Effects of heavy metals (Fe$^{3+}$/Cr$^{6+}$) on low-level energy generation in a microbial fuel cell

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Abstract. A dual-chamber microbial fuel cell (MFC) was constructed with *Pseudomonas aeruginosa* as biocatalyst to facilitate substrate conversion and, consequently, low-level energy generation. To simulate a wastewater situation with BOD and heavy metals contamination, glucose and Fe$^{3+}$ and Cr$^{6+}$ were used as substrate and heavy-metal spikes, respectively. The effects of varying substrate concentrations (150 ppm, 300 ppm, 600 ppm) and heavy metal loads (10 ppm, 50 ppm, 100 ppm) on overall power generation were evaluated. The presence of Cr$^{6+}$ in the anode compartment decreased the potential from 565 to 201 mV (i.e., lowest value achieved at highest Cr$^{6+}$ concentration of 300 ppm). On the other hand, replacing Cr$^{6+}$ with Fe$^{3+}$ as electron acceptor resulted in substantial increase in measured potential (i.e., from 565 to 703 mV). Increasing glucose concentrations resulted in longer time to reach constant open circuit voltage. A maximum potential of 606 mV was achieved at 1200 ppm glucose. Incorporating *Pseudomonas aeruginosa* increased the potential from 256 to 592 mV. On the basis of these results, a microbial fuel cell feeding on wastewater can be an important potential technology for generating low-level energy

1. Introduction

There is a need to develop alternative energy source to partially contribute to addressing the impending energy crisis predicted to happen in a few decades in the future. One such technology is microbial fuel cell (MFC), an electrochemical device that can generate low-level electricity from the energy effects associated with the bioconversion of a substrate. MFC has cathode and anode separated by an electrolyte. The microbes oxidize the organic substrate anaerobically in the anode. The electrons lost via oxidation (at the anode) is transported via a resistor to the cathode, where reduction occurs. This transfer is facilitated by membrane-associated components or soluble electron shuttle [1].

MFC technology is not novel. The use of microorganisms a fuel cell started some decades ago [2, 3]. It was used in wastewater treatment [4]. However, interest on the electricity produced during the oxidation/reduction process has been of interest recently [5, 6, 7]. Current inorganic fuel cells such as polymer–electrolyte, direct-methanol, and solid-oxide systems [8] have already reached innovation phases. Such systems, however have drawbacks in cost and waste management. Among technologies for generating energy from organic waste, MFC has the following advantages: (a) direct utilization of substrate via biologically-mediated, non-temperature-sensitive chemical reactions that consequently generate energy [1, 9]; low or no energy requirement for a sufficiently ventilated cathode chamber [5, 10]; (c) a possible widespread application in regions where energy resources are wanting. However, the study concerning this technology is still on its early stage. MFCs at present show great potential in harvesting energy from wastes. But, little is known about how presence of other factors affects waste
treatment and the subsequent generation of low-level electricity. In this study, the effect of heavy metals, which may be present in the waste stream, on the low-level energy generation is in focus. It is construed that the electrons generated from the redox reactions between the heavy metals and the microorganism might affect or contribute to the electricity generation. Fe$^{3+}$ and Cr$^{6+}$ were used as models.

*Pseudomonas aeruginosa*, a biocatalyst with wide-range biosorption capability was used. *Pseudomonas aeruginosa* has attractive features: being inexpensive, simple mass cultivation, and its being capable of producing pyocyanin, a mediator that serves as electron shuttle in the anode chamber of MFC [11]. *Pseudomonas aeruginosa* catalyzes the transformation of biochemical energy to generate electrical energy. The anaerobic fermentation pathway of *Pseudomonas aeruginosa* enables the conversion of pyruvate to ethanol. When NAD$^+$ is reduced to NADH it produces two ATP molecules, two H$^+$ ions and two electrons [12]. The electrons generated at the anode can then traverse via external circuit to produce power. The electrons then move into the cathode chamber to react with the protons transported via ion exchanger. *Pseudomonas aeruginosa* can simultaneously act as a good biosorbent. It possesses metal-sequestering properties and can reduce the heavy metal ions concentration in a given solution. At high efficiency, it can remarkably sequester dissolved metal ions out of dilute complex solutions [13, 14].

The main objective of this study is to harvest energy from wastewater using MFC while simultaneously treating the heavy metal content of the wastewater. To accomplish this, determination of the following were taken into consideration: energy generated by the MFC at different time intervals; the effect of adding *Pseudomonas aeruginosa*, whether it enhances the production of energy and reduction of heavy metal by quantifying concentrations of the specific heavy metals using spectrophotometric assay. This study specifically determines the effect of heavy metals present in the substrate in the generation of energy upon reduction (by the microorganism).

This study, however, does not include attempt at storing the energy generated.

2. Materials and Method

2.1. Microbial fuel cell assembly
The microbial fuel cell used in this study was the dual chamber type, adapted from the design of Rabaey [15]. The compartments were constructed using plexi glass (600 ml volume) for anode chamber and 300 ml for cathode chamber. The anode and cathode chambers are separated using an ion exchange membrane made of 10% agar and 5% potassium nitrate solution [16]. The electrodes were constructed using carbon brass (3 in) and copper wire was used to hold each carbon rod. Minimal oxygen content was maintained in the anode using an air-tight cover. The cathode comprised of 50 mM K$_3$Fe(CN)$_6$. Electro-analysis was conducted using a Proskit multimeter.

2.2 Preparation and cultivation of the cultures
The microorganism (obtained from the Department of Science and Technology, Manila, Philippines) was grown in test tubes. The medium for the pure culture comprised of peptone (20 g/L), MgCl$_2$ (1.4 g/L), NaCl (10 g/l) and agar (10 g/L) (Sigma-Aldrich™). The medium was autoclaved at 121°C for 20 min. The *Pseudomonas aeruginosa* cells were inoculated into sterilized media at ambient temperature and incubated at 30°C. The pure culture was grown for 2 days until pyocyanin pigment appeared. A drop of concentrated HCL would confirm the presence of pyocyanin.

2.3 Microbial fuel cell (MFC) operation
In the operation of MFC, 100 ppm of glucose was used in the anode chamber. To test the effect of heavy metal reduction in the low-level energy generation, simulated sample was used. The heavy metal tested was chromium (as potassium dichromate). The concentration was varied (10 ppm, 50 ppm and 100 ppm) and a blank solution using deionized water was considered. The effects of incorporating chromium (VI) in the cathode and substituting potassium hexacyanoferrate by potassium dichromate
as electron acceptor to potassium dichromate were determined. To test the effect of the substrate concentration, glucose concentration was varied (150 ppm, 300 ppm, 600 ppm). The concentration after a 1-day study period was evaluated using atomic absorption spectroscopy. For an actual system study, mixed culture inoculum, waste water from a brook near the meat section a public market was used.

2.4 Measurement and calculations

The cell voltage across the external load was continuously measured using Proskit multimeter. Power and current will be calculated according to \( P = IV \) and \( I = \frac{V}{R_{ext}} \), where \( P \) (W) is the generated power, \( V \) (V) is the measured cell voltage, \( R_{ext} \) (Ω) is the external load, and \( I \) (A) is the current. The current and power produced will be normalized to the surface area of the electrode. The data gathered at hourly time interval (1 day) were plotted accordingly. Also the efficiency of the microbial fuel cell was evaluated via the energetic efficiency (\( \mu \)).

\[
\mu = \frac{V}{\Delta H (nF)}
\]

where \( V \) is the voltage across the load, \( \Delta H \) is the calorific value of glucose, \( n \) is the number of electron moles transferred across the load, and \( F \) the Faraday constant.

3. Results and Discussion

3.1 P. aeruginosa cultivation and pyocyanin synthesis

Mediator is critical towards the power generation in MFC as it shuttles the electrons from the bioconversion of glucose to the anode. Usually this mediator is externally supplied, but \( P. \) aeruginosa is capable of synthesizing its own pyocyanin. During the cultivation period, \( P. \) aeruginosa would form colonies surrounded by a blue-green zone due to pyocyanin production. This could be due to the composition of the growth media. The peptone provided the essential nitrogenous nutrients, carbon, sulfur and trace elements; its low phosphorous content minimized the inhibitory action on pyocyanin production. Potassium sulfate and magnesium chloride are key components to induced pyocyanin synthesis. Although the components of the media enhances the production of pyocyanin, some pigmentation like red (pyorubin), yellow-green (pyoverdine, fluorescein) or brown (pyomelanine) pigments were observed and masked the pyocyanin; hence, to validate that majority of the pigment synthesized were pyocyanin a drop of concentrated HCl was incorporated turning the media red. This, as suggested by King et al. [17, 18], is a reliable method indicating that \( P. \) aeruginosa was able to synthesize pyocyanin; this was aside from the preliminary observations (blue-green colonies).

3.2 Microbial fuel cell performance

In a given MFC, the oxidation of substrate like glucose is the main source of energy. The specific potentials of the anode and the cathode are determined through the redox potential of the reactions at the electrode. Theoretically, the maximum open circuit voltage that can be acquired due oxidation of glucose and reduction of oxygen is 1.216 V but, since the oxidation occurs within the microbial cell, which is external to the electrode, the theoretical value is not fully acquired.

Under an oxygen limited environment, \( P. \) aeruginosa proceeds a fermentation process where NADH is being oxidized by a specific enzyme. Certain amount of NADH will permeate through the extracellular environment. This will facilitate a redox couple NADH / NAD+ on the boundary of the anode where electrons and protons are being dissipated [19], but NADH is significantly hindered because of the presence of the cellular membrane. This is the primary reason a mediator such as the pyocyanin is crucial in the electron transfer (with respect to the electrode). Pyocyanin undergoes reduction by NADH and then oxidized once it is contacted with the electrode. The reactions on the anode are summarized below.
Cell: \[ \text{NADH} \rightarrow \text{NAD}^+ + \text{H}^+ + e^- \] (2)

Electrode contact: \[ \text{Pyocyanin}_{(\text{reduced})} \rightarrow \text{Pyocyanin}_{(\text{oxidized})} + 2e^- + \text{H}^+ \] (3)

On the other hand, on the cathode compartment; the electrons from the anode reduces \( \text{K}_3\text{Fe(CN)}_6 \). Then the protons (\( \text{H}^+ \)), which passes through the ion exchange membrane, reduces the oxygen molecules present in the cathode to form water molecules.

Electrode contact: \[ 4 \text{K}_3\text{Fe(CN)}_6_{(\text{oxidized})} + 4e^- \rightarrow 4 \text{K}_3\text{Fe(CN)}_6_{(\text{reduced})} \] (4)
\[ 4\text{K}_3\text{Fe(CN)}_6_{(\text{reduced})} + \text{O}_2 + 4\text{H}^+ \rightarrow 2\text{H}_2\text{O} + 4 \text{K}_3\text{Fe(CN)}_6_{(\text{oxidized})} \] (5)

Figure 1 shows that open circuit voltage increased with time. A previous growth phase study reported that this could be due to the number of microorganisms undergoing metabolism increasing as well [20]. The voltage across the membrane increased almost linearly with time until the 20th hour. This period could coincide with the logarithmic phase of the bacterial growth, where the number of bacterial cells growing in the anode chamber would have rapidly increased. In the succeeding hours, the voltage across the MFC slowly decreased, which could be a period coinciding with stationary phase of the bacterial growth, where in the number of cells dying would be equal to the cells growing in the anode compartment. Possible source of discrepancy in the overall potential could be due to biofouling of the microorganism in the membrane; it could develop an internal resistance resulting to a decrease in the overall potential.

![Voltage output upon substrate utilization with time](image)

**Figure 1.** Voltage output upon substrate utilization with time

### 3.3 Effect of heavy metal concentration in the anode compartment

Figure 2 shows that as the concentration of the metal (\( \text{Cr}^{6+} \)) increases from 50 ppm to 300 ppm the voltage across the MFC decreases. It could be that presence of such metal could disrupt the cell membrane of the microorganism once adsorbed; hence, the number of cells growing in the anode chamber would decrease and so was the voltage across the MFC [7]. In addition, the protons and electrons generated from the utilization of glucose could have reduced metal ions present in the anode chamber resulting in disruption of the ion flow gradient (from anode to cathode); hence, decreasing in the overall potential in the MFC. On this assumption, \( \text{Cr}^{6+} \) was incorporated in the cathode compartment to serve as an alternative electron acceptor.
3.4 Effect of heavy metal concentration in the anode compartment

Here, instead of using potassium hexacyanoferrate as an electron acceptor, Cr\(^{6+}\) from potassium dichromate was used instead. This was on the basis that, theoretically the oxidation potential of Cr\(^{6+}\) (1.33V) is higher compared to potassium hexacyanoferrate (0.36 V) [6]; hence, as seen in Figure 3, substitution of potassium hexacyanoferrate to potassium dichromate led to a substantial increase in voltage generated (558 mV to 702 mV). As mentioned, this could be attributed to the higher redox of potential Cr\(_2\)O\(_7^{2-}\).

The chromium ions could have electro-deposited on the surface of the cathode, decreasing the chromium concentration in the solution (pH = 2) [6]. For this experiment, the maximum percent removal of chromium was approximately 90.6%.

3.5 Effect of substrate concentration

The results indicated that an increase in the substrate concentration increased the time needed to reach constant OCV (open circuit voltage). Liu et al. [21] suggested that once the sufficient amount of
biomass is formed, the use of an excessive substrate becomes unnecessary; since, there is a specific threshold value for the metabolic reaction rate of microorganisms on electrode surface to be optimal.

As seen in Figure 4, as the concentration of the substrate increases the maximum open circuit voltage increases as well.

![Figure 4. MFC performance at varying substrate concentrations](image)

3.6 MFC performance using actual wastewater

To elucidate the performance of the MFC in an actual waste water. A sample from a creek near a food chain and a public market was considered. This water sample is believed to have lipids, carbohydrates and etc., which can serve as nutrient source for the organisms already present in the sample and to *P. aeruginosa* which is supplied.

Figure 5 shows the sample without *P. aeruginosa* was still able to produce a steady open circuit voltage; with a maximum value of 263 mV, primarily because the flora of microorganisms innately present in the sample were still metabolizing the substrates present in the sample; but due to the lack of electron mediator (like pyocyanin), the voltage output was relatively lower as compared to sample with *P. aeruginosa* (capable of producing its own mediator), which had maximum voltage output of 622 mV.

The higher voltage output can attributed to the possibility that the pyocyanin produced by *P. aeruginosa* served as an electron shuttle molecule to other microorganisms present in the sample (see Figure 6).

![Figure 5. MFC performance on actual wastewater](image)
3.7 MFC efficiency

The efficiency of an MFC is dependent on the nature of reaction with the substrate. Thermochemically, the utilization efficiency of a substrate can be deduced from Gibbs free energy and enthalpy of formation (efficiency = ΔG/ΔH).

For glucose, ΔG_{formation} = -911 \text{ kJ/mole}, and ΔH_{formation} = -1267 \text{ kJ/mole}; hence, 71.8% of energy is obtainable for utilization. Based on the experimental data, the maximum energetic efficiency per experiment was calculated and the results are shown in Table 1. Incorporation of metal in the anode compartment decreased the efficiency from 65.4 % (normal operation) to 23% (with 300ppm metal). Substitution of electron acceptor (from potassium hexacyanoferrate to potassium dichromate) enhanced the MFC performance up to a maximum energetic efficiency of 80.6 % as mentioned, this can be attributed to the higher redox potential of Cr$_2$O$_7^{2-}$. Finally the presence of *P. aeruginosa*, as biocatalyst, enhanced the efficiency from 29.4% to 67.4 %.

| Table 1. MFC efficiencies of different substrate / electrode systems systems |
|-----------------|-----------------|
| MFC System                  | Efficiency |
| Normal operation                  | 0.654       |
| Heavy metal at anode compartment (50 ppm) | 0.419       |
| Heavy metal at anode compartment (100 ppm) | 0.292       |
| Heavy metal at anode compartment (200 ppm) | 0.230       |
| Electron acceptor substitution                           | 0.806       |
| Actual wastewater, without biocatalyst added                           | 0.294       |
| Actual wastewater, with biocatalyst added                           | 0.674       |

4. Conclusion

The dual-chamber microbial fuel cell (MFC) constructed, with *Pseudomonas aeruginosa* as biocatalyst, facilitated substrate conversion and produced low-level energy. The effects of varying substrate concentrations (150 ppm, 300 ppm, 600 ppm) and heavy metal loads (10 ppm, 50 ppm, 100 ppm) on overall power generation were evaluated. The presence of Cr$_6^{6+}$ in the anode compartment decreased the potential from 565 to 201 mV (*i.e.*, lowest value achieved at highest Cr$_6^{6+}$ concentration of 300 ppm). On the other hand, replacing Cr$_6^{6+}$ with Fe$_3^{3+}$ as electron acceptor resulted in substantial increase in measure potential (*i.e.*, from 565 to 703 mV). Increasing glucose concentrations resulted in longer time to reach constant open circuit voltage. A maximum potential of 606 mV was achieved at
1200 ppm glucose. Incorporating *Pseudomonas aeruginosa* increased the potential from 256 to 592 mV. The MFC also worked well with actual wastewater. On the basis of these results, a microbial fuel cell feeding on wastewater can be an important potential technology for generating low-level energy.

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