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# On-Line Monitoring of Minor Oil Spills in Seawater Using Sediment Microbial Fuel Cells: A Preliminary Study

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## Abstract

In recent years microbial fuel cells have been studied for their biosensing properties. Consequently, sediment microbial fuel cells (sMFCs) have been found to be able to detect minor oil spills in freshwater. However, it was previously unknown whether sMFC properties as a biosensor would be able to produce the same results in a saltwater environment. Therefore, sMFCs at various external resistances (220  $\Omega$ , 300  $\Omega$ , 430  $\Omega$ , 510  $\Omega$ , 1000  $\Omega$  and 2000  $\Omega$ ) were assembled to assess their ability to detect oil in seawater. The results indicated that an ER of 1k  $\Omega$  is optimal for sMFC power generation, and that as oil was added to the cathode of the sMFCs, there was a clear and gradual decrease in the voltage output due to the oil's interference with oxygen dissolvability in seawater; However, the relationship between the change in voltage and change in time was less linear than the change observed in previous studies, and inconsistent across the different voltages. The variation observed may be due to the absence of a catalyst, such as platinum, which would have sped up the rate of the decrease in voltage. This study illustrates that sMFCs provide a cost effective and environmentally friendly method to detect minor oil spills in seawater.

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## 1. Introduction

From the mid-1800s to the present, oil has become a valuable and treasured commodity, with offshore methods of obtaining it contributing to a large percentage of global oil production, and an even larger percentage of the oil produced is transported through or on water (Cheng & Duran, 2004). Despite precautionary measures such as epoxy coating on

underwater steel pipelines, a considerable amount of oil ends up being spilled in the ocean, causing harm to numerous ecosystems, and destroying others (Chilvers et al., 2020). While processes such as photolysis, evaporation, dispersion, and bacterial biodegradation will remove the majority of oil from the water surface, the effect on marine life is still significant, with the largest concern being for the seabird population (Kingston, 2002). Despite oil being a naturally occurring substance in the earth, volatile organic compounds (VOC) and polycyclic aromatic hydrocarbons (PAH) which are found in crude oil are toxic to humans and wildlife and can lead to the death of marine life in large quantities. Oil spills have been observed to “impede agricultural productivity and fishing”, which directly impacts the economic life of populations dependent on these two industries (Osuagwu & Olaifa, 2018). This highlights the importance of developing methods to detect and mitigate the effects of oil spills, as well as taking precautionary preventative measures; however, as accidents by nature cannot be prepared for, methods of detection are paramount.

There are several remote sensing methods already being implemented to detect more prominent oil spills, such as fluorosensors (Pangilinan et al., 2016; Fuchi et al., 2022) and infrared sensors (De Kerf et al., 2020), but minor oil spills often go undetected. Minor or small oil spills generally refer to oil spills that contain a maximum volume of 6 gallons and are usually caused by leaks while refueling or discharging fuel. Despite the small amount of oil in minor spills, the toxic and flammable effects are still present, as the percentage of crude oil made up by polycyclic aromatic hydrocarbons was found to be up to 7%, and safe levels of PAHs is generally 0.2 milligrams per cubic meter (in air) (Neff et al., 2005).

The use of microbial fuel cells (MFCs) as a biosensor has become more popular overtime as it has been used for the testing and curing of wastewater, hydrogen production, seawater desalination, microbial electro synthesis, and detecting oil spills in freshwater sources (Do et al., 2020; Dai et al., 2021). Microbes placed at the anode metabolize the substrates they are placed in, releasing hydrogen ions and electrons. These electrons are transported from the anode to the cathode, and this passage is what creates an electrical current. The cathode acts as the electron acceptor, and these electrons then are used in the synthesis of water, utilizing oxygen obtained from the air, and the protons ( $H^+$  ions) from the microbial metabolism (Logan, 2009). The current created from the anode to the cathode is passed through a resistor and a data acquisition module. This method can be used for on-line monitoring, which means that the information obtain can be analyzed and recorded from a computer that is attached to the data acquisition module.

A study done by Dai et al. (2021) on the monitoring of minor oil spills in natural waters using sediment microbial fuel cells (sMFCs) proved that sMFC are capable of detecting oil spills; however, only freshwater sMFCs were used. It is important to also consider the biosensing properties of sMFC in seawater, as seawater has been established to contain varying concentrations of salts and other dissolved substances that differentiate it from fresh water. The majority of oil spills also occur in seawater, and their adverse effects are typically more prominent. Determining if there is any difference between the biosensing properties of sMFCs in seawater and freshwater would prove to be extremely beneficial as this would increase the versatility of the device, making it useable in all bodies of water.

As previously mentioned, there are numerous studies on the usage of MFCs as a biosensor, but literature on the feasibility of sMFC in detecting oil spills is extremely limited as this is a relatively new idea (Do et al., 2020). MFC-based biosensors have only started to be explored in recent decades and have been used for monitoring both water and air

quality (Cui et al., 2019; Zhou et al., 2018). The cost effectiveness and relative simplicity of sMFCs has raised interest in their usage in developing countries where water quality testing for both drinking water and other purposes is more necessary (Chouler & Di Lorenzo, 2015). In the monitoring of water quality, studies on biochemical oxygen demand and toxicants are available due to the popularity of these parameters being tested, and the high sensitivity of MFCs to minor changes (Yang et al., 2016). Despite all of these qualifications, more studies are needed on detecting other foreign objects and particles in water and air to display the true value of MFCs as a biosensor. This emphasizes the importance of studying oil spill detection using sMFC, which is fundamentally different from the other toxins studied due to the fact that in this study the presence of the oil on the surface of the water is what causes an anoxic state in the water of the sMFC due to oil preventing oxygen from dissolving in water. The monitoring of minor oil spills using sMFCs has only been done once, and this study is the first time it is being done in a seawater environment.

Although numerous studies have explored sMFC as biosensors, they often overlook the influence of different external resistors on the biosensing performance of sMFCs. Moreover, most of the existing studies have focused on assessing sMFC as biosensors in freshwater environments. To date, there are no reports of sMFC deployment in seawater for use as biosensors. Therefore, this study aims to investigate the impact of seawater on the biosensing properties of sMFC, considering that salinity levels in seawater could potentially enhance or diminish the effectiveness of sMFC. Additionally, the study aims to explore the potential influence of resistance on the biosensing properties of sMFC.

## 2. Methods

### 2.1. *Sediment and Water Sources*

The sediment (5-30 cm below sediment-water interface) samples were collected from an area 5 meters off the coastline at [25°00'56"N 77°17'56"W]. The seawater was collected from this same point. Both the water and sediment were immediately transported to the laboratory, and the sediment was wet sieved to remove as much large debris and rocks as possible.

### 2.2. *sMFC Assembly and Operation*

**Table 1.** The resistance on each group of the sMFCs in ohms.

sMFC Group	Resistance on circuit ( $\Omega$ )
A 1-3	220
B 1-3	300
C 1-3	430
D 1-3	510
E 1-3	1000
F 1-3	2000
Control group 1-3	Open Circuit

Twenty-one sMFC were constructed from the sediment and operated at different ER (2000, 1000, 510, 430, 300, and 220  $\Omega$ ) in triplicates for 45 days incubation. Out of the twenty-one sMFCs, three were assigned as the control group and were left in an open circuit configuration, which means that the anode and cathode were not connected. The assembly of all the sMFCs was carried out in accordance with a prior study conducted by Wang et al. (2015), with the following minor alterations. Briefly, a 500 mL erlenmeyer flask was used to construct each sMFC. Square sections of carbon felt with geometric surface area of 25cm<sup>2</sup> were used as anodes and cathodes. Carbon felt forms a conductive lattice structure of polyacrylonitrile fiber that supplies oxygen reduction reaction sites, and habitats for anode respiring bacteria (Gustave et al., 2018). A sediment layer of approximately 2 cm depth, weighing 138g in dry weight, was first placed at the bottom of the sMFC container. Consequently, the anode was placed on the sediment layer's surface and covered with an additional sediment layer to simulate anaerobic conditions. The cathode was placed above the sediment, on the wall of the flask, and remained entirely submerged in seawater to maintain aerobic conditions. The sediment was then flooded with 200mL of seawater. Throughout the entire operational period of the sMFC, no external carbon sources or electron acceptors were introduced.

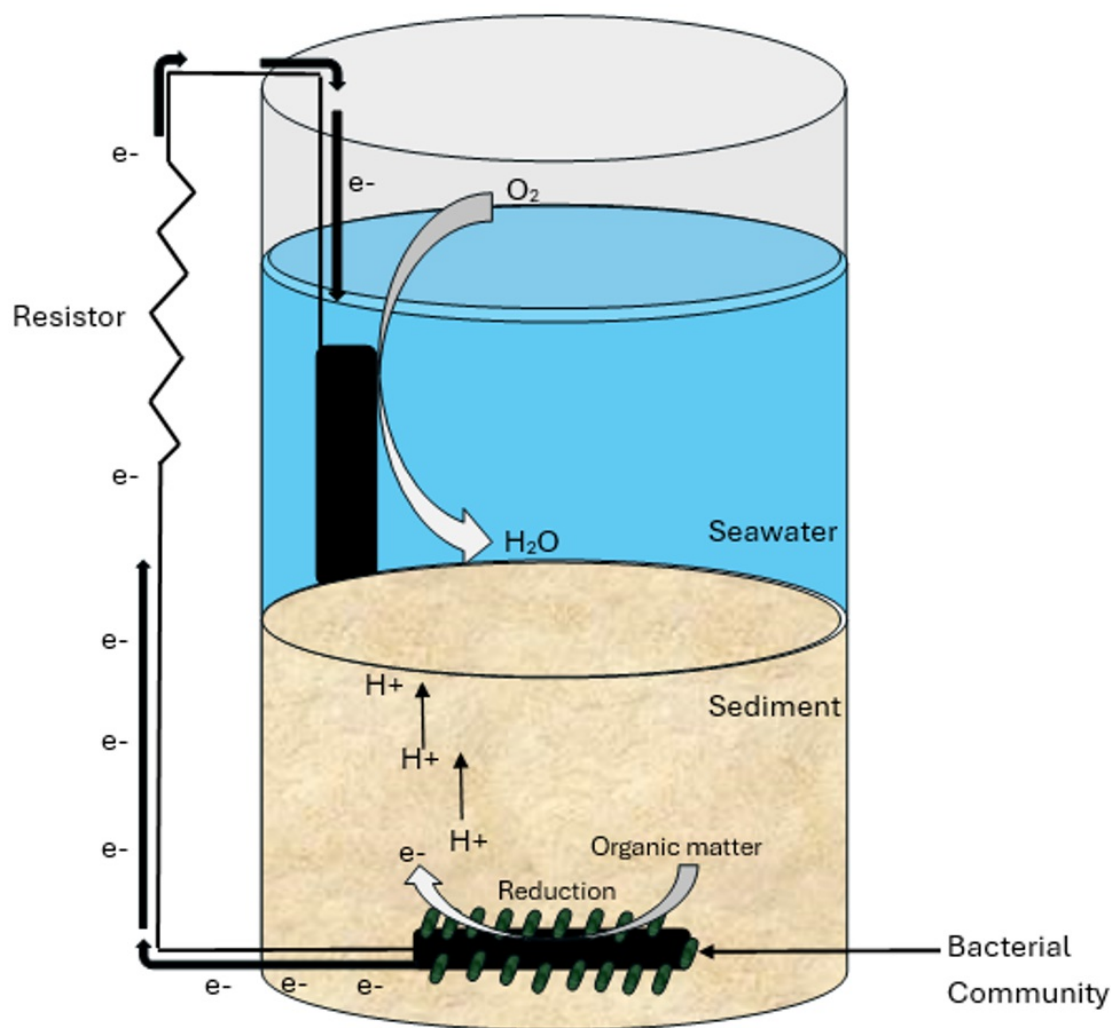


Fig. 1. A schematic diagram of the sMFC.

### 2.3. Oil Spill Monitory Experiment

On the 45<sup>th</sup> day, the sMFCs were aerated using electromagnetic air pumps until reaching a state of quasi stabilization before they were connected to a digital multimeter (BTMETER BT- 90EPC Digital Multimeter) which was used to record the current between the anode and cathode. The multimeter was synced to a laptop via PC Link that allowed real time data collection. Each volume of motor oil (1mL, 1.5mL, 2.5mL, 5mL) was drawn from a graduated cylinder filled with oil for easy access. The oil was gently added to the sMFC above the cathode via a micropipette to avoid interruption of the biofilm that had developed over the cathode. The sMFCs were then monitored for 60 minutes while the data was computed via the digital multimeter. After the 60-minute time period had been completed, oil was added to increase the volume for the next 60-minute time period, for total oil volumes of 1mL, 2.5mL, 5mL and 10mL. This process was repeated until the sMFC had been monitored for 60 minutes with a volume of 10 mL of oil at the surface of the water. This process was repeated for each volume of oil for the most stable sMFC from each group. The data collected was plotted in excel. The experiments were all done at room temperature of 25 degrees Celsius around 1 PM to avoid variation in sMFC productivity.

The power curve of the sMFCs was determined using the following formula 1:

$$P = \frac{E_{\text{cell}}^2}{AR_{\text{ext}}}$$

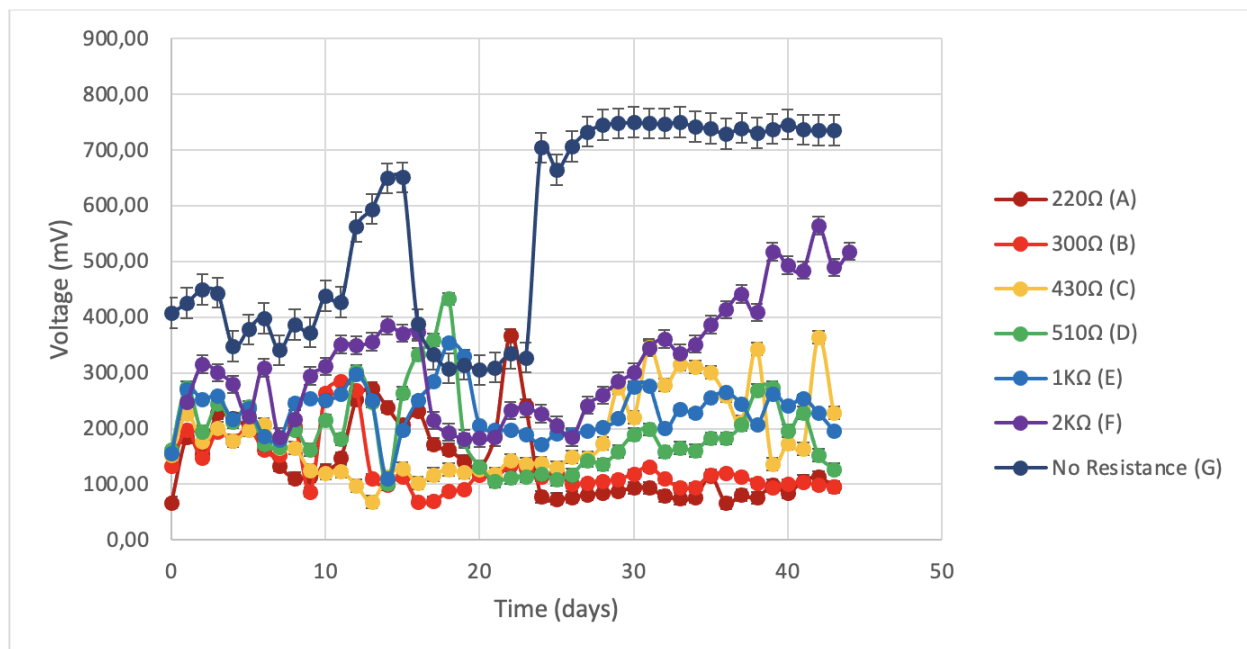
This calculation was done in accordance with the study done by Logan et al. (2006), where  $E_{\text{cell}}$  is the voltage of the cell squared, and  $R_{\text{ext}}$  is the external resistance of the cell. These values could also be used to find the power density of the sMFC, using formula 2:

$$P = \frac{E_{\text{cell}}^2}{A_{\text{an}} R_{\text{ext}}}$$

In this particular study, the area of the anode ( $A_{\text{an}}$ ) is equal to the area of the cathode, so these two values would be interchangeable. As the power curve and power density curves have the same indications, this study only displays the power curves of the sMFC.

### 3. Results and Discussion

#### 3.1. Voltage generation in the sMFC with Varied External Resistors



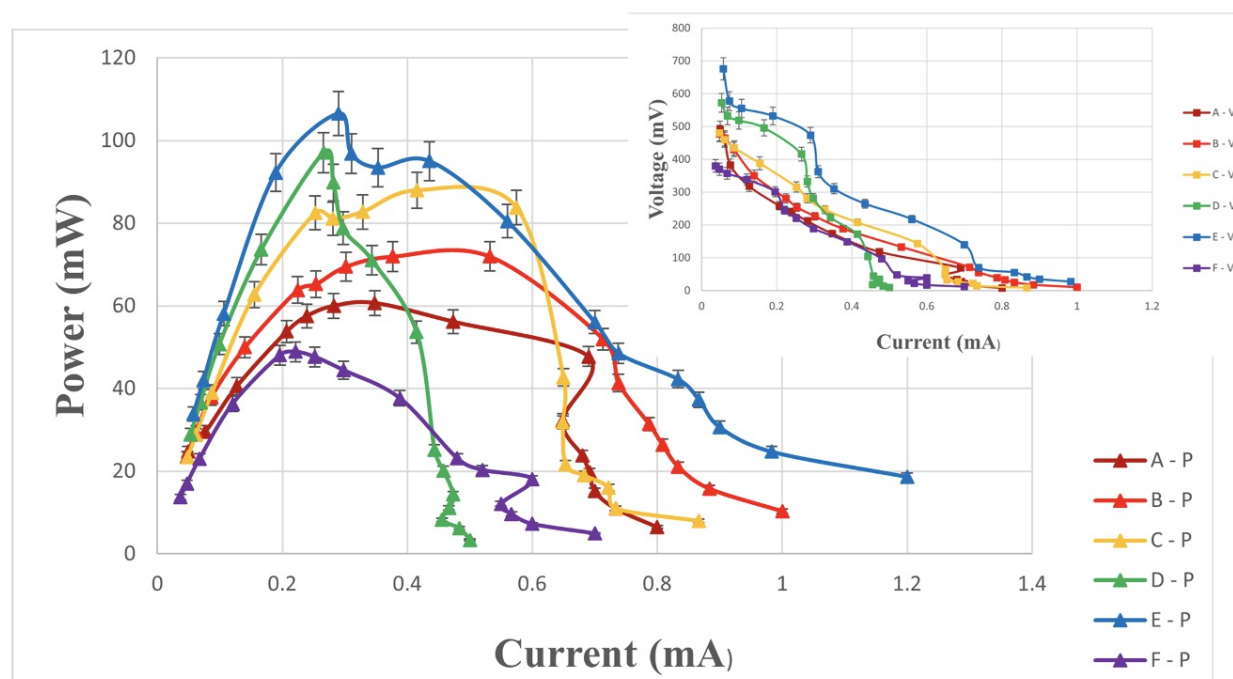
**Fig. 2.** The average voltage of each group, differentiated by voltage.

Figure 2 shows the average voltage of each resistance over the course of the experiment.

The voltage increased steadily until the constant aeration ceased, after which the voltage dropped significantly. The

control group exhibited a more drastic change at this time than the other sMFCs because they were open circuit, and colonies of electrophilic bacteria had not been cultivated. The average voltage of the control group decreased to a little over 300 mV, then suddenly jumped up to 700mV and stabilized there for the remainder of the study. This can be explained by the fact that the water in the sMFCs had been reduced after aerating ceased. When water that had not been reduced was added to the sMFC to make up for the gradual water loss to evaporation, the voltage rapidly increased to a baseline of over 700mV. After the majority of the sMFC groups had reached a semblance of equilibrium, group C still exhibited a large degree of volatility. This can be attributed to the fact that these are living colonies, and it can be speculated that minor changes in the environment of one of the sMFCs will drastically affect the average voltage of the group, especially during the experimental phase when oil was being added to the sMFCs. In this study each group of sMFCs was loaded with their own external resistor. The external resistors used were 220  $\Omega$ , 300  $\Omega$ , 430  $\Omega$ , 510  $\Omega$ , 1000  $\Omega$ , and 2000  $\Omega$ .

### 3.2. Cell Internal Resistance and Power Density



**Fig. 3.** The polarization and power curves of each sMFC.

Figure 3 shows the polarization curve of each cell and the corresponding power curves. Graphs of the polarization curves (square symbols) and the power curve (triangular symbols) of each sMFC were plotted to determine the performance of each resistance group of cells (Figure 2). In this study, the power and current curves were created by varying the external resistance of each sMFC from 10K  $\Omega$  to 10  $\Omega$  using a variable resistor, and the figures obtained were plotted in figure 2 to compare the values for each group. The maximum power curve for the sMFCs at varying resistances was 60.64, 71.97, 87.94, 97.08, 106.50, and 48.84 mW for 220  $\Omega$ , 300  $\Omega$ , 430  $\Omega$ , 510  $\Omega$ , 1000  $\Omega$  and 2000  $\Omega$  respectively. The group exhibiting the highest power density was group E, with an external resistance of 1000  $\Omega$ . This indicates that an external

resistance of around  $1000\ \Omega$  is optimal for sMFC performance. This finding aligns with previous studies done on the effects of ER on MFC activity and indicates that an external resistance of around  $1000\ \Omega$  is optimal for sMFC performance (Del Campo et al., 2014)". According to Logan et al. (2006) the maximum power output of a MFC is only achieved when the external resistance is equal to the internal resistance. Therefore, the internal resistance for groups A, B, C, D, E, and F was approximately  $500\ \Omega$ ,  $250\ \Omega$ ,  $500\ \Omega$ ,  $1250\ \Omega$ ,  $1250\ \Omega$ , and  $1000\ \Omega$  respectively, as these ERs produced the highest power point for each group.

### 3.3. Effect of Oil Doping on Voltage Output

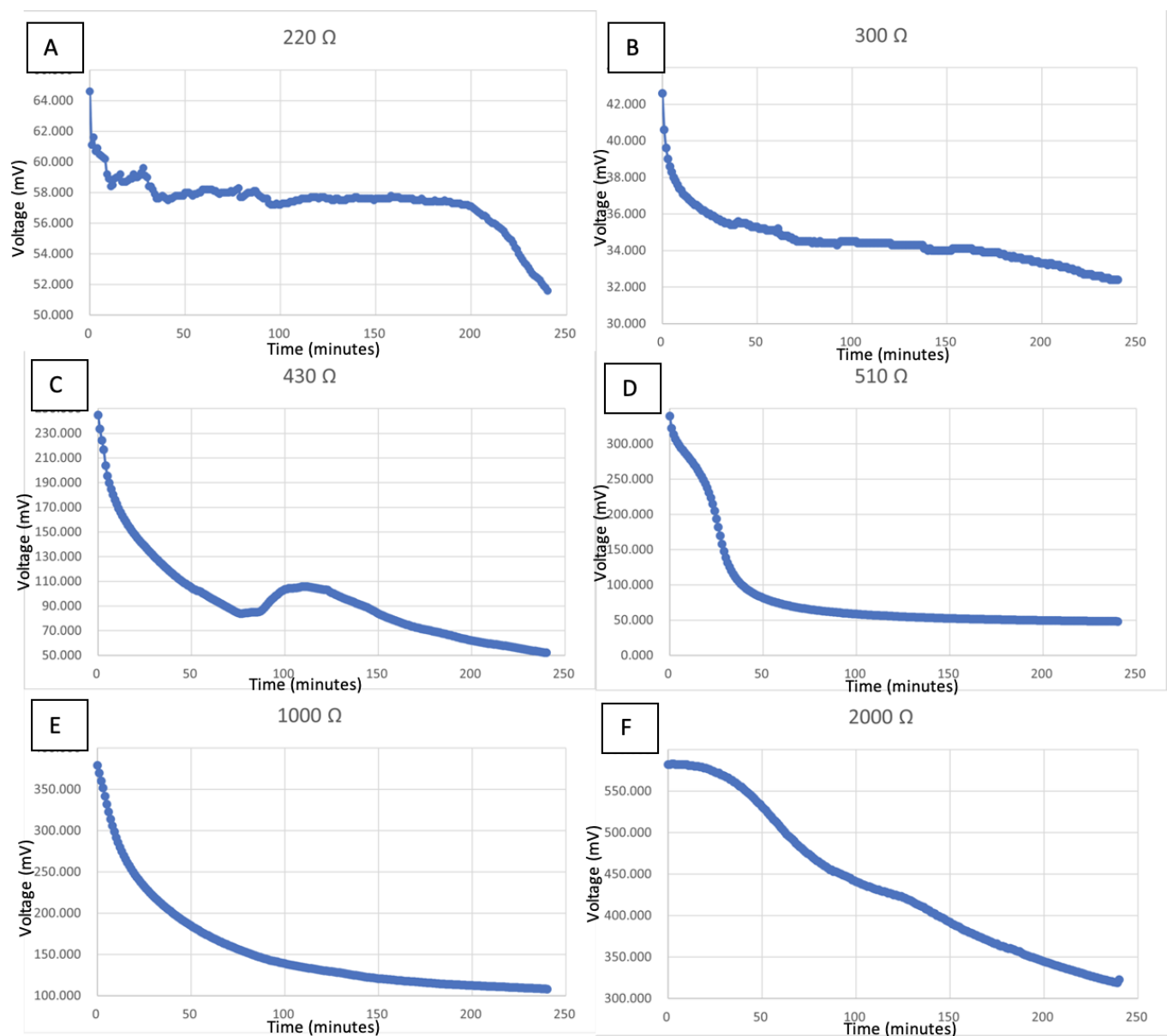


Fig. 4. A-F. The change in voltage over time as oil was injected at the cathode.

Studies have shown that the addition of oil to the cathode of a sMFC can cause the voltage to decrease over time. The



reason for this evident decrease in voltage as oil is added is because oil prevents oxygen from dissolving into the water (Dai et al. 2021). The microbes of importance in the sediment are exoelectrogenic, meaning that they transfer electrons externally (Logan, 2009). Due to this inability to internally transfer electrons, the electrons are instead transferred to the anode, which is made of conductive carbon felt. The titanium wire woven through the anode then transfers these electrons to the cathode, which was originally in an aerobic environment. As oxygen is the final electron acceptor for the respiration being carried out by microbes at the anode, when oil is covering the air-water interface, the availability of dissolved oxygen (DO) is a limiting factor for the rate at which electrons can pass through the titanium wire that completes the circuit. This means that as more oxygen molecules are turned into  $H_2O$  at the cathode as a result of binding with protons (hydrogen atoms released from catabolism of substrate at the anode) and electrons that traveled to the cathode from the microbial organisms in the sediment, there are less available oxygen molecules to accept electrons. With the constant supply of oxygen being cut off by the presence of oil, the voltage will continue to drop until it reaches 0, at which point the sMFC can be classified as dead. This is illustrated in figure 3 A-F, as the graphs all show an eventual, albeit inconsistent decrease in voltage in all the sMFCs after oil was added to the surface of the water at the cathode. This also aligns with previous studies done on DO sensing using MFCs, where a decrease in DO was observed to cause a proportional decrease in voltage production (Song et al., 2019; Zhang & Angelidaki, 2012).

However, unlike the previous studies done on this topic (Dai et al. 2021; Song et al., 2019; Zhang & Angelidaki, 2012), the relationship between increase in time and decrease in voltage was less linear. This could be attributed to the absence of a catalyst in this experiment, which would have greatly amplified the relationship as shown in the study conducted by Dai et al. (2021). The absence of a catalyst may also be the reason why the hypothesized relationship between the volume of oil used and the change in voltage over time was not consistent across all graphs. The issue with catalysts such as platinum, which was used in the experiment done by Dai et al. (2021) in the form of a powder coating over the cathode, is that they are typically toxic to the environment if not properly handled and disposed of. Furthermore, catalysts such as platinum are not only hard to transport but also fairly costly. This study serves as proof that there is a more cost effective and environmentally friendly way to use sMFC for the detection of oil spills.

In the study done by Dai et al in 2021, Service Pro 15W-40 engine oil was used, which is significantly thicker than the Pennzoil 5W-20 that was used in this research. This also could have affected the results of the study, and future research should examine a potential relationship between the viscosity of oil and oxygen insolubility in this context. Saltwater typically has a lower concentration of dissolved oxygen than freshwater, due to the high salinity levels and subsequent increase in total dissolved solids (Al-Zubaidi et al., 2021), so in this experiment it is possible that the bacteria productivity increased drastically when the water was saturated with oxygen beyond its usual capabilities when they were aerated.

The decrease in voltage was almost immediate in all graphs; however, graphs C, D and E exhibited the most notable change over a brief period of time. This is consistent with the power density curves that indicated that a resistance of 1000  $\Omega$  (group E) is optimal for sMFC efficiency, followed by 510  $\Omega$  (group D) and 430  $\Omega$  (group C). These results can be explained by the fact that as the resistance dictates microbial activity, the group with a resistance of 2000  $\Omega$  did not deplete the DO concentration as rapidly due to the resistance limiting the rate of microbial metabolism.

The sMFCs with lower resistances such as figure 3 A and B were prone to burn out faster due to the fact that a lower resistance correlates to a high current, as shown in formula 1. Without a limit to the flow of electrons, the productivity of the cells would be too great, and the metabolizable substrate at the anode would be rapidly depleted. On the other hand, if the ER was set too high, the growth of the bacterial colonies of microbes at the anode will be severely limited by the low flow of electrons due to the high resistance. In figure 3 A, the graph displays high volatility within the first hour after 1 mL of oil was added to the cathode. This can be attributed to the fact that 1 mL of oil was unable to fully cover the interface between air and water, and the amount of oxygen being dissolved varied until more oil was added, at which point it began to stabilize, then decrease rapidly as DO concentration became the limiting factor.

## 5. Implications

The results of this study show that as oil was inserted at the cathode of the sMFC, the voltage decreased. This proves that sMFCs can be used for the detection of minor oil spills in seawater. As the presence of oil directly impacts the solubility of oxygen in water, there is a direct relationship, albeit non-linear, between the decrease in voltage and the increase in time after oil has been added at the cathode. Although previous studies, such as the study done by Dai et al. (2021), have suggested that the response time may be too long to be able to mitigate all the negative effects of the oil spill, this study exhibited an immediate detection of interference via oil at the air-water interface. This shows that sMFCs still provide a cost effective, and now environmentally safe way to detect oil spills that may have gone undetected without their utilization.

## 6. Limitations

This study faced several limitations, as the absence of a catalyst may have significantly altered the effects of oil doping on the sMFCs. When further studies are conducted on this matter, more time should be allotted to the experimental phase of the experiment, and each replicate should be used. Further research should also seek to implement a safe field study, to determine the nature of the sMFC assembly for detecting oil spills in a marine environment. Finally, it is recommended that a catalyst be obtained so that the effects of the catalyst on the oil biosensing properties of the sMFC can be observed, to fully determine whether a catalyst is necessary or not. Additional research should be conducted to bring the biosensing capacities of sMFCs to their full potential.

## 7. Conclusion

This study for the first time investigated the use of sMFCs as a biosensor in the detection of minor oil spills in seawater. It was also concluded that an ER of 1k  $\Omega$  is most efficient in sMFC power generation, and thus will be the most effective at detecting the presence of oil. With further research, this system can be used for application in harbors and bays where minor oil spills from boats often occur and go undetected. Further studies into parameters such as DO decrement rate and

oxygen permeability will be conducted to ensure the success of sMFC-based oil spill detection in seawater.

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