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Many of the most pressing global challenges today and in the future center around the scarcity of sustainable energy and water sources. The innovative microbial fuel cell (MFC) technology addresses both as it utilizes bacteria to convert wastewaters into electricity. Advancing this technology requires a better understanding of the optimal materials, designs and conditions involved. The micro-sized MFC was recently developed to serve this need by providing a rapid testing device requiring only a fraction of the materials. Further, development of micro-liter scale MFCs has expanded into potential applications such as remote and self-sustained power sources as well as on-chip energy generators. By using microfabrication, the fabrication and assembly of micro-sized MFCs is potentially inexpensive and mass produced.

The objective of the work within this dissertation was to explore and optimize the micro-sized MFC to maximize power and current generation towards the goal of a usable and application-oriented device. Micro-sized MFCs were examined and developed using four parameters/themes considered most important in producing a high power generating, yet usable device:

**Anode**- The use of nano-engineered carbon nanomaterials, carbon nanotubes and graphene, as anode as well as testing semiconductor industry standard anode contact area materials for enhanced current production.
Cathode- The introduction of a membrane-less air cathode to eliminate the need for continuous chemical refills and making the entire device mobile.

Reactor design- The testing of four different reactor designs (1-75 µLs) with various features intended to increase sustainability, cost-effectiveness, and usability of the micro-sized MFC.

Fuels- The utilization of real-world fuels, such as industrial wastewaters and saliva, to power micro-sized MFCs.

The micro-sized MFC can be tailored to fit a variety of applications by varying these parameters. The device with the highest power production here was designed to be an inexpensive and robust power source in applications like point-of-care diagnostics in developing countries. This 25 µL graphene nanomaterial anode, air cathode device in an inexpensive flexible rubber architecture was powered by saliva and achieved 3.55 µW/cm² and 35.2 W/m³. The continued optimization of MFC technology promises many interesting and innovative applications.
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<tbody>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>APCVD</td>
<td>atmospheric pressure chemical vapor deposition</td>
</tr>
<tr>
<td>CC</td>
<td>carbon cloth</td>
</tr>
<tr>
<td>CMOS</td>
<td>Complementary Metal Oxide Semiconductor</td>
</tr>
<tr>
<td>CMV</td>
<td>cation exchange membrane</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotube</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammogram</td>
</tr>
<tr>
<td>CVD</td>
<td>chemical vapor deposition</td>
</tr>
<tr>
<td>CoSi$_2$</td>
<td>cobalt silicide</td>
</tr>
<tr>
<td>DRIE</td>
<td>deep reactive ion etching</td>
</tr>
<tr>
<td>EET</td>
<td>extracellular electron transfer</td>
</tr>
<tr>
<td>EIS</td>
<td>Electrical Impedance Spectroscopy</td>
</tr>
<tr>
<td>IC</td>
<td>integrated circuit</td>
</tr>
<tr>
<td>MFC</td>
<td>Microbial Fuel Cell</td>
</tr>
<tr>
<td>MWCNT</td>
<td>multi walled carbon nanotube</td>
</tr>
<tr>
<td>NiSi</td>
<td>nickel silicide</td>
</tr>
<tr>
<td>OCV</td>
<td>open circuit voltage</td>
</tr>
<tr>
<td>OECD</td>
<td>Organization for Economic Cooperation and Development</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>PEM</td>
<td>proton exchange membrane</td>
</tr>
<tr>
<td>PR</td>
<td>photo resist</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>SCCM</td>
<td>standard cubic centimeters per minute</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SOC</td>
<td>systems-on-chip</td>
</tr>
<tr>
<td>SWCNT</td>
<td>single walled carbon nanotube</td>
</tr>
<tr>
<td>TiSi$_2$</td>
<td>titanium silicide</td>
</tr>
<tr>
<td>TSV</td>
<td>through silicon via</td>
</tr>
<tr>
<td>VLS</td>
<td>vapor-liquid-solid</td>
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Chapter 1

Introduction

1.1 The Water-Energy Nexus

Today, the scarcity of potable water is a priority concern in the world. 1.2 billion people do not have access to safe drinking water; 2.6 billion people have little or no sanitation. Millions of people die every year, almost 4,000 children a day, from water borne diseases\(^1\)\(^2\). Since water greatly affects food production and the environment, economies are also affected by lack of access to clean water. Only 3% of the earth’s water is fresh water with over 60% of fresh water locked in glaciers and ice\(^3\) (Figure 1.1). It is therefore imperative we utilize efficiently the fresh water we do have available in the form of groundwater, lakes, rivers, and swamps. In order to be used, this water must be collected, transported, distributed, and treated which requires energy.

Water and energy are inextricably linked. In the US alone, it is estimated that 5% of the electricity produced is used in the transport and treatment of water\(^4\). Desalination techniques have been introduced to convert salt water to fresh water but many of the most advanced water treatment and desalination techniques, such as Reverse Osmosis, require large amounts of energy to operate\(^1\)\(^5\). Water production depends on energy and energy production depends on water. Electricity production, requiring approximately 39% of the freshwater collected in order to run thermoelectric cooling towers, is the second largest water consumer in the United States behind agriculture (Figure 1.2)\(^3\). To put it another way, on just one average day in the United States, there are more than 500 billion liters of
freshwater that are consumed through power plants throughout the country which is more than twice the amount of water that flows through the Nile\textsuperscript{3}. On the global scale, 15\% of the world’s total freshwater withdrawal in 2010 was used to produce energy and water use by the energy sector is expected to increase by 20\% over 2010-2035\textsuperscript{6}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{global-water-resources.png}
\caption{Global water resources}
\end{figure}

Overall, global energy consumption is projected to increase by 56 percent until 2040 (Figure 1.3). Developing nations outside of the Organization for Economic Cooperation and Development (non-OECD) such as India and China account for more than 85 percent of the increase in global energy demand in this time frame due to expanding populations and strong economic growth.
Although fossil fuels, including oil, gas, and coal, are forecasted to remain the primary sources of energy globally, their supply cannot last forever. Other sources of energy must be found, not necessarily as a substitute for fossil fuels, but combined with fossil fuels to create a more diversified global energy portfolio that can sustainably provide energy to the growing population in the upcoming decades. New technologies should be explored to gather energy efficiently from the sun, wind, and wastes.

The continued search for clean and renewable energy sources and energy conservation techniques is an imperative of the world population today. There is one
technology, even though it is only in laboratory phase currently, that addresses both energy and water challenges by treating waste water and simultaneously producing renewable energy, the Microbial Fuel Cell (MFC)\textsuperscript{8}. Independent of the amount of sun or wind, the MFC converts organic matter in waste waters into electricity using bacteria. Although not expected to overtake fossil fuels as a major energy supplier, the unique ability of the MFC to turn liquid wastes into electricity can play a role as one of the newest approaches in diversifying the global energy and water portfolio. Therefore, the exploration and optimization of MFCs is important in understanding the limits and possibilities of the technology and in making the mass commercialization of MFCs a reality.

1.2 Microbial Fuel Cell Background

The earliest MFC concept was demonstrated by Potter in 1910 when he produced electrical energy from bacteria using platinum electrodes\textsuperscript{9}. Since then, there was not much interest in MFC work until the 1980s when the addition of electron mediators were found to increase the current and power production in MFCs\textsuperscript{10,11}. The continuous addition of electron mediators made applications of the MFC outside of the laboratory infeasible and the real breakthrough in MFC technology came in 1999 when it was found that the addition of electron mediators was not necessary and the bacteria themselves could transfer electrons directly to the anode\textsuperscript{12}.

A microbial fuel cell is an innovative device for energy production that captures the electricity produced by bacteria during natural decomposition of organic matter\textsuperscript{8,13,14}. 
Bacteria can decompose organic matter from a wide variety of fuels that are generally abundant and non-toxic, ranging from environmental liquids like domestic and industrial wastewaters to medical liquids like urine and glucose, making the process of refueling simple and inexpensive\textsuperscript{15}. Most frequently, wastewater is used which serves a dual purpose because when the bacteria decompose the matter to produce electricity they are also cleaning the water.

![Microbial Fuel Cell Diagram](image)

**Figure 1.4 Schematic of basic components of a microbial fuel cell**

Basic components and functions of the microbial fuel cell including an anode where bacteria grow and a cathode.

Microbial Fuel Cells typically have an anode and a cathode separated by a proton exchange membrane in a two chamber set up (Figure 1.4). Both electrodes must be conductive and the anode must also be biocompatible as bacteria introduced into the system will form a biofilm on the anode. Certain bacteria found naturally in waste water or grown as a pure culture have unique electrogenic qualities in its ability to decompose organic materials and release electrons extra-cellularly\textsuperscript{8}. When an organic liquid feed is
inserted, the bacteria oxidize the substrate and produce protons and electrons. The protons pass through the proton exchange membrane to the cathode and electrons are transferred through an external circuit from the anode to the cathode driving an external load and reducing the electron acceptor at the cathode.\textsuperscript{8,16}

One of the major challenges with current MFCs is their long testing times (from weeks to months) making it difficult to evaluate new materials or conditions quickly, a necessity in determining the optimal materials for a new technology. The first miniaturized MFC was developed in 2006 with the goal of cutting down testing time to be able to “turn on” an MFC (inoculate with bacteria) in seconds or minutes instead of days and to complete a full run of the experiment in hours instead of weeks\textsuperscript{17}. Development of milli-liter and micro-liter scale MFCs have essentially continued with applications expanding into remote and self-sustained power sources as well as on-chip powering. By using microfabrication, the fabrication and assembly of micro-sized MFCs is inexpensive and can be mass-produced. It is the exploration and optimization of the micro-sized MFC that is the overall objective of this dissertation.

1.3 **Dissertation Objective and Themes**

The development of micro-sized MFCs requires an interdisciplinary understanding of environmental and water engineering, semiconductors and nanotechnology, as well as materials science. With this in mind, the objective of the dissertation research was to take a holistic approach to explore and improve upon many key features of the micro-sized MFC, developing a variety of reactors (volumes from 1- 75 µL) to test nano-engineered
materials and new fuels in an effort to increase power and current production and push micro-sized MFCs one step closer to actual implementation into usable devices and out of the laboratory. With fewer than 20 studies done on micro-sized MFCs\textsuperscript{18}, there are numerous gaps in knowledge related to this area. Therefore, many topics are explored in our effort to optimize micro-sized MFCs and we asked questions such as: Can we fabricate the smallest ever MFC? Can we make the anode a nano-sized forest instead of a flat surface? Can we make the micro-sized MFC completely mobile? Can the MFC be powered by saliva?

There are four major areas or themes of micro-sized MFCs that were explored and improved upon in the studies included in this dissertation. Each theme will be presented below with a general background explanation of its role in the micro-sized MFC as well as a brief introduction of the specific variables within that theme that were tested. The four themes are: Anode, Cathode, Reactor Design, and Fuels.

1.3.1 Theme 1: Anode

The first theme explored in the optimization of micro-sized MFCs is the anode material. The general requirements for all MFC electrodes include good conductivity, chemical stability, non-corrosivity, high mechanical strength and low cost\textsuperscript{8,19}. As the anode must also support the growth of bacteria in an MFC, it requires special characteristics to better enable biocompatibility and adhesion of bacteria to the surface, such as high surface-to-volume ratios\textsuperscript{20} and efficient electron transfer between bacteria and electrode surface\textsuperscript{21}. 

The most versatile and frequently used anode material in macro-scale MFCs is carbon-based typically due to its stability in microbial cultures, high electric conductivity and large surface areas\textsuperscript{22}. Non-corrosive metals, such as gold, have also been used, especially in micro-sized fuel cells, due to their simple fabrication requirements\textsuperscript{23}. Different carbon forms have been used to increase surface areas and promote microbial adhesion to the surface, including graphite\textsuperscript{24}, carbon cloth\textsuperscript{25,26}, carbon paper\textsuperscript{27}, carbon foam\textsuperscript{28}, and reticulated vitrified carbon\textsuperscript{29}. Higher power production in MFCs is possible with increased surface area-to-volume ratios and novel anode designs\textsuperscript{22,28}. For example, graphite fiber brushes have been used to increase surface-to-volume ratios but their use in micro-sized cells is difficult due to their bulky architecture\textsuperscript{22}. Therefore, we sought to utilize nano-engineered carbon materials into a micro-sized MFC, particularly carbon nanotubes (CNTs) and graphene. The properties of these carbon nanomaterials and their use in microbial fuel cells will be described including a note on the introduction on silicon process technology into MFC anodes. Also, a subset theme in anode exploration will be introduced, anode contact area engineering enhancements that increase the amount of electrons that can be shuttled from the anode to the cathode. Common semiconductor contact materials were chosen and compared, including nickel, aluminum, titanium, and cobalt, on two different anode locations with different fabrication methods.

**Carbon Nanotubes**

Carbon nanotubes (CNTs) are a graphene sheet of sp\textsuperscript{2} carbon rolled into tubes of tunable diameter and length that can be capped or not\textsuperscript{30,31}. The CNTs can be subdivided
into two types: Single Walled Carbon Nanotubes (SWCNTs) and Multi Walled Carbon Nanotubes (MWCNTs) which consists of multiple concentric nanotubes.

Although first found in the 1950s, the most profound CNT research occurred in the past decade (Figure 1.5). CNTs have exceptional electrical and mechanical properties with the ability to carry current up to $10^9$ A-cm$^2$ and a tensile strength of over 100 GPa, over 10 times higher than any other industrial fiber. While both types of CNTs are highly conductive and can be purified in mineral acids to remove their cap and functionalized to increase biocompatibility, the use of MWCNTs in electrochemical applications is generally preferred. MWCNTs are metallic conductors while SWCNTs can be semi-conductors depending on diameter and chirality. Also, MWCNTs can be fabricated rapidly, without requiring as much precision, and inexpensively, making production in bulk possible. The ability to align MWCNTs into CNT forests of a specified size increases control of the CNTs for use in intended applications and robustness of the surface.

![Figure 1.5 Trends in carbon nanotube research](image)

**Figure 1.5 Trends in carbon nanotube research**
Because of the unique electrical and structural properties of carbon nanotubes, recent microbial fuel cell studies have included CNTs anodes generally in a hybrid form such as carbon nanotubes integrated into carbon cloth\textsuperscript{35}, textiles\textsuperscript{36}, and polymer composites\textsuperscript{37} finding the CNT enhanced anode outperformed the control. These hybrid anodes blend CNTs with other materials and are not CMOS compatible, though, making these anodes unable to be integrated on-chip for micro-scale applications. Therefore, we integrated pure MWCNTs grown in an aligned forest on top of a silicon chip as anode as they have excellent electrochemical capabilities, can be fabricated directly on chip, and can be functionalized to improve cell adhesion.

The use of pure MWCNTs as anode can be found in Chapters 2 and 3.

**Graphene**

Although graphene is the building block of carbon nanotubes, the material, itself, was not isolated until 2004\textsuperscript{38}. Since then, there has been much interest by scientists to incorporate graphene into electrochemical applications due to its reported large electrical conductivity, vast surface area, and low production costs\textsuperscript{39,40}. Graphene is the first two-dimensional (2D) atomic crystal easily available for our use. At one atom thick, it is the thinnest known material and the strongest ever measured\textsuperscript{41}. Many of its material parameters, such as mechanical elasticity, strength and stiffness as well as high electrical and thermal conductivities are exceptional. Combining properties of graphene has allowed many researchers to envision breakthrough technologies integrating the material into current systems or disrupting the status quo technologies completely.
These properties are currently only achieved with the purest of graphene samples in the laboratory and scientists are rapidly trying to make pure graphene in an easier and mass-produced method which is expected to then attract wide-scale industrial attention (Figure 1.6)\textsuperscript{42}.

In Microbial Fuel Cells, graphene was first introduced as a cathode and then as an anode. In a cathode, graphene acts as an inexpensive replacement to precious-metal electrocatalysts which aid in the oxygen reduction reaction\textsuperscript{43,44}. As an anode, there are a few studies in which a hybrid graphene or graphene oxide modified anode was formed on other anode materials like carbon cloth\textsuperscript{45-47}, stainless steel\textsuperscript{48} or polyaniline\textsuperscript{49}. The use of pure graphene, utilized directly after growth without requiring combinations with other materials, had not been tested as an MFC anode nor had any graphene studies been performed in micro-sized MFCs.

\textbf{Figure 1.6 Methods to fabricate graphene}

Therefore, we tested three different types of pure graphene anodes in micro-sized MFCs.

Graphene grown on nickel on a silicon chip is found in Chapter 5.

Peeled graphene and nickel thin film used as a flexible anode is found in Chapter 6.

Graphene grown on a copper foil found is in Chapter 7.

**Silicon Process Technology**

As the second most abundant element in the earth’s crust, silicon is found everywhere and in its purest form, plays a critical role in the global economy and technology through silicon process technology, the most frequently used technology in fabrication\(^{50}\). Almost all of our modern day electronics require advanced circuits and functions fabricated onto a silicon chip. The importance of silicon cannot be overstated and, therefore, the building of a microbial fuel cell onto a silicon chip is essential in integrating MFC technology as a miniature power source into hand held electronics.

While a variety of micro-sized MFCs fabricate their anodes on top of glass or polydimethylsiloxane (PDMS)\(^{18}\), only two have used silicon\(^{51,52}\) leaving a large area open for optimization of micro-sized MFCs in this area.

A silicon chip was utilized in the anodes of Chapter 2, 3, 4, 5.
Contact area engineering

One of the most important features of designing a high power micro-sized MFC is the contact area to the anode. The contact area can be considered like a gate at the anode that determines how many electrons can pass through based on its inherent resistance and conductivity properties. The performance of the contact area can, therefore, be improved by material selection and position\(^{21}\). In macro-scale MFC designs, a titanium or stainless steel wire is often pressed to the electrode to provide contact with the surface\(^8\). By borrowing contact engineering knowledge from the semiconductor industry, we can improve upon contact areas to be able to increase current production as well as utilize silicon process technology to more easily access the electrons from a different location on the silicon instead of having to directly touch the anode.

When considering contact engineering options in micro-sized MFCs there are three parameters that can be varied: 1) location of the contact area 2) material of the contact 3) method of fabricating the contact material. There are two novel examples of contact engineering described in this dissertation.

In the first example found in Chapter 2, the anode is fabricated onto a 25 mm\(^2\) area on a silicon chip and the contact area is located on the topside of the silicon (same side as the anode) but 2 mm from the anode. The material used is nickel with has been annealed to make nickel silicide.

The second example which is found in Chapter 4, utilized a contact area located on the bottom side of the silicon chip (opposite side as the anode). The materials used are
aluminum, titanium, and cobalt fabricated in both thin film (Schottky) and annealed (Ohmic) methods.

1.3.2 Theme 2: Cathode

The second theme explored in the optimization of micro-sized microbial fuel cells is the cathode where we looked to optimize both the cathode and electron acceptor materials to make a practical, usable micro-sized MFC. The material selection for the cathode, like that of the anode, requires a highly conductive material and is most typically carbon in macro-scale MFCs. Micro-sized MFCs, on the other hand, have most frequently used gold as a cathode as it is easily fabricated. Gold, though, is expensive and therefore, unsustainable in any scaled up version of the micro-sized MFC. Also, silicon manufacturing foundries do not allow access of gold to silicon based devices because of its high diffusivity to shorten integrated circuits unintentionally. Therefore, we chose to utilize a carbon cathode in all of our micro-sized MFCs.

Variations on the carbon cathode were made depending on the electron acceptor chosen. MFCs, particularly micro-sized, are often constrained by the use of liquid chemical electron acceptors that require a two-chamber architectural configuration and continuous refilling when the electron acceptor is depleted making it an unsustainable design feature for use outside of the laboratory. Thus, the use of oxygen in the air has been explored in macro-scale MFCs as an electron acceptor, as it is readily available and does not need to be regenerated. The use of oxygen as an electron acceptor requires some type of air cathode in which protons can reach the cathode on the inside and air on
the outside. Typical air cathodes in macro-scale MFCs utilize carbon cloth with a painted platinum side facing inside (towards the liquid fuel) while the outside has a layer of hydrophobic carbon/polytetrafluoroethylene (PTFE) so that the liquid fuel does not leak from the device. Although air cathodes are used in larger scale MFCs, ferricyanide is almost exclusively used in micro-sized MFCs since it is generally found to produce higher power densities than more readily available electron acceptors such as oxygen. Oxygen is thermodynamically favorable to ferricyanide as seen in the following equations, but ferricyanide is still found to increase power by 1.5 to 1.8 times oxygen in larger scale MFC studies partly because it has a low over-potential making the cathode working potential close to the open circuit potential.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \text{ with a theoretical cathode potential of 1.229 V}$$

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \text{ with a theoretical cathode potential of 0.361 V}$$

In order to make sustainable micro-sized MFCs that can be scaled up or be self sustained power generators, though, the use of an air cathode must be explored at the micro-scale. Two micro-sized MFC studies have recently introduced the use of air cathodes with proton exchange membranes hot pressed onto a carbon cloth cathode. Membranes increase cost and increase internal resistance of a device which lowers overall power production. Therefore, we have introduced an air cathode in a membrane-less one-chamber micro-sized MFC system for the first time, in an effort to make a more sustainable and mobile device.

Air cathodes are found in Chapters 3-7.
1.3.3 Theme 3: Reactor design

The variety of materials discussed in themes one and two must be assembled and utilized into the final system design which ultimately affects the efficiency of the MFC as well as power and current production⁸. Therefore, the third theme of this dissertation is the building high power and current producing reactors with sustainable and usable designs in mind. Four different reactor types were tested with variations made to accommodate different applications of the micro-sized MFCs. The first is the high power and current two-chamber device that at 1.25 µL was the smallest ever fabricated at the time of its publication (Figure 1.7). Similar stacked architecture two-chamber devices exist such as the 1.5 µL MFC by Qian et al.⁵¹ and the 4.5 µL MFC by Choi et al.⁵³. As this device required the use of the chemical ferricyanide which is unsustainable due to the continual need to be refilled, the next device was made with conditions closer to larger scale MFCs using an air cathode in a larger (75 µL) plastic structure with titanium wire contacts (Figure 1.8). The contact to the nano-engineered anode was difficult in this setup and leaks were also prone to occur leading to the third design. This design continued to utilize an air cathode in a 25 µL device but with innovative bottom feed holes etched through the silicon anode to decrease leaks as well as bottom contact to the anode (Figure 1.9).

All three of the previous designs utilized anodes on silicon chips to prepare for applications integrated on the chip. We also developed a design for possible applications that did not require the complexity of integrated circuitry on a silicon chip such as a
power source for point-of-care diagnostics. Emerging medical Lab-on-a-chip devices are being developed as point-of-care diagnostics used to diagnose infectious diseases and perform drug and bacterial resistance tests specifically in the developing world where state-of-the-art laboratory facilities are not available. These applications require a device with a robust and inexpensive architecture that is easy to use and only requiring the user to input commonly used materials\(^6\). Micro-sized MFCs can play a valuable role in point-of-care diagnostics if designed accordingly. Although there are many factors that need to be addressed before a device would be ready for field application, we focused on the development of an inexpensive robust architecture. The final design (25 \(\mu\)L) was made out of inexpensive rubber to be flexible, robust, and easy to feed as syringe tips were incorporated into the device (Figure 1.10). Initial studies have been made on running micro-sized MFCs in series for increased power production and initial results, including photographs, will be included in the Outlook in Chapter 8.

The 1.25 \(\mu\)L reactor is found in Chapter 2.

The 75 \(\mu\)L reactor is found in Chapters 3, 5.

The 25 \(\mu\)L reactor with bottom feed holes is found in Chapter 4.

The 25 \(\mu\)L reactor with flexible structure is found in Chapters 6, 7.
Figure 1.7 The 1.25 μL two-chamber micro-sized MFC reactor.

Figure 1.8 The 75 μL one-chamber reactor with a plastic support structure.
Figure 1.9 The 25 µL reactor with bottom feed holes and backside anode contact.

Figure 1.10 The 25 µL flexible reactor made using inexpensive rubber as support and anode chamber.
1.3.4 Theme 4: Fuels

The last major theme explored in micro-sized MFCs was in the utilization of a wide variety of possible fuels used to power the MFC. (As a note, the term “fuel” will be used throughout to describe the liquid fed to the bacteria since the term more typically used in MFC literature, “substrate”, means the silicon wafer in microfabrication terminology.) Typically, acetate is utilized at 1 g/L in phosphate buffer solution (composed of NH₄Cl, NaH₂PO₄ • H₂O, and KCl) with vitamins and minerals as described previously since it is a simple carbon material easily able to be converted into energy by the electrogenic bacteria. A variety of fuels have been explored in the environmental and medical fields including domestic and industrial waste waters as well as urine and glucose. Here, the use of saliva as a fuel was tested which opens up the possibility of biomedical applications running on saliva or miniature power generators usable anywhere. Also, initial studies on the use of liquid yogurt whey have been done to explore the use of MFCs in new applications. The Greek yogurt industry is currently facing an environmental waste disposal dilemma with strained liquid yogurt whey waste produced from their yogurt manufacturing and the micro-sized MFC is the perfect testing venue to test this fuel before scaling up into larger scale MFCs. Preliminary results from the yogurt whey research are found in the Outlook of Chapter 8.

The use of saliva is found in Chapter 7.
1.4 Organization of Chapters

As each individual micro-sized MFC study was a combination of the four themes, every chapter in this dissertation will correspond to a first author publication/submission. Each paper will be introduced with an abstract connecting the objectives and results of the paper with the four overall themes above with a conclusion at the end of the dissertation.

As a reference, below is a table of each chapter and paper labeled with the overall thematic areas described above. As part of the Chapter 8, the Outlook section is composed of coauthored publications (in preparation) of further work in MFCs including testing novel fuels and integrated architecture designs.

Table 1.1 Dissertation organization by micro-sized MFC themes and studies corresponding to papers published/submitted.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Anode (contact)</th>
<th>Cathode (electron acceptor)</th>
<th>Reactor</th>
<th>Fuel</th>
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<tr>
<td>Chapter 2</td>
<td>MWCNTs (NiSi)</td>
<td>Carbon cloth (ferricyanide)</td>
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<td>Acetate 1 g/L</td>
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<td>Air cathode (oxygen)</td>
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<td>Acetate 1 g/L</td>
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<td>Chapter 4</td>
<td>Gold (Al, Ti, Co Ohmic and Schottky)</td>
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<td>25 µL bottom feed</td>
<td>Acetate 1 g/L</td>
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<td>Air cathode (oxygen)</td>
<td>25 µL flexible rubber</td>
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<td>Chapter 7</td>
<td>Graphene on copper (graphene)</td>
<td>Air cathode (oxygen)</td>
<td>25 µL flexible rubber</td>
<td>Saliva, Acetate 10 g/L, 20 g/L</td>
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1.5 Literature Cited


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Chapter 2


Abstract

As a new subset of microbial fuel cells, micro-sized microbial fuel cells were first introduced in 2006. In our first micro-sized MFC design, we sought to integrate semiconductor and nanotechnology concepts into the MFC in order to increase current and power production in the first step of creating self-sustained electricity generator for Lab-on-chip and similar integrated devices. Nanotechnology advancements were applied to fabricate a 1.25 µl micro-sized MFC with a nano-engineered anode containing a vertically aligned forest of pure multi-walled carbon nanotubes (MWCNT) on a silicon chip as well as a semiconductor industry standard nickel silicide (NiSi) contact area that allowed the electrons to be transferred to the cathode for the first time without requiring contact with the anode directly. The MWCNTs increased the anode surface-to-volume ratio, which improved the ability of the microorganisms to couple and transfer electrons to the anode. The use of nickel silicide also helped to boost the output current by providing a low resistance contact area to more efficiently shuttle electrons from the anode out of the device. The MFC successfully produced 197 mA/m² of current and 392 mW/m³ of power. At the time of its fabrication (2011), it was the smallest MFC by volume that had ever been manufactured and published. Unique features include its small size, comparatively high current densities, as well as the use of nickel silicide contact area. The high endurance of the MWCNT anode is highlighted at the end of the chapter.
with an excerpt from the IEEE Nanotechnology Conference Proceedings in which a replicate 1.25 µL device was run for over 50 days with repeatable current production.

This chapter was published as*


With an excerpt from:


### 2.1 Introduction

Microbial Fuel Cells (MFCs) are an innovative method for generating power that can also be used for treating wastewaters¹⁻³. Milli- to micro-liter scale MFCs provide a unique on-chip power source that could be used at a remote location or in lab-on-a-chip applications making external power sources or refined chemicals unnecessary⁴. Micro-sized MFCs can also be used for rapid screening of electrode materials and

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electrochemically active microbes\textsuperscript{4,5}. Micro-sized MFCs offer high surface area-to-volume ratios, short electrode distances, and fast response times. Many nanomaterials are electrically conductive and biocompatible with microorganisms. Micro-fabrication techniques can therefore be used not only for precise and inexpensive production of these devices, but also they allow for direct incorporation of advanced nanomaterials, like carbon nanotubes, into the reactor.

2.2 Materials and Methods

2.2.1 Carbon Nanotube Anode

The most versatile and frequently used anode materials are carbon-based. Different carbon forms have been used to increase surface areas and promote microbial adhesion to the surface, including graphite\textsuperscript{6}, carbon cloth\textsuperscript{7,8}, carbon paper\textsuperscript{9}, carbon foam\textsuperscript{10}, and reticulated vitrified carbon\textsuperscript{11}. Higher power production in MFCs is possible with increased surface area-to-volume ratios and novel anode designs\textsuperscript{10,12}. For example, graphite fiber brushes have been used to increase surface-to-volume ratios but their use in micro-sized cells is difficult due to their bulky architecture\textsuperscript{12}. Because of the unique electrical and structural properties of carbon nanotubes (CNTs), recent studies have included CNTs\textsuperscript{13-15} and CNT textiles\textsuperscript{16} in MFC anodes. Several other studies also use DC and AC electrochemical characterization of CNT-based electrodes, through cyclic voltammetry and electrochemical impedance spectroscopy (EIS), that show enhanced electrochemical properties compared with conventional materials\textsuperscript{17-21}. 
In order to enhance current generation in an MFC, we developed an anode containing a vertically aligned forest of pure multi-walled carbon nanotubes (MWCNT) in a 1.25 \( \mu \text{L} \) reactor (Figure 2.1). The vertically aligned MWCNTs were grown with controlled and uniform shapes and sizes using vapor-liquid-solid (VLS) self-assembly processes and micro-fabrication techniques. The specific materials and methods used for assembly of the entire MFC are described in the Supplementary Information in Appendix 1. The MWCNTs had a high surface area-to-volume ratio (66,000 cm\(^{-1}\) before functionalization) that was expected to be helpful for bacterial colonization, and a high electrical conductivity (resistance of 2.1 m\(\Omega\) m for a single nanotube) to enhance transfer of electrons to the anode surface. Other possible biological advantages of the MWCNT surface included increased biocompatibility, chemical stability, catalytic activity, and
resistance to decomposition\textsuperscript{16,22,23}. Surface properties such as hydrophilicity, hydrophobility, and functional groups can affect cell adhesion, growth, and metabolism. A functionalization process was performed on the CNTs in which they were cleaned in an acid treatment (nitric and sulfuric acids) to remove residual metal catalysts and other impurities. The acid treatment also has been found to improve cell adhesion by thickening the MWCNTs in an oxidation process that generates carboxylic groups in the walls and tips of the MWCNTs, and forms 3D structures using capillary tensile forces, which make the MWCNTs collapse onto each other\textsuperscript{24}. After functionalization, the average height of the CNTs was 35 mm and their diameter ranged from 200 to 400 nm (Figure 2.2).

![Figure 2.2 Scanning electron microscopic (SEM) images of CNT forest after growth and functionalization](image)

(a) top view and (b) cross section at one edge of anode trench.

An MFC electrode requires a contact area to measure the voltage generated. In macro scale MFC designs, a titanium or stainless steel wire is often pressed to the electrode to provide contact with the surface\textsuperscript{1}. Here, we integrated a low resistance nickel silicide contact area onto the silicon surface so that the electrode contact point did not directly touch the anode. This method provided good electrical contact and avoided the need to
make contact with the anode inside the solution chamber. The use of nickel silicide also enabled the entire fabrication process to be more state-of-the-art complementary metal oxide semiconductor (CMOS) compatible, allowing for rapid manufacturing and deployment as an on-chip power source.

2.2.2 Fabrication

The device was constructed using a highly doped silicon wafer in a specially designed micro-fabrication process (details and a summarized flow chart in Fabrication section of the Supplementary Information in Appendix 1; Figure A1.1). Since the process to fabricate the micro-sized MFC did not use any unconventional or expensive materials (the MWCNTs were synthesized using nickel catalysts instead of conventionally used gold, for example), the use of CMOS compatible fabrication processes substantially decreased cost and fabrication times.

2.3 Results

2.3.1 Current Generation

The MWCNT anode was tested for current and power generation using a mixed culture inoculum and a ferricyanide catholyte solution (see Supplementary Information for experimental and operational setup). MFCs were initially inoculated with wastewater
then switched to an acetate nutrient medium after at least three stable cycles had occurred, indicating growth of a functioning biofilm on the anode (Figure 2.3).

![Figure 2.3 SEM images of bacterial growth after operation](image)

**Figure 2.3 SEM images of bacterial growth after operation**
(a) cross section view, (b) top view and (c) zoomed top view show excellent compatibility with MWCNT and further colonialization for enhanced performance. Images are taken after operation showing the sustainable nature of CNTs in context of mechanical and longevity perspective.

The anode chamber was operated in fed-batch mode, with fresh medium added when the current decreased to <0.5 µA. The ferricyanide catholyte was continuously pumped through the cathodic chamber. Current started to increase immediately after initial acetate introduction but showed stabilized cycles after the second acetate feed, approximately 15 hours later (Figure 2.4). Current reached initial stable peaks of 2 µA within 5 hours after anolyte addition. Following acclimation, repeatable cycles of current generation were obtained that lasted between 10 and 13 hours. After only 1.7 days following the initial addition of the acetate medium, the maximum repeatable current was reached of 3 µA.
The total energy loss in the system can be understood in terms of the different internal resistances as seen in the equation:

$$V_{\text{Device}} = OCV - IR_{\text{int}} = OCV - I (R_a + R_m + R_c + R_e)$$

Where the internal resistance ($R_{\text{int}}$) consists of the sum of the anode ($R_a$), membrane ($R_m$), cathode ($R_c$) and electrolyte ($R_e$) resistances. Minimizing these resistances provides higher power densities from the cell. The total internal resistance was estimated to be $R_{\text{int}} = 25 \, \text{k}\Omega$, based on the slope of the linear section of the polarization curve\(^1\) (Figure 5). This value is the same as that obtained based on the peak in the power density curve\(^1\). To compare the performance with a widely used MFC anode\(^{25}\) – carbon cloth, we also set up

**Figure 2.4 Current generation vs. time plot**
Plot shows steady rise in current and short start-up time. Arrow marks show the acetate inoculation moment. Data shown 15 hours after initial acetate introduction when stable cycles began.

### 2.3.2 Energy Loss and Internal Resistance
28 mL cube- MFCs (14 mL anode chamber) in which we tested the same size anode used in the 1.25 µl cell (25 mm²) compared to a carbon cloth anode cut to the same dimensions but with an effective surface area of 50 mm² since both sides are submersed in the liquid. Power densities (normalized to the projected anode area) using CNT anode were 26% higher than those with the carbon cloth (CC) (Figure A1.3 in Supplementary Information). This result is consistent with previously published results that show improved performance with nano-engineered materials compared to carbon cloth.\textsuperscript{16,26–28}

The main resistances are expected to be due to the electrode overpotentials. The electrolyte or solution resistance can be estimated using \( R_e = \frac{d}{AK} \), where \( d \) is the electrode distance (cm), \( A \) is the geometric area available for ionic species to pass (cm\(^2\)), and \( K \) is the specific conductivity (\( \Omega^{-1}\text{cm}^{-1} \)) of the solution.\textsuperscript{29} The proton diffusion distance was 0.018 – 0.019 cm based on the thickness of the membrane (177 mm according to the manufacturer), and the distance between the CNTs and the membrane (10 and 20 mm, based on the height of the CNTs of 30–40 mm in a chamber with a depth of 50 mm). The solution had a conductivity of 0.01136 (\( \Omega \text{ cm}^{-1} \)), and the proton exchange membrane (PEM) area was 5 mm \( \times \) 5 mm. Based on these values, \( R_e = 6.4 \Omega \). Multiplying this resistance by the maximum current achieved (4.43 \( \mu \text{A} \)) results in an estimated energy loss of only 28 \( \mu \text{V} \) for the electrolyte solution. Reported area resistances for the Nafion 117 membrane are in the range of 0.09 to 0.35 \( \Omega \text{-cm}^{-1} \).\textsuperscript{1,25} Assuming the highest value for Nafion, the membrane resistance in the phosphate buffer solution is estimated to be \( R_m = 1.4 \Omega \), or a loss of 6.20 \( \mu \text{V} \) for the membrane. Therefore, the combined losses due to the membrane and solution would be only 34.2 \( \mu \text{V} \). This is negligible compared to the total energy loss at 134 mV (OCV 243 mV – 109 mV at the
maximum power). Thus, the anodic and cathodic resistances were responsible for almost the entire 25 kΩ of internal resistance. Reducing the electrode resistances is therefore a goal for improvement of future designs. Part of an improved resistance design includes improving the contact area resistances. The resistance between the MWCNT anode and the NiSi contact area was more than 15 times lower than the resistance to the silicon only contact area. Therefore, the integrated nickel silicide contact areas in our devices enabled higher maximum drive current output (197 mA/cm² from 1.25 μL device) than that possible with regular silicon-only contact areas.

2.3.3 Current and Power Densities

The current and power densities produced in this device are compared with those obtained in previous micro-scale MFCs (Table 2.1). The achieved current density at maximum power of 3947 A/m³ was more than 3 times higher than the current density of the cell most similar to our’s in size and architecture, Qian et al.’s 1.5 μL MFC30. The current density per area for our device was the highest yet attained by micro-sized MFCs5,30-32.
Current and power generation are affected by a variety of factors, including oxygen intrusion, inoculum, external resistance used to condition the MFC, and other factors. Oxygen intrusion likely played a major role in decreasing the maximum power density and increasing the anode resistance. Oxygen penetration into the anode chamber can produce an abiotic reaction at the anode that can decrease the voltage. Choi et al. found that the open circuit voltage increased by 50% when adding L-cysteine (an oxygen scavenger) to the anode chamber. Thus, the use of an oxygen scavenger may be helpful in decreasing the anode resistance, and improving current densities. Although we achieved the highest current density by area (by a factor of 2) compared to other micro-sized MFCs, our cell might further be improved through improvements in the bacterial community in the anode chamber. The 1.25 µL reactor is the smallest MFC tested so far with a mixed bacterial culture, with the next smallest at 2500 µL. Watson et al. found that mixed cultures achieved higher power densities than pure *Shewanella*, but Nevin et
al.\textsuperscript{35} found that \textit{Geobacter sulfurreducens} performed better than mixed culture. Thus, it might be possible to increase power using a pure culture of \textit{G. sulfurreducens}. Reactor acclimation at other external resistances might also increase power densities. The MFC was tested using a 100 Ω resistor, which resulted in higher power densities than most of the previous micro-sized MFC tests\textsuperscript{30,31} where resistances of 15–40 kΩ were used. The use of a resistance more closely matched to the internal resistance, such as 20 kΩ for example, would increase power although the current density would be lower.

2.3.4 Maximum Power and Applications

The power produced by the 1.25 μL MFC is sufficient for low power applications. The MFC produced a maximum net power of nearly 500 nW, with a current and voltage of 4.43 mA and 109 mV (Figure 2.5). If we consider a 50% efficient power supply in an on-chip module, then this is sufficient to run a 29.6 pW Phoenix processor\textsuperscript{36} integrated nano-biosensor\textsuperscript{37,38} or other ultra low power devices. In the past, biofuel cells using glucose\textsuperscript{39} or human plasma and \textit{S. cevevisiae}\textsuperscript{35} for power generation have been demonstrated. However, they could be sustained for less than an hour. Other micro-sized demonstrations\textsuperscript{30,32} were only tested for a few days to prove that they worked. Our device was operated in fed batch mode for 25 days, proving that the materials and design can endure long-term use.
These results demonstrate that it is possible to micro-fabricate a micro-sized MFC on a silicon substrate with forest type vertically aligned 3 million multiwalled carbon nanotubes (MWCNTs) and nickel silicide (NiSi) contact base that can produce a high current. Use of MWCNTs provided a very large surface-to-volume ratio, allowing for effective microbial interactions with the anode surface. The successful generation of power using the micro-sized MFC shows that the anode material has a good biocompatibility, and the design allows for state-of-the-art CMOS processes compatible with on-chip power generation.

Figure 2.5 Polarization plot of the 1.25 µL MFC
2.5 High Endurance Carbon Nanotube Anode

After finishing the data for the above study, we did a further endurance test of the MWCNT anode and ran another 1.25 μL two-chamber device in which we successfully ran the micro- MFC for more than 50 days (including inoculation, not shown in graph) indicating its excellent endurance as an MFC anode (Figure 2.6). The MFC achieved an average peak current at 1.9 μA. Peak current was almost always reached within minutes of inoculation with cycle times lasting between 10 and 50 hours (measured from feed time to 1% of average peak value, rounded to 0.02 μA). There were almost 20 peaks measured and as there were large lag times in between feedings, this amount could be increased by at least 20% depending on cycle time, providing further accuracy and faster endurance results of novel anode materials.

Figure 2.6 Lifetime of the 1.25 μl MFC device testing the endurance of carbon nanotube anodes over a 50 day period. Maximum currents reach about 3 μA with an average at 1.9 μA.
The 1.25 µL MFC provided an easily fabricated and assembled tool to test novel anode materials. The testing of the carbon nanotube anode over a period of about 60 days indicated high endurance of this material as an MFC anode. The endurance of an anode is an important factor in its eventual application in actual sensors or other devices which require long life of the fuel cell.

2.6 Literature Cited


Chapter 3

Sustainable Design of High Performance Micro-sized Microbial Fuel Cell with Carbon Nanotube Anode and Air Cathode

Abstract

The 1.25 µL device (Chapter 2) was a successful proof of concept indicating the large improvements nanotechnology can have in micro-sized microbial fuel cells. We next sought to further explore the two key nanotechnology enhancements of the 1.25 µL study, the MWCNT anode and contact areas, and separated them out into two different studies. The first is a comparison of the MWCNT anode with gold and nickel and will be described here while the second is a comparison of the most widely used contact area materials which will be described in Chapter 4.

One of the major drawbacks of the 1.25 µL design was the use of a two-chamber architecture which required the chemical, ferricyanide, to be continuously replenished. Therefore, in this second study in which MWCNTs were used as anodes, the more sustainable air cathode was also utilized in a one-chamber 75 µL micro-sized MFC in which the MWCNT anodes were compared to gold and nickel anodes. The same kind of architecture was used for each anode set-up, ensuring more equal comparisons than compared to other micro-sized MFC reactors in which variations in volume, materials, and design of the device can drastically change the power production. The superiority of the MWCNT anode was established with current production more than 800% higher than both gold and nickel and power production more than 600% higher. In depth discussions on possible drawbacks of using an MWCNT air cathode device are also included.

### 3.1 Introduction

Microbial fuel cells (MFCs) are devices that utilize the naturally occurring decomposing pathways of electrogenic bacteria to both clean water and produce electricity. Micro-sized MFCs are therefore essentially miniature energy harvesters requiring only the insertion of a liquid feed source containing organic materials for the bacteria to feed. Feed sources range from environmental waste waters (domestic or industrial wastes) to medical liquids (glucose or urine) making the MFC a versatile power generator for a variety of applications. As a new technology, a full range of microbial fuel cell conditions and materials must be rapidly tested to determine the optimal parameters for maximum power production and future commercialization. From that perspective, micro-sized MFCs offer a unique miniature platform for rapid testing of MFC components. In addition, as miniature power harvesters, micro-sized MFCs can be integrated onto silicon, the dominant material in the micro and nano-electronics industry, particularly in Lab-on-Chip applications or other point-of-care diagnostics.

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systems that can use the liquid itself for diagnostic or sensing purposes while also producing the energy needed to power the electronic components on the chip.\textsuperscript{9,10}

At a macro-scale, bioenergy has been growing rapidly as a major role in the renewable energy portfolio. Consisting of the use of ethanol, butanol, biodiesels and bioelectricity, bioenergy encompasses a wide range of energy sources and related technologies. Microbial Fuel Cells are an innovative technology in the bioelectricity sector. Bacteria inside of an MFC serve as catalysts that convert chemical energy in organic materials into electricity. MFCs are uniquely able to convert organic matter in waste waters and industrial wastes which is currently not being used into bioelectricity.\textsuperscript{11} Integrating nanotechnology into energy or biology sectors has vast potential for highly enhanced capabilities as we better understand and mimic these systems.\textsuperscript{12} We have taken the typical MFC system (described below) and miniaturized it as well as integrated nanomaterials to increase electricity generated.

Microbial Fuel Cells typically have an anode and a cathode separated by a proton exchange membrane in a two chamber set up. Both electrodes must be conductive and the anode must also be biocompatible as bacteria introduced into the system will form a biofilm on the anode. Bacteria used can be naturally found in waste water or grown as a pure culture but have unique electrorgenic qualities in its ability to decompose organic materials and release electrons extra-cellularly. When an organic liquid feed is inserted, the bacteria oxidize the substrate and produce protons and electrons. The protons pass through the proton exchange membrane to the cathode and electrons are transferred through an external circuit from the anode to the cathode driving an external load and reducing the electron acceptor at the cathode.\textsuperscript{1,11} In designing a more sustainable system,
we removed the membrane making it a one-chamber device and changed the cathode/chemical electron acceptor combination with an air cathode and ambient oxygen electron acceptor making the entire device mobile. Additionally, the addition of a membrane comes with extra expense ($1500/m² for Nafion membrane) and in sustainability of the device as membranes can degrade quickly. By building this mobile, sustainable device on a silicon chip we are enabling the simple integration of nanoelectronics\textsuperscript{12-13} or on-chip functions powered by the microbial fuel cell.

3.2 Materials and Methods

3.2.1 Silicon Process Technology

Silicon process technology is the most matured and frequently used technology in fabrication. The most advanced circuits and functions are integrated into a silicon chip system to run almost all of our modern day electronics. Therefore, the fabrication of a microbial fuel cell onto a silicon chip allows it to be integrated as an on-chip power source into existing systems-on-chip (SoC). The unique liquid feed source allows the MFC to essentially be a power generator recharged with the addition of organic containing liquids such as domestic\textsuperscript{14} and industrial food processing waste waters\textsuperscript{15} or urine.\textsuperscript{16} Optimizing this technology on a silicon chip is essential in the development of the MFC as an on-chip energy source.

Therefore, we have focused on making an MFC on silicon so that it could be easily integrated onto a device on the silicon chip. Using CMOS compatible processes, we have
integrated multi-walled carbon nanotubes (MWCNTs) on silicon as an anode (Figure 3.1). As carbon is the most typically used large-scale anode material, we sought to utilize carbon in a nano-structured form that can be easily assimilated into the other on-chip design processes. We compared the MWCNT anode with the conventional expensive micro-sized anode, gold, and an inexpensive anode, nickel.

![Schematic of the 75 µL micro-sized microbial fuel cell with MWCNT on silicon chip anode and air cathode](image)

Figure 3.1 Schematic of the 75 µL micro-sized microbial fuel cell with MWCNT on silicon chip anode and air cathode
(a) Gold and Nickel on silicon chip anodes were also tested and compared in the same set up; (b) Photograph of MWCNT on silicon chip microbial fuel cell in plastic encasing with titanium wire contact visible as well as the black air cathode compared to a US penny.

3.2.2 Carbon Nanotube Anode

In making the material selection for the anode, we chose carbon as many large-scale MFCs utilize carbon as an anode due to its high biocompatibility. Electrogenic bacteria require extracellular electron transfer (EET) mechanisms to transfer electrons from their cells into an anode. The fabrication of electrode materials can affect the performance of MFCs by enhancing the surface area and improving the EET between the bacteria and the anode. Large-scale MFCs have used a variety of carbon materials chosen to increase surface areas and subsequent microbial attachment with the most typical being carbon
cloth, graphite, and graphite fiber brushes. Nano-engineered carbon materials have also been utilized generally in a hybrid form such as graphene or carbon nanotubes integrated into carbon cloth, stainless steel, or polymer composites. These hybrids are not CMOS compatible and cannot be integrated on-chip for micro-scale applications. Here, we chose to test pure MWCNTs as anode as they have excellent electrochemical capabilities, can be fabricated directly on chip, and can be functionalized to improve cell adhesion.

High electrical conductivities (resistance of 2.1 mΩ-m for a single nanotube) allowed electrons to be transferred more quickly from the bacteria to the anode surface. The most important feature of MWCNTs, though, is high surface area-to-volume ratios (66,000 cm\(^{-1}\) before functionalization) which provide larger areas for bacterial colonization. Other features that aid in biocompatibility include increased chemical stability, catalytic activity, and resistance to decomposition.

Before beginning CNT growth, a 5mm by 5 mm catalyst layer was deposited onto a 4” Si wafer. The Cr/Ni (200/65 nm) catalyst layer was sputtered and patterned using a lift off process to only be within the 5mm by 5mm anode area. The CNTs were then grown in a plasma enhanced chemical vapor deposition (PECVD) system at temperatures between 650°C to 700°C with acetylene and ammonia as carbon source gases. In order to improve cell adhesion, a functionalization process was performed on the CNTs in which they were rinsed in nitric and sulfuric acids to remove residual metal catalysts and other impurities. The acid treatment also has been found to improve cell adhesion by generating carboxylic groups in the walls and tips using an oxidation process as well as encouraging the MWCNTs to collapse on each other to form 3D structures using
capillary tensile forces. After functionalization, the average height of the MWCNTs was 35 mm and their diameter ranged from 200 to 400 nm (Figure 3.2).

We previously demonstrated CMOS compatible MWCNT anode in a 1.25 µL micro-sized microbial fuel cell achieving high current densities but in an unsustainable two-chamber ferricyanide system. In order to build a more sustainable micro-sized MFC, we focused on redesigning the cathode and electron acceptor into a one-chamber membrane-less system.

![Figure 3.2](image)

**Figure 3.2 SEM images of the CNT anode with and without bacterial growth**
(a) heights of 30-40 mm and diameter ranging from 200 to 400 nm with side view, (b) and top down view of the MWCNT forest; (c) Electrogenic bacteria formed a conductive biofilm on the functionalized and biocompatible MWCNTs and transferred electrons to the anode.

### 3.2.3 Air Cathode

The material selection for the cathode, like that of the anode, requires a highly conductive material and is most typically carbon. In this case, we are using a specially designed carbon cloth air cathode. The use of an electron acceptor at the cathode is required to capture the electrons and protons produced by the anode. In micro-sized MFCs, chemical electron acceptors are almost exclusively used with ferricyanide being
most frequent.\textsuperscript{4,5} The use of ferricyanide as electron acceptor is generally found to increase power 1.5 to 1.8 times that of oxygen.\textsuperscript{1} The reason that ferricyanide has been used is that it has a low over-potential when using a plain carbon cathode, making the cathode working potential close to its open circuit potential.\textsuperscript{31} The use of ferricyanide or other chemical electron acceptors, though, is not recommended for four main reasons. First, since they cannot be re-oxidized by oxygen efficiently, chemical electron acceptors must be replaced continuously. Second, ferricyanide can diffuse through the membrane into the anode chamber which affects the long term performance of the device.\textsuperscript{31} Third, they do not produce pragmatic results that could be used for scaling up systems for a large-scale practical application, such as integrated into a waste water treatment plant, which would not be using ferricyanide.\textsuperscript{32} Lastly, the use of ferricyanide increases the cost of a system by requiring a membrane to separate the chambers as well as continuous resupply of the chemical into the system. Therefore, the use of oxygen as electron acceptor is preferred as it can be taken from the ambient air making it inexpensive and abundant. Additionally, as shown by the following chemical formulae the use of oxygen as an electron acceptor in MFCs is kinetically and thermodynamically favorable, compared to the commonly used acceptors:\textsuperscript{31}

\[ O_2 + 4H^+ + 4e^- \rightarrow 2 H_2O \text{ with a theoretical cathode potential of 1.229 V} \]
\[ Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \text{ with a theoretical cathode potential of 0.361 V} \]

Air cathodes are frequently used in larger scale MFC set ups where a carbon cloth cathode is painted with platinum as a catalyst on the water facing side and a hydrophobic carbon/ polytetrafluoroethylene (PTFE) layer on the air facing side.\textsuperscript{33} In this paper, we are presenting, for the first time, an air cathode micro-sized MFC with a nano-engineered
anode as well as a more in depth material study of the comparative performance of gold and nickel anodes in the same architecture. This is also the first time an air cathode is being integrated directly onto a silicon based MFC chip. The only other two reported studies using an air cathode in a micro-sized MFC used a highly pressurized membrane on carbon cloth cathode as well as a gold anode on glass. It is to be noted, usage of gold makes the system more expensive and for large scale manufacturing silicon foundries do not allow access of gold to the silicon based devices because of its high diffusivity to shorten integrated circuits unintentionally. In the first anode material study using an air cathode in a micro-sized microbial fuel cell, we not only showed the durability of the air cathode system under a variety of anodic conditions and showing the enhanced performance of the nano-engineered MWCNT anode in this system but by running the devices for more than 15 days, we showed a stable and reliable power supply in the longest ever run air cathode micro-sized MFC.

3.2.4 MFC Set-up and Electrochemical Measurements

The 75 µL MFC device used in this experiment utilizes a plastic anode chamber holder with a 5mm by 5mm hole drilled through the center of the plastic (thickness of 3 mm) to serve as the anode chamber. The anode was a 5 mm by 5 mm silicon chip anode with chromium and nickel deposited on top and multi-walled carbon nanotubes grown on the nickel catalyst (as described above). Two 0.5 mm holes were drilled on two of the thinner sides of the plastic, one for feeding and one for anode contact. Titanium wire was pushed through one of the 0.5 mm holes and bent to provide pressure and contact the
silicon chip anode at the bottom of the chamber. Each contact was initially measured to have only 5 ohm resistance to the anode before inoculation. We had 50 nm of gold deposited on top using an evaporator and the nickel had 300 nm nickel using sputtering. A specially fabricated air cathode was glued to the top with a small tail protruding to be used as cathode contact. All of the devices were started up using waste water from the primary clarifier of a local waste water treatment plant. After bacterial inoculation, a waste water substitute, 1 g/L acetate, was introduced and fed to each device in a batch mode at the end of each current cycle, approximately every 10-15 hours for over 15 days. Voltage measurements were taken using a continuous multimeter (Keithley).

3.3 Results

3.3.1 Current Generation

As seen from the current production graph in Figure 3.3, the initial current produced after the introduction of acetate was about 20 times greater for the MWCNT device (880 mA/m2) than for gold (29 mA/m2) or nickel (37 mA/m2) which indicates that bacteria were able to more rapidly grow and transfer electrons on the MWCNT anode than the others. Due to the increased surface area and exceptional conductivity of multi-walled carbon nanotubes, their incorporation into an anode can improve electron transfer between the bacteria and the electrode. There have been several studies using DC and AC electrochemical characterization of CNT-based electrodes, through cyclic voltammetry and electrochemical impedance spectroscopy (EIS) that show enhanced
electrochemical properties of CNTs compared with conventional materials.\textsuperscript{26-29} Interestingly, recent studies have also come out highlighting the positive effects MWCNT exposure has to cell growth. The study showed highly enhanced growth of plant cells that were exposed to MWCNTs which could also play a role in the enhanced performance of our MWCNT device and would provide interesting microbiological and genetic modification areas for future study.\textsuperscript{36}

A further analysis of the duration of the current production graph (Figure 3.3) indicates that while all three devices continued to produce current even after more than 15 days, only the MWCNT device drastically decreased current production after the first 3 cycles. The current produced in the MWCNT device decreased with each cycle from a maximum current density of 880 mA/m\textsuperscript{2} to stable reproducible cycles of about 130 mA/m\textsuperscript{2} after 10 days. The gold device reached similar stable values (115 mA/m\textsuperscript{2}) but from a start-up time of more than 3 days with minimal current production and never exhibiting current densities above 200 mA/m\textsuperscript{2} as MWCNTs. Nickel initially peaked at 39 mA/m\textsuperscript{2} and stayed relatively steady at this value.
The 75 µL MFCs were run for over 15 days, longer than any other air cathode MFC. Although the MWCNT device had a high initial peak, it dropped over a few days due to (i) oxygen intrusion into the device; (ii) clogging of the anode with bacteria; and (iii) non-optimal contact with the MWCNT anode. All devices, though, showed stable and reproducible power production by the end of the 15 day testing period. Stable power and current production, even at low quantities, is a desired feature for energy harvesting applications and indicates a promising device setup to either be set up in parallel or series to increase power to micro-devices like lab-on-chip where nano to microwatts power is required. Inset figure shows the activity of nickel and gold anode on day 1. In order to better see the data in the inset, each data point is represented as a line but still only with one fueling of the device occurring during this time at Day 0. Each peak (appearing as a line) in the larger plot represents a refueling of the device, generally once a day. A polarization plot showing the activity with carbon nanotube anode can be found in Supplementary Information in Appendix 2.
3.3.2 Analysis of Carbon Nanotube Performance

There are three main reasons we believe the MWCNT device slowly decreased in current produced over subsequent cycles before stabilizing: (i) oxygen intrusion into the device; (ii) clogging of the anode with bacteria; and (iii) non-optimal contact with the MWCNT anode. First, many of the most efficient electrogenic bacteria (such as *geobacter sulfurreducens*) are anaerobic. They are able to survive with minimal amounts of oxygen present and will then utilize this oxygen as an electron acceptor instead of the anode decreasing the overall power produced in the system. With too much oxygen, though, the bacteria will die. The usage of an air cathode system naturally introduces oxygen into the anode chamber through diffusion. The influx of oxygen from the air cathode could have killed the best electrogenic bacteria over time. The bacteria are first introduced to the device by inserting waste water as previously mentioned in which the bacteria are naturally found and are adapted to the oxygen levels in the water. After a few hours during which a biofilm begins to grow, acetate is fed to the system and current measurements begin (the first peak in Figure 3.3). Given the high surface area of MWCNTs, the bacteria were better able to attach and grow a biofilm than the flat surfaces of gold or nickel films. But with a short proton diffusion length between the anode and the cathode of 3 mm, oxygen at the cathode could diffuse through the chamber and to the anode quickly. The MWCNT biofilm that was grown in the initial inoculation produced high currents but the outer layer of the biofilm began to consume the diffused oxygen decreasing the amount of electrons reaching the electrode. Over time, the second, and perhaps combined, reason for decreased current occurred in that the high
surface area of the MWCNTs became clogged with bacteria including dead and other, non-electrogenic, bacteria species that can survive with oxygen intrusion.\textsuperscript{20,26}

The short proton diffusion length in micro-sized microbial fuel cells makes rapid oxygen intrusion an issue. There are possible options that can be implemented in order to decrease oxygen intrusion. We employed a fed batch feeding operation in which we fed the device with new acetate liquid after the completion of every cycle when current returned to zero mA/m\textsuperscript{2} but as seen from Figure 3.3 there were periods of time in which current production was insignificant for many hours before we fed the device again providing ample time for high amounts of oxygen diffusion to the biofilm. By automating the feeding as a fed batch that occurs as soon as the cycle ends or as a continuously pumped feed, oxygen diffusion to the biofilm should be minimized. There are also chemical oxygen scavengers, such as L-cysteine, that can be added to the acetate feed in order to consume the oxygen within the liquid.\textsuperscript{38} The addition of L-cysteine, though, once again makes the system unsustainable as it must be continually refilled just as the usage of the ferricyanide electron acceptor did before the introduction of the air cathode. Nevin \textit{et al.}\textsuperscript{37} developed an innovative mechanism to fill their graphite anode with liquid feed and have the feed diffuse through the anode to the bacteria providing a means for the inner bacteria in the biofilm to receive feed without relying on feed from the outer bacteria under high oxidative stress with the influx of oxygen. We are exploring future micro-sized designs that are able to be fed from below the silicon chip instead of from the top near the cathode.

Contact engineering is a very important aspect of micro-sized MFCs as establishing a good contact to both the anode and cathode is essential in efficiently transferring
electrons. Establishing effective contacts for nanomaterials is a challenge. In setting up
the architecture of our device to as closely mimic larger scale devices as possible so that
the results could be scaled up, we integrated titanium wire as direct anode contacts. The
wire was pushed into the anode chamber through a hole halfway up the plastic holder and
the wire was bent to provide pressure on the anode chip but this pressure was difficult to
maintain. Before closing the devices, we tested resistance to be below 5 ohm which
shows that initially the pressure was maintained and the titanium was functioning as an
adequate anode contact. With the introduction of liquid into the chamber, though, the
titanium wire easily could have been moved within the device and the contact with the
MWCNTs would have been compromised. We believe that it would serve us better to
fabricate a contact on a different part of the silicon chip and shuttle electrons through the
silicon instead of attempting to establish a direct contact to the MWCNTs. We have
previously explored the use of non-direct contacts in our 1.25 μL MFC where we
fabricated a nickel silicide contact on top of the silicon and contacted this area.\(^{30}\) We
chose the current set up of cutting a 5 mm by 5 mm square of MWCNTs grown on silicon
substrate anode and placing it directly into the plastic anode chamber as this was a design
that could be most easily set up to scale-up the results in future experiments. Future
designs should be made that can utilize the entire silicon chip so as to provide better
sources of contact areas than direct contact to the anode.
3.3.3 Maximum Power and Current Productions

The maximum current densities produced by the devices are seen in Figure 3.4(a) with the MWCNT anode (880 mA/m$^2$) producing about 800% more current than the gold anode (156 mA/m$^2$) and more than 2200% more current than the nickel device (39 mA/m$^2$). We also compared maximum power productions Figure 3.4(b) of the MWCNT anode (19.36 mW/m$^2$) producing more than 600% the power of the gold anode (2.96 mW/m$^2$) and 1900% the power of the nickel anode (1.12 mW/m$^2$). At nearly 500 nW, the MWCNT device is within the power range required to power ultra-low power devices such as Nanosens which developed 1 nW power hydrogen sensor$^{39}$, eXtreme Low Power PIC® Microcontrollers with XLP Technology$^{40}$, nanowatt smart temperature sensor for dynamic thermal management$^{41}$, and a low-voltage processor for sensing applications with picowatt standby mode.$^{42}$
Figure 3.4 Maximum current and power production from CNT, gold, nickel MFCs

Maximum current densities produced by the devices (a) are about 800% higher for the MWCNT anode compared to the gold and more than 2200% higher compared to the nickel anode. Maximum power densities (4b) indicate that the MWCNT anode produced more than 600% the power of the gold anode and 1900% the power of the nickel anode. Their peak power values over a 10 day period (4c) show that all devices were able to have reproducible and stable power but not at the values of their peak power achieved indicating further need for optimization within the micro-size MFC.
3.3.4 High Endurance Carbon Nanotube Device

In our efforts to make a more sustainably designed micro-sized MFC, we tested the devices for over 15 days. Almost all other micro-sized MFCs only show initial data over no longer than 2-3 days\textsuperscript{4,5,7,8,10,30,34} which does not give an indication of the endurance or durability of the design or materials used. The longest run micro-sized MFC was our 1.25 \( \mu \)l MWCNT uMFC using ferricyanide which produced stable batch fed current over more than 45 days.\textsuperscript{43} Over the 15 days we ran these devices, the MWCNT device had the most cycles with 10 over the 15 day period while both gold and nickel had 8. The average peak power height for the MWCNT device (shown for 10 days) was over 2 mW/m\textsuperscript{2} with gold (0.13 mW/m\textsuperscript{2}) and nickel (0.02 mW/m\textsuperscript{2}) at only a fraction of the power (Figure 3.4(c)). Compared to Figure 3.4(b), although all devices showed stable and reproducible power production over the entire testing period, it was not at the same value as peak power produced indicating the need for further optimization of the micro-sized MFC. Stable power and current production, even at lower quantities as seen here, is a desired feature for energy harvesting applications and indicates a promising device setup to either be set up in parallel or series to increase power to micro-devices like lab-on-chip where nano to microwatts power is required.

3.4 Conclusions

The successful integration of the carbon nanomaterial multi-walled carbon nanotubes into the anode of a completely mobile micro-sized MFC using an air cathode was
achieved. By comparing the same air cathode set up with the most commonly used but expensive gold anode as well as an inexpensive metal nickel anode we were able to confirm that air cathodes in micro-sized MFCs are feasible even without a membrane and that the devices are durable and long-lasting. The MWCNT anode outperformed the others in current and power production most importantly due to its increased surface area. This mobile environmentally-friendly micro-sized MFC already generates enough energy to begin to power ultra-low power electronics and with optimization holds potential to play a much larger role in micro-scale energy harvesting in the future.

3.5 Literature Cited


Chapter 4

Role of Metal/Silicon Semiconductor Contact Engineering for Enhanced Output Current in Micro-sized Microbial Fuel Cells

Abstract

As one of the key nanotechnology enhancements in the 1.25 μL MWCNT study (Chapter 2) was the use of nickel silicide (NiSi) as contact area, we sought to explore the use of contact engineering to extract the maximum current and power performances from micro-sized MFCs. In this contact material study, another industry standard contact material, aluminum, is tested as well as metals whose silicides have recently been recognized for their improved performance in small-scale integration requirements, cobalt and titanium. Two methods of contact area fabrication on silicon are employed, Schottky and Ohmic. The Schottky method requires a thin film deposition of the metal while the Ohmic method adds an additional annealing step to the thin film deposition creating a silicide. Nickel silicide was not used as it had been tested in the 1.25 μL MWCNT study. Also, a novel architecture was introduced to allow bottom fueling to the anode through holes etched vertically through the silicon as well as bottom-side contact areas which allows the entire topside of the silicon chip to be used for the integration of on-chip features such as circuits or batteries required for applications of the micro-sized MFC. Contact materials were found to play a large role in the performance of the micro-sized MFC with Ohmic contact materials outperforming their Schottky counterparts almost always in current produced and in some instances, power as well.
This chapter was published as:


4.1 Introduction

Microbial Fuel Cells (MFCs) are devices that use the natural pathways of bacteria inside the MFC to convert the chemical energy in organic matter within liquids into electricity\(^1\). Liquids can come from a variety of sources ranging from waste water and industrial wastes to human extracts, such as urine\(^2\). Since the bacteria decompose the organic matter in these liquids, the liquids are also being cleaned or, in the case of waste water, the water is treated (purified)\(^1,3\). Electricity produced during the process can be directly used\(^3\). Micro-sized MFCs are therefore miniature energy harvesters that use milliliters or microliters of liquid to produce electricity that can be used to power small devices like lab-on-chip or remote sensors\(^4-6\). There are many factors to consider in providing a power source for a variety of applications including the total amount of power or current produced as well as its duration and repeatable use. The micro-sized MFC can be tailored to fit certain requirements depending on the application. In that regard, the goal of this micro-sized MFC is to produce the highest amount of power and current as possible. Also, there are many possible applications of MFCs dealing with water treatment. These are generally most appropriate and effective at larger scales and
other measurements of a successful MFC that are not relevant to its utilization as an energy harvester are not included in this study.

MFCs are composed of an anode upon which the bacteria grow and generate protons and electrons when fed with liquid fuel (like waste water)\textsuperscript{1,7}. Protons flow through the MFC chamber to the cathode while electrons are shuttled from the anode across a load to reach the cathode. One of the most important features of designing a high power micro-sized MFC is the contact area to the anode. The contact area can be considered like a gate at the anode that determines how many electrons can pass through based on its inherent resistance and conductivity properties. The performance of the contact area can, therefore, be improved by material selection.

Aluminum has been the industry standard metal used for interconnects and contact areas for over 40 years\textsuperscript{8}. More recently, though, more attention has been paid to silicide contacts which are generally better suited for smaller scale integrated circuits\textsuperscript{8,9}. There are many requirements for silicide contacts for use in advanced devices including: low resistivity, good adhesion to silicon, high corrosion resistance, and compatible with other processing steps like lithography and etching. Therefore, there are three silicides generally considered in advanced integrated circuit devices: Nickel Silicide (NiSi\textsubscript{2}), Cobalt Silicide (CoSi\textsubscript{2}) and Titanium Silicide (TiSi\textsubscript{2})\textsuperscript{8}. We therefore tested the industry standard, aluminum, and other more recently used silicide materials, CoSi\textsubscript{2} and TiSi\textsubscript{2}. As we had previously used NiSi in a prior work\textsuperscript{10}, we did not include that contact material in this study.

In this letter, we report a comprehensive analysis of these commonly used contact materials fabricated in two different methods of forming contacts areas, Ohmic and
Schottky, to study the performance enhancement in micro-sized MFCs. Additionally, we have used a new 25 µL micro-sized MFC design in which we both contact and feed the device from the back side making the entire top side of the silicon chip surrounding the MFC available for integration of functions or applications for a lab-on-chip or other portable diagnostic devices.

4.2 Materials and Methods

4.2.1 Anode Fabrication

The device is a stacked architecture 25 µL micro-sized MFC (Figure 4.1) which utilizes a gold anode (5 mm × 5 mm x 300 nm) deposited onto p-type highly doped 4” silicon <100> wafer, a 1 mm thick rubber anode chamber/spacer glued on top of the anode and an air cathode prepared on top of the rubber as described before. In making the anode chip we performed two lithography steps to first etch the anode chamber before gold deposition then we etched two feed holes at the corners of the gold anode square to be used as inlet and outlet for bacterial feed.

First, we formed the anode chamber by etching 50 µm into the silicon by deep reactive ion etch (DRIE) process. To do so, photoresist (PR) was spin-coated (MicroChemicals™, ECI 3027), exposed and developed to define areas of 5 mm × 5 mm. Next, DRIE was conducted for 50 cycles at -20 °C (gases used: C₄F₈ + SF₆). Without stripping the photoresist, a metal layer of Au/Ti was sputtered (Au: 300 nm and Ti: 20
nm) on top of the patterned substrate so a consecutive lift-off process (acetone in sonicated bath for 10 minutes) removed the metals from everywhere except inside the etched anode chamber.

A second lithography step was carried out to pattern two 600 µm holes in the corners of the anode chamber. These were used as bottom feed holes. A thick photoresist was chosen to withstand the coming long etching steps (MicroChemicals™, AZ 9260). Special

Figure 4.1 Schematic and photograph of 25 µL bottom feed MFC
(A) Schematic of the stacked architecture 25 µL micro-sized MFC and (B-C) photographs of the device.
care needed to be taken with the new topography of the substrate, which can affect the uniformity of the photoresist. First, the metals were chemically wet-etched through the holes in the PR by gold and titanium standard etchants. Next, a second DRIE process was done to etch the holes through the entire wafer; a process known in the semiconductor industry as through silicon via (TSV). Finally the wafer was cleaned from PR residues in acetone for 10 minutes. At this point 25 chambers were generated out of one 4” wafer. Single cells were then cleaved from the substrate and their backsides were sputtered according to the specifications of each metal for the contact study. The as-deposited metals formed a Schottky contact whereas the post deposition annealed metals formed an Ohmic contact.  

4.2.2 Contact Area Fabrication

Aluminum was sputtered to a thickness of 400 nm. The first sample was left as sputtered to serve as our aluminum Schottky contact. The second was annealed to form an Ohmic contact.

After Al deposition, we performed controlled annealing on the Ohmic contact sample to decrease the resistance between the anode and the contact. The sample was annealed at 550 °C for 5 minutes (Ar atmosphere: 200 sccm). By using a heavily doped silicon substrate, we further improved the resistance of our annealed Ohmic contact. Sheet resistance and electrical bulk resistance were measured for both contact materials. The Ohmic aluminum contact had a resistivity of 4E-6 (Ω-cm) which is one half of the Schottky aluminum contact resistivity. With further annealing, therefore, we improved
the contact resistance and hypothesized that this would also improve the current generating capabilities of the Ohmic contact MFC.

Schottky and Ohmic contacts were also formed for cobalt. The Schottky contact for cobalt was fabricated by sputtering 50 nm of Co onto the silicon chip back side. The Ohmic contact was formed on another chip with the same amount of deposited cobalt followed by a standard two-step silicidation process (30 seconds at 550 °C followed by 30 seconds at 750 °C in 200 sccm of Ar)\textsuperscript{13}. It is to be clarified that the two-step anneal is normally necessary for a controlled cobalt silicide formation, to allow the material to go through different phases; first to a high resistivity phase and then to its low resistivity phase\textsuperscript{13,14}.

The titanium Schottky contact was formed by depositing 80 nm of Ti onto the backside of the silicon chip. The Ohmic contact to form TiSi\textsubscript{2} first used the deposited Ti followed by a two-step salicidation process (30 seconds at 600 °C followed by 30 seconds at 800 °C in 200 sccm of Ar)\textsuperscript{14}.

4.2.3 MFC Assembly and Operation

After the Schottky and Ohmic contacts were fabricated at the bottom side of the chips for all metals, we glued the chip on top of a plastic holder with tubes coming out of the side for easy feeding (Figure 4.1B). The MFC was stacked vertically with the plastic holder on the bottom then anode chip (metal contact area on back side and gold anode on top side), rubber spacer, and air cathode on top. Mixed culture bacteria were first introduced to the system using waste water (local waste water treatment plant, primary
clarifier) and given one day to acclimate before subsequent batch feedings of fuel for the device using acetate (1g/L) in a Phosphate Buffer Solution of 50 mM with vitamins and minerals\textsuperscript{15}. In order to ensure that it was not only chemical potential differences within the device leading to electricity production, we also did a control experiment with an additional cobalt Schottky device in which we ran the device with only acetate and not bacteria. Upon the addition of bacteria current output shot up to more than 600 times the maximum with just acetate (less than 0.02 $\mu$A.)

4.3 Results

4.3.1 Comparison of Ohmic and Schottky contact areas for aluminum

Both annealed and as-deposited devices were fed with acetate (1 g/L) and produced current at every feeding cycle as seen in Figure 4.2A. There was a slight increase in current production by the annealed Ohmic contact device compared to the as-deposited Schottky contact device. But the largest current increase was evident in the power density curves where the current density at the peak power was 50 times higher for the Ohmic contact device than for the Schottky contact device (current densities of 17 A/m$^3$ and 0.24 A/m$^3$) (Figure 4.2B). The current density is a reflection of the ability of electrons to be efficiently transferred from the bacteria to the anode then to the cathode. The higher current density from the aluminum indicates that the annealed Ohmic contact outperformed the as-deposited Schottky contact. In power, though, the Schottky contact was 7 times higher than the Ohmic contact. This lower power production in the Ohmic
contact device is attributed to: (a) the thermal budget induced aluminum spike related competing leakage current enhancement\textsuperscript{16} and (b) residual stress formation during annealing which eventually detached the silicide portion from the silicon substrate.

![Figure 4.2](image)

**Figure 4.2 Current and power density curves for aluminum contact**

(A) Current generated by micro-sized MFCs with aluminum anode contacts (Schottky and Ohmic) fed with acetate producing peak currents at each feeding. (B) Power density graph showing high current densities for the Ohmic contact and high power densities for the Schottky. Inset figure shows the zoomed-in projection of Al-Schottky polarization performance.
Annealed aluminum Ohmic contacts are currently undesirable in Complementary Metal Oxide Semiconductor (CMOS) fabrication since it might short the junction on the source/drain areas in smaller scale integrated circuits at temperatures above 400 °C, creating voids in the silicon substrate, which are then quickly filled by the overlying Al forming “spikes”. These spikes may compromise the quality of the underlying p-n junction and may result in enhanced leakage current and reduced breakdown voltage\textsuperscript{16,17}. However, we still used them in testing our MFC as these devices are not subject to the scaling rule needed for today’s device technology and pristine surface quality required from the polished front side of the substrate. Supplementary figure a shows the current vs. time for cobalt.

### 4.3.2 Comparison of Ohmic and Schottky contact areas for cobalt and titanium

We further tested the performance of cobalt and compared the Ohmic and Schottky cobalt contact devices. The CoSi\textsubscript{2} Ohmic contact device outperformed the Schottky device in both power and current density as seen in Figure 4.3. Producing 0.17 W/m\textsuperscript{3} of power and 0.59 A/m\textsuperscript{3} of current, the Ohmic contact generated more than 3 times the power and 1.5 times the current than that of the Schottky device. This is attributed to lower thin film resistivity (14-20 mΩ-cm) of Ohmic CoSi\textsubscript{2}. Supplementary Figure A3.1 in Appendix 3 shows the current vs. time for cobalt.
Figure 4.3 Power density curve for Co anode contact areas
The Ohmic contact out-performed the Schottky in power production by 4 times.

Figure 4.4 Current production for titanium anode contact area
The micro-sized MFC is tested using Ohmic and Schottky back side contact areas. The device is first inoculated with bacteria from wastewater (not shown) then fed with acetate (1 g/L). Each spike in the figure represents a feeding cycle and peak current produced. The Ohmic contact outperformed the Schottky contact in peak current by 66%.
The Titanium Silicide Ohmic contact device also outperformed the Schottky device in current producing $597 \, \text{A/m}^3$, about 3 times more than the Schottky counterpart (Figure 4.4). Supplementary Figure A3.2 shows polarization curve of the Ti Schottky contact and discusses the difficulties faced in getting a Ti Ohmic polarization curve.

### 4.4 Conclusions

In conclusion, contact materials play a large role in the performance of the micro-sized microbial fuel cells. As an initial study, we found that Ohmic contact materials outperform their Schottky counterparts almost always in current produced and in some instances, power as well. Although the Ohmic aluminum contact produced the highest current, it is not always to best option as mentioned previously since annealed aluminum can form spikes that short circuit smaller scale integrated circuits. For devices that do not require circuits at the smallest scales, an Ohmic aluminum contact may still be the best option. Silicides, Ohmic cobalt or titanium contacts, are also good options and can be used for many of the smallest integration requirements. A further in depth study should be performed on a larger variety of metal contact materials to better understand which Ohmic metal is best for MFCs and their subsequent intended applications.

### 4.5 Literature Cited


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Chapter 5

Choice of Electron Acceptor for Sustainable Design of Micro-sized Microbial Fuel Cell

Abstract

After the success with an MWCNT anode in micro-sized microbial fuel cells, we decided to test another nano-engineered carbon anode, the two-dimensional (2D) atomic crystal structure nano-material graphene. In the following two chapters, studies on two different types of pure graphene anodes grown on a nickel catalyst are described. First, graphene grown on nickel on top of a silicon chip followed by flexible peeled graphene from nickel. Although both studies provided a proof of concept that graphene grown on nickel could serve as an anode in a micro-sized MFC, they had further objectives as well.

The graphene on silicon chip anode was part of a 75 µL MFC that was used to provide a more in depth study of a two-chamber ferricyanide MFC with a one-chamber air cathode device. There is extensive discussion on the advantages and disadvantages of using a one chamber or two chamber system. We show that although traditional chemicals like ferricyanide can achieve higher peak power production by the additional usage of chemical electron acceptor, ambient oxygen fueled air cathode devices achieve higher power at higher current ranges making it more adaptable for electronics requiring higher currents. Also, the air cathode setup showed a stable production of current at each feed making it a durable and longer lasting power generating device on top of obvious advantages in making the micro-sized MFC chemical-free and completely mobile.
5.1 Introduction

Microbial Fuel Cells (MFCs) utilize electrogenic bacteria to convert chemical energy stored in organic matter from waste water into electrical energy and usable power\(^1\). Micro-sized MFCs are therefore similar to miniature electrical power generators powered by waste water or any other liquid that has organic matter\(^2,3\). These liquids can come from a variety of environmental and bio-medical sources\(^4\). The micro-sized MFC can be incorporated onto an integrated chip or circuits (ICs) to power specialized applications like sensors, or Lab-on-Chips. An important step in developing a usable micro-sized MFC is designing a device that is mobile, with a low-cost architecture, and does not require specialized chemicals in its operation. In applications where results from micro-sized MFC studies are to be utilized in creating a larger scale MFC design, conditions must be scalable and the same materials should be utilized in the micro-sized versions as in the macro-sized. Here, we provide a needed step in creating a mobile micro-sized MFC by eliminating laboratory-based chemicals requiring constant refilling which limits the device to the laboratory. We also utilize the same materials as commonly used larger scale MFCs for eventual scaling up. We provide a comparison study of electron acceptors in micro-sized MFCs where we compare the use of oxygen from the environment as electron acceptor (used in large-scale devices) to the chemical ferricyanide (most
commonly used electron acceptor for micro-sized MFCs) in the same micro-sized MFC reactor design. Also, we have integrated, for the first time, multilayer graphene grown on nickel/silicon substrate as an anode. As a unique material with exceptional electrochemical properties\(^5\), graphene is currently used by a variety of scientific communities and has potential to provide improved power production in MFCs if extensively used and studied now.

MFCs are composed of an anode and a cathode in a one or two chamber system. The two-chamber system has a membrane separating the anode and cathode chambers and a chemical electron acceptor on the cathode side. For both, the liquid fuel for the bacteria is inserted into the anode chamber as the bacteria grow on the anode and produce protons and electrons. The protons travel to the cathode through the liquid fuel within the anode chamber and also through the membrane and electron acceptor if in the two-chamber system. The electrons are transferred over an external load to the cathode producing usable electricity\(^1,6\). Material selection for both the anode and cathode are important in order to enhance overall power production in the MFC, but as the anode is the same for both devices here, we will describe it first.

**5.2 Materials and Methods**

**5.2.1 Multilayer Graphene Anode**

Most all large-scale MFCs utilize carbon as an anode due to its high biocompatibility.
Many forms of carbon have been utilized to increase surface areas and subsequent microbial adhesion such as carbon cloth\textsuperscript{7,8} and graphite fiber brushes\textsuperscript{9}. As an ideal anode not only has high biocompatibility as we see with carbon as a material but also high conductivity and electrochemical properties, we chose to test a novel carbon nanomaterial, graphene, as anode as it has excellent electrochemical capabilities and is also one of the few carbon materials that can be fabricated directly on chip. Graphene is a single atomic layer of carbon atoms that exhibits exceptional conductivities (> 10 S/cm)\textsuperscript{5}, high surface areas (up to 2600 m\textsuperscript{2}/g), mechanical robustness, and biocompatibility\textsuperscript{10}. Although graphene has been used in hybrid MFC anodes before\textsuperscript{11,12} such as blended in carbon cloth\textsuperscript{11} or polyaniline\textsuperscript{10}, this is the first presentation of a graphene anode grown on the most widely used bulk silicon (100) substrate.

As the second most abundant element in the earth’s crust, silicon is found everywhere and in its purest form, plays a critical role in the global economy and technology through silicon process technology, the most frequently used technology in fabrication. Almost all of our modern day electronics require advanced circuits and functions fabricated onto a silicon chip. The importance of silicon cannot be overstated and, therefore, the building of a microbial fuel cell onto a silicon chip is essential in integrating MFC technology as a miniature power source into hand held electronics.

Multilayer graphene was grown on top of 600 \textmu m nickel catalyst deposited on a silicon wafer (100) using a sputterer. The 2 inch by 2 inch nickel on silicon wafer was loaded into an atmospheric pressure chemical vapor deposition (APCVD) quartz furnace. The system was first purged with nitrogen for 5 minutes to remove any impurities inside the furnace then the temperature was ramped up to 900\textdegree C over 30 minutes while flowing
argon and hydrogen at 200 sccm and 500 sccm respectively for initial nickel cleaning. The sample is kept under 500 sccm of hydrogen for 4 minutes in order to remove oxygen and stabilize the surface of the nickel. The methane gas was inserted at a flow rate of 50 sccm for 20 minutes while the Argon gas was switched off. Finally, the growth was terminated and the furnace was cooled to room temperature under nitrogen flow. Raman spectroscopy (532 nm laser wavelength) was used to confirm the synthesis of multi-layer graphene.

### 5.2.2 Air Cathode

For the cathode, we also utilized carbon. Both devices have carbon cloth cathodes but the two-chamber device uses regular carbon cloth while the one-chamber device uses a specially designed air cathode\textsuperscript{13} (Figure 5.1). At the cathode is an electron acceptor which serves as the electron sink. Chemical electron acceptors, most often ferricyanide, are commonly used in micro-sized MFCs\textsuperscript{2,3,14}. The use of an air cathode, though, allows oxygen from the environment to be used as an electron acceptor which is advantageous in making a micro-sized MFC lower cost and mobile. Air cathodes are carbon cloth with a painted platinum side facing inside (towards the liquid fuel) while the outside has a layer of hydrophobic carbon/polytetrafluoroethylene (PTFE) so that the liquid fuel does not leak from the device\textsuperscript{13}. The oxygen in the ambient air is used as the electron acceptor providing an abundant, inexpensive electron sink for the micro-sized MFC. Although oxygen is thermodynamically favorable to ferricyanide as seen from the following equations, ferricyanide is still found to increase power by 1.5 to 1.8 times oxygen in
larger scale MFCs\textsuperscript{15}.

\[ O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \text{ with a theoretical cathode potential of } 1.229 \text{ V} \]

\[ Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \text{ with a theoretical cathode potential of } 0.361 \text{ V} \]

**Figure 5.1 Schematic and photograph of 75 µL one- and two- chamber MFCs**

Top down photograph (a) of the air cathode device (left) and the carbon cloth cathode device (right) compared to a US penny (middle). Schematics of the air cathode device (b) and ferricyanide device (c) with all components of the micro-sized MFCs labeled.
5.2.3 MFC Set-up and Electrochemical Measurement

The fabrication of micro-sized MFC (75 µL) device used here began with the outer plastic structure. The plastic anode chamber holder (thickness of 3 mm) was made by drilling a 5 mm by 5 mm hole drilled through the center of the plastic. Two holes (0.5 mm) were drilled on the thinner sides of the plastic, one to be used for insertion of acetate or liquid fuel and the other for anode contact. The anode itself began as a silicon chip (4.5 mm by 4.5 mm) with a nickel deposition followed by graphene growth (parameters as described above). The anode contact was formed by putting titanium wire through one of the holes in the anode chamber and bending it to provide pressure onto the graphene on silicon chip at the bottom of the chamber. After anode setup was complete but before bacterial inoculation, anode contact resistances were measured to have less than 5 ohm resistance.

The anode set-up was the same for both devices but the cathode set-up differed. For the ferricyanide device, a cation exchange membrane (Seleimon CMV, Asahi Glass Co, Ltd) was then glued on top of the plastic anode chamber holder and a 5 mm by 5 mm carbon cloth cathode was placed on top of the membrane before the second identical plastic chamber was aligned with the anode chamber with the membrane separating the two. The device was clamped shut to eliminate leaks. Ferricyanide (50 mM) was continuously pumped through the cathode chamber at 0.1 mL/hour. Instead of a membrane being placed on top of the anode chamber, for the air cathode device a specially fabricated air cathode was glued to the top with a small tail protruding to be used as cathode contact. The cathode had a platinum catalyst on the water facing side and
a hydrophobic carbon/polytetrafluoroethylene (PTFE) layer on the air facing side. Both devices were inoculated with bacteria from a previously working MFC and fed with 1 g/L acetate solution. Voltage measurements were taken using a continuous multimeter and polarization data was captured manually by changing the resistance of the device and letting it stabilize for 20 minutes before measuring voltage.

5.3 Results

5.3.1 Electron Transfer and Biocompatibility of Graphene

Bacteria that can transfer electrons extracellularly are called electrogenic bacteria. The mechanism used to transfer their electrons into an electrode is referred to as extracellular electron transfer (EET). Materials chosen as electrodes in MFCs can improve the performance of the device by methods such as increasing the surface area, which aid in enhancing the EET between the bacteria and anode. Nano-engineered anode materials, like graphene, with exceptional electrochemical properties and higher surface areas can, therefore, improve EET at MFC anodes. Graphene has been found to increase the electron transfer kinetics of the anode and enhance the catalytic activity of acetate oxidation increasing the power produced by graphene enriched MFC anodes. Specifically, CV plots and EIS were used to characterize a graphene biofilm to show that the graphene improved the kinetics of electron transfer between cell surface proteins and the electrode leading to an overall improvement in EET.

As we utilized pure multilayer graphene which had not been tested before, we first
conducted an experiment to ensure the biocompatibility of the graphene. First, the multilayer graphene was grown from a nickel catalyst using atmospheric pressure chemical vapor deposition (APCVD) and methane carbon source. We then inserted bacterial inoculation into the assembled graphene anode MFC and waited 24 hours for bacterial biofilm growth to begin before disassembling. SEM images were taken of the anode after bacterial inoculation. As can be seen in Figure 5.2, growth on top of the multilayer graphene indicates biocompatibility.

![Figure 5.2 SEM image of graphene with bacteria](image)

**Figure 5.2 SEM image of graphene with bacteria**

SEM images of pure multilayer graphene after first inoculation with bacteria indicating initial biofilm growth on top of graphene and biocompatibility of graphene anode.
5.3.2 Ferricyanide Analysis

Generally, the use of ferricyanide generates higher power production compared to oxygen because almost the entire cathode potential is utilized. It has a low over-potential making the cathode working potential close to its open circuit potential. Oxygen, on the other hand, does not completely utilize its cathode potential resulting in a less efficient system\textsuperscript{1,15}. Although ferricyanide can produce more power, its use in MFCs is not advised if the goal is to create a sustainable, usable, and scalable system. Since ferricyanide and other chemical electron acceptors are not self-sustaining as they cannot be re-oxidized by oxygen efficiently, they must continuously be replaced in order for the device to continue to produce power. The constant resupply of this chemical increases the cost of the entire system compared to the air cathode device which obtains its electron acceptor from the ambient air. Also, as ferricyanide must be kept in a separate chamber from the liquid fuel at the anode, its use requires a membrane. Membranes increase the cost of the system (typically used Nafion membranes are $1500/m^2\textsuperscript{18}) can add a material to the system that is prone to failure over time. As a polymer, the membrane can easily tear or be worn away allowing ferricyanide to diffuse through the membrane into the anode chamber, impairing the long-term performance of the device\textsuperscript{15}. Lastly, the use of ferricyanide in a micro-sized MFC does not produce scalable results that can be translated into a larger practical application of MFCs that would not be using ferricyanide, such as in actual waste water treatment plants. It is important to compare side by side the advantages and disadvantages of using both the one and two chamber devices for micro-sized MFCs in order to fully understand the benefits of introducing an
air cathode to a micro-sized system (Table 5.1). As even the slightest architecture or liquid fuel difference can produce huge variances between different groups’ micro-sized devices, it is very difficult to compare the results of a one-chamber air cathode in one set-up versus the results from a two-chamber ferricyanide device in another. Therefore, we designed an architecture that is the same for both devices (with the addition of a second chamber to the two-chamber device) and kept all other conditions equal in order to more fully compare the power production of a one-chamber to a two-chamber micro-sized MFC device.

Table 5.1 Design considerations for sustainable micro-sized microbial fuel cell.

<table>
<thead>
<tr>
<th></th>
<th>Cathode</th>
<th>Electron Acceptor</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>One-Chamber</strong></td>
<td>Air Cathode</td>
<td>Oxygen</td>
<td>Membrane-less</td>
</tr>
<tr>
<td></td>
<td>+ Allows entire device to be mobile as it utilizes ambient air for cathodic reactions; PTFE binder reinforces the carbon cloth making it less likely to fray - Can be expensive if using platinum as catalyst but other more inexpensive materials are being researched</td>
<td>+ taken directly from the ambient air, inexpensive and abundant - high potential energy but inefficient currently likely partially due to oxygen reaching semi-anaerobic bacteria</td>
<td>+ inexpensive and easier set-up; lower internal resistance - less protection from oxygen intrusion from the cathode to the anode</td>
</tr>
<tr>
<td><strong>Two-Chamber</strong></td>
<td>Carbon Cloth</td>
<td>Ferricyanide</td>
<td>Membrane</td>
</tr>
<tr>
<td></td>
<td>+ inexpensive; does not require specialized set-up - Not as conductive as metal cathodes; can fray and fall apart over time</td>
<td>+ higher power production (1.5-1.8 times) found in large-scale systems - liquid chemical that must be continuously refuelled making it unsustainable and higher cost to the system</td>
<td>+ barrier against oxygen intrusion to the anode - increases cost of the system, complexity of set-up, and internal resistance; can degrade allowing ferricyanide to enter anode chamber and impairing device</td>
</tr>
</tbody>
</table>
5.3.3 Current and Power Densities

Both the ferricyanide and air cathode devices were started up using bacterial effluent from a previously working MFC. After bacterial inoculation, 1 g/L acetate solution\textsuperscript{16} was introduced and fed to each device in a batch mode at the end of each current cycle, approximately every 10-15 hours. In the current production graph in Figure 5.3, the initial current produced after the introduction of acetate was about 20\% higher for the ferricyanide device compared to the oxygen device (2.7 mA/m\textsuperscript{2} and 1.7 mA/m\textsuperscript{2} respectively). The higher current value is expected as the ferricyanide maintains the cathode potential at maximum levels nearest to those at open circuit potential as described above. Interestingly, though, the subsequent acetate cycle shows dramatically decreased peaks for both devices, only reaching about 1/3 of their initial current peak indicating that the drop is due to a common feature of both devices, most likely either the bacteria or the graphene anode. As this drop after the second cycle was not due to the change in electron acceptor, it was not further explored in this paper but would be an interesting response to research further in future experiments.

The duration of the current production graph (Figure 5.3) signifies the ability of the device to produce stable current peaks over time. For the ferricyanide device, continuous current cycles could not be established past about 2 days. This is likely due to ferricyanide leakage across the membrane into the anode chamber. As the device is so small, any small leaks will reach the anode almost immediately which will disrupt the bacterial biofilm.

The membrane that was used for the ferricyanide system was a general cation
exchange membrane (Selemion CMV) which may not have been as effective preventing against ferricyanide leakage as the more typically used Nafion 117 proton exchange membrane (PEM). This could attribute to the more rapid diffusion of ferricyanide into the anode chamber which does not appear to occur so quickly using a Nafion PEM. The Selemion CMV was chosen in this case as previous studies comparing membranes in larger scale air cathode MFCs found that the Selemion CMV outperformed the Nafion 117 by having 33% lower internal resistance, less oxygen diffusion across the membrane and only one-quarter of the price ($400/m² for the Selemion and $1500/m² for Nafion) making it a suitable alternative to Nafion. Future work in this area should compare the use of various membranes in a micro-sized system over a prolonged time period as many features and characteristics of materials are expressed differently in micro and nano environments over longer time frames.

While the air cathode device decreased after the first cycle to about one third of its peak value, it maintained this current production with every feeding for more than one week (Figure 5.3). After the initial drop in current after the first cycle to 0.6 mA/m², each subsequent cycle returned to this value indicating a stable system. Stable current production, even at low quantities, is a desired feature for energy harvesting applications and indicates a promising device setup to either be set up in parallel or series to increase power to micro-devices like lab-on-chip where nano- to microwatts power is required. Many engineering companies in applied circuits today have been increasingly minimizing the minimal power requirements for their microcontrollers in both active and standby modes. For example, the eXtreme Low Power PIC® Microcontroller from Microchip Technology consumes 10 nA in deep-sleep mode and only 150 µA/MHz in active
Texas Instruments has also developed a line of “Wolverine” mixed signal microcontrollers that consume 320 nA in data retention mode and 81.4 μA/MHz in active mode.

5.3.4 Maximum Power and Applications

The power density graph (Figure 5.4) shows that the ferricyanide device did, as predicted, produce higher power with a peak of 115 μW/m² vs 60 μW/m² for the air cathode device but the air cathode device can maintain higher power values at higher currents. After the peak power is observed for the ferricyanide device at a current of...
about 2.4 mA/m², the power immediately drops while the air cathode device reaches its peak power at 3.2 mA/m² and maintains higher power densities from about 3.2 until 3.6 mA/m².

![Diagram](image.png)

**Figure 5.4 Power density curve for one- and two- chamber micro-sized MFCs**

Power density curves for the ferricyanide device compared to the air cathode device indicates a higher peak power with the ferricyanide device but a higher power production at high current values for the air cathode device.

The overall power production can be improved with better contacts made with the nanomaterial anode in particular. Contact engineering plays a key role in micro-sized MFCs as maintaining a good contact or connection to both the anode and cathode is necessary in order to efficiently transfer electrons and produce power. As a new nanomaterial, the most effective contacts for graphene are still not fully known and the
contact to graphene is considered one of the key challenges hindering further progress in graphene performance\textsuperscript{21-24}.

In setting up the architecture of our device to as closely mimic larger scale devices as possible so that the results could be scaled up, we also integrated titanium wire as direct anode contacts. The wire was pushed into the anode chamber through a hole halfway up the plastic holder and the wire was bent to provide pressure on the anode chip but this pressure was difficult to maintain. Before closing the devices, we tested resistance to be below 5 ohm which shows that initially the pressure was maintained and the titanium was functioning as an adequate anode contact. With the introduction of liquid into the chamber, though, the titanium wire easily could have been moved within the device and the contact with the graphene on silicon chip would have been compromised. We believe that it would serve us better to fabricate a contact on a different part of the silicon chip and shuttle electrons through the silicon instead of attempting to establish a direct contact to the graphene. We have previously explored the use of non-direct contacts in our 1.25 \( \mu \)L MFC where we fabricated a nickel silicide contact on top of the silicon and contacted this area\textsuperscript{14}. We chose the current set up of cutting a 4.5 mm by 4.5 mm square of graphene grown on nickel/silicon substrate anode and placing it directly into the plastic anode chamber as this was a design that could be most easily set up to compare both the ferricyanide and oxygen setups without requiring separate architectures. Future designs should be made that can utilize the entire silicon chip so as to provide better sources of contact areas than direct contact to the anode.
5.4 Conclusions

The successful integration of the carbon nanomaterial graphene into the anode of a micro-sized MFC on a silicon chip is achieved. By exploring the use of an ambient oxygen based cathode system versus the more commonly used chemical ferricyanide, we found that while peak power production was higher with the additional use of the chemical electron acceptor, the air cathode device achieved higher power at higher current ranges making it more adaptable to electronics requiring higher currents, an important subset of electronic devices. Also, the air cathode setup showed a stable production of current at each feed making it a durable and longer lasting power generating device.

5.5 Literature Cited


Chapter 6

Graphene Based Flexible Micro-sized Microbial Fuel Cell

Abstract

The second test of the 2D nanomaterial, graphene, was also utilizing a nickel catalyst but included a novel method of peeling the graphene to use as a thin film anode. The peeled graphene on nickel thin film provided a thin flexible anode that was inserted into a new sustainably designed low-cost structural architecture made of rubber that is thin and flexible allowing the device to be easily handled and inexpensively made. As part of the structure, we utilized syringe tips for ease of insertion of liquid making any fuel for the device able to be stored in syringes and used for immediate fueling. This is the first demonstration of the more sustainably designed 25 µL flexible architecture that allowed flexibility from the low-cost rubber as well as mobility provided by the air cathode. Producing 1 mW/m², the peeled graphene device outperformed the nickel control by 10 times making it nearly suitable for ultra-low power supply bioelectronic options.

This chapter was published as:

6.1 Introduction

Generally a microbial fuel cell (MFC) is a lab-based technology to treat and to purify water. It provides a unique opportunity contrary to all the known energy intensive technologies for water purification as the MFC simultaneously produces electricity while cleaning the waste water. Bacteria within the MFC naturally decompose organic wastes in water while also producing electrons which are carried across a load to generate electricity.\textsuperscript{1,2} The bacteria can decompose organic matter from a wide variety of substrates that are generally abundant and non-toxic, ranging from wastewater and industrial wastes to urine and blood, making the process of refueling simple and inexpensive.\textsuperscript{3,4} At a smaller scale, micro-sized microbial fuel cells are essentially self-powered generators that use microliters of liquids containing organic matter, like waste water, to produce nanowatts of power. Miniaturized MFCs have vast application potential in portable power devices, sensors or integrated onto Lab-on-a-Chip.\textsuperscript{5,6} Lab-on-a-Chip devices integrate a variety of laboratory functions onto a micro-sized chip making them very appealing since they can rapidly perform needed tests with substantially less volumes of liquid.\textsuperscript{7} Existing Lab-on-a-chip designs rely on microfluidic and advanced semiconductor techniques to make products that can detect anything from cancer\textsuperscript{8} to chemicals\textsuperscript{9,10} or anything in between. Advances in MFC technology are required in order to make a robust and practical device that can be used for Lab-on-a-chip applications on a mass scale.

Typical MFCs are composed of a two-chamber set up with the anode and cathode chambers separated by a proton exchange membrane. The anode and cathode must be
conductive with the additional requirement of the anode to be biocompatible as the bacteria will be inoculated into the anode chamber and form a biofilm on the anode. When an organic substrate is introduced, the bacteria oxidize the substrate and produce protons and electrons. The protons are passed through the proton exchange membrane to the cathode and the electrons are shuttled through an external circuit from the anode to the cathode driving an external load and reducing the electron acceptor at the cathode.\textsuperscript{1,4}

As part of a more sustainably designed MFC, we made changes to the typical two-chamber system to make a one-chamber MFC that is more adapted to Lab-on-a-Chip applications. Since many medical lab-on-a-chip devices are point-of-care diagnostics used to diagnose infectious diseases and test for certain drugs often in the developing world where large-scale laboratories are not available, these applications require a more robust architecture to be viable in the field, focusing more on ease of use and durable, inexpensive designs.\textsuperscript{11,12} As a recent technology, micro-sized MFCs require extensive testing under a variety of conditions and designs to make them suitable for a commercial product. Inserting specialized materials can improve the power performance of an MFC dramatically and micro-sized MFCs are ideal venues to test new anode and cathode materials like nano-engineered anodes\textsuperscript{13-16} or improved reactions at the cathode\textsuperscript{17}. With a more robust architecture in mind, we have developed an inexpensive easy-to-use 25 \( \mu \)L MFC incorporating two important sustainable design features and utilizing, a thin film atmospheric chemical vapor deposition (APCVD) based graphene grown on nickel anode for improved performance producing \( \sim 1 \) nW power directly from waste water (Figure 6.1).
6.2 Materials and Methods

6.2.1 Air Cathode

First, and the most important for a sustainable design, we integrated an air cathode into the architecture. An air cathode utilizes ambient oxygen directly from the air as cathode electron acceptor. The cathode is a specially prepared air cathode composed of carbon cloth with a thin layer of Platinum catalyst and nafion binder on the anode side and layers of polytetrafluoroethylene (PTFE) and carbon powder on the air side for
waterproofing.\textsuperscript{18,19} Previously, micro-sized MFCs used chemical electrolytes, most often ferricyanide\textsuperscript{13, 20-21}, that provided higher power but required continuous refills with the chemicals in order to function.\textsuperscript{5,6} In utilizing an air cathode, we eliminate the need for these toxic chemicals and do not need to refill the cathode electrolyte, as now oxygen is taken directly from the air, therefore, enabling the device to be completely mobile and able to function outside of the laboratory. The air cathode also reduces the structure from two chambers to one chamber and eliminates the need for a membrane. Not only does this lower the cost of the entire system as the most commonly used membranes (Nafion 117) cost more than $1500/m\textsuperscript{2}, a one-chamber system without a membrane has actually been shown to increase power density.\textsuperscript{19} By utilizing an air cathode and without a membrane, we are better able to test scale-up conditions of microbial fuel cell technology and more rapidly reach the technology’s potential in mass-scale water treatment abilities.

\textbf{6.2.2 Low Cost and Flexible Architecture}

Secondly, in our sustainable design, we included a low-cost structural architecture made of rubber that is thin and flexible allowing the device to be easily handled and inexpensively made. As part of the structure, we utilized syringe tips for ease of insertion of liquid making any fuel for the device able to be stored in syringes and used for immediate fueling.
6.2.3 Peeled Graphene Anode

We further explored the use of a thin flexible anode and used a graphene grown on nickel anode. Other microbial fuel cell studies have used graphene oxide enhanced anodes inserting graphene oxide into carbon cloth\textsuperscript{14-16} or onto stainless steel.\textsuperscript{22} Compared to previous demonstrations with graphene oxide, we used graphene grown on nickel by chemical vapor deposition. This unique process enables the thin film graphene on nickel anode to be used directly after growth without requiring any transfer eliminating possible rupture and polymer based contamination. Grown on 300 nm nickel deposited on silicon oxide (SiO\textsubscript{2}), using chemical vapor deposition, we produced multi-layer graphene (confirmed with Raman Spectroscopy, Figure 6.2) and peeled off the graphene layer/nickel (Ni)/titanium (Ti) off of the silicon substrate by etching off the silicon oxide to use directly as a thin flexible anode (~300 nm) in the MFC (Figure 6.3).

\begin{center}
\includegraphics[width=0.5\textwidth]{Figure6.2.png}
\end{center}

\textit{Figure 6.2 Raman spectroscopy confirming multi-layer graphene}
Inset shows the scanning electron microscopic images of graphene with bacteria growth.
We glued the graphene to the rubber anode chamber while leaving a space to contact the graphene. The anode area (available for bacterial growth) was 25 mm$^2$ and as the rubber anode chamber was 1 mm thick, the total volume of the device was 25 µl.

![Images of peeling and assembly process](image_url)

**Figure 6.3 Photographs of peeling and assembly process of 25 µL flexible MFC**
Flexible microsized Microbial Fuel Cell (MFC) utilizing (a) anode of graphene on nickel; (b) that is flexible; (c) and is stacked with an inexpensive rubber and sustainable air cathode; (d) to achieve the final micro-sized MFC with syringe tips inserted for easy insertion of liquid.

### 6.2.4 MFC Operational Set-up

We compared this micro-sized MFC having a flexible architecture and graphene on nickel anode with a similar device with an enclosed plastic architecture and a graphene anode grown on nickel (Ni)/titanium (Ti)/silicon oxide (SiO$_2$)/silicon stack. Due to limitations with the plastic architecture, the graphene/Ni/Ti/SiO$_2$/Si had a larger volume of 75 µL with the same anode area of the graphene on nickel MFC of 25 mm$^2$. Both
devices, though, had an air cathode to ensure design variations were not too large as the cathode size and material can cause drastic changes in the device performance.\textsuperscript{18}

The MFCs were first inoculated with domestic waste water (local waste water treatment facility, primary clarifier) to introduce the bacteria to the system and allow a biofilm to grow on the anode. Then a waste water substitute with a defined concentration of organic material (acetate (1g/L)) was fed to the device and the cycle of peak current density was recorded. When the current density dropped to 0.01 mA/m\textsuperscript{2}, we re-fueled the device with acetate.

6.3 Results

6.3.1 Current Production

![Figure 6.4 Current production of 25 μL flexible MFC](image)

Production of current by graphene on nickel anode in a sustainably designed flexible MFC. Acetate was added at approximately 1, 300, 800, and 1350 minute marks as indicated by an immediate jump in current. Peak current densities correspond to ~1nW of power produced.
The graphene/Ni/Ti MFC consistently produced between 15-34 mA/m\(^2\) current density (per anode area) (Figure 6.4). This was a two-fold increase in peak current produced compared to the graphene on SiO\(_2\) MFC whose maximum current density was only 17 mA/m\(^2\) and three to seven fold increase with all other current cycles of the graphene/Ni/Ti/ SiO\(_2\)/Si device which were below 5 mA/m\(^2\).

### 6.3.2 Power Production and Power Requirements for Use

As improvements in research on ultra-low power bioelectronics, such as Lab-on-a-chip, are constantly being made, power requirements are decreasing and sub-microwatt devices are becoming a reality\(^{23}\). The peak current of the graphene/Ni/Ti anode corresponds to power production of 0.7 nW, already almost enough to be a power source for ultra-low power electronics.\(^{24,25}\) Maximum power densities per anode area were further explored by systematically changing the resistance ranging from 1,000,000 \(\Omega\) to 100 \(\Omega\) and recording the corresponding voltage produced. At every change in resistance, twenty minutes was waited before taking measurements to ensure the device was stable at this resistance. Maximum power densities for the graphene/Ni/Ti MFC are seen in the power density curve and reached greater than 1 mW/m\(^2\) with corresponding current densities at 10 mA/m\(^2\) (Figure 6.5). The power density curve for the graphene/Ni/Ti/SiO\(_2\)/Si MFC is also shown. Both current and power densities are 10 times lower for the graphene/Ni/TiSiO\(_2\)/Si MFC than for the graphene/Ni/Ti MFC.
6.3.3 Internal Resistances

The main reason the graphene/Ni/Ti MFC outperformed the graphene/Ni/Ti/SiO2/Si MFC is the lower internal resistance of this device and more direct contact to the graphene as well as closer distance from anode to the cathode. Internal resistance of the system, as estimated by the slope of the polarization curve, was high at approximately 19 kΩ for the graphene/Ni/Ti device and 37 kΩ for the graphene/Ni/Ti/SiO2/Si. Generally, micro-sized MFCs exhibit higher internal resistances than macro-sized versions\textsuperscript{5,6} but this indicates that the contact to graphene was not ideal, particularly for the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6_5.png}
\caption{Power density curve of 25 \textmu L flexible MFC}
\end{figure}

Power density curve indicating peak power densities above 1 mW/m\textsuperscript{2} and peak current densities at 20 mA/m\textsuperscript{2} for the graphene/Ni/Ti anode device and 10 times smaller values for the graphene/Ni/Ti/SiO2/Si device. Inset shows the zoom in portion of the graphene/Ni/Ti/SiO2/Si device’s performance.
Contact with the graphene was established in the graphene/Ni/TiSiO$_2$/Si MFC using a titanium wire (0.5 mm diameter) inserted into the device and bent to provide pressure on top of the graphene. When first assembled, contact resistance was measured at less than 5 $\Omega$ but variations in the liquid entering the device could have altered the pressure of the wire or its ability to effectively connect to the graphene. In the graphene/Ni/Ti MFC, a portion of the graphene was allotted to be used as the contact area to provide direct contact to the graphene anode. As the anode itself was thin, the contact area ripped after continual use which indicates that designs for long-term use of the device will require further attention paid to graphene contact engineering but that direct external contact, in this case, was most beneficial. Contact engineering plays a key role in the ability to create an efficient fuel cell and further work in the area of graphene contact engineering is needed to truly optimize a system utilizing graphene.

The other dimension affecting internal resistance is the distance between the anode and cathode. Protons produced by the bacteria at the anode need to travel through the device to the cathode. The longer the distance the protons need to travel the more internal resistance in the device.$^1$ The set-up of the graphene/Ni/Ti device leaves a distance of 1 mm (the thickness of the rubber anode chamber) while the distance between the anode and cathode in the graphene/Ni/Ti/SiO$_2$/Si device was approximately 3 mm (the thickness of the enclosed plastic).

We would also like to note that in the handling of the device over time, there was no visual change in the layers of the air cathode. In previous large-scale air cathode devices, the air cathodes are reused multiple times without diminishing results so we would expect similar outcomes in the micro-sized device. However, this is an area which needs careful
consideration and future experiments. It is also to be noted that differences in how the device is fed (including the pressure the liquid is inserted and how much goes through the device or stays in the anode chamber) attribute to potential variations in power produced. The small size of the micro-sized MFC magnifies some of these differences. Future experiments should include testing the flexible device using continuous flow to see what the maximum continued current can be as well as a more exact measurement of liquid entering the device from the syringe.

6.4 Conclusions

In conclusion, we have designed a more sustainable and flexible micro-sized microbial fuel cell and employed a two-dimensional atomic crystal structure material graphene on nickel anode to achieve \( \sim 1 \, \text{nW} \) of power using waste water and a waste water substitute. Although only in the beginning research stages, there is a lot of potential for micro-sized microbial fuel cell applications. Such a design can be used not only as a testing array to understand conditions for scaling up the technology but also integrated into a portable sensor or Lab-on-a-chip device.

6.5 Literature Cited


Chapter 7

Energy Harvesting from Saliva in Micro-Sized Microbial Fuel

Abstract

After showing that graphene could serve as an anode, ambient oxygen as electron acceptor in an air cathode micro-sized fuel cell all within the flexible inexpensive rubber architecture, the next step was to develop a device capable of high power with readily available fuel sources. Here, we show a pragmatic step in advancing MFC applications through the fabrication of a mobile and low cost micro-sized device that can be fueled with human saliva. The 25 μL MFC was made with two-dimensional atomic crystal structured anode material, graphene, on copper foil for efficient current generation and an air cathode to enable the use of oxygen in air, making its operation completely mobile and free of the need for laboratory chemicals. With saliva as a fuel, the device produced higher current densities (1190 A/m$^3$) than any previous air-cathode micro-sized MFCs. The use of the graphene anode generated 40 times more power than that possible with a carbon cloth anode. Additional tests using acetate, a conventional organic material, at high organic loadings comparable to those in saliva demonstrated a linear relationship between organic loading and current. These findings open the door to saliva-powered applications of this fuel cell technology for Lab-on-a-Chip devices or portable point-of-care diagnostic devices.
7.1 Introduction

Microbial Fuel Cells (MFCs) rely upon the decomposition of organic matter by bacteria which transfer electrons to the anode. Electrons are then delivered through an external load to the cathode to produce current\(^1,2\). While one of the most unique advantages of MFCs is that they can be used to both clean water and produce electrical power, the objective of the micro-sized MFC is to maximize power production for use as viable liquid fuelled miniaturized energy harvesters\(^3,4\). Since micro-sized MFCs utilize less electrode area as well as less volume of liquid fuel than macro-sized versions, optimizing the electrodes and the fuel sources are the most important factors in designing a micro-sized MFC for maximum power production. Therefore, electrode material selection is crucial and an ideal micro-sized MFC device needs to have: a high surface area-to-volume anode to enhance the collection of electrons from the bacteria; and an air cathode integrated into a single chamber MFC to lower the internal resistance of the entire system, and makes the device mobile as it does not require the use of unsustainable chemicals at the cathode. Another important aspect of an ideal micro-sized MFC is an ability to utilize an abundant and concentrated fuel source that can generate high power productions with minimal liquid volumes. We therefore developed a micro-sized MFC
with a carbon nanomaterial graphene anode and air cathode and powered it using human saliva (Figure 7.1).

**Figure 7.1 MFC process diagram**

MFC process diagram describing the main components of an MFC and reactions occurring within the device. Organic liquids are broken down by bacteria into protons, electrons, and carbon dioxide at the anode and oxygen from the air is utilized at the cathode.
7.2 Materials and Methods

7.2.1 Multilayer Graphene Anode

The materials and configurations of the anode and cathode were examined to meet the goal of increased power production using a 25 µL MFC. Electrodes require high conductivity, chemical stability, corrosion resistivity, and high mechanical strength. Since the anode must also support the growth of bacteria, it must additionally have good biocompatibility. Enhanced anodes have high surface-to-volume ratios in order to provide the most surface area as possible for bacteria and excellent electrical properties to enable efficient electron transfer.

Anode materials are usually carbon-based. Due to the exceptional electrical and structural properties of nano-engineered carbon materials, such as carbon nanotubes (CNTs) and most recently graphene oxides, studies have incorporated these materials into MFC anodes. The specific surface areas of CNTs and the two-dimensional atomic crystal structure material graphene are at least three orders higher than that of conventional carbon-based electrodes (<1 m²/g) and have intrinsic high electrical-conductivities (>10 S/cm), making them potentially ideal anode materials to enhance power densities. Most studies have integrated CNTs into existing anode materials, such as carbon cloth or textiles. In our previous work, we used multi-walled carbon nanotubes directly as an anode to produce high current density MFCs. Recently, graphene oxide has also been introduced as a blended anode material in carbon cloth and stainless steel. For the first time in any type of MFC, we show here that anodes can...
be composed of pure multi-layer graphene, not a graphene oxide hybrid. To make these anodes, graphene was grown using atmospheric pressure chemical vapor deposition (APCVD) on top of a copper foil. Multi-layer graphene was confirmed by Raman spectra as seen in Supplementary Information Figure A4.1 in Appendix 4 where detailed graphene growth parameters are also found.

### 7.2.2 Air Cathode

Micro-sized MFCs are often constrained by their reliance on a two-chamber configuration, where the cathode and anode are separated by a membrane and the cathode chamber must be refilled with fresh electron acceptors. Ferricyanide is almost exclusively used in micro-sized MFCs as an electron acceptor since it can generally produce higher power densities than more readily available electron acceptors such as oxygen, as ferricyanide maintains the cathode potential closer to the theoretical value\(^5\). Ferricyanide must be continuously refilled, though, making it an unsustainable design feature for use outside of the laboratory\(^1,5\). Thus, the use of oxygen in air has been explored as an electron acceptor, as it is readily available and does not need to be regenerated. Air cathodes, which use ambient oxygen as the electron acceptor, are commonly used in large-scale MFCs with a hydrophobic carbon/polytetrafluoroethylene (PTFE) layer on the air side of the cathode to prevent water leakage and to control oxygen diffusion, and thin platinum catalyst on the water side\(^23\). However, air cathodes have only just recently been introduced into micro-sized MFCs with proton exchange membranes (PEM) that are hot pressed onto a carbon cloth cathode\(^6,7\). Membranes increase cost, and increase internal
resistance of a device which lowers overall power production\textsuperscript{5,23}. Therefore, we designed a one chamber membrane-less micro-sized MFC with an air cathode which makes the device completely mobile as it does not require any chemicals but the oxygen in the air.

\section*{7.2.3 MFC Assembly and Operations}

The graphene foil was then cut to a 1 cm by 1 cm square. The spacer used between the cathode and the anode was 1 cm by 1 cm rubber with a thickness of 1 mm and a 5 mm by 5 mm square hole cut in the center which served as the anode chamber. Syringe tips were inserted into the rubber on either side to serve as the inlet and outlet for feeding or inoculation of the anode. The graphene foil anode was glued to one side of the rubber spacer and the 1 cm by 1 cm cathode was glued to the other side. They were adjusted so as to leave one side of the spacer where the anode and cathode did not overlap so the contact could be made with the anode. Another rubber piece was glued to the bottom of the anode as a support for the thin foil anode. Space was kept on the contact side so that an alligator clip could directly clip onto the spacer-anode to contact to the graphene on the anode. A second clip was attached to the air cathode to provide contact to the cathode. Both clips were attached to a 1000 $\Omega$ resistor then to a multimeter for voltage recordings every 20 minutes. The same set up procedure was also done as a control substituting a carbon cloth anode for the graphene foil. The devices were inoculated with waste water from the local waste water treatment plant primary clarifier and after 2 insertions of inoculation were switched to the fuels being tested which will be described below.
The micro-sized MFC system created here has a bottom support and liquid chamber that are both composed of low-cost flexible rubber making the MFC bendable and inexpensive (Figure 7.2). The flexible rubber structure can be attached to a variety of surfaces and can be easily produced without difficulty making, the whole device easily deployable.

Figure 7.2 Schematic and photographs of the flexible MFC with graphene on copper anode, air cathode, and syringe tips for easy insertion of fuel.
7.2.4 Use of Saliva as Fuel

We utilized this graphene anode/air cathode micro-sized MFC to test whether human saliva could be a viable fuel source for powering such a device. Although predominantly water, saliva is composed of inorganic and organic components, such as glucose, that can be utilized for fuel by bacteria\textsuperscript{24}. Saliva was collected from the same individual (a healthy 16 year old) at the same time every day and without eating or drinking for at least an hour beforehand as described in typically used salvia collection protocols\textsuperscript{24}. Enough saliva was gathered to run a Chemical Oxygen Demand (COD) analysis for each sample with organic loadings between 13,400- 15,500 mg/L, conductivity 2.2-2.8 mS/cm, and pH of 7. Bacteria were first inoculated into the device using wastewater (primary clarifier from local wastewater treatment plant) before saliva samples were inserted (same saliva for each device).

7.3 Results

7.3.1 Current Generation using Saliva

As can be seen from the current cycles over time in Figure 7.3a, with introduction of saliva, current cycles for the graphene anode first peaked at 130 A/m\textsuperscript{3} and then increased to 1190 A/m\textsuperscript{3} with subsequent fuelling cycles. At a current density of 1190 A/m\textsuperscript{3}, the graphene anode MFC produced higher current densities than any other previous air cathode micro-sized MFCs as seen in Table 7.1.
We also tested the performance of saliva with a commonly used MFC anode, carbon cloth, in an otherwise identical device. The maximum peak for the carbon cloth device...
was 770 A/m³. Therefore, with only saliva as fuel, the graphene anode outperformed the carbon cloth by 55%. From the power density curve (Figure 7.3b), graphene generated higher power (60×) and current densities (40×) than that obtained with carbon cloth, confirming the proof of concept that saliva could power micro-sized MFC devices as well as enhance performance of the graphene anode.

One of the reasons graphene outperformed carbon cloth is that graphene has been found to increase the electron transfer kinetics (EET) of the anode and enhance the catalytic activity of oxidation, increasing the power produced by graphene enriched MFC anodes25. Specifically, cyclic voltammogram (CV) plots and electrical impedance spectroscopy (EIS) were used to characterize a graphene biofilm to show that the graphene improved the kinetics of electron transfer between cell surface proteins and the electrode, leading to an overall improvement in EET21,25.

### Table 7.1 Table of Air Cathode micro-sized MFCs

<table>
<thead>
<tr>
<th>Anode chamber volume (µL)</th>
<th>Membrane</th>
<th>Inoculum</th>
<th>Anode (Area cm²)</th>
<th>Substrate</th>
<th>Catholyte</th>
<th>Pmax reported W/m³ (mW/m² by anode)</th>
<th>Imax at Pmax A/m² (aA/m² by anode)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>650</td>
<td>Proton Exchange membrane (Nafton 117)</td>
<td><em>Shewanella</em> sp.Hac353</td>
<td>Gold (0.385)</td>
<td>Triptic soy broth</td>
<td>Air</td>
<td>0.17 (26.9)</td>
<td>0.38 (6)</td>
<td>Hou et al. 2011⁹</td>
</tr>
<tr>
<td>25</td>
<td>Proton Exchange membrane (Nafton 117)</td>
<td><em>Shewanella oneidensis</em> MR-1</td>
<td>Gold (0.126)</td>
<td>Lactate</td>
<td>Air</td>
<td>15 (29)</td>
<td>32 (65)</td>
<td>1080 (2148) @1000Ω</td>
</tr>
<tr>
<td>25</td>
<td>None</td>
<td><em>Mixed Culture</em></td>
<td>Graphene (0.25)</td>
<td>Saliva</td>
<td>Air</td>
<td>6 (6)</td>
<td>54 (54)</td>
<td>1190 (1190) @1000Ω</td>
</tr>
<tr>
<td>25</td>
<td>None</td>
<td><em>Mixed Culture</em></td>
<td>Graphene (0.25)</td>
<td>Acetate (10 g/L)</td>
<td>Air</td>
<td>8 (8)</td>
<td>49 (49)</td>
<td>488 (488) @1000Ω</td>
</tr>
</tbody>
</table>
7.3.2 Energy Losses

An analysis of energy losses was performed to determine the specific contributions of the various components of the device to the total internal resistance of the system. The overall energy loss in an MFC system can be estimated using:

$$V_{device} = OCV - IR_{internal} = OCV - I(R_a + R_c + R_m + R_e)$$

where the voltage of the device is equal to the difference between the open circuit voltage ($OCV$) and maximum current ($I$) times the internal resistance ($R_{internal}$) which is composed of resistances from the anode ($R_a$), cathode ($R_c$), membrane ($R_m$), and electrolyte used ($R_e$). The entire energy loss of the graphene system was approximately 110 mV (OCV 220 mV – 90 mV at maximum power) and 53 mV (OCV 119 mV – 66 mV at maximum power) for the carbon cloth. As we do not have a membrane in our system, an entire source of resistance is eliminated ($R_m$). The electrolyte or solution resistance can be estimated using $R_e = d/(AK)$, where $d$ is the electrode distance (cm), $A$ is the geometric area available for ionic species to pass (cm$^2$), and $K$ is the specific conductivity ($\Omega^{-1} \text{ cm}^{-1}$) of the solution. The distance between anode and cathode is approximately 0.1 cm over an area of 5 mm by 5 mm in a saliva solution, with conductivity of approximately 0.0022 ($\Omega^{-1} \text{ cm}^{-1}$). The electrolyte resistance is nearly 180 $\Omega$ and when multiplied by the current at the maximum power, 1.4 $\mu$A, results in a loss of approximately 0.252 mV.

By calculating the energy losses in the graphene and carbon cloth systems, we can better understand the maximum potential energy output under these conditions as well as the probable causes of energy losses in these systems. The components of energy loss
include calculated resistances for the anode, cathode, membrane, and fuel source. Membrane resistances are eliminated in our one chamber system, and saliva resistances were minimal leaving electrodes as the major source of energy losses in the devices. As the cathodes were the same for both the graphene and carbon cloth devices, the major contributor to the difference in energy losses between the systems was the anode. Although the graphene anode device had a higher open circuit voltage (OCV), and therefore higher potential maximum power production (200 mV for graphene, 119 mV for carbon cloth), the energy loss in the system was 50% compared to only 44% for carbon cloth. We estimate that the larger portion of the difference in energy loss is from contact engineering issues which are natural in a low density of state material like graphene. There are additional contact challenges associated with difficulty in depositing electrodes directly onto graphene because of the low interaction energy between the metal and carbon atoms\textsuperscript{26}.

7.3.3 Comparison of Graphene with a Copper Control

In order to confirm that graphene was acting as an MFC anode and it was not, instead, relying on the copper foil alone as an anode or as a wholly galvanic cell\textsuperscript{27}, we fabricated two identical micro-sized MFCs and inserted copper foil with and without graphene as anodes. Both devices were run under the same conditions with the graphene anode outperforming the copper control by more than 260% in power density and 160% in current density (See Supplementary Information Figure A4.2 in Appendix 4). As both anodes were made with the same copper foil, both would have experienced similar
amounts of additional current from galvanic reactions which does not diminish the
difference in power and current values seen between the graphene anode and the copper
control but may exhibit slightly higher values in maximum power produced\(^5\). The
improved performance of the graphene device supported our assertion that graphene was
acting as an MFC anode.

### 7.3.4 Other Fuels at High Organic Loadings

After establishing that saliva could be used to power micro-sized MFCs, we tested
whether micro-sized MFCs could be operated with a more conventional organic fuel at
similarly high organic concentrations. Other studies have found that power densities
increase with higher organic contents but only at substrate concentrations up to 2 g/L
(mass of the organic material)\(^12,28\). We therefore set up two more sets of identical
graphene anode devices to test under high acetate loadings (20 g/L and 10 g/L measured
as mass of sodium acetate and confirmed by concentrations of organic matter based on
chemical oxygen demand, COD). We inoculated the devices with the same wastewater
source as the saliva devices and then began feeding with the new acetate loadings as seen
in Figure 7.4a. As expected, the device with 20 g/L outperformed the one fueled with 10
g/L, with peaks at 490 A/m\(^3\) and 300 A/m\(^3\), respectively.
Figure 7.4 Current generation and power density curves using high concentrations of acetate as fuel
(a) Current production following startup with saliva using graphene or carbon cloth anodes, where each peak in current reflects the refueling with saliva. (The initial first two cycles of bacterial inoculation are not shown.) The maximum peak for the graphene anode was 55% higher than that obtained with carbon cloth. (b) Power density, demonstrating graphene outperformed carbon cloth, with 60x more power and 40x higher in terms of maximum current density. The inset shows the peak for carbon cloth at less than 0.1 W/m$^3$. 
Power density curves were also measured (8 W/m$^3$ for 10 g/L) and compared to saliva (6 W/m$^3$) (Figure 7.4b). The micro-sized MFC that was fueled with 20 g/L failed after 10 days due to the clogging by powder from dried acetate that clogged the feeding syringes of the device. These results showed that high concentrations of non-conventional (saliva) and conventional (acetate) could serve as fuel sources in micro-sized MFCs, but further studies should examine other organic sources as well as look at potential methods to avoid reactor clogging.

7.3.5 Applications of a Saliva-Powered Device

In considering the use of saliva, and the well known positive correlations between solution conductivity and improved power generation in MFCs, a possible application of the micro-sized MFC is an ovulation predictor based on the conductivity of a woman’s saliva. It has been found that five days before ovulation there is sharp decrease in conductivity in saliva most likely caused by a peak in estrogen and subsequent decrease in electrolyte concentration in saliva$^{29}$. The MFC could be used to measure this change in conductivity to identify the fertility period of a woman, while at the same time use the power generated to send the data to a readily available device, such as a smart phone. This application could thus help to maintain a woman’s health as well as help couples for better family planning in a non-invasive easy to use method.
7.4 Conclusions

Producing nearly 1 µW, this saliva powered micro-sized MFC already generates enough power to directly be used as an energy harvester in microelectronic applications. With the emergence of the field of ultra-low power chip-level biomedical electronics, devices able to operate at sub-microwatt power outputs are becoming a reality\(^8,\)\(^30\). For example, an electroencephalograph (EEG) seizure detection system-on-chip integrating a novel circuit architecture was demonstrated requiring less than 1 µW power\(^31\) making our production of 0.9 µW a competitive device for energy harvesting for such applications. Micro-sized MFCs are an interesting and practical energy harvesting option for health and environmental monitoring system-on-chips. An advantage of the MFC as an energy harvester is the ability to utilize abundant liquid fuel sources complementing other widely known energy harvesters like photovoltaic, thermoelectric and piezoelectric that employ a different set of fuel resources. This study is a first step in utilizing saliva and other highly concentrated organic fuels to power bio-electronics and holds great potential for further applications in this field.

7.5 Literature Cited


8.1 Summary of key findings

The micro-sized Microbial Fuel Cell has been described here using four themes central to the development of making a high power, yet sustainable and practical device. The micro-sized MFC can be tailored to fit the requirements of the intended application of the technology and there is no “perfect” micro-sized MFC that fits all applications. I will first summarize the key findings within the four themes as well as some possible applications of the micro-sized MFC describing design features that would be most suited.

8.1.1 Summary of the four themes

Anode- The use of nano-engineered carbon nanomaterials, carbon nanotubes and graphene, can enhance the performance of a micro-sized MFC, particularly in current. The carbon nanotube anode produced higher peak current (880 mA/m² in Chapter 3) compared to any of the graphene devices using the same fuel. This is likely due to the higher surface area-to-volume of the CNTs which enabled a 3D interface to increase bacteria adhesion while the 2D graphene was less conducive for as much bacterial adhesion. Anode contact materials were also found to enhance current production with a variety of ohmic silicides able to improve the peak current of the device compared to Schottky non-silicide materials. The low resistivity of NiSi makes it superior but the
usage of annealed aluminum or TiSi$_2$ were also found to produce high currents as well as long as aluminum spiking is not a concern.

**Cathode**- The introduction of a membrane-less air cathode to micro-sized MFCs was a large advancement to the field as it truly allows micro-sized MFCs to be powered outside of the laboratory. By eliminating the need for continuous chemical refills and making the entire device mobile, the inclusion of air cathodes to micro-sized MFCs is advantageous for most applications of the device.

**Reactor design**- Reactor designs can be varied to accommodate for the materials being integrated into the device as well as the intended application. The four different reactor designs (1-75 µLs) tested here added to the field of micro-sized MFCs by increasing sustainability with the introduction of a one-chamber air cathode, improving cost-effectiveness by utilizing inexpensive materials like rubber and eliminating costly membranes, making easy to use, durable structures.

**Fuels**- In an effort to push the micro-sized MFC out of solely laboratory based experiments and proof-of-concept, we sought to utilize real-world fuels to power the MFC. With the successful introduction of saliva as a power source, we showed that micro-sized MFCs can be powered with easily accessible fuels which opens up numerous possible applications of the device.

### 8.1.2 Possible applications and their design features

**Testing array for scaling up**- For micro-sized MFCs intended to be used as testing arrays for scaling up of the materials and conditions, it is logical to use the same
materials as in the scale-up. Anodes on silicon chip are therefore, not required but microfabrication can still be utilized in the manufacturing of multiple devices which are useful in testing numerous conditions at the same time. The exploration of inexpensive anode and architectural materials, like bulk graphene and rubber, should continue in micro-sized MFCs. The use of an air cathode is necessary as larger scale MFCs could not economically use the quantities of ferricyanide required to operate. The testing of larger varieties of fuels should be done in the micro-scale as proof of concept for the larger scale and to test the best materials to handle certain fuels.

**On-chip power generators**- Micro-sized MFCs intended for use as miniature power generators on a micro-chip where high power and current are the most important features, should be explored using silicon as a base. Nano-engineered anodes should also be utilized as they can increase the amount of power produced by area and volume of liquid which is important since the physical size of the MFC device limits the amount of other features that can be included on the silicon chip. The utilization of anodes with high surface-area-to-volume ratios, like MWCNTs, should be included as well as optimal contact area materials and locations which are extremely important given the need for the highest current as possible. The best contact area found here was nickel silicide but aluminum or titanium silicide could also be used for increase current if they fit better into the fabrications process flow. Perhaps easy to refill packages of ferricyanide or other high power electron acceptors can be explored but, the constant need to refill makes applications in which the use of anything other than ambient oxygen difficult. Therefore, further exploration of using air cathodes in higher power producing architectures would be fruitful. As high power and current are most important in these applications, cost is not
as large of an issue so the introduction of better catalysts on the air cathode than platinum could be looked into, especially the possibility of air cathodes that can be fabricated on silicon making the entire device easily fabricated and integrated onto a chip.

**Self-sustained remote power sources** - Micro-sized MFCs for use in point-of-care devices or self-sustained power sources in remote locations may not need the maximum power production but would definitely require the integration of an air cathode so that the device is mobile and without need of laboratory chemical refills. The exploration of an entire MFC composed of inexpensive materials for point-of-care devices that could be disposable would be an interesting area to research. If the MFC is not designed to be disposable, the endurance and durability of all materials is an important consideration.

### 8.2 Comparison to other Micro-scale Renewable Energy Devices

The highest power produced by the micro-sized MFCs studied here by volume was the 1.25 µl two-chamber MFC with carbon nanotube anode at 392 W/m³ and 1.96 µW/cm² by anode area. By area, though, the saliva powered flexible one chamber MFC with an air cathode produced 3.55 µW/cm² and 35.2 W/m³. The power output of micro-sized MFCs, though, is much lower than other micro-scale renewable energy devices (Table 8.1) making their optimization a top priority in order to better compete with other energy sources. MFCs, though, do have many unique features in their favor making their use in niche applications highly likely. MFCs are self-sustained devices utilizing bacteria to decompose waste materials whose energy would otherwise be lost. The entire process is non-toxic and can be completed at near room temperatures and at neutral acidity.
Table 8.1 Comparison of power output by micro-scale renewable energy devices.

<table>
<thead>
<tr>
<th>Micro-scale Renewable Energy Devices</th>
<th>Energy Source</th>
<th>Power by area or density</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photovoltaic</td>
<td>Photons/light</td>
<td>35 mW/cm²</td>
<td>Cook-Chennault et al.¹</td>
</tr>
<tr>
<td>Thermovoltaic</td>
<td>Heat</td>
<td>2.2 W/cm²</td>
<td>Cook-Chennault et al.¹</td>
</tr>
<tr>
<td>Piezoelectric</td>
<td>Vibration</td>
<td>5.5 W/cm³</td>
<td>Cook-Chennault et al.¹</td>
</tr>
<tr>
<td>Electromagnetic</td>
<td>Vibration</td>
<td>8.24 mW/cm³</td>
<td>Cook-Chennault et al.¹</td>
</tr>
<tr>
<td>Methanol Fuel Cell</td>
<td>Methanol</td>
<td>100 mW/cm²</td>
<td>Cook-Chennault et al.¹</td>
</tr>
<tr>
<td>Microbial Fuel Cells (in series, using Ferricyanide)</td>
<td>Bacteria</td>
<td>33 µW/cm²</td>
<td>Choi et al.²</td>
</tr>
<tr>
<td>Microbial Fuel Cells (single unit, sustainable air cathode)</td>
<td>Bacteria</td>
<td>3.6 µW/cm²</td>
<td>Mink et al. (our saliva powered MFC in Chapter 6)</td>
</tr>
</tbody>
</table>

The MFC by Choi et al.² is set-up as multiple MFCs in series in order to maximize power output, one of a few key future directions of micro-sized MFCs that will be discussed in the outlook below.

8.3 Outlook and Future Work

Future work in micro-sized MFCs should center upon making high power devices that can be used in applications and should therefore be concentrated in three areas:
1. The continued development of reactors that produce higher power outputs.
2. The usage and testing of more application specific fuels.
3. The integration of other features such as energy storage components and circuitry for applications onto an energy chip.

We have started exploring some of this work in preliminary studies on the usage of micro-sized MFCs in series and the ability of the micro-sized MFC to test new waste waters in the yogurt industry which is currently causing disposal challenges in the United States.

In an effort to further produce higher power micro-sized MFCs, future work needs to be done on MFCs in series. Single MFC units can be connected in series to increase power output. In larger scale MFCs, MFCs stacked in series have been demonstrated that increase both power and working voltage\(^3\). There is only one study on a stacked series of micro-sized MFCs that produced 100 µW in series, an increase in power output by a factor of 10 compared to a single MFC of the same set-up\(^2\). This micro-sized MFC was constructed on glass and using ferricyanide as electron acceptor that must be continuously refilled.

Therefore, future work in optimizing micro-sized MFCs in series on silicon using a more sustainable air cathode is required to truly increase the usability of MFC technology as a power generator at the micro-scale. We have fabricated an in-series MFC connecting three, six, and nine micro-sized MFCs with each single MFC having a volume of 12.25 µl. As an initial test, we utilized gold as anodes (3.5 mm × 3.5 mm) for simplicity but can
insert nanomaterials in future designs. The structural schematic for the nine MFC series is seen in Figure 8.1.

Figure 8.1 Schematic of micro-sized MFCs in series
a) Schematic of nine micro-sized MFCs set up in series showing the gold anode and smaller gold squares for gold anode contact area on the bottom layer with a plastic gasket etched as seen in (b) and then a carbon cloth air cathode on top.

We have assembled the first device (three MFC series) as seen in the photograph in Figure 8.2 and the basic flow diagram in Figure 8.3 and plan to test using commercial waste products, such as liquid yogurt whey.
Liquid yogurt whey is produced in the Greek yogurt industry as a runny, acidic waste product after straining regular yogurt in the manufacturing of the thicker, Greek yogurt\textsuperscript{5,6}. The yogurt industry in the United States has tripled in size over the last five years with companies in New York state alone producing more than 150 million gallons of liquid whey in 2012\textsuperscript{6}. The safe disposal of liquid whey is a huge concern for the yogurt industry.
as it cannot be dumped into rivers and streams due to its toxicity to the natural environment as it depletes oxygen required for wildlife to survive.

As it is composed mostly of lactose, minerals and proteins we predict that liquid whey and other food processing liquids can be used in microbial fuel cells. Miniature MFCs can be utilized to test the efficiency of these new fuels to produce power and the devices can be scaled up with optimal materials into larger MFC devices.

Further work on the optimization of MFCs in series should be done in future micro-sized MFC studies as well as more work with usable, readily available fuels. Next steps should also be taken in integrating the in-series or high power micro-sized MFCs with energy storage options, such as miniature lithium batteries, and application-required circuitry to create a self-sustaining energy chip.

In conclusion, the micro-sized microbial fuel cell is a unique device that utilizes waste to create energy. The micro-sized MFC can be used as both a testing bed for new materials and conditions to scale up and forward the progress of larger scale MFC technology or as a self-sustaining power generator for applications in Lab-on-Chip, sensors, or energy source in remote locations. The continued optimization of this technology promises a range of innovative and commercial devices.

8.4 Literature Cited


A1.1 Fabrication

The anode chamber and contact area were constructed on a 4” <100> P-type highly doped silicon wafer. First, we formed a nickel silicide contact path as a low resistance contact area for the ohmic contact from the anode to the external load. A layer of Ni was then evaporated and patterned using a lift-off process (Figure A1.1a). The wafer was annealed to a temperature of 450°C for 30 seconds, forming a silicide with a low sheet resistance 6.8 $\Omega/\square$ (Figure A1.1b). Next, by using deep reactive ion etch (DRIE) process...
the 50 µm deep anode chamber was etched into the silicon over a 5 mm × 5 mm area (Figure A1.1c). In order to prepare for CNT growth, first a Cr/Ni (200/65 nm) catalyst layer was sputtered and patterned using a lift-off process to be only inside the etched anode chamber (Figure A1.1d). Afterwards, the CNTs were grown at a temperature between 650°C to 700°C using a plasma enhanced chemical vapor deposition (PECVD) system,, with acetylene (C₂H₂) and ammonia (NH₃) as carbon source gases. By controlling the time, CNTs were grown to heights of 30 to 40 µm. The CNTs were functionalized in H₂SO₄ for 2 hours (Figure A1.1e).

The cathode chamber was formed on a separate 4” Si wafer. SU8 photoresist was first patterned forming a mold which was then replicated onto the PDMS by pouring a mixture of elastomer (Sylgard 184) and curing agent (10:1), and curing at room temperature for approximately 24 hours. The PDMS stamp was then peeled off and cut to the required size. The PEM (1cm × 1cm) was pretreated with boiling H₂O₂: H₂O (35% w/w) for 1 hour, boiling water for 2 hours, boiling H₂SO₄: H₂O (0.5 M) for 1 hour, and boiling water for 2 hours.

**A1.2 Experimental and Operational Setup**

Acetate media (1g/L) was fed to the anode chamber using two 500 µm diameter tubes placed on opposite sides of the anode chamber, on top of the silicon wafer, with one tube used for feeding the anode manually with a syringe and the second as outlet. The membrane (Nafion 117) was placed on top of the anode chamber and glued (Al Fares Silicone G1200) for an anaerobic sealing. A 5 mm × 5 mm carbon cloth cathode (Fuel Cell Earth) was placed on top of the membrane with a string of carbon cloth protruding to
be used as cathode contact. Tubes for the ferricyanide inlet and outlet were passed through the PDMS stamp horizontally to reach the cathode chamber. Ferricyanide was pumped (Fresenius Kabi Injectomat 50 mL syringe pump) continuously through the system at 0.1 mL/minute (Figure A1.2). The PDMS was then stacked on top of the carbon cloth so that tubes protruded from all 4 sides of the device, providing the inlets and outlets for the electrode chamber solutions. The MFC was held together by two plexiglass pieces clamped on either side to reduce oxygen transfer into the assembly. A 100 Ω resistor load was used in the circuit, with the copper wires attached to the silicided contact of the anode and carbon cloth cathode.

Figure A1.2 Digital photograph of the experimental setup.
A1.3 Polarization plot comparing carbon nanotube anode and carbon cloth anode

Figure A1.3 Polarization plot comparing MWCNT and carbon cloth (CC) anode
Appendix 2

Sustainable Design of High Performance Micro-sized Microbial Fuel Cell with Carbon Nanotube Anode and Air Cathode: Supplementary Information

A2.1 Polarization plot for carbon nanotube anode with air cathode

Figure A2.1 Polarization plot of carbon nanotube anode with air cathode
A3.1 Current production of Ohmic and Schottky cobalt contact areas

Figure A3.1 Current over time for cobalt Ohmic and Schottky devices
A3.2 Polarization plot of titanium

Unfortunately, in the fabrication of the Ti Ohmic anodes, the corner required for contact was not fully deposited with Ti as shown in (B). The device was able to be contacted during the current vs time measurements but by the time we did the polarizations, there was a small leak and acetate buildup on the bottom of the device not allowing a good contact to the small portion of Ti that was available to us. Therefore, we only have the polarization curve for the Ti Schottky which you see in (A) and not the Ti Ohmic. As this is the first time a bottom fed micro-sized MFC has been utilized, there are many lessons that can be implemented in how to make an improved version for future experiments.
Appendix 4

Role of metal/silicon semiconductor contact engineering for enhanced output current in micro-sized microbial fuel cells: Supplementary Information

A4.1 Detailed Graphene Growth Parameters

The MFC anode was made using multi-layer graphene synthesized by the decomposition of methane over copper using atmospheric pressure chemical vapor deposition (APCVD). First, 25 µm thick copper foils (99.8%, Alfa Aesar) were loaded into a 4 inch quartz tube inside the horizontal furnace. The system was then purged with nitrogen for 5 minutes to remove any impurities inside the tube. Then, the system was ramped to 1000°C in 30 minutes while flowing argon and hydrogen at 200 standard cubic centimeters per minute (sccm) and 500 sccm, respectively, for initial Cu cleaning. The sample was then kept under 500 sccm of hydrogen for 4 minutes in order to remove oxygen and stabilize the surface of the Cu foils. Methane gas at a flow rate of 50 sccm was introduced into the tube for 1 minute. Thereafter, the growth was terminated and the furnace was cooled to room temperature under the flow of nitrogen.

Raman spectroscopy (532 nm laser wavelength) was used to evaluate the synthesized multi-layer graphene by the aforementioned process. Graphene was confirmed by optical image and the corresponding Raman spectrum (Supplementary Figure A4.1).
Figure A4.1 Optical image and Raman spectrum of graphene
(a) Optical image of the sample. (b) Raman spectrum of the grown multi-layer graphene on of copper foils. In the Raman spectrum the D band is centered at 1355 cm\(^{-1}\), indicating low disorder induced-defect. The G and 2D bands are positioned at 1389 cm\(^{-1}\) and 2704 cm\(^{-1}\) respectively. The Raman spectrum revealed an intensity ratio of 0.4 and asymmetric 2D peak with full width at half maximum (FWHM) of 70 cm\(^{-1}\), confirming the presence of multi-layer graphene.
A4.2 Graphene on copper compared to copper control

The graphene and copper control devices are from a previous experimental set up where they were first inoculated with bacterial effluent from a previously working mixed culture MFC operating at 1000Ω to begin biofilm growth then acetate was introduced. Power density curves of both revealed that the graphene anode outperformed the copper control by more than 260% in power density and 160% in current density. As both anodes were made with the same copper foil, both would have experienced similar amounts of additional current which does not diminish the difference in power and current values seen between the graphene copper anode and the copper control but may exhibit slightly higher values in maximum power produced. As these devices were set up with bacteria acclimated to 1000Ω, they can not be directly compared with the devices from the main experiment which were inoculated with waste water.