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Relationship between electrogenic performance and physiological change of four wetland plants in constructed wetland-microbial fuel cells during non-growing seasons

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ABSTRACT

To find suitable wetland plants for constructed wetland-microbial fuel cells (CW-MFCs), four commonly used wetland plants, including *Canna indica*, *Cyperus alternifolius* L., *Acorus calamus*, and *Arundo donax*, were investigated for their electrogenic performance and physiological changes during non-growing seasons. The maximum power output of 12.82 mW/m^2 was achieved in the *A. donax* CW-MFC only when root exudates were being released. The results also showed that use of an additional carbon source could remarkably improve the performance of electricity generation in the *C. indica* and *A. donax* CW-MFCs at relatively low temperatures (2–15°C). However, *A. calamus* withered before the end of the experiment, whereas the other three plants survived the winter safely, although their relative growth rate values and the maximum quantum yield of PSII (Fv/Fm) significantly declined, and free proline and malondialdehyde significantly accumulated in their leaves. On the basis of correlation analysis, temperature had a greater effect on plant physiology than voltage. The results offer a valuable reference for plant selection for CW-MFCs.

Introduction

The microbial fuel cell (MFC) is an emerging technology gaining significant attention among researchers worldwide because of its bioelectricity generation potential (Du et al., 2007; Logan, 2005). In a conventional MFC, organic and inorganic substrates are oxidized by bacteria, generating electrons that are transferred to the anode through a conductive material and a resistor. They then flow to a higher potential electron acceptor at the cathode, such as oxygen (Logan et al., 2006; Rabaey et al., 2007).

Constructed wetlands (CWs), recognized as a green and low-cost technology, have been used to treat domestic (Lin and Han, 2012), industrial (Khan et al., 2009), urban (Reyes-Contreras et al., 2012), and agricultural (Speer et al., 2009) wastewaters, to treat stormwater runoff (Choi et al., 2015), leachates (Rustige and Nolde, 2007), and mine drainage water

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(Sheoran and Sheoran, 2006), and to dewater sludge (Magri et al., 2016) through a combination of physical, chemical, and biological processes (Imfeld et al., 2009; Zhi and Ji, 2012).

Yadav et al. (2012) were the first research group to investigate the possibility of combining CW with MFC to treat wastewater containing a synthetic azo dye. The compatibility and combination of CWs and MFCs are based on the fact that they are both biological systems engaged in the degradation of organic matter. Additionally, an MFC requires a redox gradient, which can be naturally found in a CW, depending on flow direction and wetland depth (Doherty et al., 2015). More and more attention has been focused on the constructed wetland-microbial fuel cell (CW-MFC) as it is a novel, promising, and scalable technology for harvesting bioelectricity during wastewater treatment (Villasenor et al., 2013). Most studies have focused on improving anodic and cathodic efficiency or reducing the internal resistance of the CW-MFC to generate more energy or improve nutrient removal efficiency from wastewater (Fang et al., 2017; Liu et al., 2014). For example, the electrical and azo dye decolorization performance in a CW-MFC system was studied (Fang et al., 2017). It was found that increasing the cathode diameter promoted both decolorization and electricity generation performance.

Plants growing in a wetland environment have a large biomass, developed roots, and robust stems. They can be planted in natural environments with a minimal disturbance of scenery and without being competitive with agricultural lands that are needed for food production (Holm, 2009). Wetland plants, as an important part of a traditional constructed wetland, play an important role in the process of wastewater treatment. Their roots can remove pollutants directly from sewage by absorption, adsorption, and enrichment (Fang and Tan, 2011). Plant roots can also secrete oxygen, allowing different oxygen levels to occur in different regions of the system, helping the survival of different microorganisms (Gagnon et al., 2007). Wetland plants also play an important role in the operation of MFCs. Plant roots secrete large amounts of organic matter (root exudates) to the growth medium and provide the matrix for the generation of electricity (Timmers et al., 2010). More interestingly, some researchers have found other effects of plants in CW-MFC systems. The internal resistance could be reduced by planting *Ipomoea aquatica* in the cathode of a CW-MFC (Fang et al., 2013) and plant-mediated methane accounted for 71%–82% of the total methane flux in CW-MFC emissions (Liu et al., 2017).

However, studies on wetland plants in CW-MFCs are still few, especially regarding the selection or adaptability of plants. *Spartina anglica*, *Arundinella anomala*, and *Arundo donax* have been used to construct P-MFCs (plant-microbial fuel cells) (Helder et al., 2010) and very different electrogenic performance was found for the three plants. MFCs with *S. anglica* obtained the highest power density (222 mW/m^2 membrane surface area), which was more than 10 times than that of MFCs with *A. anomala*. Clearly, the selected plants seem to have a great effect on the yield of bioelectricity. More work needs to be done to find the proper plants for CW-MFCs and to confirm that the plants grow well without physiological damage for a long period or at least one life cycle. Timmers et al. (2010) found that a continuous micro-current environment caused no fatal injury to *S. anglica* during a study lasting 119 days. However, that result may be questionable because only shoot density and the number of living shoots were monitored in his study. Even if the current does not cause any injury to the morphology and structure of plants, biochemical and physiological alterations may occur. However, no literature clearly reports whether voltages and micro-currents have an effect on plant physiology or what the physiological response of plants will be to micro-currents.

Temperature, as an important environmental factor, affects not only the growth of plants but also the root exudates of plants (Uselman et al., 2000). The research on CW-MFCs has been mainly carried out at room temperature (Fang et al., 2013; Wang et al., 2016b) or during the growing seasons of plants (Wang et al., 2016a), during which the temperature is suitable for plant growth. However, the performance of wetland plants in CW-MFCs at low temperatures remains unknown. In fact, most aquatic plants will die or wither at a relatively low temperature or during non-growing seasons. Identification of plants that can maintain the normal operation of CW-MFCs at low temperatures will be of great help in the application of these systems.

Therefore, the main objective of this study was to (1) investigate the performance of electricity production of four different wetland plants in CW-MFCs during non-growing seasons, (2) measure the growth and physiological changes of the plants during the process of electrogenesis, and (3) choose a suitable wetland plant for CW-MFCs for practical application based on the first two objectives.

1. Materials and methods

1.1. Wetland plants

In this study, four widely used wetland plants, *Canna indica*, *Cyperus alternifolius* L., *Acorus calamus*, and *A. donax*, were chosen to construct CW-MFC systems. Plants with 40–60 cm stems were collected from an experimental station in Wuhan City, Hubei Province, China. After being thoroughly rinsed with tap water, they were then cultivated with the nutrient solution before being transplanted into the CW-MFCs. The main nutrient solution consisted of 0.31 g/L NH_4Cl, 0.13 g/L KCl, 2.75 g/L $NaH_2PO_4 \cdot 2H_2O$, and 16.42 g/L $Na_2HPO_4 \cdot 12H_2O$.

1.2. Experimental setup and operation

Prior to assembly, all anodes were inoculated in the same reactor without plants by using anaerobic sludge taken from an urban wastewater treatment plant in Wuhan. The cathodes were suspended in the reactor by a nylon mesh and aerated with an oxygen pump. The anodes and cathodes were connected using a resistance of 1000 Ω. Once the voltage reached 400 mV or higher and had been maintained for about a week, the electrodes were taken out, gently rinsed with deionized water to remove residual sediment, and placed into the CW-MFC reactors.

Fourteen PVC cylinders (diameter 150 mm, height 300 mm) were used as the CW-MFC reactors, above which there was a transparent rain-shelter to avoid the interference of rain. Twelve reactors were used as for the experimental groups

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