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Redox zones stratification and the microbial community characteristics in a periphyton bioreactor

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HIGHLIGHTS

- Periphyton bioreactor greatly improved water quality of a water tank.
- Stratification of five redox zones was observed from top to bottom in a water column.
- Periphyton auto regulated its structure to adapt to aerobic/anaerobic environments.
- Periphyton kept high species richness and metabolic activity at various redox zones.

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ABSTRACT

Bioremediation techniques based on microorganisms have been widely applied to treat polluted surface water, but the efficiencies have been limited, especially in deep and static waters. Microbial aggregates, known as periphyton, were introduced into a tank bioreactor to improve pollutants removal and a periphyton bioreactor with an 84 cm column was built to investigate microbe–wastewater interactions. Periphyton greatly improved water quality and produced a distinct stratification in the water column into five redox zones with slight overlaps. From top to bottom these were: oxygen reduction, nitrate reduction, iron reduction, sulfate reduction and methanogenic zone. Periphyton communities had high species diversities (767–947 OTUs) with the facultative zone (middle layer) having higher species richness and functional diversity than the aerobic (top layer) and anaerobic zones (bottom layer). A good knowledge of interactions between periphyton and water column stratification could benefit from integration of periphyton to improve bioremediation of deep and static water.

1. Introduction

Surface water pollution is one of the major problems facing the industrialized world today (Alessi et al., 2014). The need to remediate the polluted sites has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal. Bioremediation generally refers to the use of organisms (e.g. plants, animals and microorganisms) to eliminate or reduce the concentrations of hazardous wastes at a contaminated site, such as water, soil and waste streams (Boopathy, 2000; Erickson et al., 1994; Wu et al., 2014). Bioremediation technologies can be broadly classified as *in-situ* and *ex-situ*.

In-situ bioremediation techniques refer to those where the treatment of the contaminated material takes place 'on site' and have been widely used in treating natural water ecosystems (Boopathy, 2000). For instance, these could include, planting a floating treatment bed (Hu et al., 2010), floating microalgal mat (Patidar et al., 2015) or using a column bioreactor (Alessi et al., 2014). Bioavailability of contaminants, bioactivity of organisms and characteristics of the polluted sites are major factors to be considered for *in-situ* bioremediation (Boopathy, 2000). For example, water column stratification, which is primarily caused by temperature gradients in the water column, can cause large differences in pH, DO, chemical composition and microbial community at different layers of the water column. Consequently, such stratification can reduce the convective mass transfer, microbial functions and finally the *in-situ* bioremediation efficiency of water ecosystems (Tadesse et al., 2004).

Ex-situ bioremediation techniques refer to those being applied to contaminated materials after having been transported from the site at which they were contaminated (Boopathy, 2000), for instance biological aerated filter (BAF), stabilization pond

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(Abou-Elela et al., 2015; Martins et al., 2013). However, long retention time, deep water column and relatively static flow conditions are the limitations suffered by these technologies, since, for example, suspended solid pollutants can easily settle to the bottom of these systems rendering them ineffectual (Abou-Elela et al., 2015; Martins et al., 2013). Consequently, it would induce differences in the chemical and/or microbial community composition at different sites or water layers and thereby limit the removal efficiency of contaminants.

Periphyton, composed of photoautotrophic microalgae, bacteria, fungi, protozoa and small multicellular animals, is an important ecological component of surface water and plays a major role in primary productivity, nutrient recycling and self-purification of surface water ecosystems (Larned, 2010; Wu et al., 2014). Periphyton is capable of assimilating and degrading various kinds of pollutants including inorganic nutrients, metals and organic matter (Li et al., 2012; Wicke et al., 2008; Wu et al., 2014). Frequently, periphyton has been used and/or incorporated in various types of bioreactors, such as hybrid bioreactor (Yan et al., 2011) and spiral periphyton bioreactor (Shangguan et al., 2015) for both *in-situ* and *ex-situ* bioremediations.

Moreover, periphyton with their variously complex composition can adapt to and also affect the mutable external environments (e.g. broad ranges of pH, salinity, temperature and light availability, aerobic/anaerobic conditions) and maintain a high metabolic activity by auto regulating its community structure (Larned, 2010; Shangguan et al., 2015; Wu et al., 2014; Yan et al., 2011). For instance, the photoautotrophic microalgae can consume CO_2 and produce O_2 through photosynthesis, which causes an increase in pH and dissolved oxygen in the water (Kesaano and Sims, 2014). The diverse species of bacteria can take up or degrade various kinds of pollutants from wastewater. *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Sphingobacteria* can efficiently degrade organic matter (Shangguan et al., 2015). Other groups of bacteria including species of *Achromobacter*, *Alcaligenes*, *Bacillus*, *Hydrogenophaga* and *Pseudomonas* have heterotrophic nitrification and aerobic denitrification capabilities, and thus can convert ammonium ion to nitrogen aerobically (Feng et al., 2015). Polyphosphate (polyP) bacteria such as *Pseudomonas* can accumulate large amounts of phosphorus (Oehmen et al., 2007). Thus, integration of periphyton with complex compositions into an *in-situ* and *ex-situ* bioremediation system (e.g. a column bioreactor) can probably improve the bioremediation efficiency of a water column.

With this background in mind, tank and column bioreactors with periphyton attaching to stuffing substrate were built with the objectives: (1) to investigate the potential of periphyton for bioremediation of a deep water column; (2) to determine the stratification and chemical characteristics of a water column; (3) to investigate the microbial characteristics (e.g. species composition and functional diversity) of periphyton at different layers of a water column.

2. Methods

2.1. Set-up of the tank periphyton bioreactor

To evaluate the nutrient removal efficiency of periphyton from wastewater, a periphyton tank bioreactor was built using a glass water tank (length 30 cm, width 20 cm, height 50 cm) and PVC elastic stuffing substrate (length 50 cm, diameter 8 cm, Yixing Yongda Environmental Protection Company, Suzhou, China, Fig. 1A). For periphyton formation, the stuffing substrate was immersed in 0.1 M HCl for 12 h for disinfection, and then lake water rich in microalgae and bacteria collected from Xuanwu Lake, Nanjing, China was added to the tank. The chemical composition of the lake water was: total nitrogen (TN) 1.90 mg L^{-1}, NO_3^-—N 0.73 mg L^{-1}, NH_4^+—N 0.53 mg L^{-1}, total phosphorus (TP) 0.1 mg L^{-1}, PO_4^{3-}—P 0.035 mg L^{-1}. Its pH was 7.8. After seven days, a green periphyton community on the substrate was visible to the naked eye. The lake water was replaced by BG-11 medium and the periphyton was enriched for two months until a dense brown biofilm had formed. Thereafter, periphyton on the substrate was taken out of the tank and washed with distilled water three times for use in the nutrient removal experiment.

For the nutrient removal experiment, water from the watershed of Dianchi, a hypereutrophic lake in Yunnan, China, was used as the wastewater and added to the water tank. The physical and chemical characteristics of the water was as follows: Transparency 18 ± 3 cm; pH 8.11 ± 0.20; dissolved oxygen (DO) 5.46 ± 1.20 mg L^{-1}; chemical oxygen demand (COD) 8.46 ± 2.31 mg L^{-1}; TP 0.52 ± 0.23 mg L^{-1}; TDP 0.16 ± 0.05 mg L^{-1}; TN 8.01 ± 0.34 mg L^{-1}; NH_4^+—N 2.65 ± 0.2 mg L^{-1}; NO_3^-—N 1.71 ± 0.18 mg L^{-1}. After one day of settlement, 1200 g wet periphyton (moisture content 90%) with substrate was placed in the tank. The four sides of the water tank were covered with opaque canvas to simulate the natural light condition of water column. This experiment was performed outdoors for 30 days with an average water temperature of 26 °C (20–32 °C), and this was done in triplicate. Another three water tanks without periphyton were used as controls.

2.2. Experimental design for mechanism consideration

To investigate the interactions between water column stratification and periphyton community, a vertical column bioreactor was designed. The bioreactor consisted of a water tank (length 30 cm, width 30 cm, height 45 cm) for storing wastewater or growth medium, a glass cylinder (internal diameter 14 cm, external diameter 15 cm and height 100 cm), 1 L glass tank for collecting waste liquid, supporting stand and connecting silicone tube (see schematic drawing in Fig. 1B and a physical map in Fig. 1C). On one side of the cylinder, there were 8 valves with an internal diameter of 0.5 cm placed at 12 cm intervals between each valve. Stuffing substrate (length 84 cm, diameter 8 cm) was installed in the cylinder for enriching and growing periphyton.

For periphyton growth in the column bioreactor, all the valves were closed and the lake water (as per Section 2.1) was added to the cylinder. After seven days, the lake water was replaced. First, fresh BG-11 medium was added to the water tank. Then, the top and bottom valves (Fig. 1C) were opened and thus the lake water was gradually replaced by BG-11 medium at a flow rate of 1 L h^{-1}. After replacement, these two valves were closed for two days and then reopened to create a continuous flow in the cylinder with a flow rate of 900 ml d^{-1}. To simulate the light conditions of a natural water ecosystem, the bottom part of the cylinder was partly covered with aluminum foil. This dynamic process lasted 30 days until obvious stratifications of periphyton were observed in the column.

2.3. Samples and analyses

2.3.1. Water samples

To evaluate nitrogen and phosphorus removal by periphyton in water tank, and to measure pH, DO, TN, NH_4^+—N, NO_3^-—N and TP, samples were collected on days 1, 4, 8, 14, 22 and 30.

To investigate the chemical composition of water from different layers of the column bioreactor during the dynamic cultivation, samples were collected every day from day 25 to 30 as below to measure NO_2^-, Fe^{2+}, S^{2-} and HCO_3^-. Initially, all the valves were closed. The valve at 12 cm (Fig. 1C) was opened first and after 3 s