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# Bioelectricity generation, contaminant removal and bacterial community distribution as affected by substrate material size and aquatic macrophyte in constructed wetland-microbial fuel cell



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# ABSTRACT

Integrating microbial fuel cell with constructed wetland (CW-MFC) is a novel way to harvest bioelectricity during wastewater treatment. In this study, the bioelectricity generation, containment removal and microbial community distribution in CW-MFC as affected by substrate material sizes and aquatic macrophyte were investigated. The planted CW-MFC with larger filler size showed a significant promotion of the relative abundance of electrochemically active bacteria (beta-Proteobacteria), which might result in the increase of bioelectricity generation in CW-MFC (8.91 mW m<sup>-2</sup>). Additionally, a sharp decrease of voltage was observed in unplanted CW-MFC with smaller filler size in Cycle eight. However, the peak COD (86.7%) and NO<sub>3</sub>-N (87.1%) removal efficiencies were observed in planted CW-MFC with smaller filler size, which was strongly related to the biodiversity of microorganisms. Generally, the acclimation of exoelectrogens as dominant microbes in the anode chamber of planted CW-MFC with larger filler size could promote the bioelectricity generation during wastewater treatment.

#### 1. Introduction

Under the background of natural resources depletion and increasingly severe water pollution, many scholars are starting to pay attention on the bio-electrochemical technology to harvest bioelectricity in the process of wastewater treatment. It has estimated that 1.5 billion tons of oil equivalent (TOE) of energy will be required and two-thirds of the global individuals will face the aquatic environment problems by 2025 (Eliasson, 2014). Recently, integrating microbial fuel cell with constructed wetland (CW-MFC) is a novel way into developing technical platform for recycling bioelectricity production during the wastewater treatment, which has been widely studied on the domestic and industrial wastewater treatment through the combinations of physical, chemical and biological processes under different conditions due to its advantages of low cost, easy operation, and simple maintenance (Yadav et al., 2012).

Recently, previous studies have been on focused on the effects of the operation mode (batch and continuous flow), photosynthate of wetland plants, electrode materials, and location of plant roots (anode or

cathode compartment) on wastewater treatment and bioelectricity generation in CW-MFC (Doherty et al., 2015; Fang et al., 2015; Zhao et al., 2013). Yadav et al. (2012) integrated the MFC technology with CW realized the maximum dye removal percentage of 93.15%, and power density of 15.73 mW m<sup>-2</sup>. In addition, the bioelectricity generation could be promoted by 142% based on the plant photosynthate of CW-MFC (Liu et al., 2013). In the CW-MFC system, the peak power densities in the continuous and batch reactor were  $9.4 \text{ mW m}^{-2}$  and 12.83 uW m<sup>-2</sup>, respectively (Zhao et al., 2013). Furthermore, the accelerated transfer of electrons by micro-electric field in CW could enhance the removal efficiency of nitrogen and phosphorous pollutants (Ju et al., 2014). A moderate amount of hydrogen produced around the surface of graphite electrode with external electric current could enhance the effectivity of autotrophic denitrification (Puig et al., 2012). However, the denitrification efficiency was inhibited when the current density exceeded 290 mA  $m^{-2}$ , therefore, a micro electrolysis with the current density of 20–80 mA m<sup>-2</sup> provided by CW-MFC might be propitious to nitrogen removal. Additionally, the application of cathode material around the water surface of wetland demonstrated that the

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bioelectricity generation has been significantly improved in CW-MFC under batch mode operation (Wang et al., 2016).

In CW-MFC, macrophytes play a key role in maintaining a high oxidation-reduction potential between the up and low parts of wetland due to the exudation of root oxygen. In addition, root exudates could provide carbon resource and energy to support the growth of rhizosphere microorganisms. It has been reported that the root exudates potentially could fuel a denitrification rate of 94–267 kgN ha<sup>-1</sup> year<sup>-1</sup> (Zhai et al., 2013). Oxygen diffusion from the atmosphere and exudates from the plants could ensure the aerobic condition of cathode chamber. while with the increasing of depth, water saturation contributes to anaerobic condition in the bottom of wetland. It was found that the dissolved oxygen (DO) concentrations in the depth of 20 and 40 cm below the CW surface, where the anode was installed, was nearly  $0.24 \text{ mg L}^{-1}$ , which allowed the anaerobic bacteria, especially, electrochemically active bacteria, to thrive. Especially, the connection of external circuit in the CW-MFC reactor could increase the growth of exoelectrogens such as Geobacter Sulfurreducens and beta-Proteobacteria and inhibit the activity and growth of Archaea in the anode (Fang et al., 2013). Also, some study have reported that the substrate material with small size have owned a better absorption potential and provided a larger surface for the growth of microbes (Adhikari and Lin, 2016). CW-MFC with different substrate material sizes also show a significant influence on the transfer efficiency of protons because of the rapidly proliferating of microbes might result in the depress of hydraulic conductivity. However, the effect of substrate material size or macrophyte on the bioelectricity generation and microbial community structure in CW-MFC have been rarely addressed by other authors.

In this study, four CW-MFC reactors were constructed with different substrate material sizes under planted and unplanted condition to treat synthetic wastewater under fed-batch mode. To use the oxygen from atmosphere and rapidly proliferate microorganisms in CW-MFC, the cathode electrode was placed overlying the water surface of influents and the anaerobic digested sludge was inoculated in the CW-MFC reactor, respectively. The aim of this study was to evaluate the effects of substrate material size and aquatic macrophyte on the bioelectricity generation, contaminant removal and microbial community distribution in CW-MFC reactor.

## 2. Materials and methods

#### 2.1. Construction, inoculation, and operation of CW-MFC

Four microcosms (System 1, 2, 3, and 4) were constructed using polyvinyl chloride with the height of 0.52 m and the diameter of 15.2 cm to simulate the CW-MFC reactor as shown in Fig. 1. Each

reactor was filled with quartz sand as the supporting material and planted with disease-free Canna indica (2 rhizomes per unit). The average diameter of quartz sand filled in System 1 (unplanted) and 2 (planted) was 2.8 mm, while the average diameter of quartz sand filled in System 3 (unplanted) and 4 (planted) was 5.2 mm. The total volume of each CW was 9.4 L; the liquid volume of System 1 and 2 was 2.7 L; while the liquid volume of System 3 and 4 at the initial stage of experiment was 2.9 L. Carbon fiber felt (CFF, diameter of 10 cm, surface area of 154 cm<sup>2</sup>, thickness of 3 mm, Beijing Jixingshengan Industry & Trade Co., Ltd. China) was embedded into CW-MFC as the anode and cathode electrode. The anode electrode was 12 cm above the bottom of reactor and the cathode electrode was around the water surface (5 cm below the top of reactor) of wetland. Meanwhile, the cathode and anode electrodes connected with insulated copper wires crossing an external electrical resistor with the resistance value of 1000 Ohm and the distance between the anode and cathode electrode was 30 cm. The sampling port of each reactor was 7 cm above the bottom of the microcosm.

The composition of synthetic wastewater was listed as follows (in mg/L):  $C_6H_{12}O_6H_{2}O$  (120.04 ± 0.04),  $KH_2PO_4$  (7.70 ± 0.02),  $Na_2HPO_{4'12}H_2O$  (17.80 ± 0.02),  $MgCl_26H_2O$  (17.71 ± 0.04),  $ZnCl_2$  (7.53 ± 0.04),  $CaCl_2$  (17.52 ± 0.03),  $CH_3COONa$  (100.05 ± 0.1), and  $NaNO_3$  (55.00 ± 0.02). The pH values of influents ranged from 7.1 to 7.4. Each reactor was started with adding synthetic wastewater under the sequencing batch mode and inoculating an equal volume of concentrated anaerobic digested sludge (2.5 L), which was collected from the Municipal Wastewater Treatment Plant of Songjiang in Shanghai, China. All experimental systems were installed outdoors and sheltered by veranda and the average temperature was 30 ± 7 °C. Hydraulic residence time (HRT) of synthetic wastewater was 2 days. The whole operation period was 80 days, including a 30-day acclimation for the growth of microorganisms and macrophytes.

## 2.2. Bioelectricity monitoring

To characterize the bioelectricity generation performance of CW-MFC, the cell voltage of each reactor with the resistance of 1000 Ohm was recorded every 2 min with an automatic recorder (Hangzhou Bright Technology Co., Ltd., China). The polarization curve of each reactor was determined by ranging the external electrical resistance from 50 to 80,000 Ohms in the end of experiment. Voltage (*U*) and current (*I*) were measured with a digital multimeter module (VICTOR 86E, Shenzhen, China). The power density (*P*, mW m<sup>-2</sup>) and current density (*J*, mA m<sup>-2</sup>) were calculated by dividing the power (*UI*, mW) or current (*I*, mA) by the surface of the anode electrode (*S*, m<sup>-2</sup>). The coulombic efficiency ( $\eta_{CE}$ ) of sequencing batch mode flow MFC was determined



Fig. 1. Schematic diagram of the CW-MFC system with different substrate material sizes.

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