HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 1 (2022)

Nannochloropsis Algae Biocathodes Improve Cathodic Energy Losses in Two-Chamber Microbial Fuel Cells

Sahand Adibnia[1] and Kevin George[2#]

[1]Dublin High School
[2]Amyris, Inc.
[#]Advisor

ABSTRACT

Microbial fuel cells (MFCs) are currently being researched as alternative energy sources with promising applications in wastewater treatment. However, in two-chamber designs, cathodic oxygen reduction is slow and limits MFC voltage. Biocathodes, cathodes containing microorganisms, show great promise for improving MFC performance. This study investigated how the microalgae *Nannochloropsis* affects cathodic oxygen reduction via a thermodynamic analysis of energy losses. Voltage, cathode pH, and cathode pO_2 (partial pressure of oxygen) were measured in experimental MFCs containing *Nannochloropsis* biocathodes and compared to controls containing distilled water or abiotic algae media catahodes. Isolated *Nanochloropsis* cultures were also assayed. Under open circuit conditions, cathodic energy losses in experimental MFCs were 15% ($p = 0.038597$) and 19% ($p = 0.042435$) lower than distilled water and algae media controls, respectively. Experimental MFCs produced 73% higher power at 37% higher current density than distilled water MFCs. While the pH and pO_2 of isolated *Nannochloropsis* cultures increased linearly each day, these measurements were constant in experimental MFC cathodes. This result suggests that participation in oxygen reduction reactions induces a change in *Nannochloropsis* metabolism, leading to reduced oxygen production and limiting pH changes. Taken together, this work presents a promising new type of two-chambered MFC with lower energy losses and greater power production that can also maintain a constant cathode pH and reveals a new behavior of *Nannochloropsis* algae in response to oxygen reduction reactions in such MFCs.

Introduction

Microbial fuel cells (MFCs) are a type of fuel cell that use microbes to produce electricity and simultaneously treat wastewater (Rahimnejad, et al., 2015). Two-chamber MFCs consist of an anode chamber, where oxidation occurs, and a cathode chamber, where reduction occurs. The anode chamber contains wastewater rich with organic substances and bacteria which oxidize the organic fuel and provide electrons to an electrode, while also producing H^+. The bacteria in the anode chamber treat the wastewater by removing the organic substances. As long as the organic fuel is provided, the cell can run and provide power. The H^+ produced in the anode chamber travels through a membrane, typically a proton exchange membrane (PEM), to the cathode chamber. These electrons power an external load and arrive at the traditionally abiotic cathode chamber, where they combine with oxygen and H^+ to form water. This is known as the oxygen reduction reaction (ORR).

Equation 1: Oxygen reduction reaction equation

$$O_2 + 4H^+ + 4e^- = 2H_2O \qquad E^o = 1.229\,V$$

In the US, 3% of all electrical power produced is used to treat organics-rich wastewater (Maktabifard et al., 2018). At the same time, wastewater contains roughly 333 GWh of potential energy per day (Dolfing et al., 2011). In this way, microbial fuel cells have the potential to effectively turn wastewater treatment plants into power plants, which could be advantageous in the search for new sources of alternative energy.

In the last decade, MFC researchers have explored many methods to increase power production and make two-chamber MFCs commercially viable, several of which concern the cathode reaction. Platinum-based (Pt) catalysts efficiently catalyze ORR and increase power production, however high costs limit their economic viability. Numerous studies have tried using new oxidizing agents at the cathode, such as MnO_2 or $Fe(CN)_6^{3+}$. However, these oxidizing agents are often toxic and need to be replaced over time, rendering these MFCs unsustainable (Logan et al., 2006).

Microorganisms, such as microalgae, provide an alternative method to catalyze the cathode ORR directly (i.e., through different reaction mechanisms) or indirectly (i.e., by increasing oxygen levels). One study showed that in-situ oxygen production by photosynthetic organisms in biocathodes increase the power production of MFCs by up to 42% (Gajda et al., 2013). While these studies have investigated the effect of microalgal biocathodes on general MFC performance (e.g., total power production and voltage), no study has specifically described how microalgae affect energy losses that occur at the cathode. The relationship between ORR and microalgal metabolism has also remained unclear. A more thorough understanding of these topics is necessary for the commercialization of two-chamber biocathodic MFCs.

This study evaluated the effect of *Nannochloropsis* microalgae on cathodic energy losses in a two-chamber MFC. It was hypothesized that the addition of a live culture of microalgae to the cathode chamber of a two-chamber microbial fuel cell would decrease cathodic energy losses. It was also suspected that participation in the oxygen reduction reaction at the cathode would induce the microalgae to increase metabolic oxygen production in response to the consumption of oxygen by ORR. We compared the cathodic energy losses in MFCs with microalgal biocathodes and those of MFCs with distilled water and abiotic algae media cathodes. We also compared the cathode oxygen levels and cathode pH of MFCs with biocathodes to those of isolated microalgae cultures over time. The results not only suggest *Nannochloropsis* decrease cathodic energy losses, but also indicate that ORR induces a change in microalgal metabolism, as indicated by the differing oxygen levels and pH in the biocathodes compared to isolated microalgae cultures.

Methods

Experimental Design

This investigation was split into two parts. The first part involved measuring the pH and pO_2 of three isolated cultures of *Nannochloropsis*. The second part involved constructing six microbial fuel cells, three experimental MFCs with *Nannochloropsis* biocathodes and two control MFCs.

The *Nannochloropsis* genus (culture supplied by Algae Research Supply) was selected for several reasons. For one, few previous studies have used this genus for a biocathode, as many have focused on *Chlorella vulgaris* (Huarachi-Olivera et al., 2018). *Nannochloropsis* also provides greater potential for lipid biofuel production compared to other commonly researched species, as it can produce up to 37% of dry weight as lipids (Ma et al., 2016). Furthermore, wastewater treatment plants are typically located at low elevations near the coastline (Hummel et al., 2018). *Nannochloropsis* can tolerate a wide range of salinities, and can be found in freshwater, brackish water, and seawater. Considering that MFCs will be likely used in wastewater treatment plants, it would be convenient to be able to obtain and grow the microalgae from a nearby body of water, and *Nannochloropsis* can act as this alga due to its versatility.

The goals of the first part included developing linear regressions that describe how pH and pO_2 changed over time in an isolated culture of *Nannochloropsis* and deriving an equation that describes how the maximum cathode potential of the cell will change over time based on pH and pO_2 regressions (see Equation 2). This equation would be

synonymous with the trend of the maximum cathode potential if the cell had no effect on algal metabolism. The pH and pO_2 linear regressions would describe how the cathode pH and pO_2 in a *Nannochloropsis* biocathode MFC would change if the cell did not affect algal metabolism. Therefore, these regressions were compared to the actual pH and pO_2 measured in biocathode MFCs to determine the effect of the cell on algal metabolism. Three cultures of *Nannochloropsis* were grown for 21 days, during which time dissolved oxygen, pH, and temperature were measured daily. pO_2 and pH regressions were derived as a function of time based on the average of these three cultures. All cultures were 350 mL in volume, with 276 mL distilled water, 69 mL f/2 algae media and 5 mL of *Nannochloropsis* inoculum. This results in ~5.0 g/L salinity.

The goals of the second part included comparing, between experimental and control MFCs, open circuit cathodic energy losses, power production, internal resistance, ohmic resistance, the nature of energy losses, and determining pH and pO_2 in cathodes of experimental MFCs over time to compare to linear regressions developed in the first part. The control group included one distilled water cathode MFCs and one abiotic f/2 algae media cathode MFC (media supplied by Algae Research Supply). The experimental group included three *Nannochloropsis* biocathodic MFCs. One abiotic algae media MFC was constructed to determine how the added ions and nutrients in the algae media solution may affect ORR and/or cathode potential. The composition of the f/2 algae media mainly included $NaHCO_3$, Na_2CO_3, and NaCl, with low concentrations of nitrates, phosphates, and trace metals like iron, zinc, copper, manganese. While the trace metals ions likely did not impact ORR due to their low concentrations, it is possible that the increased salinity, particularly due to the presence of carbonates, could affect cathode potential. This was the reason why one controls included an algae media cathode.

All cells were run across 1 MΩ resistor for 21 days. The voltage across the 1 MΩ resistor, cathode dissolved oxygen, cathode pH, and temperature were measured for all cells bidaily. Open circuit voltage was measured every three days. Polarization curves for cells were made 18 days after inoculation to observe internal resistance and the nature of energy losses. Using polarization curves, power density curves were also calculated.

Specifications of Control and Experimental MFCs and Controlled Variables

Variables controlled in experimental and control groups included specifications of the salt bridge (alternative to proton exchange membrane), the anode and cathode volume, the electrodes, and light exposure. The salt bridge consisted of a 10 cm long ¾" diameter PVC pipe (~28.5 mL) with 1 M KCl (Soomro, 2015) and 100 g/L agar (Sridevi et al., 2020). All cells were run in the same location with same sun exposure at a temperature of approximately 21˚C.

The anode and cathode chambers were 350 mL each. The anodes contained 300 mL distilled water and 50 mL soil as the inoculum and were covered in black construction paper as many soil microbes are known to be photophobic (Khater et al., 2017). The anode solutions of all cells were identical, because they were made using the one batch of mixed inoculum. This way, it could be assumed that anodic overpotentials are the same in both experimental and control groups, which is important because it allows for the derivation of Equation 9 (see following section).

The electrodes were graphite rods (Gregory et al., 2004), 10 cm in length and 3 mm in diameter (8.553 cm^2 surface area). Current and power produced by MFCs was normalized to cathode surface area to simplify comparisons of this study with others.

The cathode solutions of each cell acted as the independent variable, as they were different. Experimental cells contained 276 mL distilled water, 69 mL f/2 algae media, and 5 mL initial *Nannochloropsis* inoculum (~5.0 g/L salinity). The distilled water cell contained 350 mL distilled water. The algae media control cell contained 280 mL distilled water and 70 mL algae media (~5.0 g/L salinity).

Calculation of Cathodic Overpotential Differences

In fuel cells, anode and cathode reactions have individual standard reduction potentials (E°). Maximum reduction potentials in non-standard conditions can be thermodynamically calculated using the Nernst equation. For an oxygen-reducing cathode, this potential is given by the following equation (Logan et al., K. 2006):

Equation 2: Cathode Potential for an Oxygen-Reducing Cathode

$$E_{cat} = 1.229 + \frac{RT}{4F}\ln\left(pO_2[H^+]^4\right)$$

where T is temperature, R is the universal gas constant (8.31447 J mol^{-1} K^{-1}), F is Faraday's constant (96485.3 C mol^{-1}), pO_2 is partial pressure of oxygen (atm), and $[H^+]$ is the concentration of hydronium ions in solution (mol L^{-1}). The difference between this maximum cathode potential and the actual cathode potential is called a cathodic overpotential. This is a type of energy loss that occurs under open-circuit conditions. The sum of overpotentials can be described as overall energy losses.

Microbial fuel cell performance is commonly described either by open circuit voltage (OCV) and internal losses (IR_{int}) or by maximum potential (E_{emf}):

Equation 3: MFC performance described by OCV and internal losses

$$E_{cell} = OCV - IR_{int}$$

Equation 4: MFC performance described by anode and cathode potential

$$E_{emf} = E_{cat} - E_{an}$$

Polarization curves can be used to calculate the internal resistance of a microbial fuel cell, in accordance with Equation 3. They measure the voltage of a fuel cell as a function of current density. These curves can reveal the nature of energy losses in cells. At low current densities, the rapid drop in voltage is caused by activation losses, or energy losses caused by the activation energy of the anode and cathode reactions. At moderate current densities, when the curve is most linear, ohmic losses dominate. The slope of the best-fit line in this region is the internal resistance (R_{int}). At high current densities, concentration losses, energy losses due to hindered transport of reactants to the reaction site, dominate. Like the activation losses region, it is typical to observe a steep drop in voltage in this region.

Cell potential can also be described as the difference between the theoretical potential (E_{emf}), the sum of anodic and cathodic overpotentials, and ohmic losses (Logan et al., 2006):

Equation 5: Cell potential as function of theoretical potential, overpotentials, and ohmic losses:

$$E_{cell} = E_{emf} - (\Sigma\eta_{an} + |\Sigma\eta_{cat}| + IR_{\Omega})$$

Under open circuit conditions, the current (I) is 0, therefore we obtain:

Equation 6: Equation 5 when $I = 0$

$$E_{cell} = E_{emf} - (\Sigma\eta_{an} + |\Sigma\eta_{cat}|)$$

Equation 7: Equation 3 when $I = 0$

$$E_{cell} = OCV$$

Setting Equations 6 and 7 equal to each other, substituting E_{emf} from Equation 4, and then solving for $E_{cat} - OCV$, we obtain:

Equation 8: Formula for $E_{cat} - OCV$ derived from Equations 4, 6, and 7

$$E_{cat} - OCV = E_{an} + \Sigma\eta_{an} + |\Sigma\eta_{cat}|$$

Actual cathode potential, which is required to determine cathodic overpotentials ($\Sigma\eta_{cat}$), can only be directly measured using a reference electrode. However, it is possible to calculate cathodic overpotentials without measuring actual cathode potential if comparing two fuel cells with identical anodes. MFCs with identical anode chambers are assumed to have identical anode potentials; that is, identical $E_{an} + \Sigma\eta_{an}$ terms ($E_{an,1} + \Sigma\eta_{an,1} = E_{an,2} + \Sigma\eta_{an,2}$). Therefore, a comparison of difference between the theoretical maximum cathode potential (E_{cat}) and the open circuit voltage (OCV) of MFCs with identical anodes results in a comparison of the sum of cathodic overpotentials of the two MFCs ($\Sigma\eta_{cat}$):

Equation 9: Formula used to calculate cathodic overpotential differences between MFCs

$$|\Sigma\eta_{cat,1}| - |\Sigma\eta_{cat,2}| = (E_{cat,1} - OCV_1) - (E_{cat,2} - OCV_2)$$

Cathodic overpotential differences between experimental MFCs with biocathodes and control MFCs were calculated using Equation 9.

Calculation of pO_2 consumed over time by ORR

The oxygen in MFC biocathodes spontaneously reacts in ORR. Therefore, it is possible that some of the oxygen produced by the microalgae was not measured as it was immediately consumed. Considering this, pO_2 consumed after a certain number of days needed to be added back to measured pO_2 to determine the true oxygen output by the algae in MFC biocathodes. pO_2 consumed after a time t (seconds) was calculated using Equation 10. The integral of the running current with respect to time, $\int_0^t I\,dt$, provides the Coulombs of charge consumed by ORR after time t. Since voltage and current are related linearly by Ohm's law ($V = IR$), the integral can also be computed with voltage, resulting in $\int_0^t \frac{V}{R} dt$ with $R = 10^6\ \Omega$ (a constant). To obtain moles of electrons consumed, the integral is then divided by F (Faraday's constant). According to Equation 1, for every 4 moles of electrons, 1 mole of O_2 is consumed, and thus the integral is divided by 4 to obtain moles of O_2 consumed. To obtain $[O_2]$ consumed, expression must be divided once more by the cathode volume (0.350 L). Multiplying the expression by the approximated Henry's Law constant for O_2 in aqueous solution at 21°C (778.846 atm/M), pO_2 (in atm) is obtained:

Equation 10: Calculation of pO_2 consumed by ORR after time t

$$pO_2 = \frac{778.846 \int_0^t V dt}{1400000}$$

Results

Overview of Methods

The effect of *Nannochloropsis* microalgae on the size and nature of energy losses of a two-chamber MFC was examined by observing cathodic energy losses, or overpotentials, in these cells. Cathodic energy losses in MFCs containing algal biocathodes (experimental MFCs) were compared to two different controls, one with a distilled water (DI) cathode and another with an f/2 media (algae nutrient solution) cathode. The nature of energy losses was evaluated by constructing polarization curves and power curves. Additionally, the effect of the cathode ORR on microalgal metabolism was examined indirectly by comparing the oxygen levels (in partial pressure of oxygen, or pO_2) and pH of MFC biocathodes to the oxygen levels and pH in three isolated microalgae cultures over time. These isolated cultures represented the theoretical change in oxygen levels and pH with time in MFC biocathodes given that ORR has no effect on microalgal metabolism. Using the changes in pO_2 and pH observed with time in isolated cultures, an equation describing the theoretical cathode potential as a function of time was computed using Equation 2. The voltage trend of experimental and control MFCs over time was compared to this theoretical trend.

Cathodic Overpotentials Differences and Summary Measurements

Experimental MFCs outperformed both controls in all regards: they had higher power production, greater current density at maximum power, lower internal resistance, and significantly lower cathodic overpotentials ($p = 0.038597$ vs DI, $p = 0.042435$ against algae media). Lower internal resistance accords with the larger range of power-producing current densities displayed on power curves (Fig. 2), along with the larger range of the ohmic losses region in polarization curves (Fig. 1). The lower cathodic overpotential also indicates that the microalgae, on average, decrease the energy losses at the cathode in open circuit conditions.

Table 1. Comparison of summary measurements of MFCs with microalgal biocathodes and distilled water control MFCs. Metrics of experimental MFCs are averaged across the three MFCs. The mean cathodic overpotential describes the mean of cathodic overpotential measurements that were taken every other day. On average, experimental MFCs outperformed the distilled water cathode MFC in all regards: they have higher power production, greater current density at maximum power, lower internal resistance, and significantly lower cathodic overpotentials ($p = 0.038597$, $n_{experimental} = 30$, $n_{control} = 10$, two-tailed T-test).

Cell	Maximum Power Production	Current Density at Maximum Power	Internal Resistance (R_{int})	Mean Cathodic Overpotential ($E_{cat} - OCV$)
Experimental	4.98 mW/m^2	29.09 mA/m^2	4.306 kΩ	0.3686 V
Distilled Water Control	2.880 mW/m^2	21.30 mA/m^2	9.444 kΩ	0.4344 V
Percent difference	69.61%	36.57%	–54.40%	–15.15%

Table 2. Comparison of summary measurements of MFCs with microalgal biocathodes and algae media control MFCs. Experimental MFCs outperformed the algae media MFC in all regards: they had higher power production, greater current density at maximum power, lower internal resistance, and significantly lower cathodic overpotentials ($p = 0.042435$, $n_{experimental} = 30$, $n_{control} = 10$, two-tailed T-test).

Cell	Maximum Power Production	Current Density at Maximum Power	Internal Resistance (R_{int})	Mean Cathodic Overpotential ($E_{cat} - OCV$)
Experimental	4.98 mW/m^2	29.09 mA/m^2	4.306 kΩ	0.3686 V
Algae Media Control	0.7923 mW/m^2	5.745 mA/m^2	31.93 kΩ	0.4564 V
Percent difference	528.55%	406.35%	–86.51%	–19.24%

Polarization and Power Curves

As evident in Figure 1, all three experimental MFCs produced voltage over a greater range of current densities than both the DI and algae media controls, reflecting the lower internal energy losses experienced by experimental MFCs. This is shown by the shallower slopes of the linear regions of the polarization curves (Fig. 1). The open circuit voltage (OCV) of each cell is the same as the voltage at approximately zero current (i.e., the first data point). From the polarization curves, it appears that Experimental Cells 2 and 3 had lower OCVs than the DI control. However, this is not the case when comparing all OCV measurements taken every other day, as the difference in OCV is not significant ($p = 0.08291$, $n_1 = 10$, $n_2 = 10$, two-tailed T-test). Interestingly, Experimental Cell 1 seemed to have exclusively greater concentration losses, as evident from its rapid dip in voltage at high current densities.

Overall, experimental MFCs produced more power over a larger range of current densities (Fig. 2, Table 1, Table 2). Experimental Cells 2 and 3 exhibited similar change in power density with current density, but Experimental Cell 1 saw a sharper increase in power density at low current densities, and subsequently, a sharper decrease at high current densities (Fig. 2). This reflects both the greater internal losses of this MFC and the smaller activation losses, as evident in polarization curves (Fig. 1).

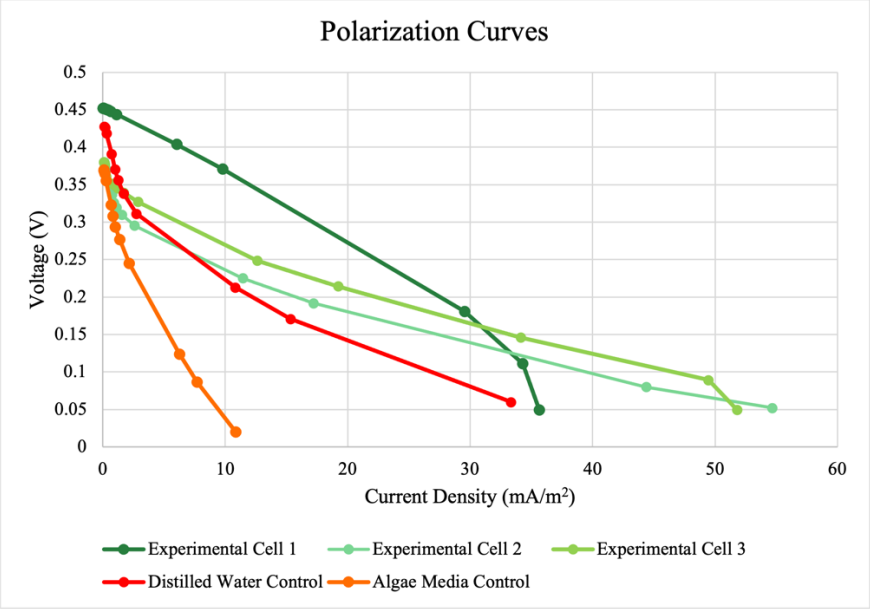

Figure 1. Polarization curves of all microbial fuel cells. At low current densities, significant activation losses were observed for all MFCs except Experimental Cell 1. At moderate current densities, when ohmic losses dominate, linear regressions were constructed to approximate the internal resistance. Only Experimental Cells 1 and 3 exhibited some concentration losses at high current densities, as evident from the steep decrease in voltage at high current densities.

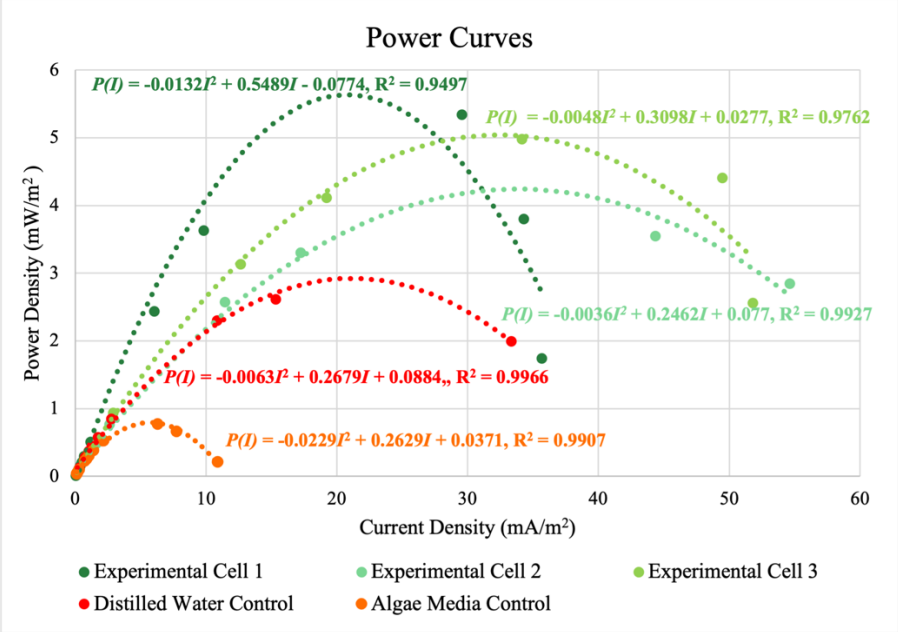

Figure 2. Power curves of all microbial fuel cells. Experimental Cell 1 appeared to have the highest maximum power, but also lower current densities than the other experimental cells, which reflects both its greater concentration and ohmic losses. Power curves were computed using data from polarization curves. Quadratic regression curves were constructed to estimate maximum power production and current density at maximum power (P is power density in mW/m^2, and I is current density in mA/m^2).

Cathode pH and pO_2 vs Isolated Algae Cultures

In isolated cultures of *Nannochloropsis*, pH and pO_2 increased linearly with time (Fig. 3). This was not observed in experimental MFC biocathodes, as the average pH and pO_2 of experimental MFCs matched the trend of controls (Fig. 4). pO_2 levels in experimental cells did not deviate significantly from that of control cells ($p = 0.4987$), while on average, the predicted pO_2 from the isolated culture regression was 42.67% higher than the measured pO_2 in biocathodes ($p = 2.411 \times 10^{-9}$). To confirm that consumption of oxygen by ORR did not cause the maintenance of constant pO_2 levels observed in experimental MFCs, the pO_2 consumed by ORR after 21 days was calculated using Equation 10. It was found that the amount of pO_2 consumed by ORR was negligible compared to the pO_2 present in the cathodes.

On average, pH levels in experimental cells also did not deviate significantly from the algae media control ($p = 0.9231$) but did deviate significantly from the DI control ($p = 1.414 \times 10^{-22}$), which is expected because the algae media solution is supposed to be buffered around pH = 8.5.

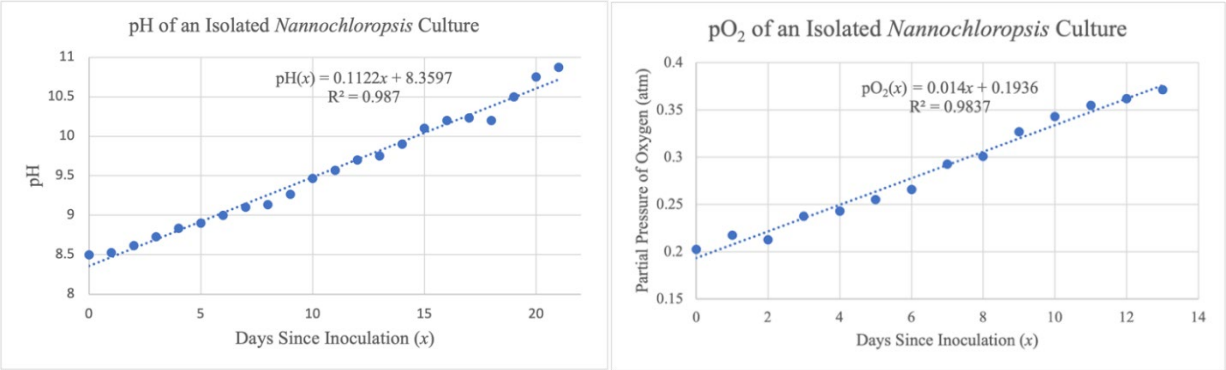

Figure 3. Regression lines of pH and pO_2 over time in isolated *Nannochloropsis* cultures**.** Both pH and pO_2 showed strong linear associations with time. Measurements were averaged across the three cultures each day.

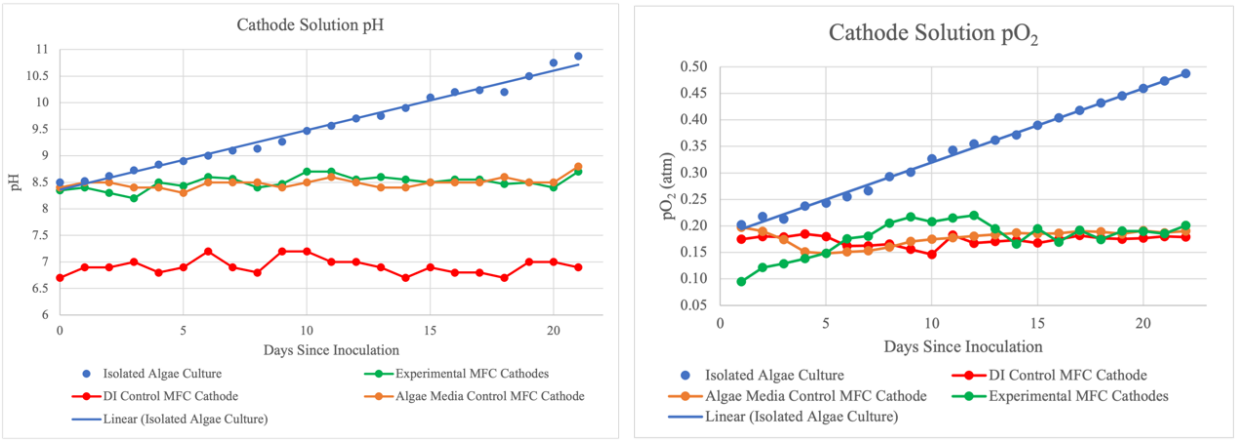

Figure 4. Comparison of average experimental MFC biocathode pH and pO_2 to control MFC cathode pH and pO_2 over time and predicted pH and pO_2 trends in biocathodes. pH and pO_2 of MFC biocathodes do not follow the predicted trend in isolated cultures. pH and pO_2 of the three experimental cells were averaged each day for this comparison.

MFC Voltage Over Time

Substantial deviations in the trend of MFC voltage over time were observed from the trend of the theoretical cathode potential equation (Fig. 1). Assuming that the microalgae do not directly impact ORR in MFC biocathodes, the equation for theoretical cathode potential suggests that the growth of microalgae in MFC biocathodes will decrease the voltage of the experimental MFCs by decreasing the cathode potential, a net result of increased oxygen production but also increased pH. However, the voltage of these MFCs did not decrease as predicted by the equation, and instead matched the trend of controls. On average, there was no significant difference between the running voltage of experimental cells and the DI control ($p = 0.65797$), however the 41.16% average running voltage difference between experimental cells and the algae media control was statistically significant ($p = 0.00406808$). Furthermore, the DI control cell showed a 36.86% higher average running voltage than the algae media control ($p = 0.04623$).

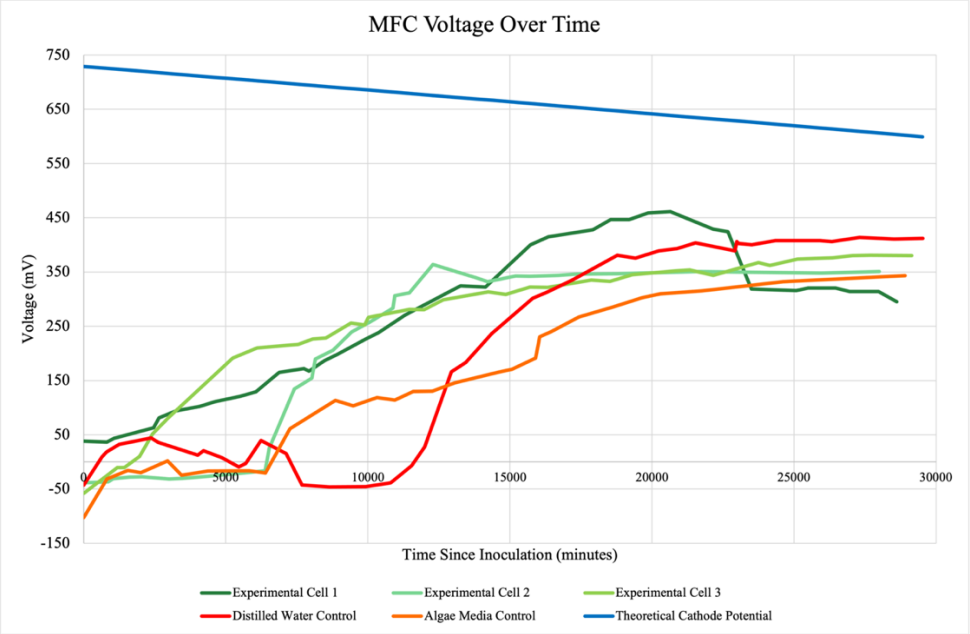

Figure 5. Voltage of all MFCs across a 1 MΩ resistor over time. The equation for the theoretical cathode potential was $E_{cat}(x) = 0.7123 + 0.006315\ln(x + 13.935) - 0.006592x$, where x is days since inoculation and E_{cat} is the thermodynamically calculated maximum cathode potential. It was calculated using linear regressions for pO_2 and pH inputted into Equation 2.

Qualitative Observations

In Experimental Cell 1, the microalgae appeared to grow on the graphite electrode after Day 14. Additionally, in isolated cultures, after around 10-13 days, immiscible, non-buoyant bubbles would form. These bubbles were not observed in MFC biocathodes. Experimental Cell 1 also experienced corrosion of its copper wire, forming an insoluble green substance that was speculated to be cooper (II) carbonate considering the high carbonate concentration in the algae media solution.

Discussion

Effect of Nannochloropsis biocathode on MFC performance

Based on the data obtained, the effects of a *Nannochloropsis* biocathode on an MFC are generally beneficial, as measured by metrics noted in previous studies (Gajda et al., 2013; Gajda et al., 2015; Gajda et al., 2018; Revelo Romo et al., 2019) and new metrics based on thermodynamic analyses.

The effect of the algae on overall MFC performance is best described through the maximum power production. Algal MFCs produced 69.61% higher maximum power than the DI control, and 528.55% higher than the algae media control. This 69.61% increase in power is greater than the 42% increase in power noted by Gajda et al., 2013 for similar biocathodic MFCs.

The thermodynamic analyses performed in this study revealed the nature of energy losses in biocathodic MFCs. For one, *Nannochloropsis* appears to decrease internal resistance and ohmic losses. In experimental cells, on average, R_{int} was 54.40% lower than the DI control, and 86.51% lower than algae media control. The ohmic losses range was also, on average, 84.78% greater than DI control and 496.30% greater than algae media control. A larger range of current densities at which ohmic losses occur indicates that the ohmic losses are generally lower. *Nannochloropsis* does not appear to affect activation or concentration losses. However, the power curves show that experimental cells on average, had 36.57% higher current density at maximum power than the DI control, and 406.35% higher than the algae media control. Generating power at higher current densities indicates that the cell can run when the rate of ORR is higher, suggesting that the reactants of ORR (i.e., O_2 and H^+) are more efficiently transported to the reaction site (cathode electrode). Along with that, the algae seemed to decrease cathodic overpotentials on average. In experimental cells, they were 15.14% lower than the DI control (p = 0.038597), and 19.24% lower than the algae media control (p = 0.042435).

Effect of MFC on Nannochloropsis microalgae

In an MFC, the metabolism of *Nannochloropsis* seemed to be altered to favor little to no oxygen production. This is evident from the fact that pO_2 in experimental cells followed the same general trends as the pO_2 in control cells. Since the pO_2 consumed by ORR was negligible compared to pO_2 present in biocathodes, it can be concluded that the inability of experimental cell pO_2 to match the isolated culture trend is not a result of spontaneous consumption of the oxygen by ORR and is instead more likely due decreased oxygen production by the algae. The cell also decreased the augmentation of pH by the algae. The microalgae seemed to generally help buffer the pH of the cathode, which can solve the problem of pH splitting in MFCs (Logan et al., 2006). Taken with the power production analysis, this indicates the *Nannochloropsis* increases the power of an MFC by greater amounts as previous studies indicated (Gajda et al., 2013) without acting as an in-situ oxygen producer.

Additionally, interesting conclusions can be drawn from the qualitative observations. Concentration losses were greatest in Experimental Cell 1, which could have been a result of the algae growing on the cathode that inhibited oxygen or proton transport to the electrode. The bubbles observed in isolated *Nannochloropsis* cultures were most likely lipids, as *Nannochloropsis* is known to be a heavy lipid producer. This accords with the fact that the bubbles were immiscible with water and did not rise out of the aqueous solution. Based on this conclusion, *Nannochloropsis* seemed to produce less lipids in MFC biocathodes than in the isolated cultures. This is not favorable for biofuel production, and thus it should be considered that *Nannochloropsis* cultures in MFCs may not be able to provide biofuel. In general, the DI control outperformed the algae media control. This indicates that, without the microalgae present, the ions in the algae media solution decrease the performance of the MFC.

Next Steps

Based on the results obtained, further research should look to determine how *Nannochloropsis* changes its metabolic pathways to affect the cell in the ways observed in this study. This would include determining the specific metabolic mechanism that *Nannochloropsis* switches to in an MFC, which would require a better understanding of its normal metabolic pathways. This type of research would require instruments like scanning electron microscopes (SEM), which could help determine how they attach to the graphite electrode. The cells could also be run at higher and more varied currents to do kinetics analysis of ORR in *Nannochloropsis* biocathode MFCs, which can determine if this alga also acts as a biocatalyst for ORR. Other studies could also investigate the exact effect of the cell on *Nannochloropsis* growth rates using more advanced technology, such as spectrophotometer, to measure algae concentrations. Furthermore, the results of this study cannot be generalized to all genera of algae, and thus the same experiment should be conducted with different types of algae.

Conclusion

Overall, it can be concluded that *Nannochloropsis* decreases the cathodic energy losses in two-chamber MFCs, while the cell hinders the algae's natural metabolism to favor a lower pH and reduce in-situ oxygen production by the microalgae.

Limitations

There are several limiting factors in this experiment that could have provided sources of error. As stated in the previous section, the cells were run at relatively low current densities over their entire lifespan. This was done to prevent anodic concentration losses from affecting the cell voltage but may have also limited the impact of ORR on microalgal metabolism, potentially giving more variable or unreliable results. Additionally, while it is assumed the ORR was occurring at the cathode, there did not appear to be definitive evidence that this was the case. It was difficult to prove so mainly due to oxygen diffusion into the cathode solution, which could have replenished any oxygen consumed by ORR. Although f/2 media ions should not have been reduced considering the high reduction potential of oxygen, there are still other reduction pathways that may have occurred simultaneously with ORR, such as the hydrogen peroxide variation of the same reaction (Tartakovsky & Guiot, 2006).

Acknowledgements

Special thanks to my mentor, Dr. Kevin George, who helped edit my manuscript, and the Dublin High School Science Department, which provided the dissolved oxygen meter used in this study.

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