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ORIGINAL PAPER

Evaluation of long-term performance of plant microbial fuel cells using agricultural plants under the controlled environment

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Abstract

Plant microbial fuel cell (PMFC) is a novel bioelectrochemical system that integrates the photosynthetic reaction from the living plants to generate electricity via microorganisms at the rhizosphere of the plant roots. To elucidate factors which are critical for PMFCs operation, this study investigated the effects of different plants and soil conditioners on PMFCs performance. The experiment was done in a controlled lighting incubator at 27 °C and 75% of humidity for 200 days. Two waterlogged agricultural plants, paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*), were applied in PMFC systems; besides, the compost made from food waste and biochar made from waste wood biomass were selected as soil conditioners. Results showed that varied electricity generation during the operation was observed for different PMFC systems, but the Paddy-PMFC with compost (PC-PMFC) demonstrated relatively more stable electricity generation for 200 days (15.57 ± 8.15 mW/m^2) and significantly higher voltage production, reaching the highest output voltage of 894.39 ± 53.44 mV (34.78 mW/m^2) among all PMFCs. It was observed that the output voltage of PMFCs was significantly higher than soil-MFC, and the output voltage of P-PMFC was significantly higher than water bamboo-PMFC, implying rhizodeposition of different plant roots could be important for the performance of electricity production in PMFCs. However, Paddy-PMFC with biochar (PB-PMFC) demonstrated significantly lower voltage production than those without biochar, likely due to the inhibitory effect of biochar made by waste wood biomass. The taxonomic identification of the microbial community at the anode showed that *Proteobacteria* was the most abundant phylum, and *Gammaproteobacteria* and *Deltaproteobacteria* were the most dominant classes of the microbial communities. Further analysis showed that the PB-PMFC had the most distinct anode microbial community structure, with the predominant family of *Gallionellaceae*, instead of *Geobacteraceae* as in other PMFCs. *Geobacter* was the major genus of the microbial population in all samples and showed the highest relative abundance in PC-PMFC, suggesting that it was the main exoelectrogen involved in electricity generation in our PMFC systems. This study has demonstrated that the power output of PMFC systems can be influenced by different agricultural plants and soil conditioners made from waste biomass, which warrants the need to better understand the underlying interaction among the anode microbial community, the rhizodeposition of different plant roots, and electrochemical mechanisms for the future scale-up application of PMFCs.

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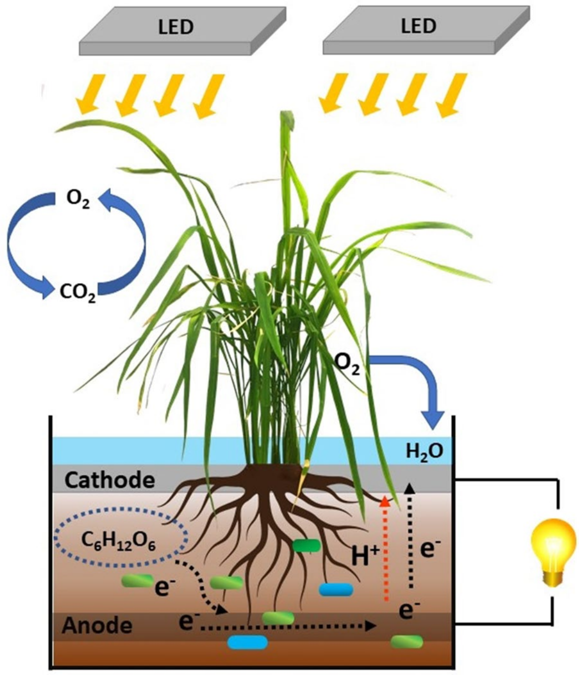
Graphic abstract



Keywords Plant microbial fuel cells · Agricultural plant · Compost · Biochar · Rhizodeposition · Microbial community

Abbreviations

EAB	Electroactive bacteria
MFC	Microbial fuel cell
Soil-MFC	Soil microbial fuel cell
P-PMFC	Paddy plant microbial fuel cell
W-PMFC	Water bamboo plant microbial fuel cell
PC-PMFC	Paddy plant microbial fuel cell with compost
PB-PMFC	Paddy plant microbial fuel cell with biochar
OCV	Open circuit voltage

Introduction

Nowadays, the demand for renewable energy or sustainable energy sources has been increasing. Bioenergy is considered as one of the renewable energy sources. Plant microbial fuel cell (PMFC) is a novel biotechnology which converts solar energy to electrical energy via plants and microorganisms. In the PMFC, the photosynthetic reaction from a living plant is integrated to generate the electricity via microorganisms at the rhizosphere of the plant roots (Guan et al. 2019a). During the photosynthesis process, a wide variety of organic compounds or rhizodeposition such as root exudates, secretions, lysates, and gases can be released to the rhizosphere (Gregory 2008). The rhizodeposition from plant photosynthesis could function as the self-sustained organic compounds, and the oxidation of the rhizodeposition at the plant roots via electrochemically active bacteria plays a key role in PMFC systems to generate electricity (Timmers et al. 2013a). The electricity can be collected through electrodes with an external circuit.

Wetland plants or the waterlogged plants are suitable to use in PMFCs (Guan et al. 2019b) because the soil subsurface remains anaerobic when the soil is submerged in water, and the community of anaerobic microorganisms (comprised of sulfate-reducing bacteria, iron-reducing bacteria, fermentative bacteria, and methanogenic archaea, etc.) will be established (Chin et al. 1999). Several wetland plants have been used to generate bioelectricity in PMFCs, for instance, reed mannagrass (*Glyceria maxima*) (Strik et al. 2008), cattail (*Typha latifolia*) (Oon et al. 2015), Chinese pennisetum (*Pennisetum alopecuroides*), and common reeds (*Phragmites communis*) (Guan et al. 2019a). To date, the maximum power output achieved was 679 mW/m^2 in PMFCs with *S. anglica*, and more efforts are still underway by different researchers to achieve a higher power output (Santos et al. 2018). It is suggested that the high root biomass (Timmers et al. 2013b) and the plant growth medium or nutrients could improve the power output of PMFCs (Helder et al. 2013).

PMFCs also show the potential to integrate with agricultural plants. The rice plant is one of the most important crops, particularly in Asian countries. Rice plants are typically cultivated in flooded land in which the soil can be under different redox zones, including oxic zone, anoxic or anaerobic bulk soil, and rhizosphere. These redox zones could cause microscale chemical gradients and a heterogeneous spatial distribution of microbial communities (Liesack et al. 2006), which could be observed also in the PMFCs (Guan et al. 2019b). The paddy PMFC has been demonstrated (Kaku et al. 2008), but the voltage generation was relatively small and faced the limitation for the growth of the roots by the electrode materials. Moqsud et al. (2015) studied factors which influence the power output of rice paddy PMFCs with paddy field soils. They found that the highest electricity production from rice paddy PMFCs was around 700 mV when rice paddy soil was mixed with additional compost.

Although PMFCs have been developed for a decade, it is still difficult to conclude the factors which are critical for PMFCs operation, since most of the study reported highly varied electricity generation during operation. We were uncertain whether the varied electricity generation was mainly due to the changing environmental conditions in the greenhouse or outoor environment in the previous studies (Helder et al. 2013; Guan et al. 2019a). Therefore, in this study, we systematically investigated effects of different plants and soil substrates on the electricity generation and microbial community of PMFCs in the controlled lighting incubator. We used semiaquatic crops to compare the electricity production, including rice paddy (*Oryza sativa*) and water bamboo (*Zizania latifolia*), which were widely planted and used as food in Taiwan. Moreover, since the addition of soil conditioner has been reported to stimulate rice paddy growth (Khan et al. 2013), in order to improve the efficiency of bioelectricity generation in PMFCs, Paddy-PMFC (P-PMFC) operation was compared under the addition of waste-based soil conditioners, including compost, and biochar, which were converted from food waste and waste wood biomass, respectively. Therefore, the information from our results will be beneficial for the further improvement of PMFCs toward feasible applications.

Material and methods

Soil preparation

The soil was collected from a natural paddy field in Taoyuan City (24° 53′ 21″ N, 121° 17′ 20″ E) at the topsoil (0–15 cm depth). The rocks and the plant debris were manually removed via screening. The screened soil was air-dried to remove the moisture content and sieved through 2 mm sieved-mesh. Before setup of soil-MFCs and PMFCs, the soil was incubated by adding tap water until the saturated condition and mixed with NH_4NO_3 (120 mg N/kg soil) and K_2HPO_4 (30 mg P/kg soil and 75 mg K/kg soil), which function as the essential nutrients for crop farming (Khan et al. 2013).

Paddy and water bamboo cultivation

The two species of waterlogged agricultural plants, including paddy plants (*Oryza sativa*) and water bamboo (*Zizania latifolia*), were used to evaluate the electrical energy generation in PMFCs. Rice seeds were obtained from the National Taiwan University farm. The rice seeds were cultivated at 27 °C in the incubator without light. After the root shooting, the paddy was transplanted into the soil until the 3-week old and then transplanted in PMFCs. Meanwhile, the water bamboo plants were obtained at the ages of 2 weeks from Honglin Garden Company, Changhua, Taiwan, and continuously cultivated from their stem until they had 5 cm length, and they were set up in PMFCs afterward (Fig. S1).

Experimental design and construction

In this study, round polyvinyl chloride (PVC) buckets (24 cm height and 17 cm diameter) were designed in all cases and set up in the light incubator (LG-600RH, LIAN SHEN ENTREPRISE CO., LTD., Taiwan). Each bucket contained 3 kg of soils and 1.5 L of water. The experimental setup included soil-MFCs without plants and different PMFCs, including two species of plants (paddy and water bamboo) and two soil conditioners (biochar and compost) for P-PMFC. The soil-MFC was set up as a control for comparison. P-PMFC and water bamboo-PMFC (W-PMFC) were set up to evaluate the impact of different waterlogged agricultural plants on electricity production in PMFCs. Two additional sets of P-PMFC which were mixed with 10% (*w*/*w*) of compost (Moqsud et al. 2015) and 1% (*w*/*w*) of biochar (Khan et al. 2013), respectively, to test the effect of soil conditioners. The compost, which was made from food waste, was obtained from Musta Refuse Incineration Plant, Department of Environmental Protection, Taipei City government. The commercial biochar made from waste wood biomass was obtained from the GreenPros CO., LTD, Taiwan. The basic properties of soils, compost and biochar are reported in Table S1 (Supplementary Information). In this study, the soil-MFC and PMFCs were labeled as shown in Table S2, and all experimental setups are triplicated.

The electrode material for both anode and cathode was made from carbon felt in a round shape (Gansu Haoshi Carbon Fiber Company, China) with 13 cm of diameter and 3 mm of thickness. The cathode was placed on the top of the soils but in the waterlogged condition, while the anode was buried in the soils. The distance between cathode and anode was about 5 cm. Both cathode and anode were connected via titanium wire, and the circuit was connected with

1 kΩ of an external resistor. The experimental configurations are showed in Fig. 1. All experiments were conducted in the controlled environment in the lighting incubator with 27 °C and 75% of humidity and carried out for a period of 200 days (December 2018–June 2019). An artificial light which includes fluorescent and LEDs lighting was controlled at 12/12 h of the light and dark cycle. The average light density monitored via a light sensor (UA-002-64, HOBO, USA) was 2095.4 Lux within the 63 × 65 × 50 cm space of the incubator. To keep all experiments in the waterlogged condition, all cases were irrigated with tap water every day.

Analytical methods

To monitor power performance, the voltage across the resistor of all PMFCs was monitored every 5 min via connection to the data acquisition system (2700, Keithley, the USA) controlled with KE 302 Kick start data logger software, and the data were saved to a computer. After 60 days, the polarization tests were made by using different resisters. Internal resistances and power density were calculated as described in the previous literature (Logan et al. 2006). The electricity output was measured in voltage (V) against time, and the current was calculated by using Ohm's law. The current density was calculated based on the anode surface area according to Eq. (1) (Moqsud et al. 2015).

$$I = V/\alpha R \tag{1}$$

where V is the measured voltage in volts (V), R represented the value of the external load resistor in Ohms, and α is the electrode surface area. The power output (P) was calculated following Eq. (2)

$$P = V \times I \tag{2}$$

The internal resistance (R_{int}, Ω) was calculated by the peak power density method with the aid of polarization and power density curves. When the maximum power density (P_{max}, mW/m^2) is acquired, the internal resistance is equal to the external resistance (R_{ext}, Ω) following Eq. (3) (Logan et al. 2006)

$$R_{int} = R_{ext} = P_{max}/i^2 \tag{3}$$

where i represented the current corresponding to the maximum power density.

The organic matter content of the soils and compost was determined by "loss on ignition" method (LOI). The dry weight of the sample was weighted before and after combustion at 600 °C for 2 h. The LOI was calculated according to Eq. (4)

$$\text{LOI}\ (\%) = ((\text{DW105} - \text{DW600})/\text{DW105}) \times 100 \tag{4}$$

where DW105 is the dry weight of sample at 105 °C to constant weight, and DW600 is the weight of dry sample after combustion in the furnace at 600 °C for 2 h (Zhao et al. 2016).

The measurement of pH and the electrical conductivity (EC) of all treatments were performed every week using a pH meter (HQ40d, HACH USA) and an electrical conductivity meter (multiHQ40d, HACH USA). Both soil pH and EC were analyzed at the soil surface near the cathode. The soil samples (approximate 2 g) were mixed with distilled water according to 1:2.5 of the soil–water ratio (Thomas 1996). After waiting for the suspension to precipitate, pH meter was used to measure the pH value. For the EC measurement, the same ratio of the soil–water was provided into the shaker for 1 h at 140 rpm (Rhoades 1996). After filtered by Whatman filter #5, the EC meter was used to measure the EC of soil samples.

16S rRNA gene amplicon high-throughput sequencing and statistical analysis

After 120 days of the operation, the anodes were carefully removed to analyze the microbial community using 16S rRNA gene amplicon high-through sequencing. The DNA was extracted from the anodes of soil-MFCs and PMFCs by using the commercial DNA extraction kit (DNeasy

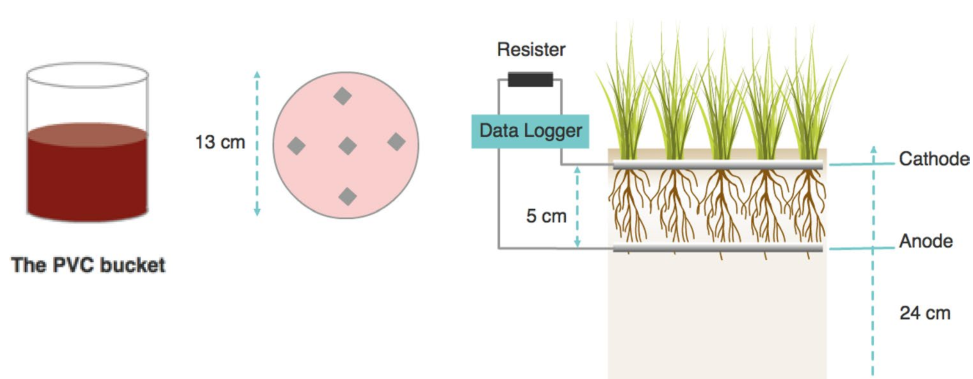


Fig. 1 Schematic diagrams of the PMFCs configuration

PowerSoil Kit, QIAGEN, Germany). The fragments of 16S rRNA genes were amplified using the universal primers: 16S V3–V4; 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′GACTACHVGGGTATCTAATCC-3′). The DNA was amplified using the thermal cycling with the initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, elongation at 72 °C for 30 s, and the final extension at 72 °C for 5 min. All 16S rRNA gene amplicons were sent to sequencing (Genomics company, Taiwan). The sequencing libraries were generated using Truseq nano DNA Library Prep Kit (Illumina, the USA) following the manufacturer's recommendations, and index codes were added (Caporaso et al. 2010; Schloss et al. 2009). The mothur (Caporaso et al. 2010) and QIIME (Schloss et al. 2009) softwares were used to analyze raw sequencing data as mentioned previously. Every representative sequence was described as an operational taxonomic unit (OTU) using the RDP classifier with the SILVA database version 132 (Quast et al. 2013).

The descriptive statistics and analysis of variance (ANOVA, IBM SPSS Statistics version 22.0) with Tukey post hoc test were used to compare the electrical voltage, pH, and EC values of P-PMFC, W-PMFC, soil-MFC, PMFCs with conditioners. Using $\alpha = 0.05$, statistical significance (P value) was provided to compare the different treatments of soil-MFCs and PMFCs. UniFrac principle coordinate analysis (PCoA) which is a distance metric using phylogenetic information was also used to compare microbial communities among the soil-MFC and PMFCs (Lozupone et al. 2011). Meanwhile, the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering was used to classify the average linkage of the microbial community in soil-MFCs and PMFCs based on their pairwise similarities (Saitou and Nei 1987). The method can be more effective in revealing ecological patterns than taxon-based methods (e.g., use of lists of species, genera, and OTUs) used in the previous study (De Schamphelaire et al. 2010). In this study, UniFrac coupled with PCoA and UPGMA hierarchical cluster was carried out by PALSTAT software package version 3.21.

Results and discussion

Electricity generation in PMFCs

The output voltages of the multiple PMFCs tested in this study are shown in Fig. 2. After operating soil-MFC and PMFCs for 10 days, the closed circuit voltage of all treatments increased gradually and showed varied voltage outputs, which were similar to the previous study (Timmers et al. 2010). For the soil-MFC, the highest output voltage was 468.57 ± 34.64 mV, while the P-PMFC and W-PMFC

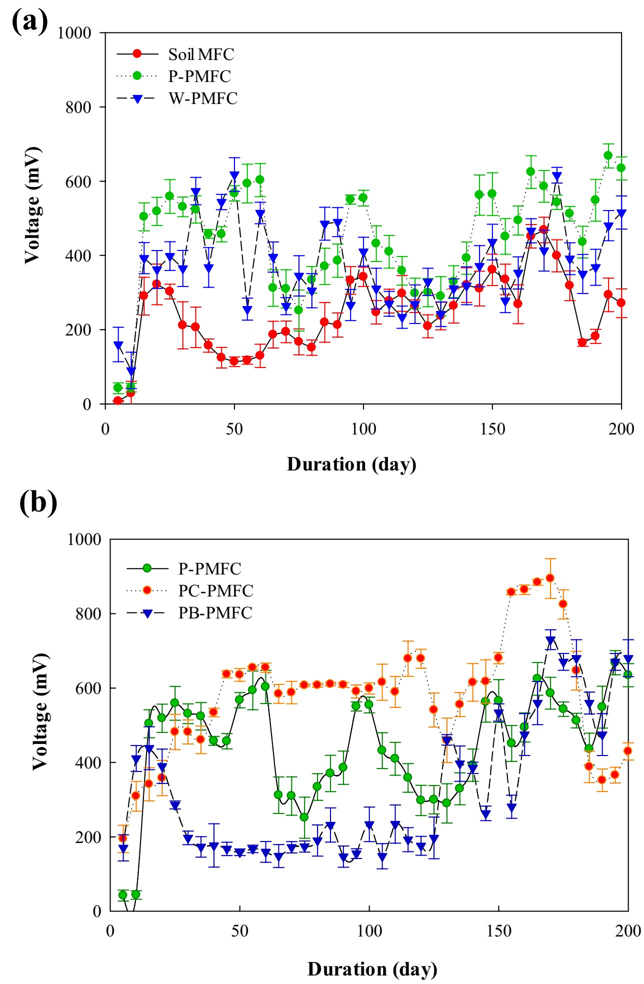


Fig. 2 Variation of voltage generation versus time. **a** Soil-MFC and PMFCs with different plants; **b** P-PMFCs with or without soil conditioners

had the highest output voltage of 668.28 ± 32.53 and 618.11 ± 45.38 mV, respectively, as shown in Fig. 2a. Comparing soil-MFC with PMFCs, PMFCs with paddy and water bamboo had significantly higher voltage values ($P < 0.05$) than soil-MFC (Table S3). The significantly higher output voltage in PMFCs should be related to the available carbon sources from rhizodeposition, which is the root excretion of organic compounds to the soils including sugars, organic acids, polymeric carbohydrates, enzymes, and dead-cell material (Timmers et al. 2010). As also shown in the previous study, it revealed that PMFC with paddy was still able to generate electricity even using inert vermiculite as the growing medium, where the plants were the only sources of organic compounds (De Schamphelaire et al. 2010). These results suggest that living plants like rice paddy and water bamboo used in this study are important to enhance the bioelectricity generation, since electroactive bacteria (EAB) could access more carbon sources from the rhizodeposition in PMFCs. In addition, the declining trend of output voltage in soil-MFC after 20 days in Fig. 2a implied the depletion of readily biodegradable organic matter in soil. However, after 60 days, there was a gradual increase in output voltage in soil-MFC again, which might suggest after a period of acclimation, microorganisms were likely able to use the complex organic matter in soil as the new substrate for electricity generation. Overall, plants are critical to supply the organic compounds through rhizodeposits in PMFCs, especially in the long-term operation. From the result in Fig. 2a, the P-PMFC showed significantly higher output voltage than W-PMFC ($P < 0.05$), suggesting that different plants could cause the different performances of electricity production in PMFCs. Although plant roots in PMFCs could fuel the EAB at the anode by providing rhizodeposits (Md Khudzari et al. 2018), previous studies mentioned that the root architecture, quality and the quantity of plant rhizodeposition would vary over the growth stages and plant species (Aulakh et al. 2001). The variation in amount and speciation of rhizodeposits from different plants probably resulted in different performances of electricity production in P-PMFC and W-PMFC. Furthermore, the varied plant rhizodeposition over the growth stages likely caused changes in the flux of organic compounds and therefore caused fluctuation in anode potential as a consequence cell potential of PMFCs (Timmers et al. 2010), since all environmental conditions were maintained under constant conditions throughout the operation.

As shown in Fig. 2b, it is found that P-PMFC with compost (PC-PMFC) reached the highest voltage at 894.39 ± 53.44 mV (34.78 mW/m^2), which was the highest voltage values among all treatments. The previous study of outdoor paddy PMFCs observed the increase involtage generation with the addition of compost made from kitchen and yard waste (Moqsud et al. 2015). However, the compost experiments in the previous study were conducted based on one single PMFC without replicate, and the observation was not yet statistically confirmed. Our PMFCs were triplicated and clearly demonstrated that PC-PMFC with the addition of compost made from food waste could produce significantly higher voltage than those without compost ($P < 0.05$) (Table S3). In addition, compared with other treatments with largely varied electricity production, P-PMFC showed more consistent output voltage with the addition of compost. A stable output voltage suggested that the organic substrates in compost could provide sufficient foods for EAB to minimize the impact from varied plant rhizodeposition and resulted in additional capacity to enhance the bioelectricity in P-PMFC. Food waste management has been a critical environmental issue in different countries (Gustavsson et al. 2011). Therefore, our results demonstrate that compost converted from food waste can be considered as an efficient organic fertilizer to apply in paddy PMFCs to enhance electricity production.

On the other hands, the P-PMFC with biochar (PB-PMFC) had quite low electricity generation in the first and second month of the experimental duration. Even though it largely increased after 120 days, the output voltage still showed high fluctuation and significantly lower than PC-PMFC ($P < 0.05$). This result implied that adding the biochar in soils might cause some adverse effects to PMFCs, e.g., the higher EC after adding the biochar in the soils could impact some microorganisms which have a low tolerance to higher EC (Tremouli et al. 2010). In addition, although the previous study reported the addition of biochar produced by sewage sludge significantly stimulated rice growth (Khan et al. 2013), our study observed the inhibited rice growth after the addition of biochar produced from waste wood biomass (Fig. S2). One study has shown that free radicals can be detected in biochar produced from biomass charring, and these free radicals were persistent and could inhibit the germination and growth of rice seedlings (Liao et al. 2014). The authors also found that lignin in the biomass played an important role in the free radicals generation during biochar production. As mentioned earlier, the biochar used in our experiments was made from waste wood biomass, which may contain sufficient amount of lignin to produce free radicals and inhibit rice growth, and further influence the performance of electricity production in PMFCs. Furthermore, the low electricity generation also might be caused by the electrical resistivity of biochar granules and the oxygen intrusion occurred through the porous of the biochar near the cathode (Md Khundzari et al. 2019). According to biochar made from numerous and abundantly feedstock, e.g., forest, agricultural residues or even wastewater sludge (Huggins et al. 2014), the chemical and physical properties of biochar could have different effects on the crops and soil microorganisms when added into the soil. Therefore, our results warrant the need for future studies to identify biochar quality requirements for PMFC application.

The changes of pH and EC in PMFCs

The pH and EC values of the surface soils close to the cathode of soil-MFC and PMFCs varied with time as shown in Fig. 3. After operating for 15 weeks, pH values of soil-MFC showed the range of 7.23 ± 0.65 to 8.57 ± 0.57, while pH of the P-PMFC and W-PMFC varied from 6.00 ± 0.69 to 7.89 ± 0.44 and 6.95 ± 0.41 to 8.30 ± 0.12 as shown in Fig. 3a, respectively. Comparing pH values of P-PMFC, PC-PMFC and PB-PMFC, it is found that adding compost significantly increased pH values ($P < 0.05$) (Table S3). The pH values of PC-PMFC and PB-PMFC as demonstrated in Fig. 3b were at the range from 7.5 ± 0.11 to 8.3 ± 0.29 and 6.9 ± 0.15 to 7.8 ± 0.34, respectively. Generally, pH values showed increasing trend in the top soil close to the cathode, although a variation of pH was also observed during

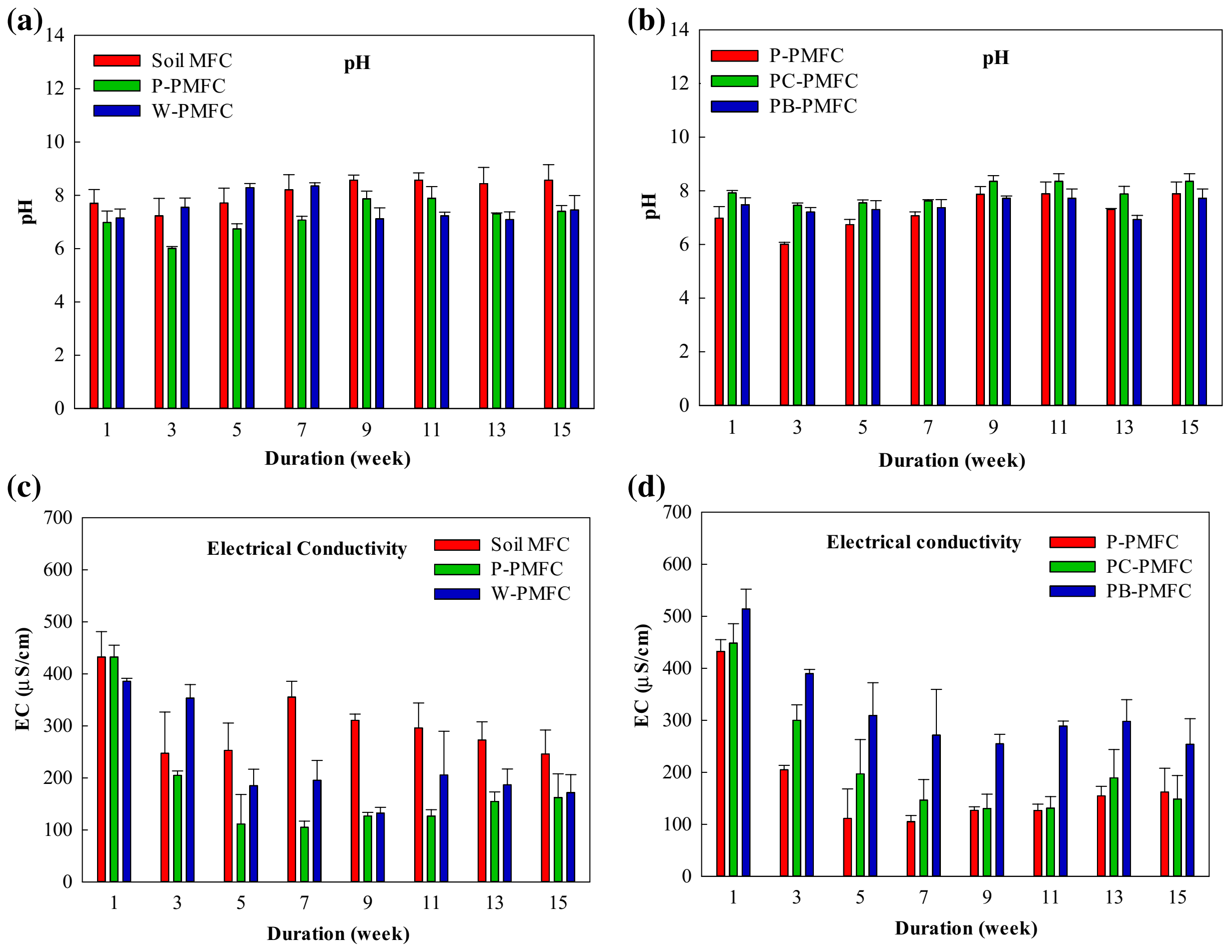


Fig. 3 Variation of pH and EC of the soil-MFC and PMFCs versus time. **a** pH of soil-MFC and PMFCs with different plants; **b** pH of P-PMFCs with or without soil conditioners; **c** EC of soil-MFC and PMFCs with different plants; **d** EC of P-PMFCs with or without soil conditioners.

operation, likely due to the heterogeneity of soil samples. Similar findings were also reported in the PMFCs study of remediation of metal contaminated soils, which showed significantly higher pH values of soils close to the cathode than those close to the anode after long-term operation (Guan et al. 2019b). As shown in the previous study, rapid consumption of H^+ in the oxygen reduction reaction in the cathode could cause the increase in pH values in the cathode chamber of MFCs (Wu et al. 2019), and therefore, increasing pH values in the top soil close to the cathode could be due to the redox reaction effects driven by the electrochemical reactions through the relationship among microorganisms, plants, soils, substrates, and electrode systems in the soil-MFC and PMFCs.

The weekly EC was monitored at the soil surface near the cathode in the experiment. Generally, EC values of all treatments showed a decreasing trend since the 1st week of operation, which was similar to the previous study (Guan et al. 2019a), as shown in Fig. 3c, d. After 15 weeks of operation, the EC of soil-MFC, P-PMFC, and W-PMFC decreased to 246 ± 46.11, 162.30 ± 45.57, and 171.80 ± 34.55 µS/cm, respectively. The EC of PC-PMFC and PB-PMFC were 115.75 ± 40.77 and 254.01 ± 49.11 µS/cm, respectively. From the results, the EC of soil-MFC was significantly higher than that of P-PMFC and W-PMFC ($P < 0.05$) (Table S3), indicating that the electrokinetics mechanism of PMFCs driven by the bioelectrochemical process (Guan et al. 2019b) and the ion absorption by plant root systems could cause the decrease of EC in soils. In addition, compared with W-PMFC, P-PMFC had significantly lower EC ($P < 0.05$). As mentioned earlier, P-PMFC had better performance in electricity production than W-PMFC, and therefore, the stronger electrokinetic effects in P-PMFC might cause the more significant migration of soluble ions. Compared with other treatments, PB-PMFC had the highest EC values (254.01 ± 49.11 to 514.00 ± 38.18 µS/cm) from the beginning till the end of the operation as shown in Fig. 3d, although the weekly EC of PB-PMFC decreased during operation. The high EC values should be caused by biochar, whose properties have changed the soil physiochemical properties such as pH, EC, cation exchange capacity, salinity, and redox potential in the PMFC systems (Palansooriya et al. 2019). Even though sometimes the high soil EC could imply more nutrients in the soils, the high EC or saline soils could likely affect the plant growth (Corwin and Lesch 2005) and consequently affect the bioelectrochemical performance of the anodophilic bacteria with a lower tolerance and cause lower coulombic efficiency and electricity generation of PMFCs (Tremouli et al. 2010; Lefebvre et al. 2011).

The polarization curve of PMFCs

The polarization curve, which presented the voltage as a function of current, was provided as the characteristics of our P-PMFC to compare with the previous study. However, as shown in Fig. 2, the output voltage of P-PMFC varied during operation and could achieve around 600 mV on the 60th day, which was roughly the highest values achieved by P-PMFC in our study. Therefore, the obtained polarization curve could represent the better performance of P-PMFC. As shown in Fig. 4, the polarization curve of P-PMFC started with initial open-circuit voltage (OCV), and then, the voltage was evaluated across several different resistors (10, 39, 68, 100, 320, 510, 820, 912, 1 K, 1.5 K, and 22 KΩ). Starting with the OCV of 608.8 mV, this point means no current in the system, and during the polarization, the cell potential decreased stepwise. After measuring with different resistors, the power density reached the peak value with decreasing external resistance. The maximum power density of P-PMFC was 8.66 mW/m^2. At the maximum power density point of polarization, the internal resistance was estimated to be 328.85 Ω according to Eq. (3), which indicates the internal resistance will be equal to the external resistance at the maximum power density. Afterward, the power density began to drop with an increasing current density which indicated typical fuel cell behavior. The polarization trend of P-PMFC showed similarity to the polarization curves reported in previous MFC studies and PMFC studies (Logan et al. 2006; Moqsud et al. 2013, 2015). The

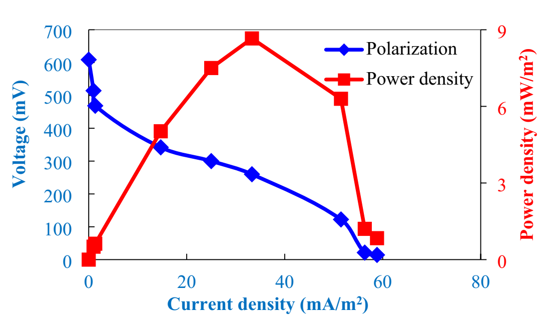


Fig. 4 Polarization curve of the P-PMFC at the 60th day of the experiment

previous reed mannagrass (*Glyceria maxima*) PMFCs study reported the internal resistance in the range of 450–600 Ω, and their maximum voltage was at 253 mV (Strik et al. 2008). It indicated that the high internal resistance could affect the electron transfer in the bioelectrochemical systems and could influence the power density of PMFCs. Kaku et al. (2008) estimated that the maximum power density, internal resistance, and OCV via polarization curve of the rice paddy PMFCs were 5.75 mW/m^2, 156 Ω, and 701 mV, respectively. Watanabe et al. (2017) reported paddy PMFC using carbon graphite felt as electrodes achieved power density around 12 mW/m^2. In this study, the polarization curve was generally similar to most of the previous PMFCs studies, with the compatible maximum power density and internal resistance values. However, this study used the simple setup of PMFCs, and the tubular PMFCs with biocathodes have been reported to improve the power generation to 82 mW/m^2 (Wetser et al. 2017). Therefore, the better design of PMFCs can be considered to further increase the electrical performance.

Microbial community structure

After 120 days of long-term incubation, anode samples of all experimental setups were analyzed for their constituents of the microbial community. High-throughput sequencing of 16S rRNA genes amplified using 16S V3–V4:341F-805R primers was adopted for the microbial community analysis. Overall, total 406,044 high-quality 16S rRNA gene sequences were obtained and classified into OTUs with 97% of similarity. Species richness and evenness of community distribution indicated by Shannon index and Chao1 are showed in Table S4. The results of taxonomic classification demonstrated that *Proteobacteria* was the most predominant phylum with relative abundance ranging from 20.25 to 34.10% followed by *Patescibacteria*, *Bacteroidetes*, *Chlorflexi*, *Verrucomicrobia,* and *Planctomycetes*, accounting for 16.39–21.02%, 9.56–15.17%, 5.02–13.24%, 4.40–16.09%, and 3.54–8.13%, respectively (Fig. 5a). The most abundant classes were *Gammaproteobacteria* and *Deltaproteobacteria* which comprised 5.44–14.62% and 5.48–11.54% of the microbial communities as showed in Fig. 5b. A previous study showed that *Proteobacteria* (31.7–38.7%), *Chlorflexi* (8.1–8.9%) and *Bacteriodetes* phyla (1.7–6.9%) were enriched at the anode of *Canna indica* PMFCs, and the results also showed the dominance of *Gammaproteobacteria* at the class level (Lu and Xing 2015). Furthermore, the other study found that *Proteobacteria* was the most abundant phylum of the anode rhizosphere bacterial community in *Glyceria maxima* PMFCs (Timmers et al. 2012). In addition, the phyla of *Bacteriodetes* and *Chlorflexi*, which were considered as the rhizosphere bacterial groups, were also found enriched on the anodes of the previous study of paddy PMFCs (De Schamphelaire et al. 2010). Generally, our

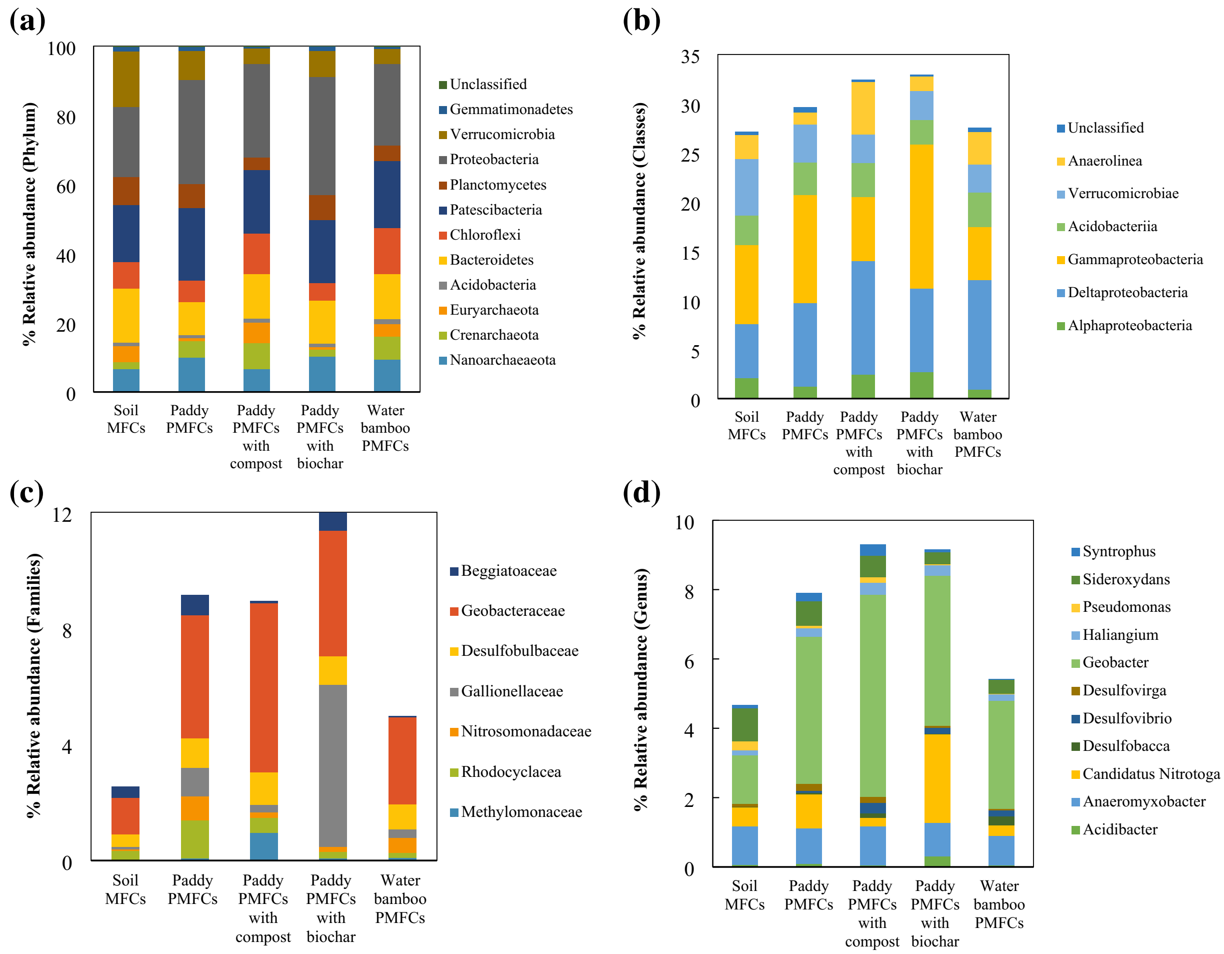


Fig. 5 Microbial communities at the anode after 120 days of operation. **a** Relative abundance of phyla of the soil-MFC and multiple PMFCs; **b** relative abundance of classes of the soil-MFC and multiple PMFCs; **c** relative abundance of families of the soil-MFC and multiple PMFCs; **d** relative abundance of genera of the soil-MFC and multiple PMFCs

results of microbial communities in the anodes of PMFCs were in agreement with the previous PMFC studies at the phylum and class levels.

Geobacteraceae, which consisted of the commonly known EAB genus *Geobacter*, was the most abundant family of the whole microbial communities and was found more abundant in PMFCs (2.99–5.82% of the total microbial community) than the soil-MFC (1.3%) as showed in Fig. 5c. In addition, the family *Desulfobulbaceae*, containing the sulfate-reducing bacteria, was also enriched at the anode. *Desulfobulbaceae* is known to contain filamentous bacteria, cable bacteria, and mesophilic sulfate-reducing bacteria, which can use sulfate, thiosulfate and sulfite and nitrate as electron acceptors (Kuever et al. 2005). *Desulfobulbaceae* was also found more abundant in PMFCs (0.85–1.13%) than soil-MFC (0.43%). *Geobacter* was the most dominant genus which accounted for 6% of the whole microbial communities followed by *Anaeromyoxobacter*, *Candidatus Nitroga*, and *Sideroxydans* as shown in Fig. 5d. Since *Geobacter* is the well-characterized EAB with high electrical production capacity (Bond and Lovely 2003), the result indicated that *Geobacter* should be involved in electricity generation in PMFC systems in this study. *Anaeromyoxobacter* has been reported to use acetate, lactate, and pyruvate as the electron donor (Hwang et al. 2015). Since the root exudation or rhizodeposition of PMFCs could provide acetate or other organic compounds as electron donors for microorganisms, whether the functions of microbial population found in this study were related to current generation or related to the carbon, nitrogen, sulfur, and iron cycling in paddy soil (Liesack et al. 2006) needs further validation. Competition for electron donors among the different microorganisms could result in the decrease in electron donors available for EAB and thus lower current generation (Timmers et al. 2012).

The microbial community structure was analyzed by using UniFrac analysis coupled with PCoA and UPGMA hierarchical clustering to compare the linkages of microbial community among different samples of soil-MFC and multiple PMFCs based on beta diversity and their phylogenetic assignments as shown in Fig. 6. Figure 6a showed the comparison of microbial communities among five samples by PCoA, and the dendrogram cluster analysis of the microbial communities (at the family level) of the anode is showed in Fig. 6b. From the results, PCoA and cluster analysis of microbial community structure roughly showed three different groups, i.e., (1) soil-MFC and W-PMFC, (2) P-PMFC and PC-PMFC, and (3) PB-PMFC (Fig. 6). Thus, the results suggest that microbial communities could be influenced by the soil fertilizers and conditioners, the plant root systems, and root exudates. However, the PB-PMFC had the most distinct anode microbial community from other samples (Fig. 6). From the microbial community analysis in PB-PMFC, we found *Gallionellaceae* as the predominant family, which is considered to be involved in the iron cycling (Hallbeck and Pedersen, 2014), but *Geobacteraceae* was the most dominant family in other PMFCs (Fig. 5c). As mentioned above, the electricity generation of PB-PMFC was significantly less than that of P-PMFC and PC-PMFC (Table S3). Therefore, these results demonstrated that the performance of electricity generation by PMFC systems would be influenced by the dominant microbial community, not only including EAB, e.g., *Geobacter* but also other microbial population with different pathways of electron transports in PMFC systems.

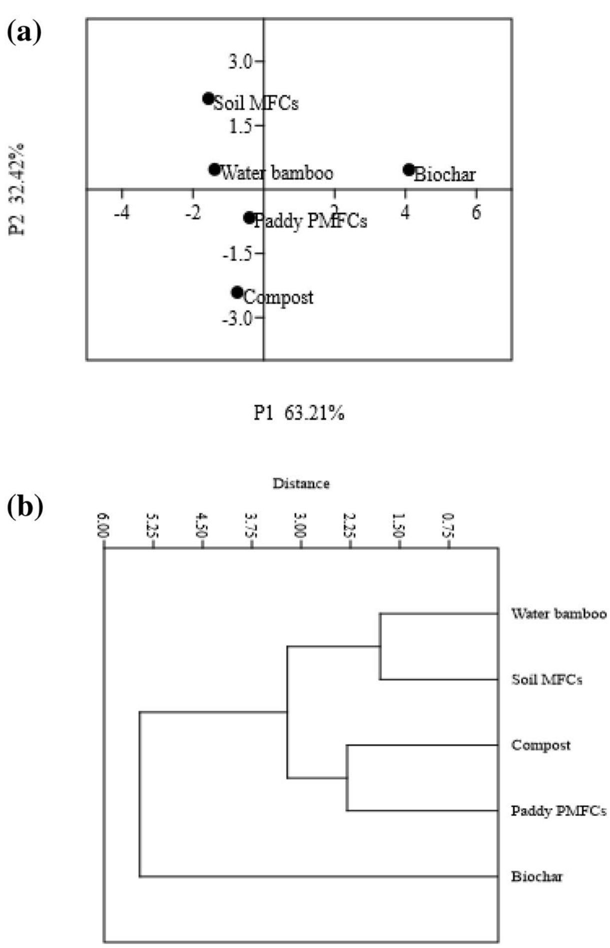


Fig. 6 Analysis of microbial community structures of the soil-MFC and multiple PMFCs. **a** PCoA of microbial community structures in the soil-MFC and different PMFCs; **b** cluster analysis of the microbial community structures in the soil-MFC and different PMFCs

Conclusions

Multiple PMFCs were operated under the controlled environments, and the voltage output of PC-PMFC, which was added with compost made from food waste, reached the highest value of 894.39 ± 53.44 mV (34.78 mW/m^2). PB-PMFC demonstrated significantly lower voltage production than those without biochar, likely due to the inhibitory action of biochar made from waste wood biomass. All PMFCs had significantly higher voltage outputs than soil-MFC. The significantly higher output voltage of P-PMFC than W-PMFC indicated that plant species would affect electricity generation of PMFCs. The 16S rRNA gene high-throughput sequencing revealed *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were the most abundant phyla of the anode microbial community. The exoelectrogen *Geobacter* was the most dominant genus of anode microbial communities and showed the highest abundance in PC-PMFC. This study has demonstrated that the power output of PMFC systems can be influenced by different agricultural plants and soil conditioners, and soil conditioners made by suitable waste biomass could be applied in PMFCs to enhance the performance of electricity production.

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Author's contribution NT, C-PY were involved in conceptualization; NT, C-YG, C-PY contributed to methodology; NT, W-SC were involved in formal analysis; NT, C-YG contributed to visualization; NT was involved in data curation, investigation and writing—original draft; C-YG, W-SC, C-PY contributed to writing—review and editing; C-PY was involved in funding acquisition and supervision.

Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration

Conflict of interest The authors declare that they have no competing interests.

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