depth of the device was 55 cm, and the total volume was 34 L. Device 1 (D1) had only fiber fillers in it, and device 2 (D2) had only *Iris pseudacorus* L. Device 3 (D3) and 4 (D4) were containing *Iris pseudacorus* L. and *Canna indica* L. along with fiber filters. A styrofoam plate was placed in each device as a floating bed, and the size was 20 cm × 20 cm × 80 cm. Four planting holes approximately 3 cm × 3 cm large were made on each bed, and the distance between each hole was 10 cm. One to two plants were planted in the hole. Four strings of combination fillers were hung under the planting hole. There were five disks on each string, and the diameter of the disk was 8 cm. There were eight fiber fillers on each disk with a length of 5 cm, and the distance between each plastic disk was 8 cm. The water tank was built following the gravity flow design. All influent entered the reactor from bottom to upper, using rubber hoses to connect the water tank and reactor. There were five sampling sites on the side of each device and the distance between sampling sites was 10 cm. The experiment started after the plants and fiber fillers were cultured in the device for two weeks, when new roots grew out and created a biofilm on the fiber fillers.

2.2. Experiment conditions

Experiments were conducted from April to July of 2015, and the effects of eutrophic water quality improvement were monitored. Each pilot device could hold 8.5 L/d water, and the hydraulic retention time was 4 d. The flow velocity was controlled by adjusting the water stopping clips on the effluent of the water tank on each device. The influent water quality of the four pilot devices was the same.

Raw water was taken from an urban lake. The water quality of the raw water and the water quality standards (The national surface water environment quality standards, GB3838-2002) are shown in Table 1 (three parallel samples were monitored for each sample). It shows that the water quality of the raw water is worse than class V.

2.3. Sampling and analyses

The symbol of mature biofilms is that rotifers and *Vorticella* can be seen under a microscope. Microscopy was done before each experiment. The result showed that the density of rotifers was 1800a/g in dry membrane and that the biofilms were mature.

The growth of tertiary roots packing in with the filler showed that the ecosystem of enhanced ecological floating beds was constructed well. This type of structure has great surface area and is able to grow biofilms.

Water samples were collected in triplicate from both the inlet and outlet of each device and were analyzed immediately after collection. The pH value and dissolved oxygen (DO) concentration were immediately measured using a portable HACH DO/pH/Eh meter (HACH SensION + DO6). NO_3^--N, NH_3-N, total nitrogen and total phosphorus were measured following standard methods (APHA, 2005). COD_{Cr} was measured through a Hach DR2800 colorimeter according to standard calibration and operation.

The height and root length of plants were measured every week.

Plate counting of soil bacteria populations on Nutrient Agar medium was used to determine the total number of bacteria in the samples.

2.4. DNA extraction and PCR–DGGE analysis

At the beginning and the end of the experiment, bacterial DNA on plant roots and fiber fillers of four pilot devices were extracted with Soil DNA kits D5625-00 (Omega, USA) following the manufacturer's protocol. Extracted genomic DNA was detected by 1% agarose gel electrophoresis and stored at −20 °C. The bacterial 16S rRNA genes were amplified using universal forward primer EUBf933: 5′-GCCCGGGAAC GTATTCACCG-3′ and reverse primer EUBr1387: 5′-GCACAAGCGGTGGAGCATGTGG-3′, and the GC-clamp was connected to primer EUBf933 (Iwamoto et al., 2000).