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The enhanced survival of submerged macrophyte *Potamogeton malaianus* by sediment microbial fuel cells

Yan-Li Zhou[a,b], Hui-Fang Wu[c], Zai-Sheng Yan[a], Hai-Yuan Cai[a], He-Long Jiang[a,*]

[a] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China
[b] Graduate University of Chinese Academy of Sciences, China
[c] College of Environment, Nanjing University of Technology, Nanjing, China

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ABSTRACT

Submerged macrophytes can promote the water quality and then facilitate the maintenance of good ecological status in aquatic environments, but have been subjected to severe reduction due to eutrophication. This study investigated the effect of sediment microbial fuel cell (SMFC) on the growth of submerged macrophyte *Potamogeton malaianus* in degenerated sediments taken from a shallow eutrophic lake. Over 6 weeks, it was found that the growth of *P. malaianus* was significantly enhanced by both closed-circuit SMFC and open-circuit SMFC in term of survival rate and biomass contents. Survival rate of plants were 103.7% and 107.4% in closed-circuit and open-circuit SMFC treatments respectively, but only 18.5% with only plants without SMFC due to unfavorable environments in sediments. Obvious difference in physico-chemical properties of sediments was not observed in the three treatments. However, high-throughput sequencing showed higher abundances of iron-reducing *Geobacter*, *Desulfuromonas* and *Geothrix* in bulk sediments with closed-circuit SMFC, and plant growth-promoting *Pandoraea* and *Methylophilus* in sediments with open-circuit SMFC at the end of experiment. These distinct bacterial communities might regulate nutrients/trace metals availability to plants and then make some contribution to plant growth. SMFC employment thus provided an alternative strategy for re-establishment of aquatic macrophytes in sediment-contaminated aquatic environments.

1. Introduction

Submerged macrophytes serve as important functional and structural elements in lake ecosystems (Sondergaard et al., 2010). As reported previously, submerged macrophytes can promote the water quality and then facilitate the maintenance of good ecological system by stabilizing sediments and preventing them from re-suspension, providing fish and shrimp with refuge and places for laying eggs, impeding phosphate and ammonium release in the water column, and enhancing degradation of persistent toxic organic compounds (Jiang et al., 2008; Yan et al., 2011). Compared to emergent species, however, submerged macrophytes display lower resistance to pollution (Pulido et al., 2011).

In many lakes, submerged macrophyte abundance and species diversity have been dramatically reduced due to eutrophication. In order to ensure the successful restoration of these aquatic ecosystems, re-vegetation of macrophytes, especially submerged macrophytes, has become the most essential work. The growth and reproduction of submerged macrophytes are widely found to strongly depend on environmental factors such as sediment types. Sediments act as an anchoring substrate and a source of nutrients for rooted plants, but fertile sediments with high contents of nutrients and organic matters cause stress for submerged macrophytes and can substantially reduce the growth of submerged macrophytes (Cao et al., 2004; Pulido et al., 2011). Indeed, the mineralization of organic matter requires oxygen, and this process leads to a greater demand for oxygen within the sediments. Oxygen depletion also results in a series of chemical changes in the sediments, leading to the accumulation of several compounds that can be phytotoxic (Bornette and Puijalon, 2011). As a result, the sediment anoxia limits the growth of plants and induces a loss of biomass (Lemoine et al., 2012).

In eutrophic lakes, sediments are always found with high nutrient content and anoxia prevalence, and thus should be treated prior to macrophyte restoration (Handel et al., 1997). Currently, a few attempts like capping and dredging have been implemented for remediation and removal of contaminated sediments to create critical habitats for a variety of submerged macrophytes

* Corresponding author at: State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China.
E-mail address: hljiang@niglas.ac.cn (H.-L. Jiang).

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(Geurts et al., 2011; LaSalle, 1991), but these conventional methods were relatively costly (Jacobs and Förstner, 1999) and sometimes not environmentally friendly. Alternatively, *in situ* diverting the anaerobic metabolism pathways in sediments and lowering the production of phytotoxic compounds might be an effective and non-invasive approach to create a favorable environment for macrophyte growth.

As one of recently-developed technology, sediment microbial fuel cell (SMFC) altered oxidation-reduction potential and anaerobic metabolism pathways in sediments, and decreased the production of adverse and/or harmful compounds from sediments. Beside acting as power sources deployed in aquatic environments (Tender et al., 2008), SMFCs could also be explored as a new technology for the removal of organic pollutants from sediments (Huang et al., 2011).

Additionally, a plant sediment microbial fuel cell (plant-SMFC), which is a type of microbial fuel cell (MFC) with an anodic electrode embedded in a planted sediment layer and a cathode in the overlying water layer with closed electrical circuit, was developed to continuously utilize rhizodeposits excreted from living plants in the anode area and increase power production (De Schamphelaire et al., 2008; Strik et al., 2008). Up to now, plants like rice plants (De Schamphelaire et al., 2008), and emergent macrophytes *Spartina anglica* (Helder et al., 2012) and *Reed mannagrass* (Strik et al., 2008), have been utilized as model plants combined with MFCs. However, these previously studies just focused on power production rather than plant growth, and the effect of SMFC on the growth of submerged macrophytes in degenerated and harsh sediments was not known.

In this study, *Potamogeton malaianus*, one of the dominant submerged macrophytes in one shallow eutrophic lake Taihu in China (Dong et al., 2014), were selected to investigate how SMFC employment affected the growth status of submerged macrophytes. Laboratory macrophyte-SMFCs were constructed with sediment samples taken from heavy-contaminated zone in Taihu Lake. High-throughput illumina sequencing was applied to analyze the bacterial communities in bulk sediments, sediments around the roots, and on anodes. This study would be helpful in developing practical strategies to restore submerged macrophytes in aquatic environments.

2. Materials and methods

2.1. Sediment and lake water sampling and preparation

Samples of surface sediments were taken manually using a Pedersen grab sampler from the Meiliang Bay (31°30′ N, 120°11′ E) of Taihu Lake, a typical eutrophic shallow freshwater lake in China. As the most seriously polluted bay of the Lake Taihu, most area in Meiliang Bay had no macrophyte (Ye et al., 2007). Lake water was also collected from the same site. Then the samples were transported to laboratory within several hours. Plant debris and macrofauna in sediments were removed by hand-sieving through a 2-mm sieve. Then all sediments were homogenized by mixing with a big stainless steel ladle prior to use.

2.2. SMFC construction

SMFC in the experiment was constructed using plastic containers which was filled with 5 kg of lake sediments and lake water with a depth of 20 cm. In SMFC, two electrodes were placed horizontally, with anode 5 cm below the surface of sediments and cathode immediately above the sediment surface. Both anodes (20 cm diameter) and cathodes (20 cm diameter with three 7 cm diameter holes distributed as a triangle) were made of graphite felt with a thickness of 5 mm. These electrodes were firstly soaked in 1 M HCl for 24 h and were then washed thoroughly with deionized water before employment. The electrodes were connected to an external circuit using copper wires, and joints were sealed with conductive silver epoxy glue and insulating epoxy glue successively. The circuit was connected through a resistance of 1000 Ω for SMFC, and not connected for open-circuit SMFC.

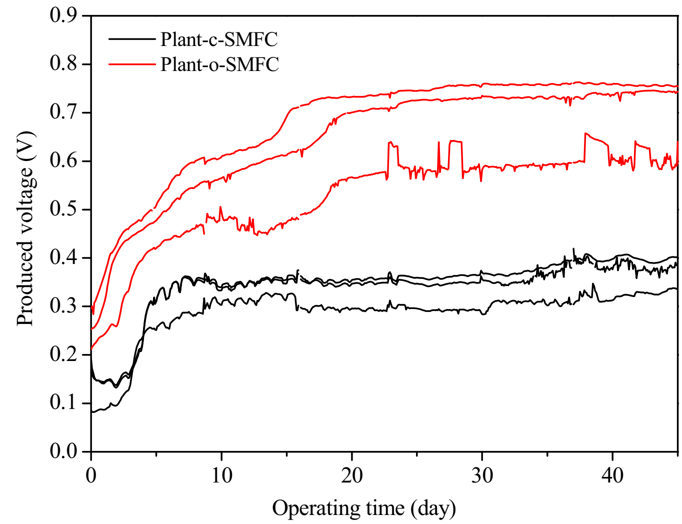

Fig. 1. Voltage generation from Plant-c-SMFC and Plant-o-SMFC (3 replicates) during experiments. Plant-c-SMFC means plant with closed-circuit SMFC, while Plant-o-SMFC means plant with open-circuit SMFC.

2.3. Experiment setup

Submerged macrophyte *P. malaianus* belonging to *Potamogetonaceae*, was used because it is one of the dominant species in Lake Taihu (Ye et al., 2007). Seedlings of *P. malaianus* were washed to remove sediment residues and pre-incubated in a plastic bin with lake water for acclimation prior to use. In each experimental container, 9 seedlings were planted.

Experiments in this study lasted for 6 weeks (from 17 October 2013 to 2 December 2013), which was illustrated to be adequate for the development of treatment-related differences in plant growth as well as minimization of tissue deterioration association with senescence (Barko and Smart, 1983). For experiments, seedlings with almost similar size and growth status (fresh weight, 0.78 ± 0.28 g; average stem height, 16 cm) were transplanted into sediments. Experiments included three treatments: plant with closed-circuit SMFC (Plant-c-SMFC) and plant with open-circuit SMFC (Plant-o-SMFC), and plants grown in the plastic barrel without electrodes (plant only) as control. All experiments were done in triplicate. Artificial illumination consisted of six time-switch-controlled fluorescence lamps (25 W) which were positioned above barrels and had an illumination: dark cycles of 12 h:12 h day^{-1}. The artificial illumination resulted in average light intensity of 320 lux at the plant level. The ambient temperature was not controlled. Water loss was mainly caused by evaporation from the water surface and was weekly replenished with lake water.

Prior to the above experiment, preliminary experiment were conducted over nine months (from 1 September 2012 to 1 June 2013) for the overall observation. The detailed description about the preliminary experiment was provided in Supplementary material.