Simultaneous phytoremediation of Cu$^{2+}$ and bioelectricity generation in a plant-microbial fuel cell assembly growing Azolla pinnata and Lemna minor

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Abstract. The relationship between power generation in a Plant-Microbial Fuel Cell (PMFC) and phytoremediation were observed in this paper. It is hypothesized that both power generation and metal uptake capacity will be enhanced synergistically upon the combination of the two processes. To test the hypothesis, the experiment was composed of three set-ups: (A) PMFC only, (B) phytoremediation only, and (C) PMFC combined with phytoremediation. A maximum power density for set-up A of 3.43 μW/m$^2$ and 247.11 μW/m$^2$ for Azolla pinnata and Lemna minor, respectively, while a maximum power density for set-up C of 17.82 μW/m$^2$ and 1076.16 μW/m$^2$ for the two plants were observed. The values showed a significant increase in power density for set-ups where copper ions are present (set-up C). The internal resistance of the PMFCs were also investigated. It was observed that internal resistance continuously decreased through time and the presence of copper ions in set-up C of both plants resulted to a lower internal resistance, equating to higher power densities. In terms of phytoremediation potential, the results showed that the presence of electrodes in the hybrid set-up induced the plants to absorb more copper ions than the control. The copper ion uptake increased by about 18% for A. pinnata and 38% for L. minor in phytoremediation tests in PMFCs.

Overall, the combination of PMFC technology and the concept of phytoremediation has been shown to benefit both power generation and metal uptake capacity. The results of this study can be used to enhance the clean-up of metal contaminants while simultaneously providing power in-situ.

1. Introduction

Electricity is a necessity for a country to prosper in this age of technology. The Philippines relies mostly on coal for its energy needs. Only 24% of the total electricity generated came from renewable energy (RE) (i.e. hydrothermal, geothermal, solar, and wind). However, these types of renewable energy are highly dependent on geographic location, and they contribute to landscape transformation and may even compete with agriculture for land. A recent type of emerging renewable energy is the Plant-Microbial Fuel Cell (PMFC) which utilizes the rhizodeposits being produced by the plant and metabolized by the bacteria found in the root zone of the plant. The rhizosphere represents an area of symbiotic interaction between the soil’s microorganisms and plant roots. Simple sugars and amino acids are produced as a bioprocess in plants and then purposefully excreted through the roots to attract microbes that are beneficial to the plant. As a consequence of microbial metabolism of root exudates,
Electrons and hydrogen ions are released in the substrate and can subsequently be harvested as electricity by the proper placement of electrodes. The result is the continuous generation of electricity by the electrogenic bacteria which continue to do so as long as the plant is living and is giving them a share of its photosynthetic products [1].

Plants are also widely used in environmental remediation processes. Phytoremediation is the process of using plants to treat different organic and inorganic pollutants through accumulation, extraction, destruction, modification, and other modes of action. The different types of process in phytoremediation are phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization [2]. Given that plants are already being utilized in phytoremediation, it is of interest if this waste treatment process can be combined with another technology involving plants to increase its efficiency and effectiveness. One such candidate for technology hybridization is the incorporation of electrodes in a phytoremediation set-up to produce a combined PMFC-phytoremediation hybrid.

A study on *Eichhornia crassipes* (water hyacinth) in a wastewater-fed PMFC showed a maximum power density of 80.08 mW/m² and emphasized that the organic loading in the wastewater is directly proportional to the power density generated by the fuel cell [3]. A study on *Lemna minuta* (duckweed) in a PMFC without supplementation of organic waste generated a power density of 380 mW/m² which is relatively higher compared to other aquatic plants experimented up to date [4]. The anode is a rectangular piece of carbon felt while the cathode is graphite granules which are exposed to air. Other species of duckweed (*Lemna aequinoctialis* and *Spirodela polyrhiza*) were assessed for their toxicity threshold and metal uptake for different metal concentrations. One of the results from *L. aequinoctialis* shows that it can withstand copper accumulation but can pose harm to its cell membrane [5]. The study on *Azolla caroliniana* showed that the presence of copper is toxic for this plant. *A. caroliniana* has a weak removal rate of copper containing waste, but it can remove a significant amount of Mn, Zn, Pb, Cr, and Cd [6].

Studies on duckweed in both phytoremediation and in PMFCs have already been reported. However, the simultaneous process of metal removal in phytoremediation and electricity generation in PMFC is yet to be tested on *L. minuta*. A previous study has investigated the effect of combining heavy metal (Ni²⁺) remediation and electricity generation using *E. crassipes* in a PMFC with promising results [7]. Therefore, it is of interest in this study to replicate the observed effects on a different set of plants and metal ion pollutant. The main objective of this study is to investigate the possible synergistic relationship between bioelectricity generation in a PMFC growing *Lemna minor* and *Azolla pinnata*, and the Cu²⁺ ion uptake capacity through phytoremediation. It was hypothesized that both processes, when combined, would increase each other’s effectiveness in terms of power density (PMFC) and metal uptake capacity (phytoremediation). This study also aimed to determine the behavior of the internal resistances of PMFCs through time and with the presence of dissolved metal ions.

## 2. Materials and methods

### 2.1. PMFC set-up

Acrylic plastic sheets were cut to the specified dimensions (5.5 in x 3 in x 3 in) and were formed as containers (figure 1). The electrodes used were woven carbon fiber with dimension of 5 in x 2 in for both anode and cathode. An Etekcity digital multimeter was used to read the voltage in each set-up. Copper wires was used to connect the electrodes to each external resistor (1000 Ω). A plastic separator was also used to prevent the plants from reaching the cathode region to prevent short-circuiting the whole set-up. Grow lights (E27 LED Plant Grow light bulbs, 3 W) were used as a source of light for the entire experiment at a constant photoperiod of 12 hours.

*L. minor* and *A. pinnata* were sourced from an aquaponics center in Manila, Philippines. The plants were washed and acclimated in shallow basins with dechlorinated tap water with 0.5% dissolved complete (20-20-20) inorganic fertilizer for 10 days under grow lights before transferring in the PMFCs. After acclimation, 5 grams (wet mass) of the plants were transferred in each PMFC set-up.
and were floated on top of the anode.

![PMFC assembly](image)

**Figure 1.** PMFC assembly (A: anode with floating plants; B: cathode; C: physical separator; D: resistor; E: multimeter).

### 2.2. Experimental set-up

The experimental design was patterned from a similar study [7] and is shown in Table 1. Two control systems and a combination of the two were prepared to fulfil the objectives of the study. The first control set-up is a traditional PMFC set-up (system A) which was described in the previous section. The second control (system B) is a phytoremediation cell wherein the plants are grown in a media spiked with copper (II) sulfate to give a resultant initial Cu$^{2+}$ concentration of 10 ppm. The combined set-up (system C) is a PMFC assembly with the media containing 10 ppm Cu$^{2+}$. This arrangement allows for the analysis of power density and Cu$^{2+}$ uptake with their separate control set-up. All systems were done with at least 5 trials.

| System | Description | Parameters determined |
|--------|-------------|-----------------------|
| A      | PMFC only   | Power density         |
| B      | Phytoremediation only | Cu$^{2+}$ uptake |
| C      | PMFC + Phytoremediation | Power density and Cu$^{2+}$ uptake |

### 2.3. Data acquisition and analysis

Voltages of systems A and C were measured daily against a 1000 Ω resistor for 30 days. From the voltage, current (V/R), power (V$^2$/R), and power density (P/A) were computed. Polarization curves were done every 5 days to monitor the internal resistance of the system. Varying resistance values (10 Ω to 50000 Ω) were connected to systems A and C to determine the maximum power obtained at the value of the internal resistance.

At the end of the experiment, the plants from all systems were gathered and dried for the determination of Cu$^{2+}$ content. Plant samples were separately dried at 100°C until constant weight was achieved. The dried samples were digested with concentrated nitric acid for 30 minutes or until the formation of brown fumes ceased. The digested samples were diluted to 50 mL. The Cu$^{2+}$ content of the samples were determined using Atomic Absorption Spectroscopy (AAS) after the construction of a standard calibration curve.
3. Results and discussion

3.1. Voltage and power density of PMFCs

The average voltage and power densities for systems A and C for both *A. pinnata* and *L. minor* are shown on figures 2 and 3.

![Figure 2](image1.png)

*Figure 2.* (a) Voltage of systems A (○) and C (■) in PMFCs with *A. pinnata* and (b) Voltage of systems A (○) and C (■) in PMFCs with *L. minor*.

Set-up A of *L. minor* has shown spikes in voltage readings which can be attributed to the fast asexual reproduction of the fronds. Since *L. minor* and *A. pinnata* has fast growth rates and short lifespans, the root biomass increases at a faster rate than decaying of old fronds which increases total rhizodeposition that is responsible for the increased bioelectricity generation. In principle, when plants had aged or began to die, rhizodeposition decreases [8]. A decrease in power output attributes to
physiological changes in the plants when the plants reaches the end of its life cycle and bacterial populations become stagnant or die as well. The voltage readings and power density of the constructed systems were less than those of previous studies involving *Lemma* [4,9] due to differences in growing conditions (indoor growing). Nevertheless, the voltage generated by set-up C (PMFC and phytoremediation) was significantly higher than that of set-up A (control, PMFC only) ($\alpha = 0.05$). This may be attributed to the presence of $\text{Cu}^{2+}$ in the media as it increases the electrolytic conductivity of the set-up [7]. There were no observed trends of voltage increase with respect to substrate pH, relative humidity, and temperature.

Figure 3. Voltage (●) and power density (□) of system C (PMFC+phytoremediation) growing *A. pinnata* (left) and *L. minor* (right).

3.2. Polarization

Figure 4. Day 20 polarization curves for *A. pinnata* grown in system A (control, left) and system C (hybrid, right); points represent experimental values, while the solid lines are fitted polynomials.

Polarization curves evaluate the steady-state power density across values of current densities and can show the maximum possible power density at an optimum resistance or current density. PMFCs utilize bacteria in the rhizosphere, which catalyze the oxidation of simple organic substances to generate current from the substrate, which can be form of renewable energy. The slope of a voltage versus current polarization curve determines both internal and polarization resistances [10]. In a power versus current polarization curve (figure 4), power density increases with increasing current density until a
maximum power density is reached. Beyond that, any increase in current density will result to a lower power density. Maximum power densities are dependent of substrate concentration and materials of the electrode [11].

![Image](image_url)

**Figure 5.** Day 10 polarization curves for *L. minor* grown in system A (control, left) and system C (hybrid, right); points represent experimental values, while the solid lines are fitted polynomials.

It is shown in figures 4 and 5 that the power generation in a PMFC coupled with phytoremediation resulted in higher maximum power densities, about 183% increase for *A. pinnata* and 4600% increase for *L. minor*. The main contributor of this phenomena was seen in the presence of copper ions, wherein additional ions facilitated faster transport of electrons from the anode to the cathode. This result is similar to a previous study where PMFCs growing *E. crassipes* (water hyacinth) in the presence of Ni$^{2+}$ exhibited elevated power densities compared to the control [7]. The set-up produced a higher amount of electricity when nickel solution was added in their set-up. Maximum power densities were observed for *A. pinnata* at 3.43 µW/m$^2$ and 9.71 µW/m$^2$ for systems A and C, respectively.

![Image](image_url)

**Figure 6.** Power density versus resistance plots (day 10) for *L. minor* (left) and *A. pinnata* (right) grown on system C.

### 3.3. Internal resistance

It has been established in the previous section that the internal resistance of a PMFC can be determined from the polarization curve. This method was used to plot the power density versus
resistance, with the maximum point indicating the resistance value wherein the internal and external resistances applied on a cell are equal (figure 6). The value of the internal resistance is an important parameter that can dictate the overall performance of a fuel cell.

![Figure 7](image)

**Figure 7.** Behavior of internal resistance of PMFCs through time, growing *A. pinnata* (left) and *L. minor* (right), comparing the resistances of system A (□) and system C (●).

As evident in figure 7, the internal resistance of PMFCs steadily decreases. The internal resistance of replicates in system C are generally lower than those in system A, meaning that the presence of ions in the substrate do facilitate faster ion transport, thereby decreasing ohmic resistances. The internal resistance is composed of anodic resistance, cathodic resistance, substrate resistance, and membrane resistance [12]. For this study, no membrane was used, thereby reducing the source of resistance to three. The developing biofilm in the electrodes are vital for electricity generation, however, it also contributes to higher resistances [13]. Thus, the main mode of action for the improvement of internal resistance in a hybrid PMFC-phytoremediation system is the lowering of the substrate resistance, resulting to higher power densities.

### 3.4. Cu²⁺ uptake capacity

The copper content of digested plant samples were determined spectrophotometrically with the aid of a standard calibration curve for copper (figure 8). Quadratic fitting was applied to have a better fit (R²=0.9998). The copper content of all samples are summarized in figure 9.

Both plants have significantly increased (α = 0.05) metal uptake capacity when placed in a hybrid PMFC-phytoremediation set-up (system C). The uptake capacity increased by 18% and 38% for *A. pinnata* and *L. minor*, respectively. A possible explanation for this is that the current passing through the system stimulates the plant to absorb more metal ions, in the same way that being polarized stimulates the plant to grow faster and accumulate more intracellular content [9]. Furthermore, the positive copper ions can be attracted to the negatively charged anode where the plants are, effectively forming a copper ion concentration gradient with higher concentrations at the anode. This forces the plant to absorb more copper to compensate for the higher copper ion concentrations in the bulk media [14]. Both plants have the capability to absorb copper through their roots and frond surface [15]. When metals are absorbed by the plants, they can be trapped by the negative charges of the cell walls or be taken up into cell cytoplasm and compartmented in some organs like vacuoles, or they can be extracellularly excreted [16]. The uptake of excess metals can cause plants to display their stress pigmentation which was evident on the yellowing of experimental plants [17]. The net effect is the plants die faster in exchange of absorbing more copper ions and generating more power in the hybrid system.
4. Conclusions

This study has demonstrated the benefits of combining PMFC technology with phytoremediation of copper ion using *L. minor* and *A. pinnata* as model plants. It also supported a previous study using water hyacinth in PMFC and nickel ion as a contaminant. Both plants grown in system C (PMFC with phytoremediation) exhibited significantly higher power generation and copper ion uptake compared to their respective controls. Therefore, both plants grown in the hybrid set-up outperformed standalone systems in terms of power density and copper ion uptake capacity. Using the hybrid system helps to reduce the ohmic resistance and introduce an alternative terminal electron acceptor which ultimately increases the power output and increases the metal ion uptake of the plants.

*A. pinnata* and *L. minor* exhibited 183% and 4600% increase on their respective maximum power densities, while increasing Cu$^{2+}$ ion uptake by 18% and 38%, respectively. Therefore, the combination of PMFC technology and phytoremediation can enhance both processes simultaneously.

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