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The Effect of Cobalt Biomineralization on Power Density in a Microbial Fuel Cell

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Summary

Microbial fuel cells produce electricity while breaking down organic waste. However, they have low power output because of the poor kinetics of oxygen reduction at the cathode under neutral pH conditions. In this project, we developed a novel microbial fuel cell with a biocathode using cobalt oxidation and reduction, to determine whether a cobalt biocathode could increase power density more than the conventional manganese biocathode. We built three types of fuel cells; the control had only oxygen reduction in the cathode chamber. The second and third cells included manganese and cobalt, respectively, with oxygen reduction. *Shewanella oneidensis* was used to oxidize organic material at the anode chamber and *Leptothrix cholodnii* was used in the biocathode. We measured power output when the fuel cells had reached peak voltage under a limited glucose supply. We found that the cobalt biocathode resulted in a higher power density than the control group and the manganese biocathode, but took the longest to reach peak voltage, indicating that microbial biofilm formation takes longer when oxidizing cobalt than it does when oxidizing manganese.

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Introduction

For the past few years, microbial fuel cells (MFCs) have been touted as a way to purify water at wastewater treatment plants while simultaneously generating electricity. These fuel cells work by using electrochemically active bacteria at the anode. In anaerobic conditions, these bacteria conduct cellular respiration by oxidizing organic compounds in the water and using the anode as the final electron acceptor [1]. The end products of this process are H^+, an electron, and CO_2 [2]. After the bacteria oxidize organic compounds, they reduce the anode using cytochromes and bacterial nanowires [3]. These electrons are then transferred to the cathode, which reduces O_2 to $2O^{2-}$, which then reacts with the H^+ produced at the anode to form H_2O. A proton exchange membrane separates the cathode chamber and the anode chamber; this allows H^+ to diffuse to the cathode chamber while keeping the oxygen from the cathode chamber out of the anode chamber.

While MFCs are promising solutions to energy issues at wastewater treatment plants, there is a major problem with their market viability: they have low power output [4]. One reason for the low power output is that oxygen is reduced at the cathode, and oxygen reduction has a slow reaction rate [5]. This results in a lower cathode potential [6]. This problem can be remedied through the use of a biocathode, in which microbes oxidize and deposit insoluble ionic compounds on the cathode, increasing cathode potential. The manganese-oxidizing bacterium *L. cholodnii*, has been used in biocathodes because it oxidizes Mn^{2+} to $MnO_{2(s)}$ in solution [7]. The MnO_2 then serves as the final electron acceptor as it is reduced by the cathode. This cycle of redox reactions continues so that electricity generation by the fuel cell remains constant. The use of manganese in biocathodes therefore leads to increased voltages.

Both manganese oxidation and cobalt oxidation in microbes have been linked to the enzyme manganese peroxidase [8]. *L. cholodnii* contains manganese peroxidase and can oxidize cobalt and manganese [9]. Oxidation of Co^{2+} to CoO(OH) is beneficial because CoO(OH) has a higher reduction potential than that of MnO_2 [10]. In addition, cobalt use is extremely widespread, especially in nuclear power plants, metallurgy, mining, pigments, paints, and electronics [11]. Manganese has fewer uses, mainly in steel manufacturing, paints, alkaline batteries, and ceramics [12]. As a result, harnessing cobalt oxidation can lead to increased voltage in microbial fuel cells that are used for industrial waste.

The purpose of this research was to determine whether the power density of a cobalt-based biocathode can exceed that of a recently developed manganese-based biocathode. We hypothesized that cobalt oxidation and reduction in a biocathode will increase power density to the level of biocathodes currently in development, because the reduction potential of Co^{3+} is higher than that of Mn^{4+}, which is currently being used. Because power density is defined as the product of voltage and

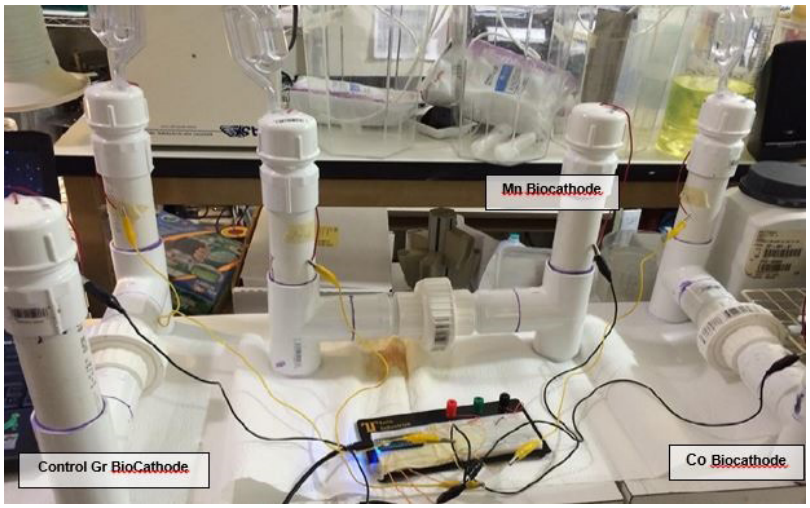

Figure 1: The setup of all three types of MFCs connected to a voltmeter.

current per square meter of electrode surface area, the rise in electrode potential caused by the deposition of cobalt would increase power density.

Experimentation revealed that voltage and power output were higher in the cobalt biocathode than in either the manganese biocathode or the control group, a microbial fuel cell with only oxygen as the final electron acceptor. However, the microbial fuel cell with a cobalt biocathode took a longer time to reach peak voltage.

Results

To test the power density of a microbial fuel cell with a cobalt biocathode, we built three fuel cells. All three used *S. oneidensis* to reduce the anode. The control group had an abiotic cathode that reduced oxygen. The second fuel cell had manganese(II) and *L. cholodnii* in the cathode chamber. *L. cholodnii* oxidizes manganese(II) to manganese(IV), which has a positive reduction potential, and deposits it on the electrode. The third fuel cell had cobalt(II) and *L. cholodnii* in the cathode chamber. The enzymatic pathway in *L. cholodnii* allows it to oxidize cobalt(II) to cobalt(III), which has a positive reduction potential, and deposit it on the electrode.

For the control group, glucose oxidation occurred in the anode chamber and oxygen reduction occured in the cathode chamber. *S. oneidensis* oxidized glucose in the following process:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

The cathode reduced oxygen in this process:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

For the manganese biocathode fuel cell, the anode reaction was the same. In addition to the oxygen reduction, manganese(IV) was reduced in the cathode chamber. *L. cholodnii* oxidized the manganese(II) that was added to the solution and deposited it on the electrode through this process:

$$Mn^{2+} + 2H_2O \rightarrow MnO_{2(s)} + 4H^+ + 2e^-$$

The cathode reduced the manganese(IV) in the insoluble manganese(IV) oxide in this process:

$$MnO_{2(s)} + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$$

For the cobalt biocathode fuel cell, the anode reaction remained the same. In addition to the oxygen reduction, there was cobalt(III) reduction in the cathode chamber. *L. cholodnii* oxidized the cobalt(II) added to the solution, and deposited it on the electrode through this process:

$$4Co^{2+} + O_2 + 6H_2O \rightarrow 4CoOOH_{(s)} + 8H^+$$

The cathode then reduced the cobalt(III) in the insoluble cobalt(III) oxyhydroxide using this process:

$$CoOOH_{(s)} + 3H^+ + e^- \rightarrow Co^{2+} + 2H_2O$$

We used an Arduino Micro microcontroller board to measure the open circuit voltage from each fuel cell every 12 hours (**Figure 1**). Because a limited amount of glucose was present, the voltage rose, reached peak voltage, and then declined. In order to estimate the average power density in industrial conditions, in which the voltage rises, reaches peak voltage, and remains at that state, we measured power output when each fuel cell had reached peak voltage. The control group and manganese biocathodes reached peak voltage on day 4 while the cobalt biocathode reached it on day 6 (**Table 1,**

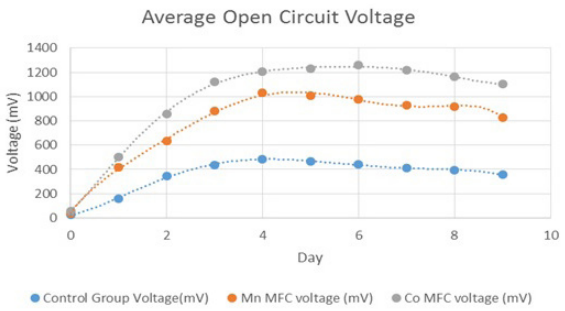

Figure 2: Average open circuit voltage per day for each fuel cell across all three trials.

Day	Control Group Voltage(mV)	Mn MFC voltage (mV)	Co MFC voltage (mV)
0	25	39	58
1	158	415	502
2	345	632	860
3	438	879	1123
4	484	1029	1204
5	465	1007	1228
6	441	978	1262
7	412	931	1217
8	395	918	1164
9	355	830	1106

Table 1: Average open circuit voltage per day for each fuel cell across all three trials.

Figure 2). In **Table 1**, we took voltage readings twice a day for 10 days for each fuel cell. We used the two daily readings from all three trials to find the average voltage per day.

Because of the higher potential of the microbial fuel cells with the cobalt biocathode, power density was higher, as well (**Figure 3, Tables 2–4**). We closed the circuit with an ammeter and a 25-ohm resistor. We recorded power output for 30 seconds; this was the time it took for power output to stabilize. Because of the limited supply of glucose, the power density continued to decline slowly, even after stabilization. As a result, the power output after those 30 seconds was not used to calculate the power output for each fuel cell. We determined power density using the electrode surface area, which was 0.015806 m^2. The control group produced a power density of 0.81 mW/m^2, whereas the power densities from fuel cells with the manganese biocathode and cobalt biocathode were 2.97 mW/m^2 and 5.63 mW/m^2, respectively.

While the manganese biocathode allowed for an average power density 3.6 times that of the standard oxygen cathode, the cobalt biocathode resulted in a power density 6.95 times that of the control group. We took voltage readings over the course of ten days, and the voltage dropped after the fuel cells reached peak voltage because of the limited supply of glucose.

Discussion

The results indicate that the equilibrium concentration of cobalt(II) is low enough to ensure a higher cathode potential than both a standard oxidation reduction cathode and a manganese biocathode. Cobalt(III) has a higher standard reduction potential than oxygen and manganese(IV), so the only factor that can lower its actual reduction potential is, according to the Nernst equation, the cobalt(II) concentration. The cobalt(II) concentration, in turn, is determined by the rate at which cobalt(II) is oxidized to cobalt(III) by manganese-oxidizing bacteria. However, it took seven days for the cobalt(II) concentration to reach equilibrium, while it only took 5 days for the manganese(II) concentration to decline to a similar level. This means that a bacterial biofilm forms more slowly when the bacteria are oxidizing cobalt(II) than when they are oxidizing manganese(II).

Both manganese and cobalt increased the electrode potential to a much higher level than that of the control group because manganese(IV) reduction and cobalt(III) reduction reactions are much faster than oxygen reduction.

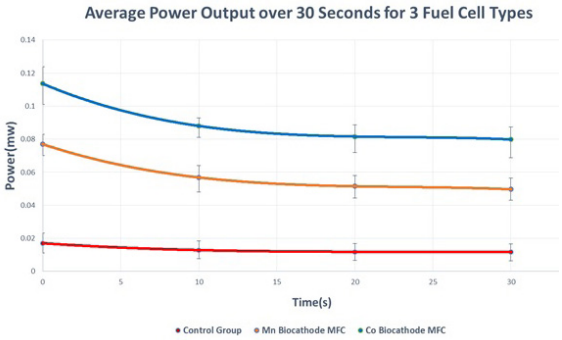

Figure 3: The average power output for each of the three fuel cell types over three trials.

The concentration of organic compounds in the anode medium and the cathode medium affected the peak voltage for every fuel cell. By the time the control group and the fuel cell with the manganese biocathode had reached peak voltage on day 4, much of the organic compounds had been consumed, decreasing cell voltage. Concentration of glucose determines the potential of the anode, and organic compounds are necessary to sustain *L. cholodnii* as it oxidizes manganese and cobalt. The fuel cell with the cobalt biocathode reached peak voltage on day 6, so the concentration of organic

Time(s)	0	10	20	30
Power(mW)	0.0170232	0.012851	0.011832	0.0116256
Voltage(mV)	20.76	18.1	17.4	17.3
Current(mA)	0.82	0.71	0.68	0.672
Circuit Resistance(Ω)	25.3170732	25.4929577	25.5882353	25.7440476
Work Done in 30 seconds(mJ)	0.384			
Average power output(mW)	0.0128			
Average power density (mW/m^2)	0.80981906			

Table 2: Average power density in the control group over 30 seconds, determined using data in Figure 3.

Time(s)	0	10	20	30
Power(mW)	0.077	0.05685	0.051465	0.050457
Voltage(mV)	44	37.9	36.5	36.3
Current(mA)	1.75	1.5	1.41	1.39
Circuit Resistance(Ω)	25.1428571	25.2666667	25.8865248	26.1151079
Work Done in 30 seconds(mJ)	1.41			
Average power output(mW)	0.047			
Average power density (mW/m^2)	2.97355435			

Table 3: Average power density in the fuel cell with the manganese biocathode over 30 seconds, determined using data in Figure 3.

Time(s)	0	10	20	30
Power(mW)	0.113652	0.0874	0.082482	0.0812
Voltage(mV)	54.12	47.5	46.6	46.4
Current(mA)	2.1	1.84	1.77	1.75
Circuit Resistance(Ω)	25.7714286	25.8152174	26.3276836	26.5142857
Work Done in 30 seconds(mJ)	2.6685			
Average power output(mW)	0.08895			
Average power density (mW/m^2)	5.62760977			

Table 4: Average power density in the fuel cell with the cobalt biocathode over 30 seconds, using data in Figure 3.

compounds had diminished even further by then. If there were to be a constant supply of organic compounds, the cell potential of the control group and the fuel cell with the manganese biocathode would increase, and the potential of the fuel cell with the cobalt biocathode would increase even more.

The calculation of maximum power density for each fuel cell is an estimation of the stable power density in an industrial fuel cell, in which there is a constant supply of carbon compounds. Although this stability was absent in the experiment due to the limited supply of glucose, the 30-second interval minimizes any drop in power output due to this limitation.

The cobalt biocathode can be further optimized by increasing the cobalt(III) reduction rate by the cathode, or by increasing the cobalt(II) oxidation rate by the manganese-oxidizing bacteria. Both of these processes can be facilitated through the use of redox mediators, which have been used to facilitate reduction of the anode in microbial fuel cells [13].

Methods

Building Fuel Cells

We built three fuel cells, each made of 2 PVC flat plugs, 2 PVC tees, 4 PVC male adapters, 2 PVC thread caps, 1 PVC coupling unit, 2 pieces of 8-inch long PVC piping, and 2 pieces of 4-inch PVC piping. All PVC components were 1.5 inches in diameter. We put each fuel cell together with PVC primer and glue, and then covered the threads of the male adapters and the coupling unit in Teflon tape to prevent leaking.

We drilled a hole in the top of each thread cap, which we used to close off the cathode chamber and anode chamber. We drilled these holes with a #47 drill bit to let No. 13 AWG copper wires through. Holes were also drilled in the 5x1x0.187-inch carbon electrodes, which were used as both the cathode and the anode. We fastened No. 13 AWG wires to loop crimp connectors; each connector was then fastened to the electrode using a #2 size screw, two washers, and a nut. We looped the wire through the hole drilled in each thread cap, and kept the wires in place using hot glue. We also covered the hole drilled for each thread cap above the anode chambers in order to ensure anaerobic conditions. We drilled a second hole on the each cap used for the anode chambers, and then inserted an S-Bubble airlock into each hole to allow the exit of gases produced by the microbes while keeping conditions anaerobic. We also fastened a piece of 1.5-inch by 1.5-inch Nafion membrane in the middle of the PVC coupling unit.

Preparing Media

For the fuel cells with the *L. cholodnii* biocathodes, we inoculated the cathode chamber using the ATCC 1917 MVSP medium; this medium was also used to culture *L. cholodnii*. We used a total of 701 mL of 1917 MVSP medium. To prepare ATCC 1917 MVSP medium, we added 0.24g of $(NH_4)2SO_4$, 0.06 g of $MgSO_4 \cdot 7H_2O$, 0.06 g of $CaCl_2 \cdot 2H_2O$, 0.02 g of KH_2PO_4, 0.03 g of Na_2HPO_4, 2.383 g of HEPES, and 1.0 mL of 10 mM $FeSO_4$ to distilled water for a final volume of 984.0 mL. We added NaOH until the pH became 7.2, and autoclaved the solution at 121 °C for 15 minutes. At 50 °C, we added 1.0 mL of a vitamin solution and 5.0 mL of a 20% Sodium pyruvate solution.

The vitamin solution consisted of Biotin (20.0 mg), Folic acid (20.0 mg), Thiamine Hydrochloride (50.0 mg), D-(+)-Calcium pantothenate (50.0 mg), Vitamin B12 (1.0 mg), Riboflavin (50.0 mg), Nicotinic acid (50.0 mg), Pyridoxine hydrochloride (100.0 mg), p-Aminobenzoic acid (50.0 mg), and distilled water to 1.0 L.

We inoculated the anode with a medium made of 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1.825 g of Na_2HPO_4, 0.35 g of KH_2PO_4, 1 g of glucose, 1 mL of the vitamin solution, 1 g of $NaC_2H_3O_2$, and distilled water to 1 L. The anode medium was the same as the medium used by Rhoads et. al. [14].

We cultured and subcultured *S. oneidensis* using tryptic soy broth and agar. We prepared a solution of 250 mL of tryptic soy agar solution by adding 10g of tryptic soy agar (BD 236950) and 0.625 g of dextrose to 250 mL of distilled water. We prepared a total of 100 mL of tryptic soy broth solution with 3 g of tryptic soy broth (BD 211825) and 100 mL of distilled water. We autoclaved both solutions at 121 °C, and used tryptic soy agar to prepare agar plates.

Inoculating the Fuel Cells

We propagated *S. oneidensis* in aerobic conditions 24 hours before fuel cell inoculation. In order to do so,

we hydrated the pellets from ATCC with 7 mL of tryptic soy broth using a micropipette. We separated 7 mL into two tubes using a serological pipette; one was used to inoculate 10 agar plates. We incubated these plates at 30 °C for 24 hours.

We propagated *L. cholodnii* in aerobic conditions 4 days before fuel cell inoculation. To culture *L. cholodnii*, we hydrated the pellets from ATCC with 125 mL of MVSP medium using a micropipette and put the solution into a flask. We incubated this flask at 20 °C for 24 days before use in a microbial fuel cell.

After incubation, we transferred *S. oneidensis* on the plates to 450 mL of anode medium with inoculation loops. We filled each anode chamber with 150 mL inoculated anode medium and 200 mL fresh anode medium.

After incubating *L. cholodnii*, we transferred 1 mL of the inoculated MVSP medium to a tube using a micropipette. Of the remaining 124 mL, we added 62 mL to 288 mL of fresh MVSP medium. We added the remaining 62 mL to another flask containing 288 mL of fresh MVSP medium. In one flask, we added 0.069 g of $MnCl_2 \cdot 4H_2O$ so that the manganese(II) concentration would be 1mM. In the second flask, we added 0.083 g of $CoCl_2 \cdot 6H_2O$ so that the cobalt(II) concentration would be 1 mM. We added the contents of the flask with manganese to the cathode chamber of the second fuel cell; we then added the contents of the flask with cobalt to the cathode chamber of the third fuel cell. For the first fuel cell, the control group, we filled the cathode chamber with 350 mL of MVSP medium.

Microbial Fuel Cell Performance

We built three fuel cells. The first type was the control group, with *S. oneidensis* in the anode chamber and an abiotic cathode. The electron acceptor for the cathode was only oxygen. The second and third types of fuel cell tested the biochemical composition of the cathode chamber. The second cell consisted of *S. oneidensis* at the anode chamber and *L. cholodnii* with manganese solution in the cathode chamber. The electron acceptors at the cathode were oxygen and manganese. The third cell consisted of *S. oneidensis* at the anode chamber and *L. cholodnii* with cobalt solution in the cathode chamber. The electron acceptors at the cathode were oxygen and cobalt. *S. Oneidensis* reduces the anode under anaerobic conditions, and *L. cholodnii* deposits oxidized cobalt and manganese on the cathode.

The purpose was to determine whether or not the biomineralization of cobalt increased cell power density as much as the biomineralization of manganese. In order to do so, we connected the fuel cells to an Arduino Micro microcontroller board, which we programmed to record voltage once every 12 hours. On the day 4, when voltage had more or less stabilized for most fuel cells, we recorded power output. In order to do so, we changed the Arduino program to record voltage every ten seconds, and recorded power output for each fuel cell separately. After closing the circuit with a 25 ohm resistor and an ammeter, we measured voltage and current for 30 seconds for each fuel cell, the time it took for the power output to stabilize. Because the voltage for the fuel cell with the cobalt biocathode continuously increased until the third day, we repeated the power output recording process for the cobalt biocathode on day 6. The testing process ended on day 9.

After testing the three types of fuel cells, we took them apart and poured their contents into flasks. We used bleach to sterilize the contents of the flasks, and sterilized the PVC components of the fuel cells with isopropyl alcohol. We threw out the electrodes, but reused the PVC. We repeated the whole process two more times for a total of three trials.

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