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Electricity production and evolution of microbial community in the constructed wetland-microbial fuel cell

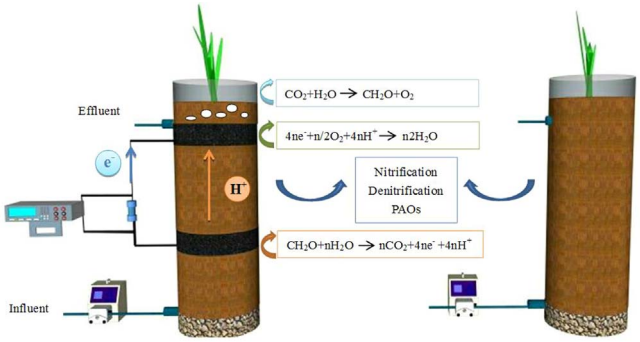
Fei Xu[a,1], Fu-qian Cao[a,1], Qiang Kong[a,*], Lu-lu Zhou[a], Qing Yuan[a], Ya-jie Zhu[a], Qian Wang[a], Yuan-da Du[a], Zhi-de Wang[b]

[a] College of Geography and Environment, Collaborative Innovation Center of Human-Nature and Green Development in Universities of Shandong, Shandong Normal University, Jinan 250014, PR China
[b] Rushan No. 2 Middle School, Weihai 264500, PR China

HIGHLIGHTS

- CW-MFC could generate electricity combined with purification of wastewater.
- TN removal rate in CW-MFC was highly significant ($p < 0.01$) higher than that in CW.
- Voltage was 265.77 ± 12.66 mV and power density was 3714.08 mW·m^{-2} in CW-MFC.
- The diversity and richness of the CW-MFC were higher than those of the CW.

GRAPHICAL ABSTRACT



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ABSTRACT

Constructed wetlands combined with microbial fuel cell (CW-MFC) could purify the wastewater while using microorganisms to generate electricity. Our study investigated pollutant removal and microorganism evolution in CW and CW-MFC. The average removal rate of total nitrogen (82.46 ± 4.74%) in the CW-MFC was highly significant ($p < 0.01$) higher than that in the CW. The average removal rate of chemical oxygen demand (82.32 ± 12.85%) and total phosphorus (95.06 ± 5.45%) in the CW-MFC were higher than those in the CW. In the CW-MFC, the average voltage was 265.77 ± 12.66 mV and the highest power density was 3714.08 mW·m^{-2}. The microbial community diversity and richness of the CW-MFC system were higher than those of the CW system. The read number of ammonia oxidizing (149 ± 7), nitrite-oxidizing (144 ± 8, 132 ± 18) and anammox bacteria (281 ± 8) were the highest in the CW-MFC (Anode). The contents of denitrification, dissimilatory nitrate reduction to ammonium, and electrochemically active bacteria in the CW-MFC (Cathode) were significantly ($p < 0.05$) higher than others.

1. Introduction

Constructed wetlands (CWs) have been successfully applied to secondary processing treatments of domestic sewage, leachate, rainfall runoff, and industrial effluent [1,2]. CWs have the advantages of low cost and easy operation, have been thoroughly studied and widely used

* Corresponding author.
E-mail address: kongqiang@sdnu.edu.cn (Q. Kong).
[1] These authors contributed equally to this work.

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[3]. The integration of CWs with a microbial fuel cell (MFC) technologies shows promise as a new type of wastewater treatment, which is considered to be an economical and effective way of harvesting bioenergy [4]. CWs and MFCs can be combined because CWs can provide the redox conditions, anaerobic anodes and aerobic cathodes, required by the microbial fuel cells [5]. The chemical transformations occurring at the anode is $CH_2O + nCO_2 \rightarrow nCO_2 + 4ne^- + 4nH^+$. From anode to cathode, electrons pass through the external circuit while protons move inside the system. Then occurring at the cathode is $4ne^- + n/2O_2 + 4nH^+ \rightarrow n2H_2O$, completed the whole electrochemical cycle [6,7]. Thus, CW-MFC systems have been examined in recent years with the goal of improving the capacity of wetland wastewater treatment while generating electricity [8].

In a previous study, CW-MFC systems were developed based on three macrophytes, namely, *Juncus effuses*, *Typha orientalis,* and *Scirpus validus*, for treatment of sanitary sewage and production of bioelectricity. The average COD removal efficiencies of CW-MFC planted with *J. effuses*, *T. orientalis* and *S. validus* macrophytes were respectively 77%, 80%, and 80%, and the total nitrogen (TN) removal efficiencies were respectively 65%, 71%, and 74% [9]. Currently, Liu et al. obtained the maximum power density in a CW-MFC planted with *Ipomoea aquatic*, the performance of which was 142% as high as that of an unplanted reactor [10]. To date the effects of pH, vegetation types and the organic substrates in CW-MFC systems have been the main focus of research [11]. Electrochemically active bacteria (EAB) are generally considered to be the major factor leading to fluctuation of bioelectricity and changes of the microbial community structure in CW-MFCs [4]. Some studies have suggested that more attention should be paid to the distribution of microorganisms to better understand the mechanism of bioenergy production and bio-degradation in environmentally friendly CW-MFC systems [12]. However, the community structure of functional microorganisms associated with the removal of pollutants such as COD, TN, and TP, and electricity generation, and the effects of this structure on the CW-MFC performance have yet to be investigated.

In this study, the performances of closed-circuit CW-MFC and CW systems were studied. The reactors were operated under continuous influent mode. We examined the electrical performance of the CW-MFC and compared the sewage treatment performance between CW-MFC and CW devices. The aim of this study was to evaluate the relationships among bioenergy production, water quality removal efficiency, and microbial community structure in CW-MFCs and CWs.

2. Material and methods

2.1. Experimental apparatus construction

As represented schematically in Fig. 1, the two reactors consisted of Perspex containers (20-cm diameter, 55-cm height) filled with uniform quartz sand, haycite, activated sludge, and activated carbon (3–5 mm grain size). The locations of components in different parts of the device are indicated in Fig. 1. The wetland plant *Phragmites australis* was planted at a density of 10 rhizomes per reactor. The reactors were operated under continuous upflow mode with a hydraulic retention time (HRT) of 3 days. The synthetic wastewater flowing into the device was simulated polluted river water. The composition of the synthetic wastewater included sucrose (53.483 mg/L), $(NH4)_2SO_4$ (37.714 mg/L), KNO_3 (50.500 mg/L), and KH_2PO_4 (6.581 mg/L). All other micronutrients required for normal growth and development of the plants were as follows (mg/L): 21 Ca, 10 Mg, 14 S, 0.8P, 0.3 Fe, 0.03 Zn, 0.01 Cu, 0.03 Mn, 0.03B, and 0.002 Mo. The same level of these minerals was maintained in the two devices by adding $CaCl_2$, $MgSO_4$, Fe-EDTA, $ZnSO_4$, $CuSO_4$, H_3BO_3, H_2MoO_4, and $MnCl_2$ [13]. The pump (Longer Precision Pump Co., Ltd, Baoding, China) pumped the synthetic wastewater to the device at a feed rate of 1.5 ml/min. Synthetic wastewater in the influent was fed into the lower part of the reactor and recollected at the surface of reactor at a pipe installed 5 cm above the bottom of the wetland. The anode was situated at the lower part of the device and the cathode was horizontal placed at the upper part of the device. Two electrodes were placed parallel, and the distance between them was about 17.5 cm. The projection area of the two electrodes is 254.34 cm^2. The cathode consists of a single-layer titanium mesh with a diameter of 18 cm. The anode is a cylinder enclosed by a titanium mesh (18 cm in diameter, 5 cm in high) which filled with a mixture of activated carbon. The mesh size of titanium mesh was 3 mm × 8 mm and the purity of titanium mesh was 99.99%. The two electrodes were integrated in a closed-circuit system by copper wires and cascaded with an external electrical resistor (1000 Ω), which can reduce the influence of the device's internal resistance on the voltage detection [14].

2.2. Reactors operation and sampling

Two reactors were inoculated with activated sludge (2.0 L) collected from a municipal wastewater treatment plant in Jinan and acclimated for a fortnight in a refrigerated anaerobic environment. The experiment began on June 2017 and lasted to August 2017. During the operation period, the water pump pumps synthetic wastewater into two reactors through the water inlet (H in Fig. 1) of the device, while samples of inlet (H in Fig. 1) and effluent (G in Fig. 1) were collected every three days. A digital multimeter (Shenzhen post victory Technology Co., Ltd., China) was used to monitor the fluctuating voltage at both ends of the electrical resistor every ten min. The multimeter monitored the data of voltage. The current and the power data was calculated by Ohm's law. The two reactors were sheltered on the balcony and the air temperature was varied from 18 to 25 °C. At the end of the operation period, the external electrical resistance ranged from 50,000 to 5 Ω, and the corresponding voltage and current values were recorded to acquire the polarization curves of each reactor.

2.3. Water-quality determination

Analysis of the water quality was performed according to the standard method based on water quality indexes [15]. NH_4^+-N, total phosphorus (TP), and total nitrogen (TN) were determined by a colorimetric method with a sodium reagent, a spectrophotometric method with ammonium molybdate, and spectrophotometry with alkaline potassium persulfate ultraviolet, respectively. The soluble chemical oxygen demand (COD) was measured by the potassium dichromate method (APHA method).

2.4. DNA and 16S rRNA polymerase chain reaction (PCR) amplification high-throughput sequencing

At the start of the experiment, we collected the activated sludge sample that was initially used as the inoculate (control). At the end of the operation of the reactors, activated sludge samples in the CW-MFC were collected from the surface of the anode electrode material (CW-MFC(A)) and cathode electrode (CW-MFC(C)). For CW samples (CW(C) and CW(A)) were collected at positions corresponding to locations of the cathode and anode in the CW-MFC system (10 g, each). Each sample was performed in triplicate for high-throughput sequencing analysis of the microbial community. Total DNA extraction was performed with the OMEGA Soil DNA kit (Omega Bio-Tek, Norcross, GA, USA).

Universal 16S bacterial primers 515F (5′-GTGCCAGCMGCCG CGG-3′) and 907R (5′-CCGTCAATT CMTTTRAGTTT-3′) were chosen to amplify approximately 420 base pair (bp) fragments within the hypervariable regions V4-V5. The high-throughput sequencing analysis was performed on an Illumina Miseq platform supported by Personal Biotechnology Co., Ltd. (Shanghai, China). For the 16S rRNA polymerase chain reaction (PCR) amplification reaction an initial denaturation was performed at 98 °C for 3 min; followed by 25 cycles of 98 °C denaturation for 30 s, 50 °C annealing for 30 s, and 72 °C extension for 30 s, and a final extension at 72 °C for 5 min [4]. The final