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Bioenergy generation and rhizodegradation as affected by microbial community distribution in a coupled constructed wetland-microbial fuel cell system associated with three macrophytes

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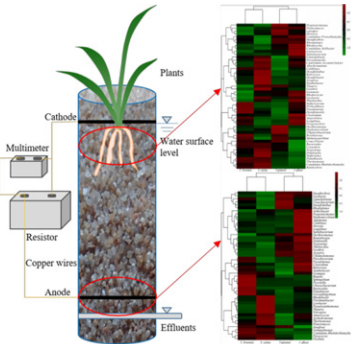
HIGHLIGHTS

- *T. orientalis* and *S. validus* contributes to the bioelectricity production in CW-MFC.
- Biodiversity observed in rhizosphere is slightly higher than that of anode material.
- Oxygen depletion in planted unit is higher than that of unplanted during dark cycle.
- Relative abundance of exoelectrogenic bacteria apparently promoted in planted CW-MFC.

GRAPHICAL ABSTRACT



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ABSTRACT

Rhizodeposits excreted by various macrophytes might lead to the potential discrepancy of microbial community distribution in constructed wetland coupled with microbial fuel cell (CW-MFC), which has been considered as main factors for the variations of bioelectricity generation during wastewater treatment. In this study, CW-MFC has been associated with three macrophytes (*J. effuses*, *T. orientalis* and *S. validus*) for domestic sewage treatment, also unplanted CW-MFC was performed as a control system. Macrophyte *T. orientalis* and *S. validus* can significantly strengthen the bioenergy output in CW-MFC. Highest current (94.27 mA m^{-2}) and power densities (21.53 mW m^{-2}) were obtained in CW-MFC planted with *T. orientalis*. Removal efficiencies of COD, NO_3-N and NH_3-N in CW-MFC planted with *S. validus* was respectively 5.8%, 7.2%, and 23.9% higher than that of unplanted system. Notably, the oxygen depletion in *S. validus* CW-MFC reactor during the dark cycle was higher that of other reactors. Results of high-throughput sequencing analysis showed that higher biodiversity was observed in rhizosphere than that of anode material, and the relative abundance of *Desulfobulbus* sp. and *Geobacter* sp. has been apparently promoted in the samples of rhizosphere. However, a higher relative abundance of electrochemically active bacteria (*Proteobacteria*) was observed on the surface of anode electrode material. In addition, microbes (*Cytophagales*, *Clostridium* sp., and *Dechloromonas* sp., and so forth) found in rhizosphere show a

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capability to decompose refractory contaminants. These contaminants and death roots in the upper part of wetland could be oxidized to fat acids, which may be used as the electrons acceptors for promoting the bioelectricity generation during wastewater treatment.

1. Introduction

As a low energy consumption system, constructed wetland (CW) has been successfully applied to the secondary treatment of domestic sewage, landfill leachate, storm water runoff and industrial wastewater (Kim et al., 2016; Vymazal, 2002; Vymazal and Kröpfelová, 2011). Integrating with microbial fuel cell (MFC), CW system contributes to bioelectricity generation reaction during the biodegradation of organic matter (Corbella et al., 2015; Doherty et al., 2015; Gogoi et al., 2014; Villasenor et al., 2013), while a multitude of electrochemically active bacteria (EAB) act as biocatalysts at the lower part of wetland (Liu et al., 2013). In the CW-MFC reactor, the electrons produced by EAB can flow through a conductive material to higher redox electron acceptors, such as NO_3-N and oxygen, at the substrate or cathode (Logan, 2009). Direct electron transfer is based on the conductive nanowires, c-type cytochromes, or pili, which has been played an important role in the electron transfer of microbes (Holmes et al., 2003). Herein, the distribution of oxygen and microorganisms should be gaining more attention to understand the mechanisms of bioenergy output and biodegradation in an environmentally friendly system of CW-MFC (Yadav et al., 2012; Zhang et al., 2016).

Currently, Liu et al. (2013) obtained that the maximum power density in the CW-MFC associated with *Ipomoea aquatic* was 142% higher than of unplanted reactor, plant photosynthesis and substrate degradation enhanced were the main reasons for the promotion of power output. However, the different macrophytes planted in wetland can lead to a graded distribution of biomass because of the concentration of diverse oxygen and dissolved organic carbon (DOC) produced by wetland plants. It has been indicated that the below-ground biomass of *Canna indica*, *Typha angustifolia*, and *Scirpus triqueter* was 4.30 ± 0.83, 0.50 ± 0.33, and 0.12 ± 0.00 g plant^{-1} with the root diameter below 1 mm (Lai et al., 2011). The aquatic macrophytes have significantly different oxygen release and transfer rates to CW does [*Scripus* sp. (0.005–0.011 g m^{-2} d^{-1}) and *Typha* sp. (0.45 g m^{-2} d^{-1})] according to the measurement of root respiration and model simulation, respectively (Bezbaruah and Zhang, 2005; Nivala et al., 2013). Notably, the average DOC release rates of *Phragmites australis*, *Iris pseudacorus* and *Juncus effusus* was 12.2, 9.0 and 4.3 μg g^{-1} root dry mass h^{-1}, respectively (Zhai et al., 2013). In addition, the root activity, higher radial oxygen loss (ROL), transpiration rate, photosynthetic rate, and removal rates of total nitrogen and phosphorus of fibrous-root plants was significantly higher than that of the thick-root plants (Lai et al., 2011). The plant uptake process has also been considered as one of the major removal mechanisms in CW system (Vymazal, 2014). Thus, the root features of different kinds of macrophytes may show a vastly different influence on the oxygen transfer and bioelectricity generation in CW-MFC.

Previous studies on the microbial community distribution of in plant microbial fuel cell associated with *Glyceria maxima* indicate that the abundant of EAB located on the graphite granular electrode of cathode was higher than that on the root surface (Timmers et al., 2012). It was also reported that most of EAB contribute to nitrification and denitrification process, and the fermentative bacteria and EAB could be used for syntrophic interactions (Lu et al., 2015; Wang et al., 2016b). In the literature, COD removal in CW-MFC was 27–49% higher than that of normal CW may be resulted from the anode of CW-MFC have been used as a temporary electron acceptor to contribute the process of anaerobic treatment (Srivastava et al., 2015). The use of anaerobic treatment for municipal treatment in the bottom of CW-MFC is based on the anaerobic microorganisms including *Proteobacteria*, *Firmicutes*, *Acidobacteria* and *Actinobacteria* (Logan, 2009; Xing et al., 2010), and many bacteria among these phyla can show the ability of electron acceptors and hydrogen production. However, little is known that the effects of different macrophytes on the structure and biodiversity of microbial community on the surface of anode and cathode electrode in CW-MFC reactor.

In this study, saturated CW-MFC systems planted with three different macrophytes, *Juncus effuses* (*J. effuses*), *Typha orientalis* (*T. orientalis*) and *Scirpus validus* (*S. validus*), as well as unplanted controls, were constructed and investigated with the purpose to assess the bioelectricity generation performance and pollutants removal in the rhizosphere. To transfer more oxygen from the atmosphere, the cathode electrode was positioned over the water surface level of CW-MFC reactor. The bacterial DNA templates collected from the rhizosphere and anode material were conducted high-throughput sequencing analysis to identify the bacteria structure associated with different wetland plants.

2. Materials and methods

2.1. Experimental apparatus construction

The three macrophyte species, *J. effuses*, *T. orientalis* and *S. validus* with stem lengths of 21 ± 3, 32 ± 4 cm, and 25 ± 4 cm, respectively were propagated for one month in a culture solution multiplication, which contained macro-nutrient elements [500 mg L^{-1} KNO_3, 150 mg L^{-1} KH_2PO_4, 950 mg L^{-1} $Ca(NO_3)_2$ and 500 mg L^{-1} $MgSO_4$] and 1.0% Hoagland's trace elements (Hoagland and Arnon, 1950). Then the wetland plants were rinsed for five times using tap water and transplanted to CW-MFC with a density of 6 rhizomes per unit. As shown in Fig. 1, the CW-MFC reactors consisted of 8 polyethylene plastic containers (52 cm length by 16 cm diameter) filled with uniform quartz sand (2–4 mm size with an average porosity of 29.2%). The containers for each plant species and controls CW-MFC were prepared with two replicates ($n = 2$), and reactors were operated under fed-batch mode with a hydraulic retention time (HRT) of 2 days. Synthetic wastewater with COD concentration of 207.3 ± 4.2 O_2 mg L^{-1} in influents was fed onto the surface of CW-MFC and recollected at lower part of CW-MFC with a perforated pipe (20 mm in diameter with 12 regularly distributed 6 mm-diameter holes), which was installed 7 cm above the bottom of wetlands. The anode and cathode electrodes [carbon fiber felt (CFF), 10 cm in diameter] were embedded in the CW-MFC, and the two electrodes were 35 cm in distance and connected by an external electrical resistor (1000 Ω). The cathode electrode, 5 cm below the surface of media and surface level of influent wastewater, was placed 1–2 cm above the rhizosphere of macrophytes.

2.2. Reactors operation and sampling

Each CW-MFC was inoculated with diluent activate sludge (2.0 L) collected from a wastewater treatment plant in Shanghai and stabilized for one month. During the acclimation and operation period, synthetic wastewater (Table S1) was fed onto the surface of CW-MFC at about 9 o'clock a.m. in each cycle, meanwhile samples of influents and effluent were collected. A digital multimeter (Hangzhou Bright Technology Co., Ltd., China) was employed to monitor the fluctuated voltage across the electrical resistor for every minute. The experiment began on April 2016 and lasted to August 2016. All reactors were sheltered in the veranda and the air temperature varied from 17 °C to 38 °C. At the end of operation period, the external electrical resistor ranged from 80,000