ALGAL BIOFILMS, MICROBIAL FUEL CELLS, AND IMPLEMENTATION OF STATE-OF-THE ART RESEARCH INTO CHEMICAL AND BIOLOGICAL ENGINEERING LABORATORIES

by

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ABSTRACT

Alternative energy technologies become more attractive as the price of energy from fossil fuels becomes more expensive and the environmental concerns from their use mount. While a number of biological alternative energy technologies currently exist, a complete understanding of these technologies has yet to be developed. This dissertation characterizes an aspect of biological alternative energy technologies: the production of algal biofuels and energy conversion in microbial fuel cells. Specifically, this dissertation addresses the characterization of microalgae as a biofilm and the characterization of the power limitations of microbial fuel cells.

The attachment and detachment of algae were observed using temporal microscopic imaging in a flow-cell with autofluorescence and staining techniques as part of a collaborative Montana State University and Idaho National Laboratory project. Colonies of algae exhibit many characteristics seen in bacterial biofilms: adherence; detachment and sloughing; difference in structure of an attached colony; varying strength of attachment on different surfaces; association of other organisms in an EPS matrix; and the heterogeneous nature of attached colonies.

The characterization of a microbial fuel cell was completed in less than 30 minutes using an empirical procedure to predict the maximum sustainable power that can be generated by a microbial fuel cell over a short period of time. In this procedure, the external resistance was changed incrementally, in steps of 500 Ω every 60 seconds, and the anode potential, the cathode potential, and the cell current were measured. This procedure highlights the inherent limitations of energy conversion in a microbial fuel cell. A voltage/current characterization of the microbial fuel was also completed from the data collected.

This dissertation also includes the evaluation of *A Hands-On Introduction to Microbial Fuel Cells*, a laboratory developed for an introductory chemical and biological engineering course. The experiment has been updated to include a voltage/current characterization of the microbial fuel cell. Learning objectives have been identified and pre- and post-laboratory activities have been developed for further implementation into a chemical and biological engineering curriculum.

INTRODUCTION

Biological Energy Conversion

Energy conversion facilitated by biological sources has traditionally been limited by the significant expense as compared to traditional fossil fuel sources. As these fossil fuels become scarce and energy becomes more expensive, alternative energy technologies become more attractive. While a number of biological alternative energy technologies currently exist, a complete understanding of these technologies has yet to be developed. They are limited by the relatively few optimization processes that have been employed thus far. Many of these technologies are relatively new and unproven at full-scale production (Dresselhaus and Thomas 2001).

Moreover, the conversion of energy from biological sources is complex in that one not only needs to collect and process the energy source, but also needs to cultivate the organism and optimize the biological system for the maximum energy conversion possible (Schenk, et al. 2008). This is the case both in the production of biofuels from microalgae or the production of electricity in a microbial fuel cell (MFC).

The following sections of the introduction summarize the dissertation, which has two major sections. The first two sections outline experimental work on algal biofilm formation and characterization of microbial fuel cells, and the final two sections describe the restructuring of a freshman level Introduction to Chemical and Biological engineering course and the implementation of a microbial experiment into this course.

Algal Biofilms

Chapter 2 examines *Botryococcus braunii* cell colony clusters as the basic structure of a biofilm, not as an addition to a bacterial biofilm. *B. braunii* is a green microalga which generally exists as a colony of individual cells supported by a colonial matrix. *B. braunii* generally forms a colony unit by forming adhering "cups" and the units vary in size from a few cells to thousands (Largeau, et al. 1980). A colony usually consists of densely-packed conical cells radiating and branching from the center of a roughly spherical unit. The increasing cost of traditional petroleum based fuels and the worldwide interest in renewable energy sources has recently pushed the production of algal biofuels into the forefront of biological energy conversion processes. Algal biofuels, unlike some other biofuels, do not require current food sources for feedstock for production. *B. braunii* is noted for the production of hydrocarbons that are readily crackable into gasoline fractions (Hillen, et al. 1982); hydrocarbons are produced at a high yield relative to the mass of the microalga (Casadevall, et al. 1985). It should be noted that previous studies have indicated that hydrocarbon synthesis is not affected by cell immobilization (Banerjee, et al. 2002). The hydrocarbons are generally located in a cellular structure between an inner lamellar membrane and an outer membrane, but hydrocarbons are also found in the extracellular region which also contains extracellular polysaccharide in a trimellar region between cells (Largeau, et al. 1980). These hydrocarbons, generally with 30+ carbon atoms per molecule and termed "botryococcenes", provide the colony with buoyancy (Metzger, et al. 1985) and may help to take the algal cells to the air-water interface in an aqueous environment so the cells

preferentially are able to absorb more light from the sun. The movement of botryococcene out of the colony, shown in Figure 5, is consistent with hydrocarbon formation between the cells. For many years, *B. braunii* has been examined at as a commercial biofuels producing microalgae due to high hydrocarbon yields and carbon dioxide biofixation (Akin, et al. 1993). The ease of removal of botryococcenes from the colony, while maintaining cell viability, may have commercial potential. The findings in this chapter help characterize these algal communities in a new way so that further exploration might lead to a change in their carbon sequestration capacity and/or biofuels production ability.

It is important to note that the laboratory research in Chapter 2 was completed by multiple researchers. Specifically, any research relating to the surface attachment of microalgae was performed by Narendren Jayawickramarajah. These results, included here for context, were also included in Mr. Jayawickramarajah's thesis as well as in *Enhanced Conversion of CO_2 by Structured Microorganisms*, a technical report written by research collaborators at Montana State University-Bozeman and the Idaho National Laboratory. The information in Chapter 2 was, in part, researched, collected, assembled, and often written by the author as part of this collaborative MSU/INL effort made up of Greg Bala, Pete Pryfogle, Dr. John Sears, Narendren Jayawickramarajah and myself. It is not the author's claim that he is solely responsible for these works, but that he was an active part of this research process and that the authorship of this chapter was, in part, his.

Power Measurements in Microbial Fuel Cells

Chapter 3 presents "Procedure for Determining Maximum Sustainable Power Generated by Microbial Fuel Cells". When this paper was published in Environmental Science and Technology (ES&T), it was intended to offer a standardized method for microbial fuel cell power generation that was simple and would provide a realistic view as to the potential uses of microbial fuel cells outside of the laboratory. Although the method for the determination of the maximum sustainable power has not been widely adapted in the microbial fuel cell community, the message it presented was well received. The microbial fuel cell community recognized that the power potential of microbial fuel cells is limited and that the ultimate value of a microbial fuel cell will be tied to the device it can power.

For the purposes of this dissertation, sustainable power can be interpreted to be power that can be sustained at a relatively constant level for short periods of time (on the order of 10 minutes or less). It is important to note that power degradation is still observed over this time period (see Table 10).

In addition to the paper published in ES&T, Chapter 3 will also include a discussion of the factors that influence microbial performance, a basic comparison to other power producing devices (including traditional fuel cells and batteries), and suggested changes to the terminology and data presentation of microbial fuel cell research.

Microbial Fuel Cells in Chemical and Biological Engineering Laboratories

Also explored in this dissertation is the implementation of a state-of-the art technology into an undergraduate chemical engineering curriculum. This advancement requires not only knowledge of the technology itself, but also an understanding of what will be taught, how it will be taught, and what effect the implementation of this technology will have on the overall learning of the students.

The implementation of microbial fuel cells into a freshman-level hands-on introduction to chemical engineering course is described and a complete description of the resources needed to do so is documented. Chapter 4 gives a description of the state of the course prior to implementing this curricular improvement. It also describes the methods used to determine whether the curriculum change had an effect on student learning. In Chapter 5, the microbial fuel cell experiment that was designed and included in the course is presented and the impact of this fuel cell experiment on the course and on student learning is measured.

Since it is not standard protocol that an instructor would evaluate the impact of a curricular change in his/her course, some of the results presented here necessarily represent the work of others. Dr. Betsy Palmer collected data and conducted interviews for this evaluation of Chemical and Biological Engineering 100 (CHBE 100).

The author did, however, collect data and conduct interviews for the evaluation of the CHBE 100 course in the fall of 2008 as he was not otherwise involved in the instruction of the course that semester. He was solely responsible for the design of the

microbial fuel cell laboratory and helped in the development and instruction of individual laboratories in this course and in the departmental unit operations laboratory. He is responsible for the initial design, implementation, and instruction of a student-designed laboratory in CHBE 100.

Chapter 6 offers an updated microbial fuel cell experiment for laboratories in the Chemical and Biological Engineering curriculum. This chapter also extends the microbial fuel cell experiment to include characterization of the microbial fuel cell using the traditional voltage/current (V-I) characteristic and proposes evaluation metrics for the implementation of this experiment into the curriculum.

ALGAE AS BIOFILMS

Introduction to Algal Biofilms

Characklis and Marshall define a biofilm (Characklis and Marshall 1990) as: "cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin." Biofilms represent a natural way of immobilizing cells and developing a community response to their environment to gain an ecological advantage (Hall-Stoodley and Stoodley 2002). Conversely, algae communities have generally been described as "mats" or "colonies" (Wimpenny 2000), although the word "biofilm" has occasionally been used (Jarvie, et al. 2002, Romani and Sabater 2000, Gilbert, McBain and Rickard 2003, Crispim, Gaylarde and Gaylarde 2003, Jahnke and Priefer 2002, Paje, et al. 2002). It has usually been assumed that biofilms are bacterial and the bacterial extracellular polymeric substances (EPS) support structures act as the matrix, and algae and yeasts are opportunistic additions to a biofilm. Wimpenny defines a biofilm as a heterogeneous community of organisms that forms at a phase boundary and produces EPS for adhesion, protection, and to facilitate community interactions; an algal mat, on the other hand, is defined as a system of organisms with biology driven by photosynthesis (Wimpenny 2000). Stoodley, et al. come to the conclusion: "Possibly we should not restrict a biofilm model to certain structural constraints, but instead look for common features or basic building blocks of biofilms which could be readily incorporated into different structural models in a modular fashion (Stoodley, Boyle, et al. 1997)." Using the approach of describing a biofilm based upon common features, these features might

include attachment to a surface and development of sessile cell colonies in close proximity, including the existence of an EPS matrix; association of other materials or cells in a biofilm matrix; growth, detachment and attachment of a biofilm in a dynamic, heterogeneous manner; and cell-cell communication and gene changes as the cells change from a planktonic to a sessile state (Davies and Greensberg 1998). The microalgae *B. braunii* has been extensively examined as a possible species to form hydrocarbon biofuels photosynthetically (Akin, et al. 1993). It is interesting to determine if changes in colony structure and associations occur in sessile algae cells, as in bacterial cells, and if the resulting colonies can be called biofilms.

Materials and Methods

Organisms

B. braunii is found abundantly in fresh and brackish water (Wehr and Sheath 2003), so three different *B. braunii* isolates collected from freshwater lakes in Minnesota and Iowa were used, courtesy of the late Dr. David Czarnecki from the Loras College Freshwater Diatom Culture Collection. *B. braunii* (572 Kutz culture) from the Culture Collection of Algae at the University of Texas at Austin was also used in experimentation. Note that LB strains have associated bacterial contamination as the L indentifies the culture as liquid, and the B identifies the culture as xenic.

Media

The organisms were grown in a modified ASM-1 media (Eberly 1966) (Table 1, 2, and 3). Soil water extract (Table 4) was added to enhance growth. A simulated flue gas (15% CO_2, 2.5% O_2, 500 ppm NO, balance N_2) was bubbled through the media in order to provide the algae a carbon source for photosynthesis The gas flow was started with a tank pressure of 1500 psi and an inlet gas pressure of 2 psi (Jayawickramarajah 2003).

Table 1: ASM-1 Media Composition

Chemical	Solution(g/L)	Volume(mL)
Macro-Nutrient Solution	(see Table 2)	100
Micro-Nutrient Solution	(see Table 3)	100
Soilwater Extract	(see Table 4)	35
Water	----------	725

Table 2: Macro-Nutrient Solution (10X)

Chemical	Concnetration (g/L)
$NaNO_3$	1.6998
$MgSO_4$	0.2407
$MgCl_2$	0.1904
$CaCl_2$	0.2220
K_2HPO_4	0.1742
Na_2HPO_4	0.1420

Table 3: Micro-Nutrient Solution (10X)

Chemicals	Concentration (mg/L)
$FeCl_3$	6.4
H_3BO_3	24.70
$MnCl_2$	8.8
$ZnCl_2$	4.4
$CoCl_2$	0.1
$CuCl_2$	$1.1*10^{-6}$
Na_2EDTA	74.4
Added nanopure water for a total solution volume of 1 L	
Autoclave solution for 20 minutes	

Table 4: Soil Water Extract

Chemical	Concentration (g/L)
Volcanic Soil	20
$MgCO_3$	1.0
$CaCO_3$	2.0

Stock Culture Preparation and Wet Slide Image Analysis

10 mL of inoculum was added to 30 mL of ASM-1 media. The inoculated solution was then grown in a naturally illuminated condition for 1440 hours. Periodically, samples were mounted on wet slides and viewed under the microscope. Some wet slide procedures involved scraping samples from the interior of glass containers. This procedure proved effective in exposing microbial structures that were attached to the inner walls of the growth vessels.

Flow Cell and Reactor System

The system consisted of the simulated flue gas (Air Liquide Specialty Gases), growth tank, illumination bulb, stir plate, recycle/mixing tank, bubble trap, inoculation port, parallel-plate flow cell, peristaltic pumps, tubing, and connectors. A bubble trap was used to prevent air bubbles from disrupting biofilm formation. The entire system was placed on a mobile setting so that it could be moved to and from a Nikon Eclipses 800 microscope equipped with an Hg bulb. The parallel-plate flow cell was used to study the attachment and detachment phenomena of *B. braunii* and associated organisms. The parallel-plate flow cell used in this study was similar to the flow cell used by Rice, et al. (Rice, Hamilton and Camper 2000). Figure 1 shows the basic components of the parallel plate flow cell. The bottom plate contains a sunken well where a coupon can be inserted. Different coupons (aluminum and Teflon) were placed in the bottom of the cell to examine adherence of cells to different surfaces. The top plate of the rectangular flow conduit was a glass cover slip (43 X 61 mm). The glass cover slip serves as an observation window and also allows light to penetrate to the photosynthetic cells attached to the coupon surface.

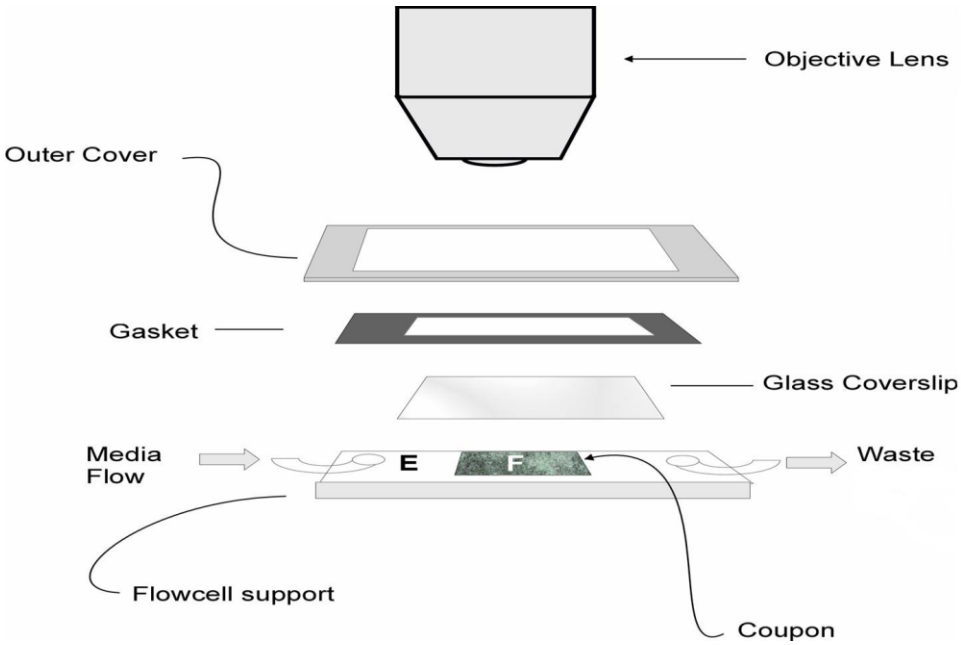


Figure 1: Parallel Plate Flow Cell with Coupon (Jayawickramarajah 2003)

The hydrodynamics and dimensions of the coupon spacing and tubing are given in Tables 5 and 6.

Table 5: Hydrodynamic Characteristics of Flow Channels (Jayawickramarajah 2003)

	Teflon	**Aluminum**	**Tubing**
Bulk Fluid Flow Rate Through Flow Cell	0.3 mL/min	0.3 mL/min	0.3 mL/min
Bulk Fluid Mean Linear Velocity	0.21 cm/sec	0.24 cm/sec	0.27 cm/sec
Reynolds Number of Flow Through the Flow Cell	0.92	0.92	4.6
Wall Shear of Flow Through the Flow Cell	0.028 N/m^2	0.036 N/m^2	0.013 N/m^2

Table 6: Dimension of Coupons and Flow Channels (Jayawickramarajah 2003)

	Teflon	Aluminum	Tubing
Length of Coupon	3.68 cm	3.75 cm	Not Applicable
Height of Coupon	0.145 cm	0.147 cm	Not Applicable
Height of Flow Channel	0.02 cm	0.018 cm	Not Applicable
Cross Sectional Area of Flow Channel	0.024 cm^2	0.021 cm^2	0.018 cm^2

Teflon and aluminum coupons were machined to fit inside the flow cell; after the coupons were machine cut, they were polished using a silicon carbide sand paper (grit size 10 microns). Polishing provided the coupons with uniform surface roughness. The polished coupons were stored in a sterile nanopure water solution to prevent adherence of surface conditioning agents.

The flow system with coupons was sterilized in an autoclave. Once the system was dry, 150 mL of ASM-1 media was added aseptically to the recycle tank in a bio-hood. The calibrated peristaltic pumps were adjusted to a flow rate of 1 mL/min allowing the media to circulate through the system for 12 hours. Then, the system was checked for leaks and cover slip cracks. The flow was turned off when the system was ready for inoculation. Prior to inoculation, the inlet stream leading to the inoculation port was blocked so the injected inoculum would proceed downstream into the flow cell. 0.4 mL of inoculum was taken from the aerated growth tank and injected into the inoculation port. After inoculation, the effluent stream leaving the flow cell was also blocked. By blocking the effluent stream, the microalgae were confined to an area within the flow cell. The cells were allowed to attach and multiply under a no-flow condition for another

5 days. Next, both pumps were activated at a flow rate of 0.3 mL/min. All experiments were run at room temperature (Jayawickramarajah 2003).

Image Acquisition and Staining

Before sampling, the system was moved to the microscope room. To prevent alteration in the system while moving, both inlet and outlet steams of the flow cell were clamped. The flow chamber was then mounted onto the microscope stage and the field of view was established. The surfaces of the coupons were viewed under the Nikon Eclipse 800 microscope. *B. braunii* autofluoresces when exposed to an excitation wavelength of 596 nanometers (emission wavelength 615 nm). This provides a means to take images of cells on a non-transparent surface without adding any harmful tags or dyes. Transmitted light was used for images on the transparent surface of Teflon. An imaging tool, MetaView, was used to acquire and produce 24-bit images of the coupon surface biofilm. The regions of the coupon that harbored the most cells were examined. The x and y coordinates of the specific regions were recorded so that subsequent views of those particular coordinates could be accomplished. Images were collected once prior to the initiation of flow, and at regular intervals afterwards. At the conclusion of the above runs, two different dyes were introduced into the flow cell to stain microbial mass in order to observe biofilm formation and bacterial cohabitation. The blue fluorescent DAPI (4',6-diamidino-2-phenylindole, dihydrochoride) nucleic acid stain, with a maximum excitation wavelength of 358 nm and a maximum emission wavelength of 461 nm, was used in this study. DAPI stains both dsDNA and RNA of cells without staining the cytoplasm and hydrocarbons. This particular stain was chosen because its blue

fluorescence stands out in contrast to the red auto-fluorescence of the algal cells. In certain flow-cell runs, the dye rhodamine B was also used to identify matrix formation.

To introduce the dyes, the tubing directly upstream of the inoculation port and directly downsteam of the flow-cell were clamped shut. Then the clamped section, including both the inoculation port and the flow-cell, were disconnected from the system. The stream leading away from the flow cell was unclamped so that any solution injected through the inoculation port would proceed through the flow channel. DAPI solution (0.8 mL) was then injected (5 mg/mL) into the flow cell. The open end was closed and the stain was allowed to fixate for 15 minutes. After fixation, the stream was reopened and 3 mL of fresh media was injected through the inoculation port. The media was used to washout unbound DAPI particles and unattached cells that would otherwise produce blurred images. The staining procedure for rhodamine B was almost identical to the procedure used for DAPI, but 10 times as much fresh media was used in the washout step.

The DAPI-stained flow cell was viewed under a mercury-arc lamp. By using the wavelengths between 596-615 nm for autoflorescence and wavelengths of 358-461 nm for DAPI, two distinct images of the same surface were obtained. The two images could be combined using the color-combine function built in to the MetaView soft-ware. The rhodamine B dye was prevalent when viewed at wavelengths between of 500-600 nm, and again color-combined images could be built (Jayawickramarajah 2003).

Results

Wet Slide Analysis and Observations

Images of *B. braunii* did not show much variation from a young culture to an aged culture. *B. braunii* is slow growing (exponential phase doubling times were found to be as fast as 1 day and regularly at 3-4 days), and sufficient sampling volumes were not present to distinguish such variations. Figure 2 illustrates a typical colony and the autoflorescence capability of B. *braunii*; individual cells within the colonies can be clearly identified. There was an apparent difference in colony structure and physiology, however, between attached cells (Figure 3A) of *B. braunii* and those grown in media without immobilization (Figure 3B). Note that the hydrocarbons were easily squeezed from a *B. braunii* colony, in this case by the slide coverslip hydrostatic pressure (Figure 4); after rehydration, the basic colony structure still exists. The colony size distribution of *B. braunii* was not examined for all strains and conditions, but colonies can have diameters of up to hundreds of microns (Zhang and Kojima 1998). The colony units examined in this study fit within this range, also shown in Figure 4. As indicated previously, the alga *B. braunii* produces unique hydrocarbons that can have a mass of up to 75% of the dry algal biomass. The type of hydrocarbon produced is related to the strain of algae. The hydrocarbons are similar to compounds found in crude oil and contain a high thermal value (30–42 MJ/kg). These compounds increase the value *B. braunii* cultures in addition to the benefits of CO_2 sequestration. Hydrocarbons were collected and concentrated by methanol extraction and analyzed with gas chromatography/mass spectroscopy (GC/MS). The analyses (see Figure 5) indicated the

material produced regularly spaced, specific peaks that indicated the presence of some shorter chained hydrocarbons (Pryfogle, et al. 2003).

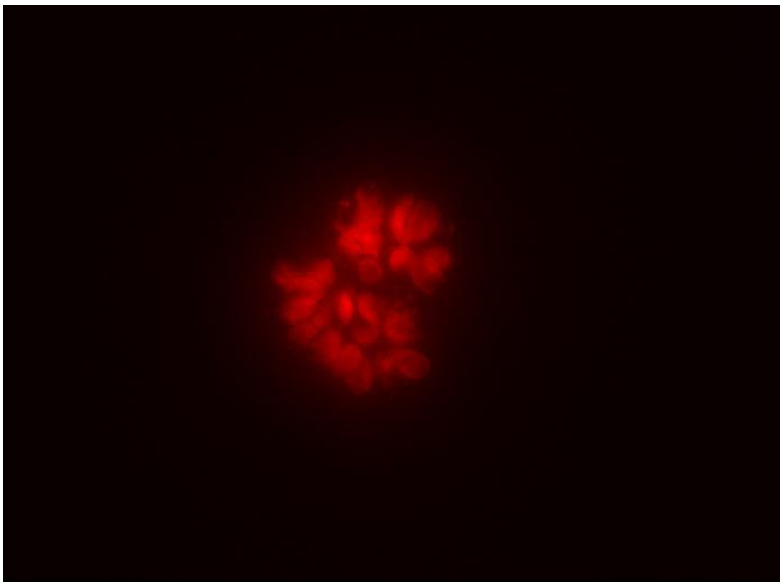


Figure 2: Autoflorescence of *Botryococcus braunii* in Loosely Connected Colonies (UTEX isolates, 600X)

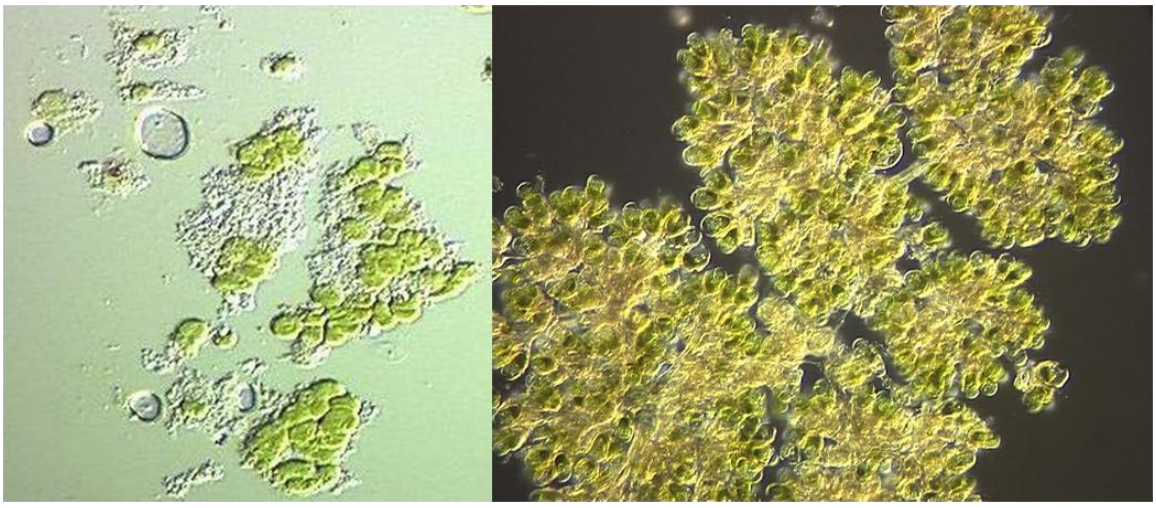


Figure 3: (A) Surface (Glass) Attached Colonies of *Botryococcus braunii* and (B) a Free-Floating *Botryococcus braunii* Colony (Czarnecki Isolates)

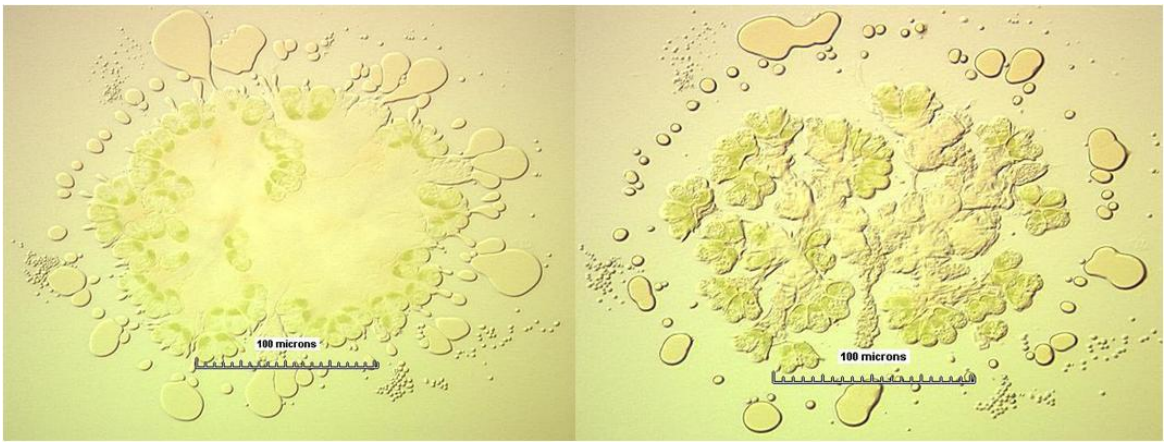


Figure 4: *Botryococcus braunii* (A) With External Pressure Causing Hydrocarbon Excretion and (B) After Rehydration of the Same Cells (Czarnecki Isolates)

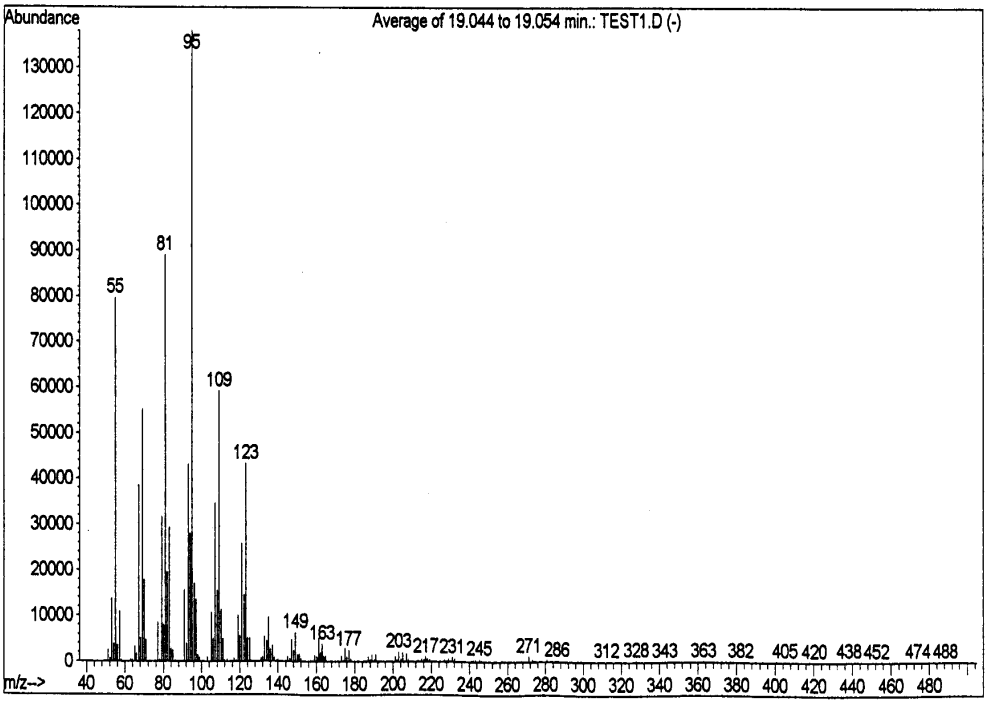


Figure 5. GC/Mass Spec Analysis of *Botryococcus braunii* Hydrocarbons

B. braunii on Aluminum

B. braunii colonies appear to be immobile under the microscope; therefore it was not easy to determine if cells were only adjacent to the substratum or if they were indeed attached to the surface. Once flow was started the extent of attachment could be observed. *B. braunii* were not present as attached single cells; they were congregated in large, independent colonies. All images on the aluminum surface were taken using florescence microscopy (Figures 6A through 6D). Images were taken at a specific location (0.65 mm^2 sampling area) on the aluminum coupon at 24 hour increments. Image 6a is a picture taken before flow was initiated to the system. If cells were not attached, then the colony would have not remained, but have been carried out of the flow cell. Figures 6b and 6c illustrate the loss of adhesion by some cells within the colony, but the colony itself remains attached to the substratum. After each 24 hour increment, the images show decreased cell numbers and the cell cluster structure seems to slowly breakdown. As the growth doubling time for the cells in the flow cell is normally 3 days, and as only the cover slip is available for light, the colony unit does not have enough activity to reproduce to replace any lost cells and EPS to this flow shear. During the last time step (between 48 and 72 hours) there was an apparent sloughing event where a large portion, almost the whole cluster, was washed out.

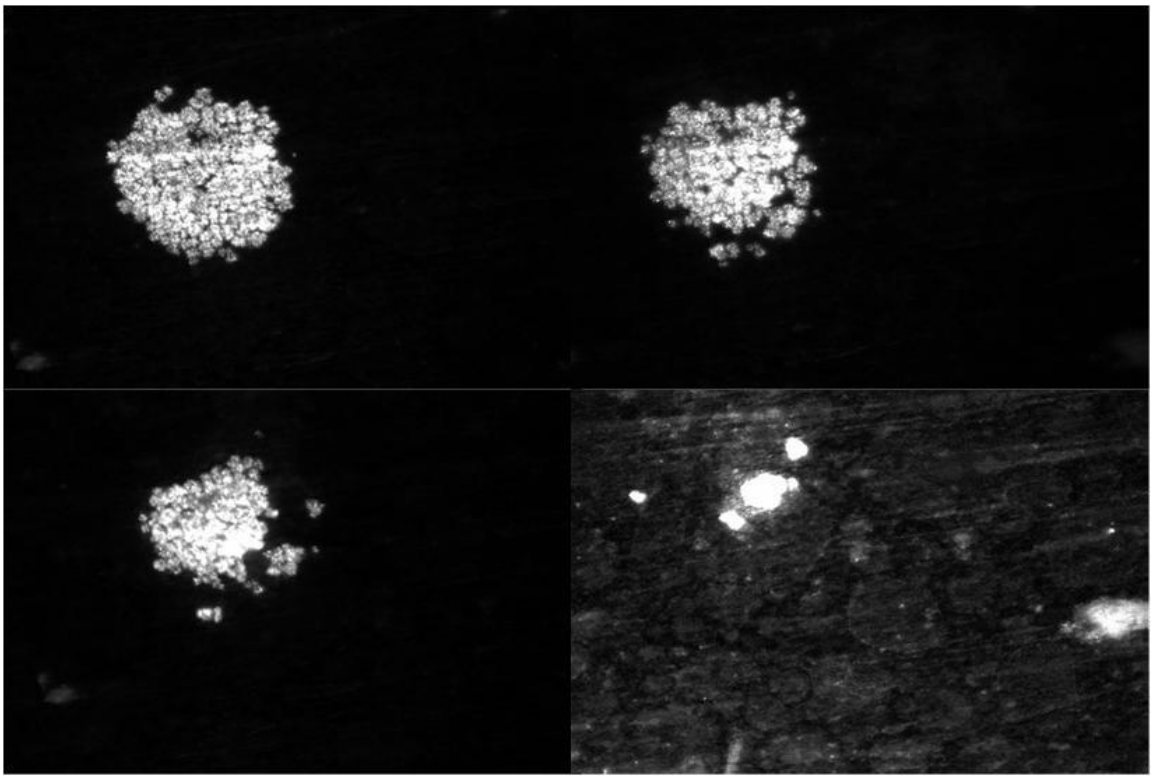


Figure 6: Colony of *Botryococcus braunii* on an Aluminum Coupon Under Flow Conditions at (A) Time 0 Hours (No Flow) (B) 24 Hours (C) 48 Hours and (D) 72 Hours (UTEX isolates, 100X)

B. braunii on Teflon

The DAPI-stained images of locations sampled on the Teflon coupon showed evidence of weak surface attachment; *B. braunii* adheres to aluminum more strongly than it adheres to Teflon due to the electronegative nature of Teflon. Within 24 hour sampling periods, most cell colony clusters were washed out after the onset of flow; only one cluster remained, and it was also lost within the next 24 hour period (images not shown).

Image Analysis with Stain

After completion of the time-dependent experiments, the flow channel was stained with either DAPI or rhodamine B dye to observe biofilm formation and bacterial cohabitation. The stain analysis was carried out on a flow-cell containing *B. braunii* adhering to aluminum. Typical images of a colony of *B. braunii* and associated organisms on the surface of the aluminum coupon are shown in Figure 7. The red images are autofluorescing algae cells, while the blue from the DAPI stain indicates the presence of other organisms. The autoflorescent images of *B. braunii* demonstrate the apparent presence of "algae-empty crevices" between the highly dense cellular regions on the colonies. Figure 7(b) shows that these "empty crevices" are actually filled with organisms. These are apparently bacteria in close association with algae. The nature of this algal/bacterial association is not known, but it symbiotic relationships between microalgae and bacteria are known to exist (Amin, et al. 2009).

Figure 8 presents a combined color image which shows that the bacteria are spread throughout the flow-cell and separately adhere to the substratum, but there remains a strong association to the algae colonies. Separate experiments with the algae *Dunaliella tertiolecta* found this association as well. Staining with Rhodamine B found biomass lightly covering the surface, with clusters of both algae and bacteria present.

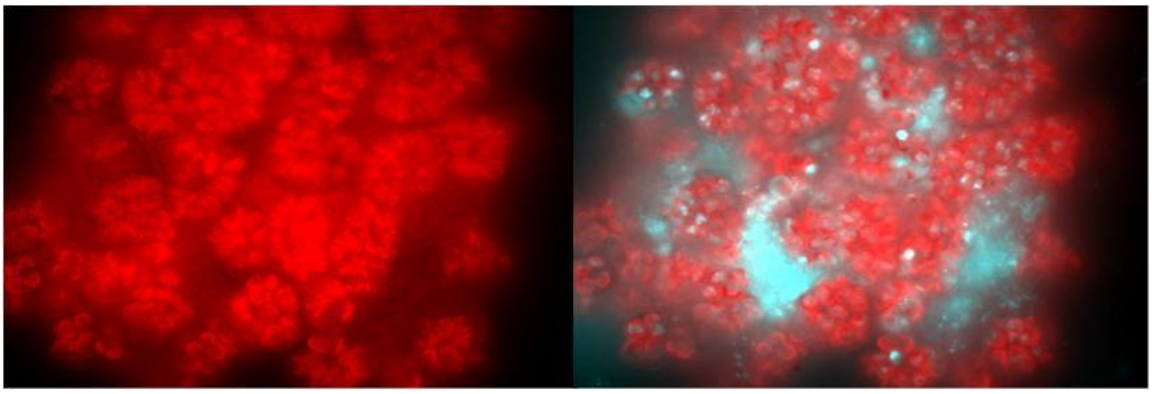


Figure 7: (a) Autoflorescent View of *Botryococcus braunii* on Aluminum and (b) *Botryococcus braunii* and Associated Organisms Stained with DAPI on Aluminum (UTEX isolates, 600X)

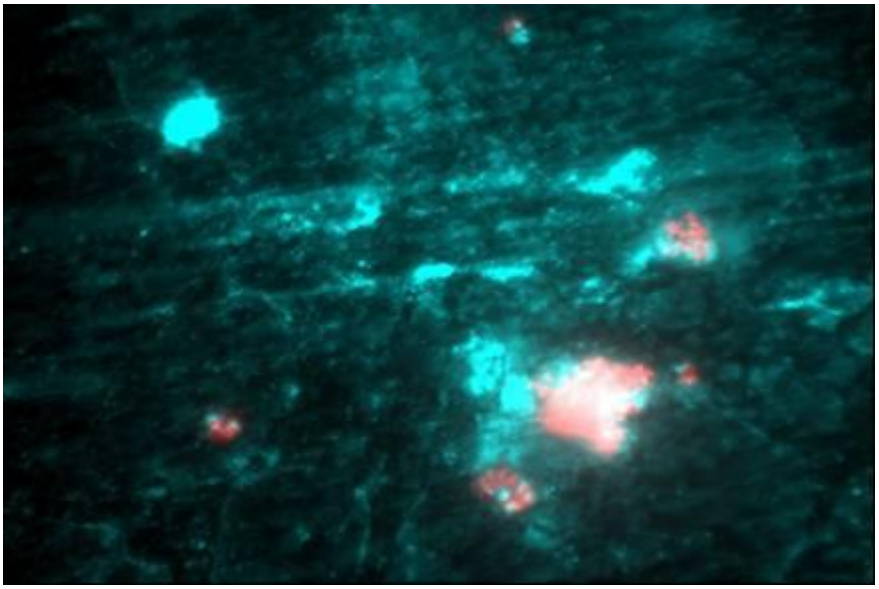


Figure 8: *Botryococcus braunii* and Associated Organisms on Aluminum Stained With DAPI (UTEX isolates, 100X)

Discussion of Algae as Biofilms

The apparent detachment and sloughing events shown in Figure 6 were the first images explicitly demonstrating this phenomenon in microalgae. The sloughing event is reminiscent of the sloughing of an entire section of bacteria observed by Stoodley, et al. (Stoodley, Wilson, et al. 2001). The detachment trend in Figure 6 is observed in most of the sampled locations, in that there was gradual erosion of cells from the periphery of the colony, followed by detachment of the colony itself. As time passes, there is likely a threshold where shear force breaks the bonds attaching the remaining colony to the substratum. Future work should include identification of the variables that cause algal communities to detach. This visual erosion and sloughing event is strikingly similar to that found in bacterial biofilms. These images present the characteristic features of a biofilm of attachment, detachment, and sloughing. The weak adherence on Teflon, a smooth, hydrophobic surface is similar to weak bacterial biofilm adherence to smooth surfaces (Mueller, et al. 1992, Scheuerman, Camper and Hamilton 1998). Figure 7 identifies bacterial association, which is to be expected as bacteria are often associated with algae and the utilized isolates also contained bacteria. The nature of this association, however, is not known at this time.

Casadevall, et al. showed that the cells of *B. braunii* are capable of both hydrocarbon production and active cell division early in the exponential growth stage (Casadevall, et al. 1985). The botyrococcene hydrocarbons are usually concentrated within the outer walls (sometimes outside) of *B. braunii* cells (Largeau, et al. 1980). As the media included no organic carbon, the primary carbon substrate for the bacteria must

come from algal photosynthesis, which would explain a close association. Some of the bacteria generally associated with *B. braunii* are species of *Pseudomonas*, *Flavobacterium*, and *Alcaligenes*. The *Pseudomonas* and *Flavobacterium* species that are known to be present in these algal cultures are capable of growing on hydrocarbons as their sole organic carbon source (Chirac, et al. 1985). It is also possible that these seemingly empty areas contain the extracellular products from algae. These products would include the algal hydrocarbon botryococcene and polysaccharides (hydrocarbons and polysaccharides will not appear in autoflorescent images). Polysaccharidic fibrils were previously shown to be attached to *B. braunii* cells (Fernandes, et al. 1991). The images from Figure 7 support the contention that the colony of *B. braunii* is the main structure, and the bacteria are associated with the biofilm structure and not providing the structure. In the absence of associated microorganisms, however, *B. braunii* has been reported to lose aspects of its colonial habitat (Murray & Thomson, 1977). Since associated organisms might affect the cell-cell adhesion within the algal colony, the idea that bacteria could also be involved in the adhesion of the algae to the substratum must be retained, although the present results indicate that bacteria are not necessarily needed in adhesion.

These results indicate that these algae exhibit many characteristics of biofilms: adherence; detachment and sloughing; difference in structure of an attached colony; varying strength of attachment on different surfaces; association of other organisms in an EPS matrix; and the heterogeneous nature of attached colonies. The examination of gene change upon attachment was beyond the scope of the present study, but this should be

examined, as such results have been reported in bacterial biofilms (Xu, et al. 2001). Although there is not indisputable evidence that microalgae form a biofilm with all characteristics normally associated with bacterial biofilms, there is enough evidence to further examine the behavior of microalgae with special attention to events related to biofilm development. Insight into behavior of *B. braunii* as a biofilm may lead to advances that will compliment contemporary research, as well as stand alone as an early study of biofilm development by microalgae.

CHARACTERIZATION OF A MICROBIAL FUEL CELL

Introduction to Microbial Fuel Cells

The microbial (or biological) fuel cell was described in 1969 as an "electrochemical energy converter" (Bockris and Srinivasan 1969). In the 1990's, Allen and Bennetto described a microbial fuel cell as able to withdraw electrons from the oxidation of a carbohydrate (glucose) as electrical energy (Allen and Bennetto 1993). A microbial fuel cell, like other electrochemical cells, has both an anode and a cathode, separated by a semi-permeable ion conducting electrolyte membrane. Bacteria in the anodic compartment facilitate the liberation of electrons through a microbial oxidation reaction. These electrons are consumed in the cathodic compartment for the reduction of another chemical species, often oxygen. The flow of electrons occurs through an external load in order to produce electric power. This flow of electrons is balanced with a flow of cations through the semi-permeable electrolyte membrane.

In a microbial fuel cell, the electrical power produced is generally low when compared to other conventional fuel cells. This is because of the inherent limitation on current in a microbial fuel cell. Because there is a limit to the electrons that can be liberated, the maximum possible current can be calculated. This calculation assumes that every mol of glucose in the growth media is used for the generation of electrons. For every mol of glucose oxidized, 24 mols of electrons are produced. This relationship is given as Figure 1 and can be found in the discussion of the anodic compartment of the microbial fuel cell later in this chapter. Because there are $6.023*10^{23}$ electrons in a mol of

electrons, there are $1.46*10^{25}$ electrons for every mol of glucose oxidized. A growth media that has 1 g/L (or 0.00555 mol/L) glucose will have $8.024*10^{22}$ available electrons in every liter. If the flow rate of growth media into the microbial fuel cell is 0.3 mL/minute, there will be $2.41*10^{19}$ electrons/minute ($4.012*10^{17}$ electrons/second) available. Again, this assumes that all of the glucose in the growth media is used for current generation. One ampere of electricity is equivalent to $6.242*10^{18}$ electrons passing through a point every second. Therefore, the maximum possible current that this microbial fuel cell could possibly produce is 64.3 mA. Though it is unreasonable to think that the microorganism uses all of the glucose in the growth media for the production of electrons, it does provide an upper bound by which one can compare the current produced to the maximum current available. In the case of this study, the maximum current that could be sustained over a short time period is approximately 125 µA. Therefore, only 0.19% of the electrons available in the growth media were actually used in current generation.

Finally, the ceiling on the current available in any microbial fuel cell is dependent on the glucose concentration in the growth media. This assumes that the concentration of glucose in a growth media should be no greater than 60 g/L in order to avoid toxicity to the microorganism. The theoretical limit of current corresponding to this maximum glucose concentration is 3.86 A. There is a possibility of stacking microbial fuel cells to increase the total voltage, but charge reversal did occur in one study, causing one cell to reverse polarity (Oh and Logan 2007). A capacitor was successfully used to store energy

in the powering of a wireless sensor (Shantaram, et al. 2005). This allows for a greater load to be powered by a microbial fuel cell, if only for a short period of time.

There are some comparisons that can be drawn between microbial fuel cells and other similar power sources, (e.g. fuel cells, batteries). For instance, the power derived from the cell is limited by the cell potential. Like all fuel cells, the performance of a microbial fuel cell, as measured by cell potential, deviates from the ideal potential. Figure 9 comes from the 7th Edition of the Fuel Cell Handbook. It shows clearly the regions in a fuel cell that deviate from ideal. These regions include a region of activation polarization, a region of ohmic polarization, and a region of concentration polarization (EG & G Services 2004).

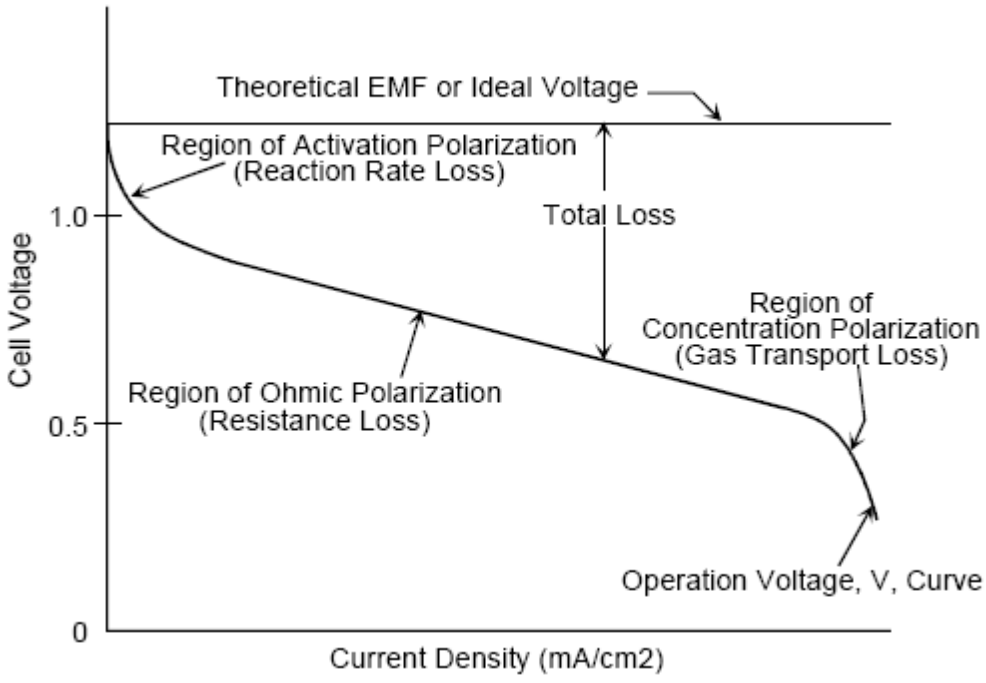


Figure 9. Voltage Losses in Fuel Cells (EG & G Services 2004)

The initial losses in Figure 9 and Figure 10 are associated with activation losses. Activation losses can be attributed to a slow electrode kinetics response in a fuel cell (EG & G Services 2004). Figure 10 shows the V-I characteristics of the microbial fuel cell described in Menicucci, et al. (Menicucci, et al. 2006). This characterization shows that a microbial fuel cell exhibits slow electrode kinetics. This is what one would expect in any low temperature fuel cell, as high temperature fuel cells generally do not have significant activation losses. Resistance losses (regions of ohmic polarization) can also be seen in microbial fuel cells (Figure 10).

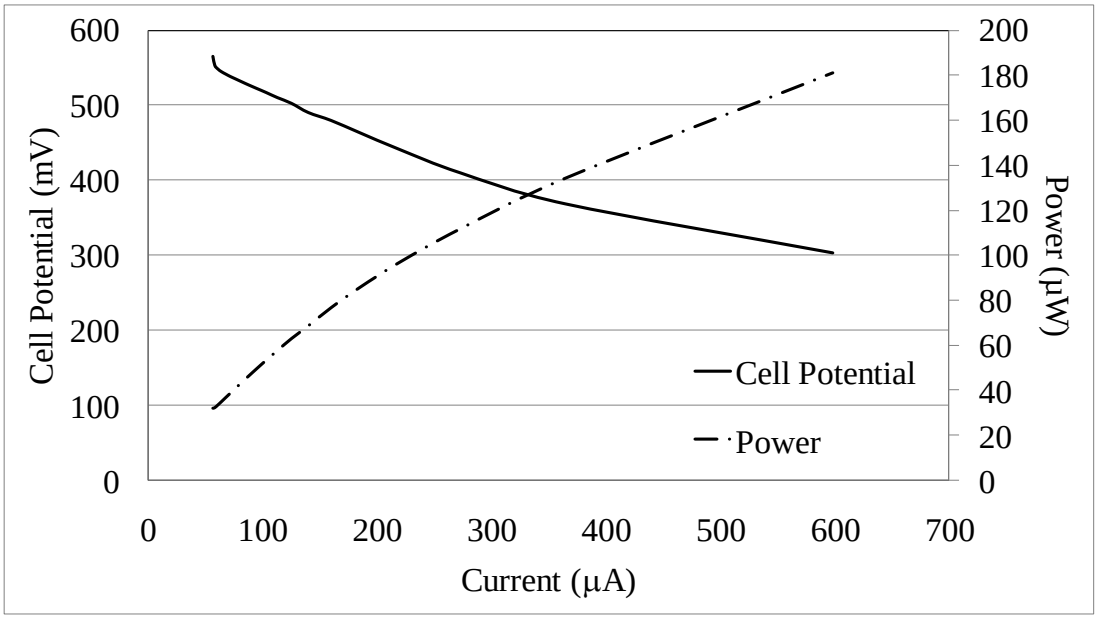


Figure 10: Voltage Losses in a Microbial Fuel Cell

He, et al. used impedance spectroscopy to characterize a microbial fuel cell. They generally attributed kinetic limitations to slow activation rates on the electrodes (He, et al.). They also indicate that ohmic limitations in microbial fuel cells are usually attributed to electrolyte resistance. This paper also includes an equivalent circuit and a Nyquist plot

of the microbial fuel cell (He, Wagner, et al. 2006). Finally, the resistance scan in this chapter did not explore the region of concentration polarization. Had this region been explored, it is expected that a significant voltage loss (and a corresponding loss in power) would have been seen.

Like a traditional fuel cell (and differing from a battery), the supply of energy in a microbial fuel cell needs to be constantly replenished. In the case of a microbial fuel cell, that energy source is the carbon source in the growth media. Unlike a battery, the conversion of the fuel source is not direct. Instead, the microorganism facilitates the conversion of chemical energy to electrical energy through the anaerobic oxidation of glucose and liberation of electrons.

It should be noted that the microbial fuel cell literature does not address concerns at the triple phase boundary because, unlike other power sources, the triple phase boundary does not play a significant role in limiting power generation in a microbial fuel cell.

A discussion of the source of possible power limitations of the microbial fuel cell can be made by looking at the biological system as well as the underlying chemical engineering principles involved. The power production in a microbial fuel cell might be limited by the metabolic activity of the microorganism. In a different microbial fuel cell the rate of electron transfer might be power limiting. In yet another microbial fuel cell, the rate of diffusion of the nutrient source through the biofilm might be power limiting. The microbial fuel cell design also has the potential to limit the power it is able to produce. This is the case if the distance between the anode and cathode is significant such

that the mass transfer of the cations from the anodic compartment to the cathodic compartment is rate limiting.

Dewan, et al. explore a number of factors that can limit the power productivity of a continuously-operating microbial fuel cell. The type of microbial fuel cell, electrode type, redox mediator used (if any), microorganism used, and carbon source can all play a role in the maximum power a microbial fuel cell can produce (Dewan, Beyenal and Lewandowski 2009). Table 7 offers examples (with the authors' research goals, when available) of the parameters tested. This table is a summary of information found in Dewan, et al. and is used with permission. This information, found in Table 7, gives representative view of the present state of microbial fuel cell research.

Finally, it is important to note that no fuel cell or battery is truly sustainable; all are subject to power degradation. This phenomenon is observed in microbial fuel cells. When the procedure to determine sustainable power was published there was no standardized method for the simple characterization of a microbial fuel cell. Since the publication of this procedure, more conventional fuel cell characterizations have become commonplace in the microbial fuel cell community. To some extent, however, the publication of this procedure and the explanation of its necessity was a driving force for standardization within the field. The maximum sustainable power (as defined below) is a metric by which different fuel cells can be compared. The power of a microbial fuel cell can be found by applying an external load (generally an external resistor), measuring the voltage and the current, and calculated as the product of the current (I) and voltage (V); P=IV. For the purposes of this procedure then, it is important to recognize that the most

promising potential uses of microbial fuel cells will involve powering a small device for a relatively short period of time. As such, when the term sustainable is used in this procedure, it refers to power generation over relatively short time periods (less than 10 minutes) when the effect of power degradation is small relative to the overall power output.

Table 7: Optimization Parameters in a Microbial Fuel Cell (Dewan, Beyenal and Lewandowski 2009)

Variable Changed	**Authors**	**Selected Variable**	**Notes**
Microorganism	(Bond and Lovley 2003)	*Geobacter sulfurreducens*	Increase efficiency of organic substrate oxidation
	(Choi, et al. 2004)	*Bacillus licheniformis* and *Bacillus thermoglucosidasius*	Power production at elevated temperatures
	(Prasad, et al. 2007)	*Hansenula anomala*	Direct electron transfer
Carbon Source	(Kim, et al. 2000)	Glucose, Galactose, Sucrose, Maltose, Trehalose	
	(Liu, Cheng and Logan 2005)	Acetate, Butyrate	
	(Oh, et al. 2005)	Food and Animal Wastewater	
Electron Transfer Mediator	(Park and Zeikus 2000)	Neutral Red	Anodic Reaction Rate
	(Schaetzle, Barriere and Schroder 2009)	Laccase	Cathodic Reaction Rate
Electrode Material	(Logan, et al. 2007)	Carbon Fiber Brush	Anode
	(Morris, et al. 2007)	Lead Oxide Catalyzed Platinum	Anode

	(Rhoads, Beyenal and Lewandowski 2005)	Biomineralized Manganese Deposited on Stainless Steel	Cathode
	(Pham, et al. 2004)	Platinum-Coated Graphite	Anode/Cathode
Reactor Design	(Liu, Cheng and Logan 2005)	Single-Chambered	Decrease Internal Resistance Caused by Membrane
	(Rabaey, Clauwaert, et al. 2005)	Tubular	Increase Power Generation
	(Reimers, et al. 2001)	Membrane-Less	Generate Power from Marine Sediment

Materials and Methods

Microbial Fuel Cell Components

The microbial fuel cell used in the experiments is shown in Figure 11. The cell casing is made out of polycarbonate and had anodic and cathodic compartments separated by a cation exchange membrane ESC-7000 (Electrolytica Corporation, 770-410-9166). Each chamber had a working volume of 250 mL. A saturated calomel electrode (SCE) is used as a reference electrode (Fisher, catalog no. 13-620-51) and is located in the cathodic chamber of the MFC. J-cloths with thicknesses of 0.1 mm (First Brands Corporation), located in front of the cation exchange membrane, are used to protect the cation exchange membrane from direct contact with the electrodes and to minimize microbial growth on the cation exchange membrane (Bennetto and Delaney 1983, Rhoads, Beyenal and Lewandowski 2005). Feed and effluent tubing is made of Neoprene (Cole-Parmer, catalog no. 148441). Glass flow breakers located in feed lines were used to prevent carboy contamination.

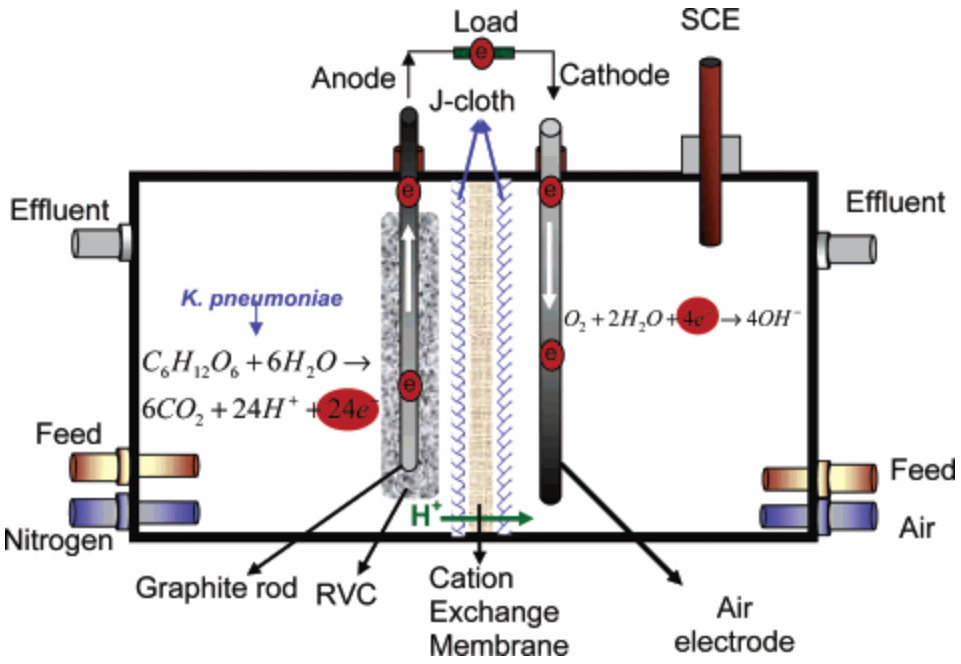


Figure 11. The Microbial Fuel Cell Used in the Experiments (Rhoads, Beyenal and Lewandowski 2005)

Anodic Compartment: In the anodic compartment, glucose in the growth media is oxidized anaerobically by *Klebsiella pneumoniae* (ATCC no. 700831) according to the following ideal metabolic reaction.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \qquad (1)$$

The microbial growth medium in the anode has the following composition: 10 g/L tryptone, 5 g/L yeast extract, 5 g/L sodium chloride, 9.12 g/L Na_2HPO_4, 1.75 g/L KH_2PO_4, and 1 g/L glucose. Equation 1 illustrates the principle of extracting electrons from organic substances. The growth medium used in the MFC is a complex solution of nutrients, and not only glucose but also organics in yeast extract and tryptone are oxidized by the microorganisms. The anode is made of reticulated vitreous carbon (RVC)

connected to graphite rods (Sigma-Aldrich, CAS no. 7782-42-5). The anodic surface area for the 80-ppi RVC is approximately 0.4 m^2, calculated from the specifications given by the vendor.

Cathodic Compartment: The cathodic reaction is the reduction of oxygen:

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

The full cell reaction is then:

$$C_6H_{12}O_6 + 3O_2 \rightarrow 6CO_2 + 12H^+ + 12e^- \qquad (3)$$

An air electrode, provided courtesy of Dr. Neal Naimer of the Electric Fuel Corporation of the Arotech Corporation (Putt, Naimer and Koretz 2000), is used as the cathode. The electrode is composed of two active layers of carbon bonded to each side of a current-collecting screen made of nickel mesh (Hamlen and Atwater 2001). Oxygen is reduced on the electrode surface.

To prevent the pH from changing in the cathodic compartment due to diffusion of H^+ through the cation exchange membrane, a phosphate buffer (1.825 g/L of Na_2HPO_4 and 0.35 g of KH_2PO_4) is used at a pH of 7.2. The surface area of the cathode is 79 cm^2 (measured).

Fuel Cell Startup and Operating Conditions

Preparation of Inoculum: 1 mL of a frozen stock culture of *K. pneumoniae* is added to 100 mL of medium and is allowed to grow overnight (18 h) at room temperature on a rotary shaker (150 rpm).

MFC Preparation: The microbial fuel cell is cleaned thoroughly in deionized water. The cation exchange membrane is soaked in 1 M NaCl for at least 24 h before installing in the cell. The cell is assembled as shown in Figure 11. Both compartments are filled with deionized water (to avoid dry autoclaving). A rubber stopper is used to protect the open port (to house the reference electrode). The MFC is autoclaved at 121 °C for at least 20 min. The rubber stopper is removed, and a saturated calomel electrode is inserted. The water in the anodic and cathodic compartments is then drained, and the growth medium is pumped into the anodic compartment of the cell while phosphate buffer is pumped into the cathodic compartment of the fuel cell.

60 mL of the *K. pneumoniae* stock culture is then inoculated into the cell, replacing 60 mL of the sterile medium. The bacteria are allowed to grow in batch mode until the anodic potential stabilized (−450 mV_{SCE}). Sterile medium is then pumped into the anodic compartment at a flow rate of 0.3 mL/min (D = 0.0012/h) for the duration of the experiments.

Measurement of Anodic and Cathodic Potentials and Current in a Microbial Fuel Cell

The anodic and cathodic potentials of the microbial fuel cell are measured against a saturated calomel electrode (Fisher, catalog no. 13-620-51) using a data logger (HP

model no. 34970A) to store the measured values regularly (every 10 s). A variable resistance box (Ohm-Ranger, Ohmite Manufacturing Co.) is used to select an applied external resistance for current measurement. Figure 12 shows the experimental setup used for logging the current and the anodic and cathodic potentials. The circuit consists of two different wiring sections: one for the potential measurement and one for the current measurement. For the current measurement, the anode and the cathode are connected to the current ct6hannel through a variable resistor box in series. For potential measurement, the anode and cathode are connected to potential channels 1 and 2 of the data logger, respectively, with the SCE connected to the common terminal of the potential channels.

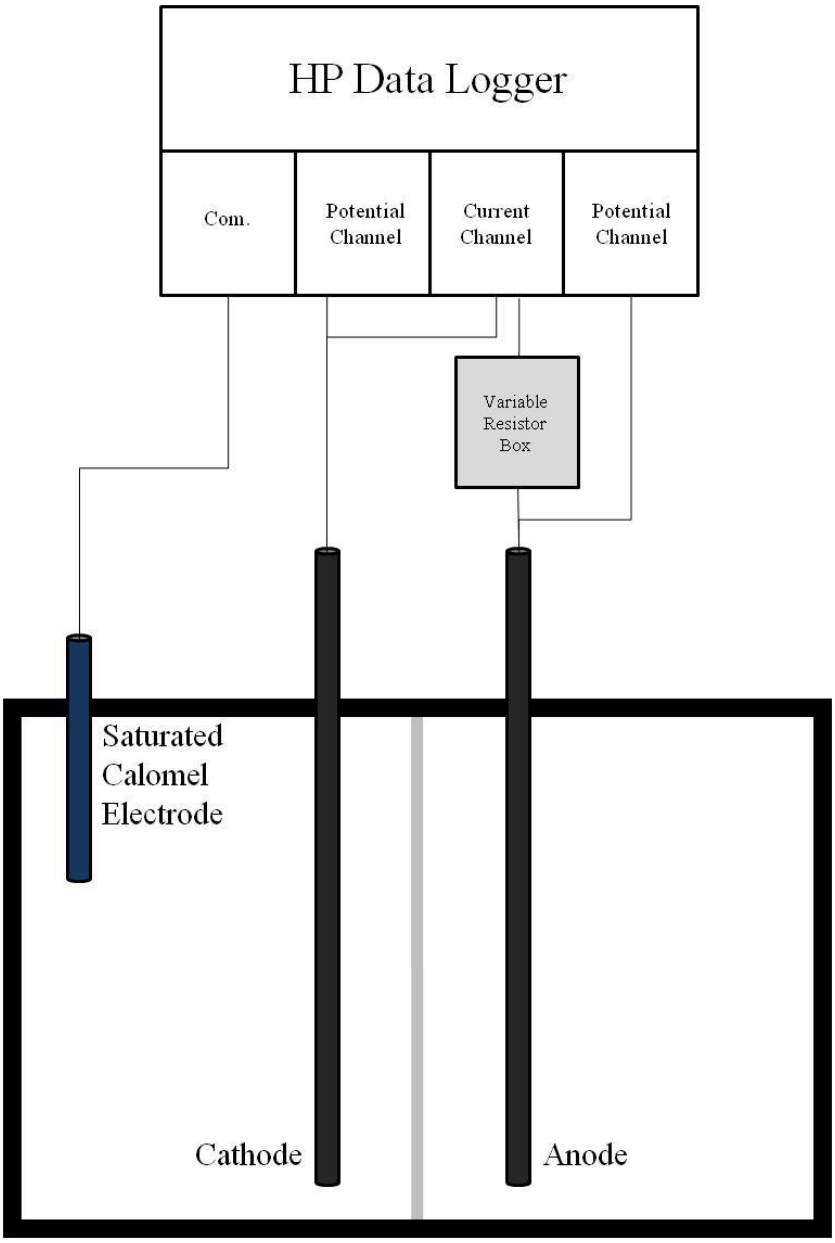


Figure 12. Setup Used for Logging the Current and the Anodic and Cathodic Potentials

Sustainable Power in Microbial Fuel Cells

At steady state, the power generated by a microbial fuel cell (MFCs) is computed as the product of the cell potential and the current in the external circuitry (Liu, Cheng

and Logan 2005). At this time, the power generated by the MFC equals the power consumption and the power production is sustainable for a short period of time. It is important to define the condition at which the sustainable current reaches a maximum and to compute the maximum sustainable power that can be generated by a microbial fuel cell as it indicates the type of device that can be powered by a microbial fuel.

Current flows when the anode and the cathode of a fuel cell are connected through a resistor (Bennetto and Delaney 1983, Allen and Bennetto 1993). The current is affected by the potential of the cell and by the electrical resistance. The electrical resistance has two components: external, the circuitry powered by the fuel cell; and internal, the fuel cell itself (Gzebyk and Pozniak 2005). Initially, before the external circuit is connected to the cell, the open circuit voltage of the cell reflects the thermodynamic equilibria of the anodic and cathodic reactions. If the external circuitry has a relatively low electrical resistance, then the equilibrium potential of the cell initially generates a high instantaneous electric current, higher than the short-term maximum sustainable rate of charge transfer to/from the current-limiting electrode. As a result, the potential across the cell decreases quickly and adjusts to the rate of charge transfer to the current-limiting electrode, effectively decreasing the current in the external circuitry. However, if the external circuitry has a relatively high electrical resistance, then the equilibrium potential of the cell generates an electric current lower than the maximum sustainable rate of charge transfer to/from the current-limiting electrode. The potential of the cell adjusts to the external resistance. In the latter case, the power generation is sustainable but lower than it could be if the load were optimized.

Each factor involved in the computation of power generated by a MFC-the cell potential and the current- depends on many other factors, such as the chemistry, concentrations of the reactants, and microbial activity. Some of these factors cannot be controlled by the operator (Man, Cheng and Logan 2005, Rabaey and Verstraete 2005, Zhang and Halme 1995). The cell can generate maximum power for a specific combination of external and internal resistances only. The external resistance can be controlled by the operator, but the internal resistance is an inherent feature of the fuel cell, and the operator cannot control it. Predicting the lowest external resistance at which the MFC yields the maximum sustainable power (MSP) from the circuit analysis is difficult because many factors affecting the internal resistance are difficult to quantify.

Most researchers studying MFCs make a point of estimating the power generated by these devices; some just measure the current by applying an arbitrarily selected external resistance and measuring instantaneous current and potential difference. Such instantaneous measurements estimate the power generated at the time of the measurement, but such data cannot be used to determine the maximum sustainable power. Also, this approach does not allow for the comparison of various devices and various operational procedures because the choice of the external resistor is arbitrary, and the power generated by the fuel cell is dependent upon that choice. Table 8 shows a selection of such measurements extracted from the available literature.

Table 8. Applied Resistor and Measured Current Values Found in Contemporary MFC Literature.

Reference	Applied Resistor (Ω)	Measured Current (or Current Density)[a]
(Liu, Cheng and Logan 2005)	70−5000	0.2−2.2 A/m^2
(Man, Cheng and Logan 2005)	1000	18−130 mA/m^2
(Chang, et al. 2004)	10	5 mA
(Chaudhuri and Lovley 2003)	1000	(31 mA/m^2)
(Jang, et al. 2004)	10−1000	2−0.4 mA
(Bond and Lovley 2003)	500	0.4 mA
(Park and Zeikus 2003)	300	14 mA
(Lee, et al. 2002)	560	0.075−0.35 mA
(Simon, et al. 2002)	not listed	(30−80 μA/cm^2)
(Pizzariello, Stred'ansky and Miertus 2002)	100 000	(5.94 μA/ cm^2)
(Park, et al. 2001)	1000	0.08−0.22 mA
(Kim, et al. 2002)	1000	0.04 mA

[a] Current densities are reported only if they are available.

To compare the power generated in various MFCs, it is useful to start by standardizing the method of measuring the power, so that different fuel cells can be compared. Selecting the external resistor is the most important task in evaluating the

power generated by a MFC, and it is the key to determining the sustainable power generated by the MFC (Rabaey, Ossieur, et al. 2005). Once the electrodes are connected through an external resistor, the cell potential decreases because of the limitations imposed on the electrode reaction kinetics, on mass transfer, and on charge-transfer processes at the current-limiting electrode (the one of the two electrodes that exhibits the slower charge-transfer kinetics). The decreasing cell potential results in a decrease in the current flowing through the external circuit, following Ohm's Law, $I = V/R$. Figure 13 illustrates a few of the factors that have the potential to affect the power generated by MFCs. Very important is the work completed by Anderl, et al. indicating that there none of the glucose in the bulk fluid phase will penetrate a *K. pneumoniae* biofilm (Anderl, et al. 2003). This indicates that the electrons are produced at the surface of the biofilm and are transferred to the anode via bacterial electron shuttling (Rabaey, Boon, et al. 2004) or through electrical conduction through nanowires in the extracellular polymeric substances in the biofilm (Gorby, et al. 2006, Reguera, et al. 2006).

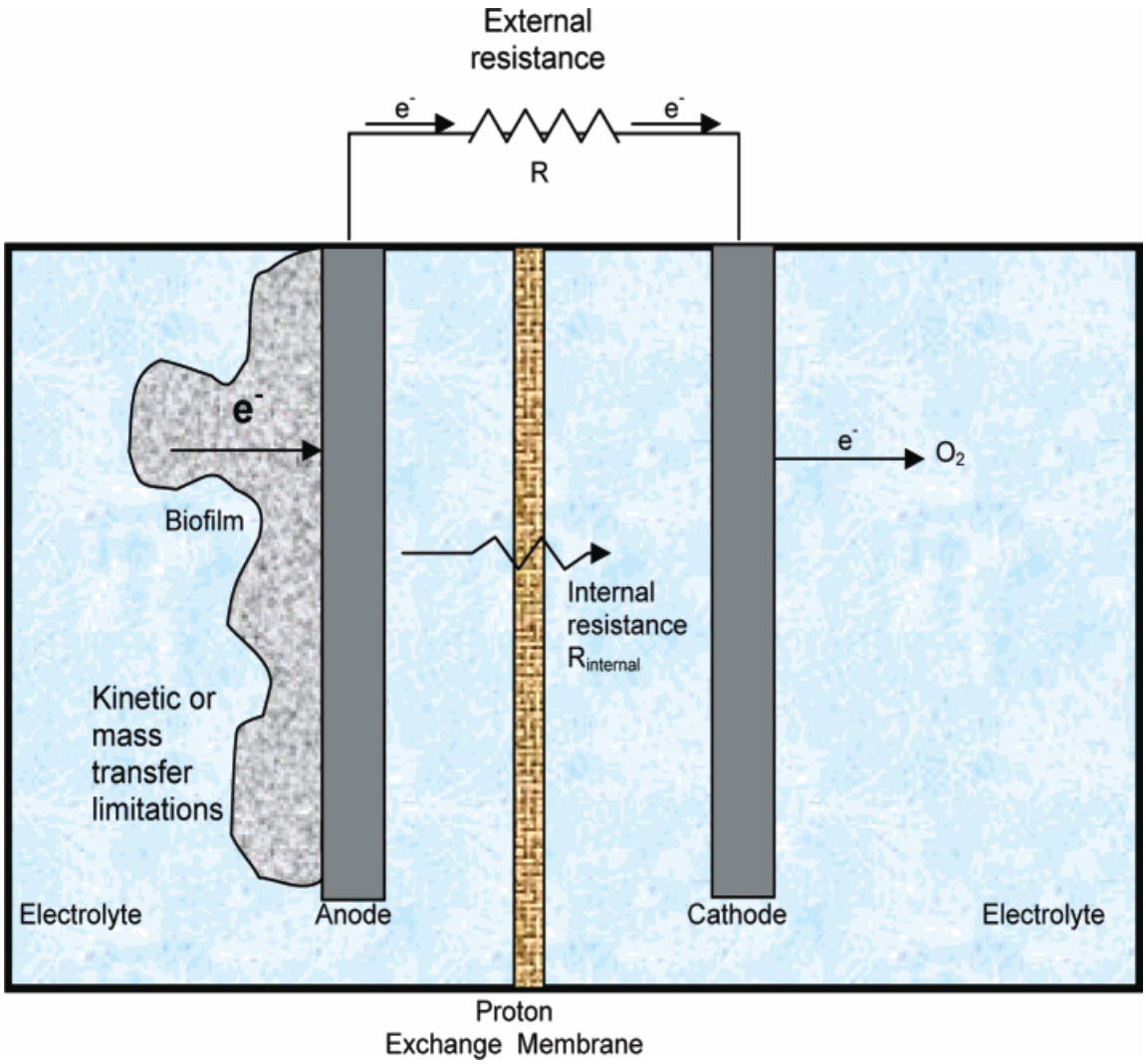


Figure 13. Simplified Charge Transfer in an MFC Depicting Factors Affecting the External and Internal Resistances

The power generated by the MFC can be limited by the charge-transfer resistance to the electrode or the external resistance. The effect of the internal mass transfer resistance on power limitation can be demonstrated by stirring the solution of electroactive species. This will increase convection and the therefore, the power. The effect of the external resistance on the power generated by the MFC is more complex and

can be visualized by short-circuiting the electrodes, which forces the cell to generate high power for a very short time. Such elevated power generation is not sustainable in the short term, and it decreases rapidly in time regardless of the effect of power degradation. This effect highlights the need for a procedure to select the correct resistance for determining the maximum sustainable power generated by microbial fuel cells.

The goal, then, is to develop a procedure for selecting the external resistance for microbial fuel cells that ensures generation of the MSP and allows for a voltage/current characterization of the MFC. To demonstrate the utility of this procedure, a microbial fuel cell is operated with *Klebsiella pneumoniae* oxidizing glucose in the anodic compartment. Oxygen is abiotically reduced in the cathodic compartment. The resistor in which the cell generates maximum sustainable power is selected empirically by changing the resistances at a predefined rate (Ohms per unit time) and measuring potentials and currents.

Evaluating Sustainable Power

To evaluate the sustainable power, a decreasing resistance is applied at a predefined scan rate and the current and cell potentials are measured for each resistance. The applied resistor, cell potential, and current are later used to calculate power and the sustainable power is evaluated from these measurements.

Example Measurements and Computations: An example measurement showing how current and cell potential change with the external resistance is shown in Figure 14. The external resistance is changed stepwise in equal time intervals. For practical reasons, this step-like variation is approximated as as a continuous change of resistance with

respect to time (dR/dt = 0.5 kΩ/min, shown by a dashed line). This data is seen in a traditional fuel cell V-I curve in Figure 10.

The data used to predict sustainable power is produced after the MFC reached a stable cell potential (550 mV). The different resistances are applied using an external resistor-ranger (Ohm-Ranger, Ohmite Manufacturing Co.). The initial external resistance is 10 kΩ, and the resistance is decreased by 0.5 kΩ every minute (hereafter referred to as the resistance scan rate) until 0.5 kΩ is reached. The average current value at each external resistance (over one minute) is used in the calculation of power.

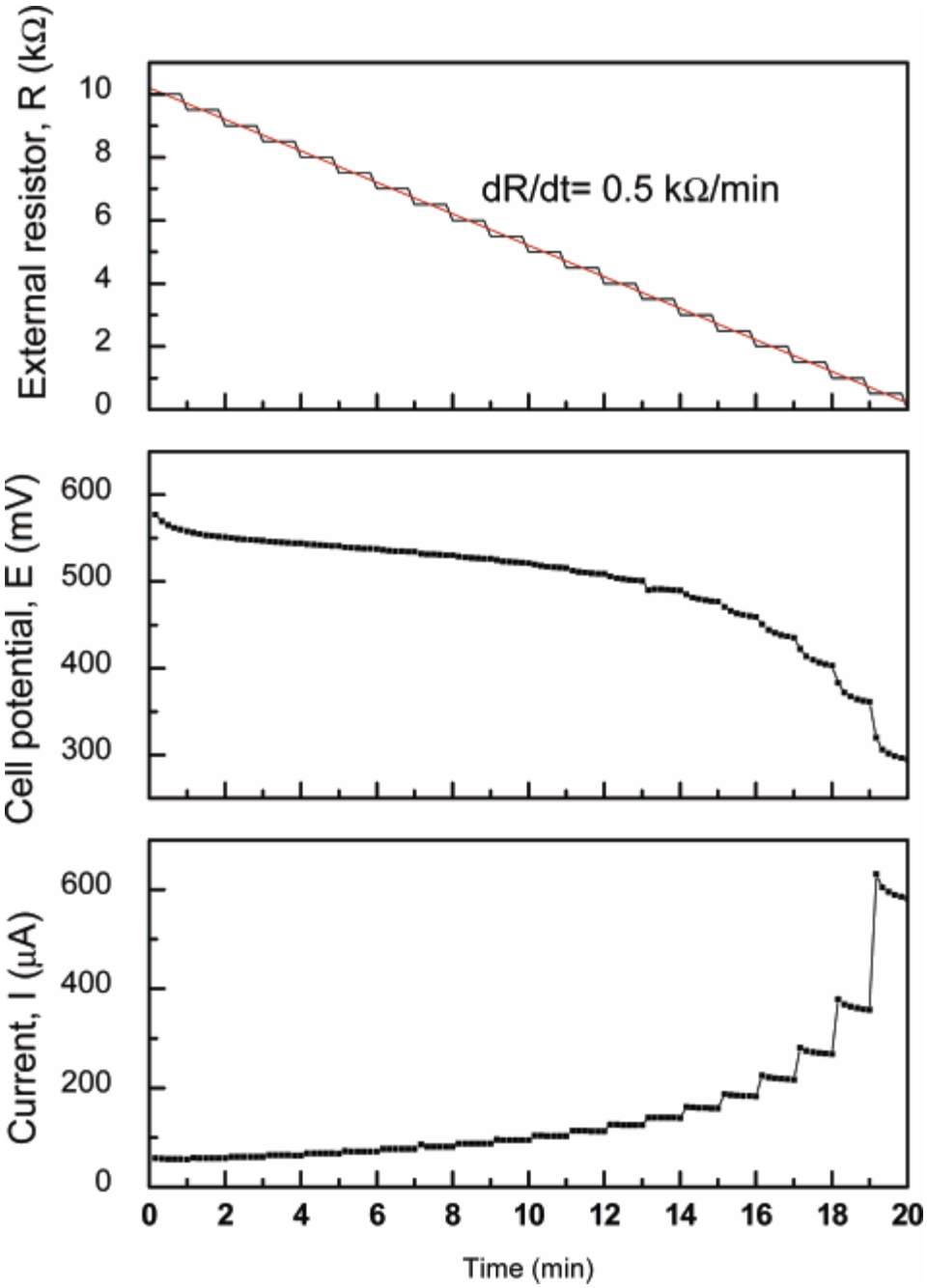


Figure 14. Resistance Scan Showing the Effect of Applied External Resistance on the Variations of Cell Potential (E, Potential Difference Between Anode and Cathode) and Current (I). The Continuous Line is an Approximation of the Stepwise Ramp of the Decreasing Resistance (dR/dt = 0.5 kΩ/min)

Effect of the External Resistance Scan Rate on the Sustainable Power: The measurements in Figure 14 are repeated starting at an initial external resistance of 10 kΩ and scan rates of 0.5 kΩ/10 s and 0.5 kΩ/180 s.

Experimental Verification of the Computed Maximum Sustainable Power: The microbial fuel cell was operated for over two weeks to verify that the maximum sustainable power computed using the developed technique was consistent with the sustainable power evaluated by operating microbial fuel cells at a single resistance. The MFC was operated at different resistances (6, 5, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.5, 0.25, and 0.125 kΩ) for a longer time period (generally greater than 6 h) at a constant applied resistance. The current and anodic and cathodic potentials were measured with respect to time.

Results and Discussion

Effect of External Resistance Scan Rate on the Evaluated Sustainable Power

Table 9 shows the evaluated sustainable power and corresponding external resistance for different resistance scan rates (0.5 kΩ/10 s, 0.5 kΩ/60 s, 0.5 kΩ/180 s). For lower external resistance scan rates, the difference between the estimated maximum sustainable power is very small (1 μW). However, there is a 20% difference in the estimated maximum sustainable powers between 0.5 kΩ/10 s and 0.5 kΩ/60 s external resistance scan rates.

Table 9. Effect of the Rate of Change of External Resistance on the Change in Sustainable Power

Rate of Change of Resistance (kΩ/time)	Evaluated Maximum Sustainable Power (μW)	Corresponding Resistor (kΩ)
0.5 kΩ/10 s	86	3 kΩ
0.5 kΩ/60 s	68	3.5 kΩ
0.5 kΩ/180 s	69	3.5 kΩ

From the given graphical procedure, an applied external resistance of 3−3.5 kΩ corresponds to a sustainable power. A slightly higher external resistor (4 kΩ) was used experimentally to be sure that the power is sustainable over the defined time period.

Experimental Verification of the Evaluated Sustainable Power

The maximum sustainable power of our microbial fuel cell was estimated from a 30 min experiment. This sustainable power is 65.51 μW, which was measured using a 4 kΩ resistor. If this prediction is correct, then a resistance value less than 4 kΩ should cause a drop in cell current with time. However, any resistance greater than 4 kΩ should not change the cell current. Figure 15 shows the temporal variation of cell current for three applied external resistors: (1) an external resistance less than the resistance able to maintain a corresponding sustainable power (0.25 kΩ), (2) an external resistance able to maintain a corresponding sustainable power (4 kΩ), and (3) an external resistance that consumes less power than the cell is capable of producing (6 kΩ). When a 0.25 kΩ resistor was used, there was a significant, immediate, and continuous drop in current and cell potential, while current and potential remained constant over many hours when a 4 kΩ resistor was used. When a 6 kΩ resistor was used, the power was below the maximum sustainable power.

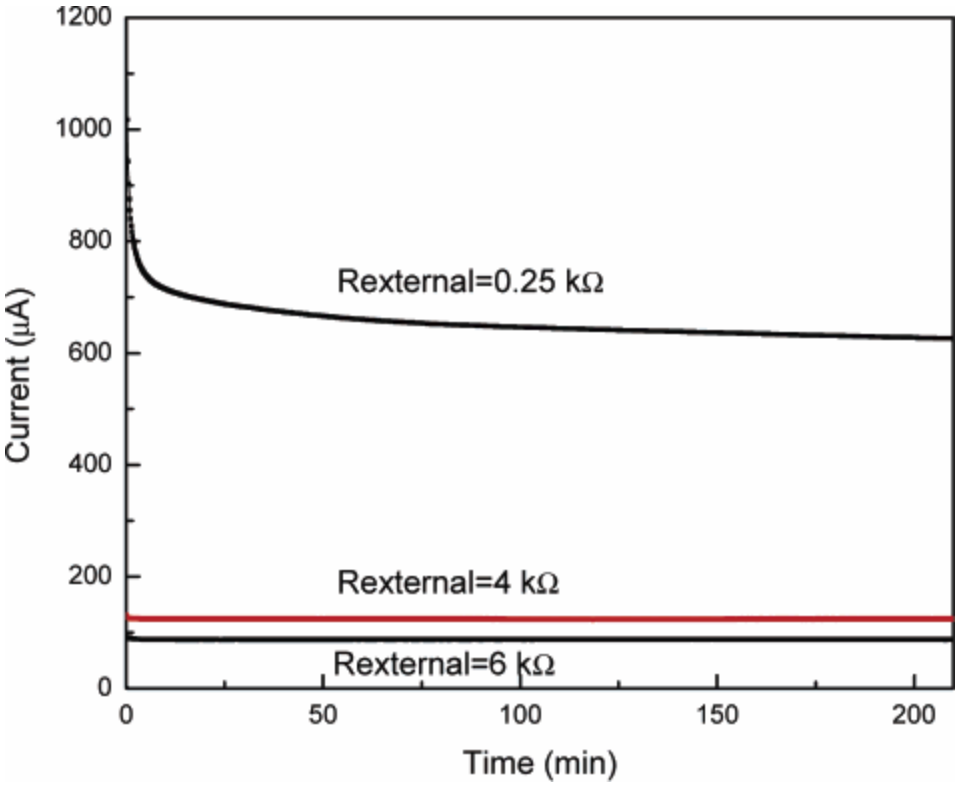


Figure 15. Current Measured Over 200 Minutes for 0.25, 4, and 6 kΩ External Applied Resistances. The Current Remains Constant When a 4 or 6 kΩ Resistance is Applied. However, the Current Decreases with Respect to Time When a 0.25 kΩ Resistance is Applied

In experiments run at a constant resistance over longer periods of time (four hours or longer), the cell potential and current decreased significantly when the applied resistance was lower than the resistance identified as yielding the sustainable power. The cell potential and current remained constant (neglecting the effect of power degradation) when the applied resistance was equal to or greater than this resistance. The microbial fuel cell was then operated for 16 h, applying a 4 kΩ resistance, and it was found that the current and cell potential remained constant. This verified the sustainable power predictions (performed in less than 30 minutes).

Comparing the Maximum Sustainable Power to the Maximum Power

The definition of sustainable power implies that the power generated does not change with time and the cell potential remains constant after applying the external resistance. However, the sustainable power obtained using higher resistances may be less than the maximum sustainable power, as shown in Figure 16. The sustainable power measurement at a resistance of 4 kΩ is 61.51 μW. The measurements in Figure 16 were taken for 1 hour after applying the external resistance. Figure 16B shows that, even after 1 hour, the power in a microbial fuel cell continues to decrease when the resistance is less than that which can maintain a sustainable resistance. The inset in Figure 16B shows the decrease in power over the first two minutes of operation.

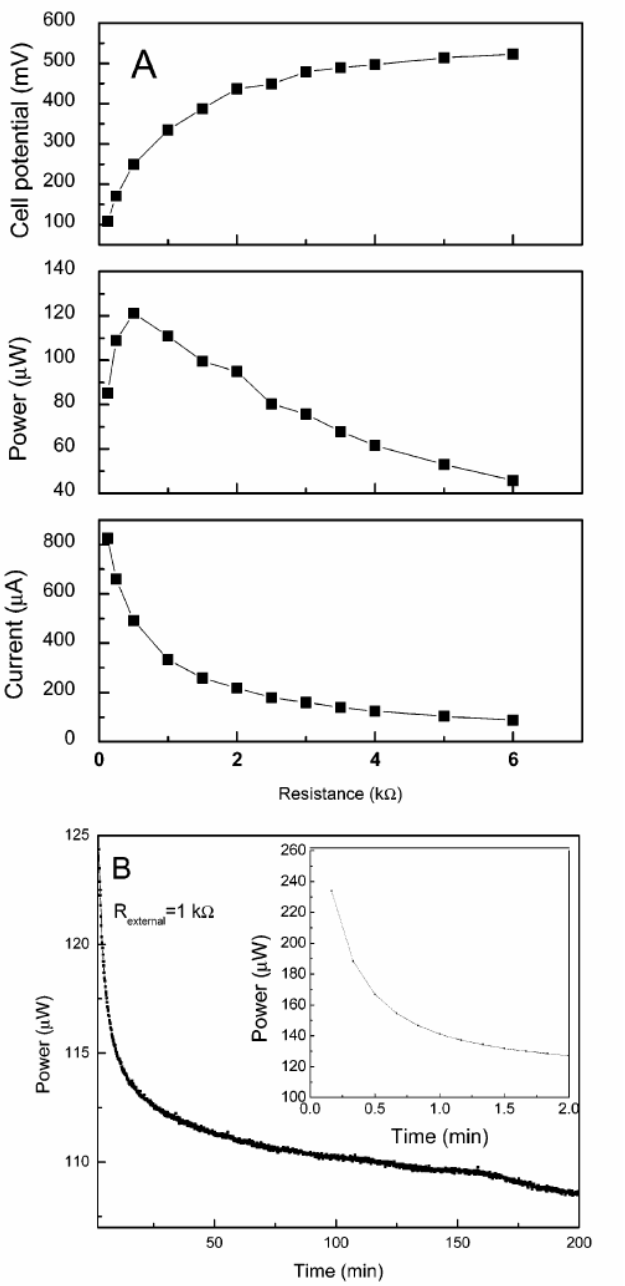


Figure 16. (A) Current, Cell Potential, and Power Measurements in a Microbial Fuel Cell (measured at t = 1 h). (B) Power Measurements in a Microbial Fuel Cell with an Applied External Resistance of 1 kΩ. Note that Power Continues to Decrease Even After 300 Minutes of Continuous Operation

Figure 17 shows that the instantaneous power generated by the MFC depends on the resistor if the power is not sustainable. The power also depends on the time that the cell is allowed to equilibrate, demonstrating transitional conditions and non-sustainable power generation. Only when the external resistance is large enough does the power generation reach a sustainable level quickly. The lower the external resistance, the longer the time needed for the cell to equilibrate and produce the sustainable power. When a large, 6 kΩ, resistor was used, power generation is sustainable after 10 seconds.

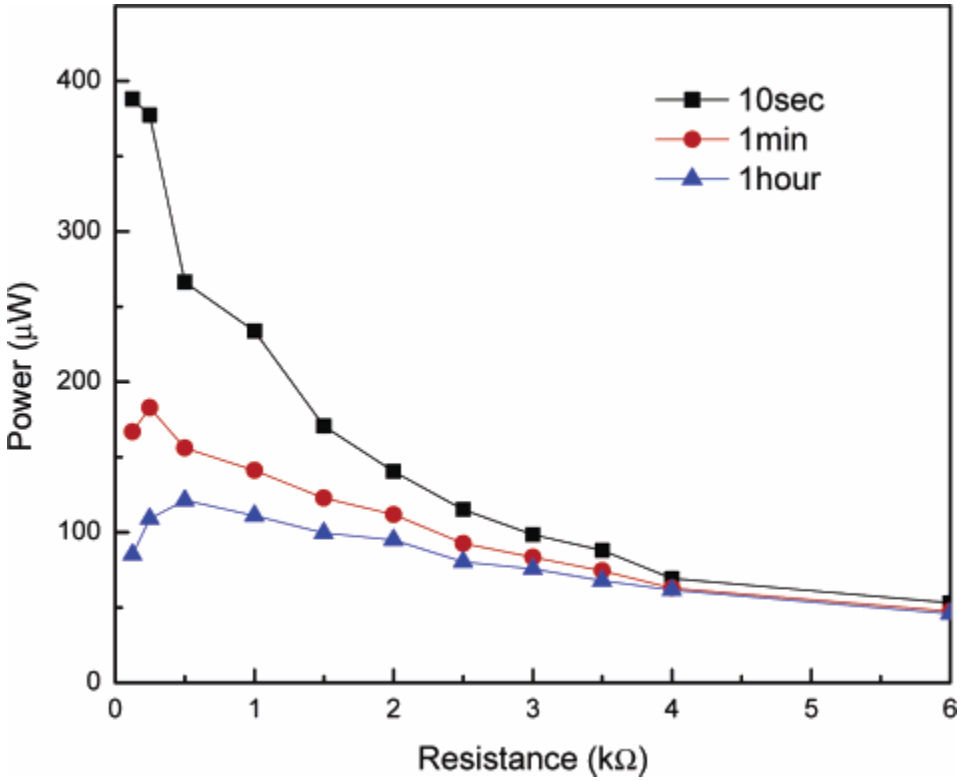


Figure 17. Power Generated by the MFC Measured Using Various External Resistors. The Power Generated by the Cell Changes with Time, Demonstrating Unstable Conditions and Non-Sustainable Power Generation. Only When a High Resistor is Used Does the Cell Reach a Steady-State Quickly; It Produces Steady-State Power After Just 10 Seconds

From Figure 17, the peak maximum power of the MFC is estimated to be 395 μW. However, the maximum sustainable power of the microbial fuel cell is only 65 μW, much smaller (6 times) than the maximum power. He, et al. reported the fuel cell power when a 66 Ω resistor was applied (He, Minteer and Angenent 2005). However, when larger resistors, 100 Ω and 250 Ω, were applied the current decreased in time. Figure 3a in *Electricity Generation from Artificial Wastewater Using an Upflow Microbial Fuel Cell* (He, Minteer and Angenent 2005) shows this phenomenon. When a 470 Ω resistor was applied, however, the current did not change, showing sustainable conditions. Figure 18 is Figure 3a from He, Minteer and Angenent that has been modified for aesthetic purposes only. The thin line in this figure is the current in the microbial fuel cell and the thick line is the voltage. These results corroborate the observations in this study and demonstrate its need. Schroder, et al. changed the external resistance and measured maximum power (similar to Figure 17) but did not indicate whether the measured maximum was sustainable (Schroder and Niesen 2003).

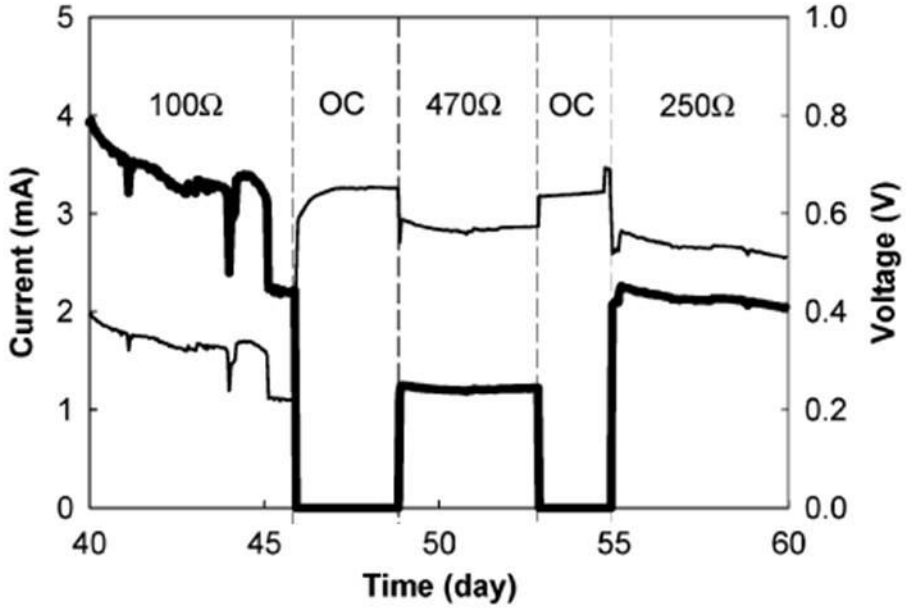


Figure 18. Current and Voltage in an Upflow Microbial Fuel Cell (He, Minteer and Angenent 2005)

Note that the resistance corresponding to the sustainable power can be roughly estimated from Figure 17. More importantly, a voltage/current characterization of the microbial fuel can be completed from the data collected using this procedure. A graphical procedure to find the sustainable power was described in Menicucci, et al. (Menicucci, et al. 2006). This description can be found in Appendix A.

The procedure for evaluating the sustainable power generated by microbial fuel cells can be executed in a short time (less than 1 h), and the results have been verified in long-term experiments (lasting more than 2 weeks). Future experimentation is needed to characterize power degradation in this microbial fuel cell.

THE COURSE BEFORE AND AFTER THE CURRICULUM CHANGE

Introduction to CHBE 100

Prior to 2003, the Montana State University course, CHBE 100: Introduction to Chemical and Biological Engineering was traditionally taught in a seminar style. The implementation of a hands-on style course was initiated in 2003 with the hope that it would better meet the needs of the students by improving their learning in the course. Previous research has indicated that students felt that a hands-on education gave them a deep commitment to an engineering career (Aglan 1996). In a review of the Rowan Clinic, it was determined that the program--which emphasizes an interdisciplinary industrial/academic "clinic", hands-on laboratory work, and teamwork--does a better job with retention and satisfaction of women in their program than do traditional engineering programs.

Also worth noting is that these pedagogical methods are actually "student-friendly" rather than just "female-friendly" (Hartman and Hartman 2006). Also interesting was the revelation that the active implementation of social issues into the Rowan curriculum led to no noticeable difference in the rate of female attrition due to an evaluation of an engineering degree as socially unsatisfying (Hartman and Hartman 2006); this is contrary to previous results for a traditional engineering programs (Seymour and Hewitt 1997).

The implementation of state-of-the-art research into an undergraduate engineering education program not only provides the instructor with another hands-on experiment

which they can choose to use in their course, but also offers the students a different perspective regarding the possibilities available to them upon completing their degree. According to the American Institute of Chemical Engineers, 16% of graduating chemical engineers go on to receive another degree in chemical engineering (Cobb, et al. 2008). The implementation of academic research technologies into an academic curriculum is not without precedent (Dahm and Hesketh 2007, Dahm, Hesketh and Savelski 2005, Lefebvre, Connell and Dahm 2009). In fact, a laboratory of this type was developed for CHBE 100 within the Department of Chemical and Biological Engineering at Montana State University-Bozeman. The Magnetic Resonance Imaging (MRI) Laboratory was developed for the course in 2004 and incorporated an introduction to MRI and a data analysis section (Seymour, et al. 2005).

Hands-on Engineering Education

Starting in 2003, in a manner similar to Rowan University's Freshman Engineering Clinic I, students in CHBE 100 were introduced during their first semester in the program to fundamental engineering concepts using a hands-on laboratory approach. Innovative laboratory modules were designed and/or adapted from published materials. These laboratories utilize common activities (such as brewing coffee, taking blood pressure, and delivery of medication) to teach fundamental engineering principles, techniques for experimental measurement, data representation and analysis, and group problem solving skills (Farrell and Hesketh 2002, Hesketh 1996, Hesketh, Slater, et al. 2002). Many of these laboratories are designed to build upon the student's current base of knowledge and experience. Through hands-on laboratories and follow-up seminars, the

students were also introduced to the breadth of traditional and non-traditional careers available to graduates in chemical engineering. A unique aspect of this laboratory based course is that most concepts are taught in an inductive learning format. Students were asked to predict experimental outcomes, perform the experiments, plot and analyze the data, and compare results to their predictions before being exposed to the underlying theory and predictive calculations. By incorporating the concepts within innovative/hands-on activities, it was expected that the students would personalize the learning, thereby leading to improved mastery, retention, and transferability (Bransford and Brown 2000). The course change was initiated fall semester of 2003 by incorporating selected laboratory modules into the existing one credit seminar-based course. In fall 2004, the course was expanded to two credits with the addition of several more laboratory units. The resulting course was formatted as a one credit seminar which meets one hour per week and a one credit laboratory which meets two hours per week.

The revised freshman seminar course that was developed is based on the findings of research on learners and learning as presented in How People Learn: Brain, Mind, Experience, and School, a National Research Council publication. The three key findings of research on learners and learning outlined in the NRC publication are (Bransford and Brown 2000):

1. Students come to the classroom with preconceptions about how the world works. If their initial understanding is not engaged, they may fail to grasp the new concepts and information that are taught, or they may learn them for purposes of a test but revert to their preconceptions outside of the classroom.

2. To develop competence in an area of inquiry, students must:
    a. have a deep foundation of factual knowledge,
    b. understand facts and ideas in a conceptual framework, and
    c. organize knowledge in ways that facilitate retrieval and application.
3. A metacognitive approach to instruction can help students learn to take control of their own learning by defining learning goals and monitoring their progress in achieving them.

Course Format

Lab/recitation sections met for two hours each week on Wednesday or Thursday and the seminar section met for one hour each week on Friday. Lab sections were limited to 9 students in order to provide the students with an opportunity to readily interact with their instructor(s). Lab groups were limited to 3 students as it was found that in groups with 4 or more students, one or more students may not fully participate in the exercise. There were typically 4-6 lab sections each semester. Prior to the lab session, each group met to discuss the lab and assign team responsibilities which consisted of a team leader, data recorder, and worker. At the beginning of most experiments, the group was asked to predict a particular outcome of the experiment and explain their prediction. The group then performed the experiment, plotted and/or analyzed the data, and compared the outcome to their initial prediction. If the outcome differed from their prediction, they were then asked to explain why the outcome differed and to formulate a new explanation. Only after the students spent time working with data and attempting to explain any trends were they exposed to the underlying theory. After discussing the theory, the groups were

asked to make predictive calculations based on the theoretical relationships and compare the predictions to the data obtained in the experiment. This format of inductive teaching is fully supported by educational research as described in How Students Learn:

> "A critical feature of effective teaching is that it elicits from students their preexisting understanding of the subject matter to be taught and provides opportunities to build on –or challenge – the initial understanding… Numerous research experiments demonstrate the persistence of preexisting understandings among older students even after a new model has been taught that contradicts the naïve understanding… For the scientific understanding to replace the naïve understanding, students must reveal the latter and have the opportunity to see where it falls short (Bransford and Brown 2000)."

Moreover, active learning techniques, group problem solving, and scientific investigations involving real-world contexts seem to be particularly advantageous for retaining all students, including women and minority students, in the engineering curriculum (NSF 2003).

The lab units have been sequenced such that the skills and concepts learned each week form a foundation for concepts developed in subsequent weeks. The order of teaching concepts in this course is modeled after that presented in the early chapters of the text *Elementary Principles of Chemical Processes* (Felder and Rosseau 2000). This is a required text for this course and is also used in the sophomore level material balance course. A schedule for the Fall 2005 course offering is shown in Table 10.

Table 10: Course Schedule

Week	Lab Topic	Seminar Topic
1	Course Introduction, Blood Pressure Lab, and Problem Solving	Pre-Assessment Surveys
2	Drug Delivery I	Significant Figures and Dimensional Homogeneity
3	Drug Delivery II	Linear Interpolation and Linear Curve-Fitting of Non-Linear Data
4	Coffee Leaching I	Elementary Statistics
5	Coffee Leaching II	Chemical Composition
6	Fluidized Bed Polymer Coating I	Industrial Applications of Leaching
7	Fluidized Bed Polymer Coating II	Nuclear Submarines
8	Biological Fuel Cells	Biological Engineering
9	Introduction to Term Project	Materials and Product Design
10	Developing the Experimental Plan	Exam
11	Presentation of Experimental Plan	No Class-Veterans Day
12	Student Designed Experiment	MRI Research in Antarctica
13	Student Designed Experiment	No Class-Thanksgiving
14	Student Designed Experiment	PowerPoint Basics
15	Student Presentations	Wrap-up and Course Evaluation

Student-Designed Experiments

For three years a significant portion of the laboratory was a student-designed experiment where groups of two to three students design their own experiments, run the experiments, analyze the collected data, run follow-up experiments, and present their results to their peers. The student-designed experiment provided a much needed component to the introductory laboratory: application of learning. The student-designed experiment offers students the opportunity to take what they've learned and apply it to an experiment of their choosing. Students had dedicated time with an instructor for experimental plan development. They were then required to propose their ideas to both

instructors and other lab groups prior to experimentation. Each lab group had up to six hours in the laboratory to complete their experiments. Finally, students gave a formal presentation about their experimental design and results. Students design their experiments as they choose, including identifying the experimental parameters and data collection methods. Projects ranged from adaptations of in-class experiments (leaching of tea, dissolving aspirin) to experiments identical to an in-class experiment but using different parameters (polymer coating with longer coat times, higher washer temperatures, etc.) or completely novel experiments (design and testing of a thermocouple, measurement of the flight of a basketball filled with various gases, etc.).

Assessment Model

An assessment model was developed for the CHBE 100 which included both quantitative and qualitative data collection (Menicucci, Duffy and Palmer 2007). For the first two years of the project, students were informed of the research procedures used to assess the curriculum changes and were asked to sign informed consent forms prior to participating in data collection. Students who participated in the first year of the course were also asked to participate in a focus group one year after their experience. In addition, the results were supported with field observations in the lab and by conducting a focus group with senior chemical engineering students who were completing their degree requirements and who had *not* experienced the hands-on introductory course during their first year curriculum. The selection of senior chemical engineering students as a focus group and not transfer students was made because the number of transfer students

entering the chemical engineering curriculum at this time would not provide statistically significant data for comparison.

Data Collection

Students completed a pre-test of chemical engineering knowledge and a pre-test survey of attitudes and self-reported skills in the first week of the course. The same test of knowledge was administered as a post-test and final exam during a regular class period just prior to the beginning of the student-designed labs. The post-test of attitudes and self-reported skills included the pre-test items and some additional items measuring student engagement in the various lab exercises and was administered in the final class meeting. These instruments were designed specifically to assess the objectives of this course. As a final assessment of the course, the student evaluations for the new course were compared with those of the previous iteration of the introductory course (without the hands-on lab component). The instructor and evaluation form for this course were the same, although a more comprehensive evaluation was completed on the hands-on laboratory after its implementation.

In addition to completing the knowledge, skills and attitudes assessment measures, students in the first two cohort years were also asked to participate in a focus group during the last class period. At the end of the fall semester in the second year of teaching the hands-on laboratory, two one-year follow-up focus groups were conducted with the first cohort of students and a focus group with seniors who had not taken a hands-on introductory course. All focus groups were conducted by Dr. Betsey Palmer or her designated assistants in the role of independent evaluator(s). This way, students could

be assured that their honest discussion of the course would not affect their grades. All focus group interviews (13 groups total) were transcribed verbatim by a paid transcriptionist and were analyzed and coded for themes. Pre-test and post-test data were analyzed using paired t-tests.

Student absenteeism on the days assessment data were collected resulted in a few missing cases. The pre-test sample size for the chemical engineering knowledge test for year one was n= 36 and for the knowledge post-test was n=34 resulting in a 94% response rate. The sample size for the pre-test of attitudes and skills for year one was n=35 and the post-test for that cohort was n=31 (response rate = 88.6%). For the second year cohort, knowledge pre-test and post-test were both n=37 (100% response rate) and the attitudes pre-test and post-test sample sizes were n=37 and n=34 respectively (91.9% response rate). The combined cohorts therefore had a pre- and post-test knowledge response rate of 97.3% (N=71 and N=73) and a pre- and post-test attitudes response rate of 90.3% (N=72 and N=65). In addition, in analyzing particular items, there was some item nonresponse, so the final N for each analysis varied from N=62 to N=72.

Assessment Results

Students scored significantly higher on the post-test of engineering knowledge than they did on the pre-test. The mean score on the pre-test was 42.3 (n=71, sd = 13.5) and on the post-test, the mean score was 84.1 (N=71, sd =12.8). The difference between these means was tested with a paired t-test which was significant well below the 0.05 alpha level (t = 23.08, df=70, p=0.000). Students in the course made significant gains in learning the required course material. An interesting and unexpected outcome from this

assessment strategy was that students commented frequently on the knowledge gained in the course based on the pre-test. One student commented,

> "I was surprised how much I learned and not even realizing it. At the beginning of the year, we took that pretest and I failed it. We had to take it again at the end of the year and I aced it. I didn't realize I had gone over all of that."

Students also self-reported their knowledge of the field of chemical and biological engineering and their knowledge of professional practices in chemical and biological engineering on the pre- and post-course assessments. On both items, students rated themselves as having gained knowledge of chemical engineering (t_{field} = 4.85, df=63, p=0.000), ($t_{profession}$ = 5.66, df=63, p=0.000).

On the six skill items (EXCEL, PowerPoint, engineering problem-solving, group participation, leadership, graphing) students rated themselves significantly higher on their post-test skill assessments in all areas except creating and reading graphs. Figure 19 shows the students' pre- and post-course mean skill assessments.

Since students will, at times, overestimate their skill level at the beginning of a learning experience, they were asked to estimate the improvement in the six different skill areas on the post-course assessment. As shown in Figure 20, students estimated that their skill in solving engineering problems showed the greatest improvement (X=4.1 on a 1-5 scale). The rated their improvement on creating PowerPoint presentations as the smallest gain. While their skill estimates from pre- to post-course assessment were not significantly different, students rated their improvement in creating and reading graphs in the mid-range of some improvement (X=3.6).

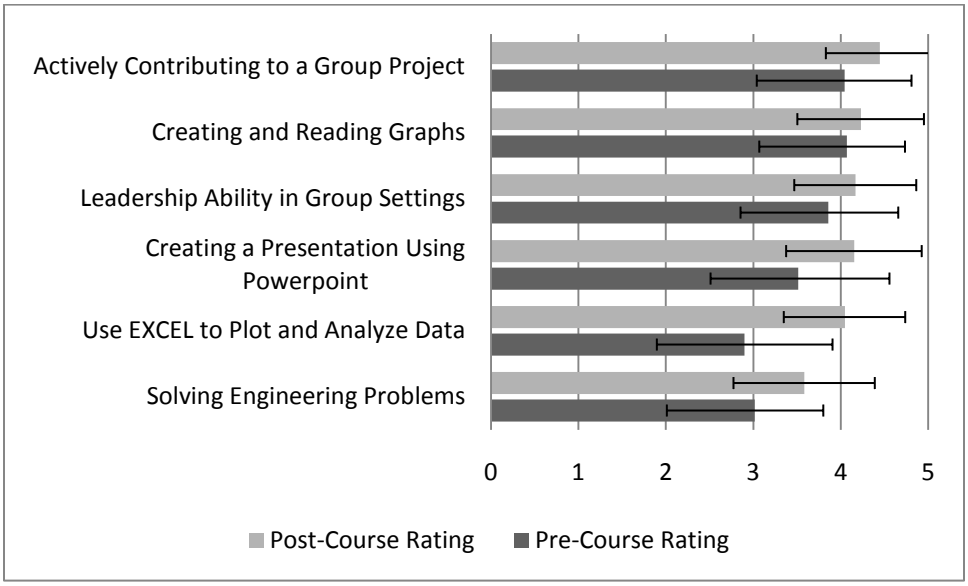


Figure 19. Student Assessment: Pre- and Post-Course Rating of Skills
Rating scale: 1 = Not skilled 3 = Some skill 5 = Very skilled

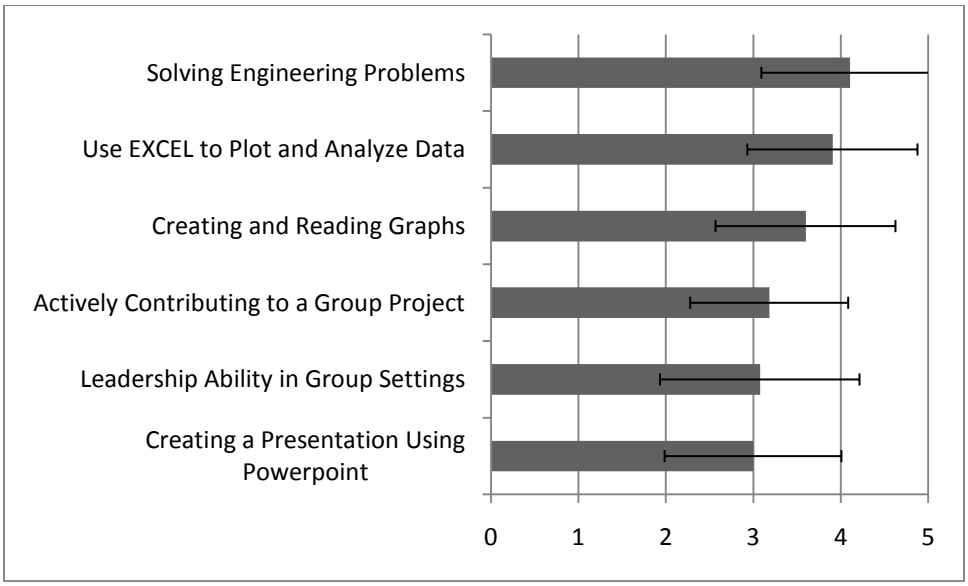


Figure 20. Student Assessment of Improvement in Skill Level
Rating scale: 1 = Did not improve 3 = Some improvement 5 = A great deal of improvement

On the post-course assessment students were asked to respond to some general attitude items which were created to correspond with overall course re-design objectives. The goal of these questions was to see if the course as a whole was having a positive effect on students. As shown in Figure 21, students showed very positive attitudes toward active learning (X=6.0 on 1-7 scale) and learning to analyze data (X=5.9). The students also responded well above neutral to items asking their comfort level in the department, the application of chemical engineering to everyday life, and learning to work effectively in groups. In essence, students responded positively to the items corresponding to overall objectives of the course.

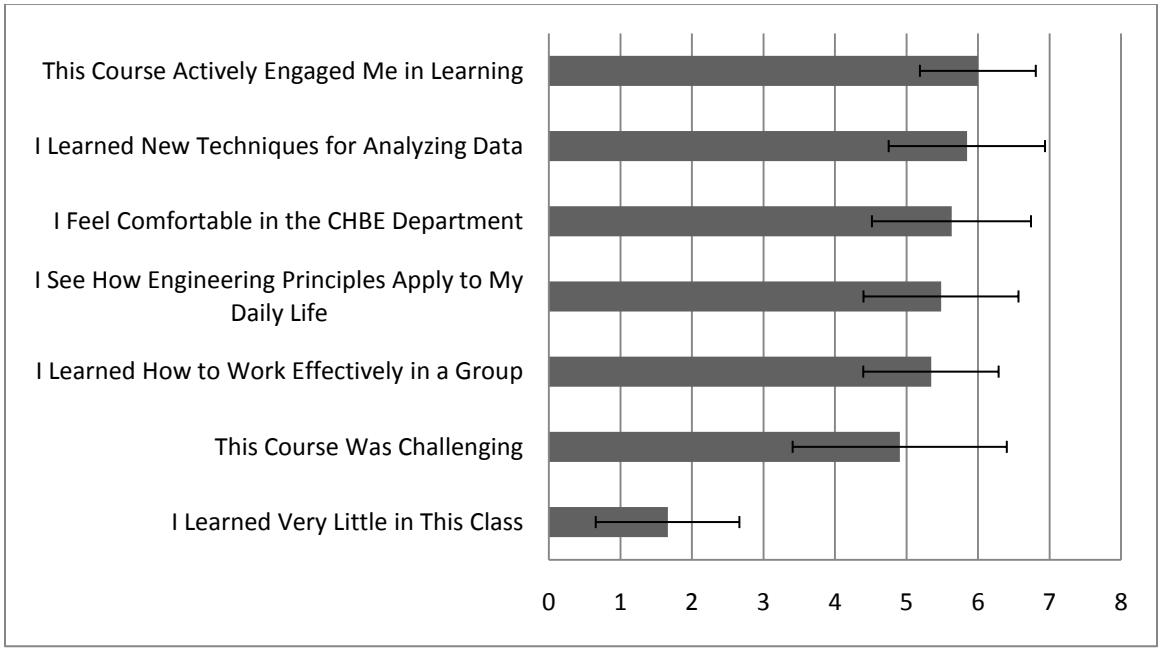


Figure 21. Student Assessment: General Impressions
Rating scale: 1 = Disagree strongly 4 = Neutral 7 = Agree strongly

Figure 22 presents the student ratings of learning in each of the main laboratory modules. Students reported learning a moderate to an extensive amount of learning in all

lab modules but rated the fluidized polymer bed lab the highest of the regular laboratories.

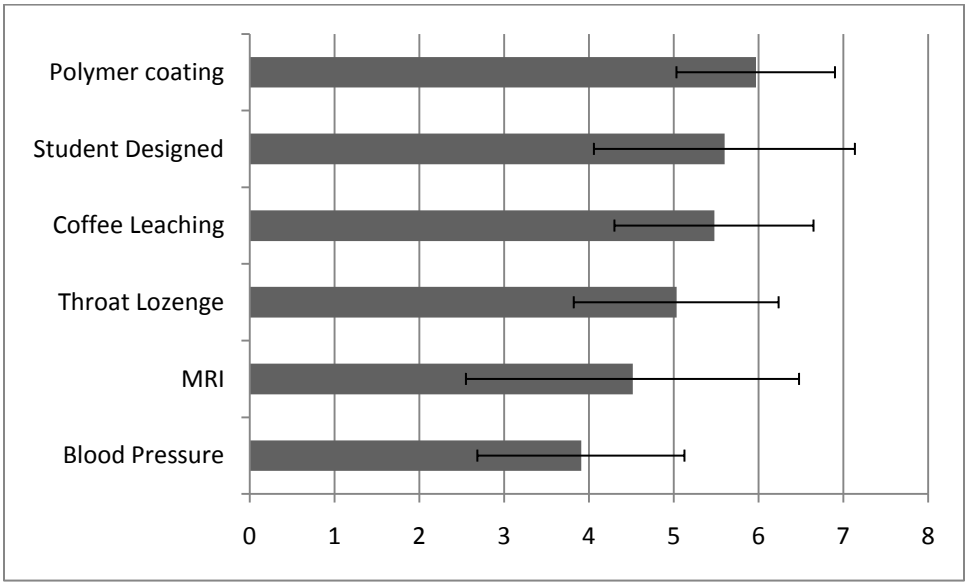


Figure 22. Student Assessment of Learning in Labs
Rating scale: 1 = Little to no learning associated with this lab 4 = A moderate amount of learning 7 = Extensive learning associated with this lab

Students rated the student-designed experiment as contributing to their learning, although for this particular lab, students responded on the full range of the scale, with some students rating the lab at a 1 and others at a 7. The average rating was 5.6. In the focus groups, students also disagreed on the value of this lab with one student commenting, "[The Student designed labs] took a little bit of extra effort. I think you got more back from it, as well, having put so much of ourselves into it. It combined all those skills we gathered throughout the semester." Other students remarked that the nature of this lab mimicked potential future experiences, both in required senior capstone projects

and in real-world work situations. They believed their experiences would be beneficial in preparing them for these future challenges.

The data in Table 11 is from the standard "end of course" student evaluation of the instructor. A comparison is made to the former "Freshman Seminar" course that consisted primarily of faculty led seminars about various aspects of chemical and biological engineering. In every category, the newly-designed course was evaluated in a more positive light than the previous, seminar-style course. One student had this observation:

> "I went to a high school with a buddy I played hockey with. He was three years older than I am. He came to MSU and he was in the chemical engineering degree as well. He said he didn't have this class or the class was a lot smaller than it is now. He really regrets not having this opportunity. I think it is a great way to start you into chemical engineering or any degree, no matter what it is. Just kind of ease you into it and give you a background of where we are going and what chemical engineers actually do instead of just throwing it all at you your sophomore year."

This anecdote corroborates the students' general impression that the course helped them feel comfortable in the department (seen in Figure 21).

Table 11. End of Course Student Evaluation.

The numerical rating system used in this evaluation is:
1 = Excellent
2 = Above Average
3 = Average
4 = Below Average
5 = Poor

Survey Question	Former Course Rating	Current Course Rating
Organization of the course was:	2.26	1.44
Presentation of the material was:	2.05	1.33
Was the instructor prepared?	1.74	1.03
Was the grading impartial and fair?	1.44	1.21
Was the instructor concerned for the student?	2.32	1.33
The instructor's ability to answer questions was:	1.79	1.21
The instructor's knowledge of the material was:	1.47	1.06
What overall rating do you give the instructor?	1.95	1.09
How useful to learning the material was the homework?	3.1	1.69
Did the course provide a good learning experience for you?	2.21	1.33
The resources (equipment, help) for the class were:	2.41	1.15
How does this course compare to other technical courses?	2.56	1.43
Average Rating for Course	2.07	1.27

MICROBIAL FUEL CELLS IN CHBE 100

The Experiment

A microbial fuel cell experiment was designed for CHBE 100 and was implemented in 2005. In the development of the initial microbial fuel cell module, it was determined that the fundamental principles to be taught would engage a student's understanding of current, resistance, power, and voltage. These concepts are generally discussed in a typical high school physics course and would later be readdressed in an introductory university level physics course.

A laboratory handout was given to students to help guide them through four workstations that highlighted different attributes and limitations of microbial fuel cells. The first station asked the students to assemble and disassemble the microbial fuel cell shown in Figure 11 (Rhoads, Beyenal and Lewandowski 2005, Menicucci, et al. 2006). The second station allowed the students to observe the operational microbial fuel cell. They identified any activity (bubbling, pumping, etc.) and used a multimeter to find the potential difference across the microbial fuel cell. Next, they found the current of the microbial fuel cell across a 10 ohm resistor. Finally, they repeated these measurements using a "D-sized" battery instead of the MFC. The third station showed a second type of microbial fuel cell being developed in the laboratory. Although this particular style of microbial fuel cell was not ultimately involved in generating any published data, the general principle of the microbial fuel cell design mimicked the multiple-anode design found in *Wireless Sensors Powered by Microbial Fuel Cells* (Shantaram, et al. 2005). The

final work station showed how voltage relates to power by lighting a small bulb using one, two, three, or four 1.5 Volt D-sized batteries connected in series. The handout for this laboratory is found in Appendix B.

Learning Objectives

The microbial fuel cell experiment was designed specifically to introduce students to a novel technology (microbial fuel cells) in a hands-on fashion. The learning objectives in this laboratory were to:

1. Demonstrate how potential, resistance, and current are related.
2. Explain how a microbial fuel cell works.
3. Explore the value and limitations of the microbial fuel cell technology.

Microbial Fuel Cell Laboratory Evaluation

The microbial fuel cell laboratory was evaluated by Dr. Betsey Palmer using the same methods as the other CHBE 100 laboratories. The microbial fuel cell laboratory is part of the 2005 evaluation cohort only, the MRI laboratory the 2004 evaluation cohort only, and all other laboratories as a combined 2004/2005 evaluation cohort. Normalization of laboratory evaluations by year and/or a comparison of the 2004 and 2005 cohort evaluations were not included in this analysis. Figure 23 shows the evaluation of all CHBE 100 laboratories in 2004-2005, including the microbial fuel cell laboratory.

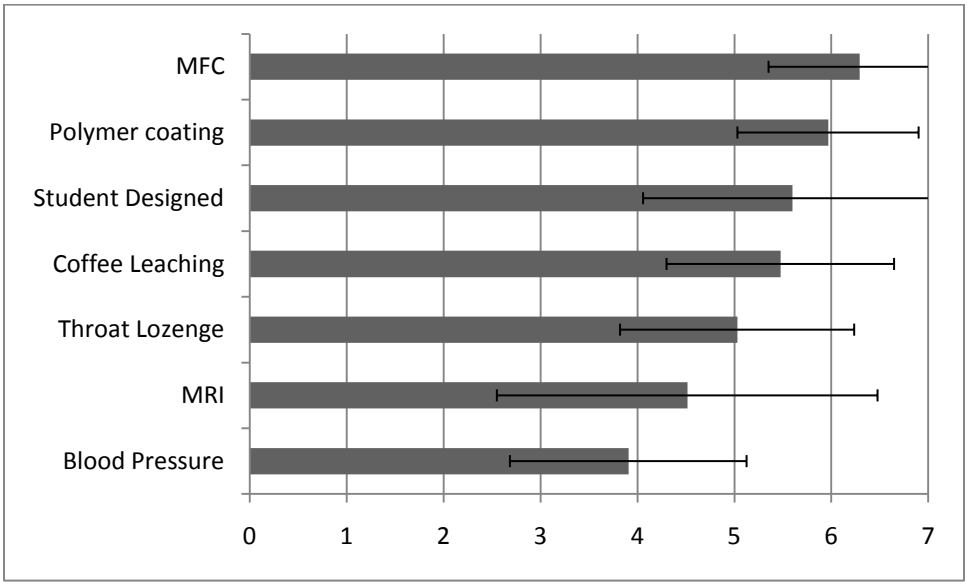


Figure 23. Updated Student Assessment of Learning in Labs
Rating scale: 1 = Little to no learning associated with this lab 4 = A moderate amount of learning 7 = Extensive learning associated with this lab

The microbial fuel cell laboratory received the highest average score from the students in 2004 and 2005, though this laboratory is not statistically different from any other laboratory except the blood pressure laboratory. One student did note about the microbial fuel cell lab, "If you are going to major in this business, it is something you could potentially be doing and it gave us a lab session where 'Hey, this is what life might look like in four years.'"

Finally, there is some legitimacy in questioning whether students learning in the microbial fuel cell laboratory can be attributed to the novelty of the experiment rather than the actual learning that takes place; however, Michael Prince makes an argument that this is not the case: "As a final cautionary note for interpreting reported results, some readers dismiss reported improvements from nontraditional instructional methods because they attribute them to the Hawthorne effect whereby the subjects knowingly

react positively to any novel intervention regardless of its merit. The Hawthorne effect is generally discredited, although it retains a strong hold on popular imagination (Prince 2004)".

Since 2005, three new laboratories have been developed and/or implemented for CHBE 100 by the new instructor and the student designed laboratory has seen significant changes. The new laboratories include a comparison of the viscosity of common household liquids, adding cream to coffee to observe Newton's Law of Cooling, and a laboratory allowing the students to produce biodiesel. The student designed laboratory has been more focused with students given specific tasks (design a vehicle and make it move using only a chemical reaction) or using only specific resources.

AN UPDATED MICROBIAL FUEL CELL EXPERIMENT

Introduction to the New Experiment

The purpose of this new laboratory is to afford the instructor the opportunity to easily implement microbial fuel cells and techniques to characterize them into an upper level hands-on chemical or biological engineering course.

The experiment described in the previous chapter offered an introduction to microbial fuel cells through demonstration in a research laboratory and work stations designed to provide a hands-on experience. This was adequate for the purpose of exposing students to new and novel research, but the next step is the design of a stand-alone microbial fuel cell experiment built into the course so that the students can truly experiment with a microbial fuel cell, and not just observe how it works.

The design of this new experiment gives students an opportunity to use state-of-the-art equipment as a motivating factor for learning and provides exposure to current research in their field. Moreover, this new experiment offers students the opportunity to characterize microbial fuel cells using traditional fuel cell metrics.

The layout of this chapter is such that the instructor can choose selections that fit with their desired experiment and can incorporate them as they see fit. As appropriate, suggestions for instruction pertaining to either chemical or biological engineering will be included. A complete description of the microbial fuel cell can be found in the research literature (Rhoads, Beyenal and Lewandowski 2005).

Supplies and Equipment Needed for Implementation in a CHBE Laboratory

Table 12 shows a list of equipment suggested for the implementation of microbial fuel cells into a chemical and/or biological engineering laboratory.

Table 12. Suggested Supplies and Equipment

For Fuel Cell Experiment	For Growth Media Preparation	For Data Collection and Analysis
Polycarbonate Microbial Fuel Cell (Rhoads, Beyenal and Lewandowski 2005)	Glucose	Saturated Calomel Electrode
J-Cloths	0.1mM NaOH	Personal Computer
Air Electrode (Menicucci, et al. 2006)	Na_2HPO_4	DataChart 1250 Data Logger
Glass Flow Breakers	Tryptone	Ohm Ranger Variable Resistance Box
Graphite Rods	Yeast Extract	Alligator Clip Connectors
Reticulated Vitreous Carbon (80 ppi)	KH_2PO_4	Multimeter
Cation Exchange Membrane	0.1 mM HCL	Stopwatch
Neoprene Tubing	Sodium Chloride	
Rubber Gaskets	*Klebsiella pneumoniae* stock culture	
Rubber Stoppers	Autoclave	
Masterflex Pumps	Autoclavable Vessels	
	Rotary Shaker	
	Flasks	
	Magnetic Stir Plates	
	Weigh Boats	
	Deionized Water	
	Balance	
	Magnetic Stir Bars	
	pH meter	

Learning Objectives

The suggested learning objectives are provided for the convenience of those instructors implementing this experiment in a hands-on chemical and/or biological engineering laboratory. They are not all-inclusive, but they do give an instructor a starting point from which to work. The specific learning objectives for students performing this experiment might include:

1. Demonstrate how potential, resistance, and current are related.
2. Explain the concept of power degradation.
3. Analyze data using a spreadsheet.
4. Perform a simple statistical analysis of data (calculate a mean and standard deviation).
5. Produce a voltage/current characterization of a microbial fuel cell.
6. Interpret analyzed data in order to "develop competence in an area of inquiry (Bransford and Brown 2000)".
7. Explain and calculate power limitation factors of a microbial fuel cell.

The implementation of this experiment not only provides a mechanism by which an instructor can address, at a minimum, seven learning objectives, but also introduces students to state-of-the-art research related to sustainable energy production.

For the sake of simplicity, the learning objectives and the suggested mechanisms by which an instructor can address them are found in Appendix C.

Pre-Experiment Activity

In order to "elicit from students their preexisting understanding of the subject matter to be taught (Bransford and Brown 2000)", a pre-experimental activity has been developed that will engage the student's understanding of resistance, voltage, and current. This exercise uses the students' knowledge of a common household device, the coffee maker, as a coffee leaching laboratory has been part of the CHBE 100 curriculum since the inception of the hands-on learning approach to the instruction of this course.

This exercise should take the students five to ten minutes to complete, but will allow a student to meet Learning Objectives 1 and 2, and will provide the framework of understanding that will later allow them to meet Learning Objective 5. The questions below should be given to the students to complete before they perform the experiment. Students will be asked to complete a post-experiment activity that will explore the difference between their expected results and the actual results. Below are the questions to be asked:

1. A coffee maker with a resistance of 10 ohms is plugged into a standard 110 V wall outlet. Calculate the current flowing through the coffee maker (remember that $V=I \cdot R$).
2. The same coffee maker has a switch that stops current from flowing (turning off the heating element). This is caused by the expansion of the metals as the temperature increases; by design the switch opens when the temperature reaches a certain point. When the temperature drops below that certain point, the metal again makes contact and current flows (this keeps your coffee warm!). Assume

that it takes 10 minutes to brew a full batch of coffee and that the switch opens immediately after the cycle has been completed. If the switch remains open for 15 minutes before turning closing for 5 minutes, plot the current used with respect to passing time for the brewing cycle and three off/on warming cycles.

3. A microbial fuel cell that has reached a steady-state cell potential of 0.5 V is to be used to power a coffee maker with a resistance of 10 ohms. Predict the current that will flow through the coffee maker immediately after you connect it to the microbial fuel cell.
4. Predict the current produced by this microbial fuel cell with respect to time if you were to attach the coffee maker and let it operate for 70 minutes (make a plot of the current with respect to time).

Step-by-Step Procedure and Timeline

After the instructor has their equipment and supplies, it will be important to prepare for the laboratory on a schedule so that the microbial fuel cells are ready for experimentation. Table 13 provides a schedule designed so that students can assemble the microbial fuel cells and prepare and/or autoclave growth media in the laboratory period a week before the microbial fuel cell laboratory if the instructor so chooses.

Table 13. Step by Step Procedure and Timeline

Task	Timeline
Soak Cation Exchange Membrane in 1 M NaCl	8 days prior to Laboratory Experiment
Assemble Microbial Fuel Cell	7 days prior to Laboratory Experiment
Prepare and Autoclave Growth Media	at least 5 days before Laboratory Experiment
Prepare Phosphate Buffer	at least 5 days before Laboratory Experiment
Begin Culture Growth	4 days, 18 hours before Laboratory Experiment
Batch Inoculation of MFC	4 days before Laboratory Experiment
Begin Continuous Flow Operation (0.3 mL/min)	1 day before Laboratory Experiment

A printable list of all of the tasks needed for the implementation of this laboratory into the course is included in Appendix D.

Post-Experiment Activity

The post-experiment activity is developed to help students meet the learning objectives. To that end, the following questions are suggested as a post-experiment activity or take-home assignment. The purpose of this activity is to "provide opportunities to build on –or challenge – the initial understanding (Bransford and Brown 2000)." The suggested questions directly address the students' initial understanding while engaging the students in a hands-on-activity representative of state-of-the-art research:

1. Look at the plot of current with respect to time at an external resistance of 10 Ω and compare it to your prediction for powering a coffee maker using a microbial fuel cell.
2. How does your prediction differ from your actual results? What is a possible explanation for this difference (Hint: look closely at your experimental data and evaluate any assumptions you made in your predictions)?

Significance of the Updated Microbial Fuel Cell Experiment

The significance of this new laboratory is found in its great teaching opportunities in both chemical engineering and biological engineering. In-class discussions following the experiment could specifically emphasize the chemical engineering aspects and/or the biological engineering aspects of operation of the microbial fuel cell. For example, a chemical engineer might indicate that mass transfer or internal resistance of the microbial fuel cell limits the current produced. A biological engineer might instead indicate that the rate of the biological oxidation of glucose is, in fact, limiting. Moreover, a

comprehensive discussion of microbial physiology would be appropriate in certain courses. Because upper level laboratories can incorporate higher level concepts of both chemical engineering and biological engineering, a discussion of mass transfer, internal resistance, and biological oxidation of glucose is appropriate. Figure 24 shows data that indicates that the activation polarization of the cell is increased by the addition of a redox mediator. Moreover, the rate of ohmic polarization is decreased with higher concentrations of redox mediators.

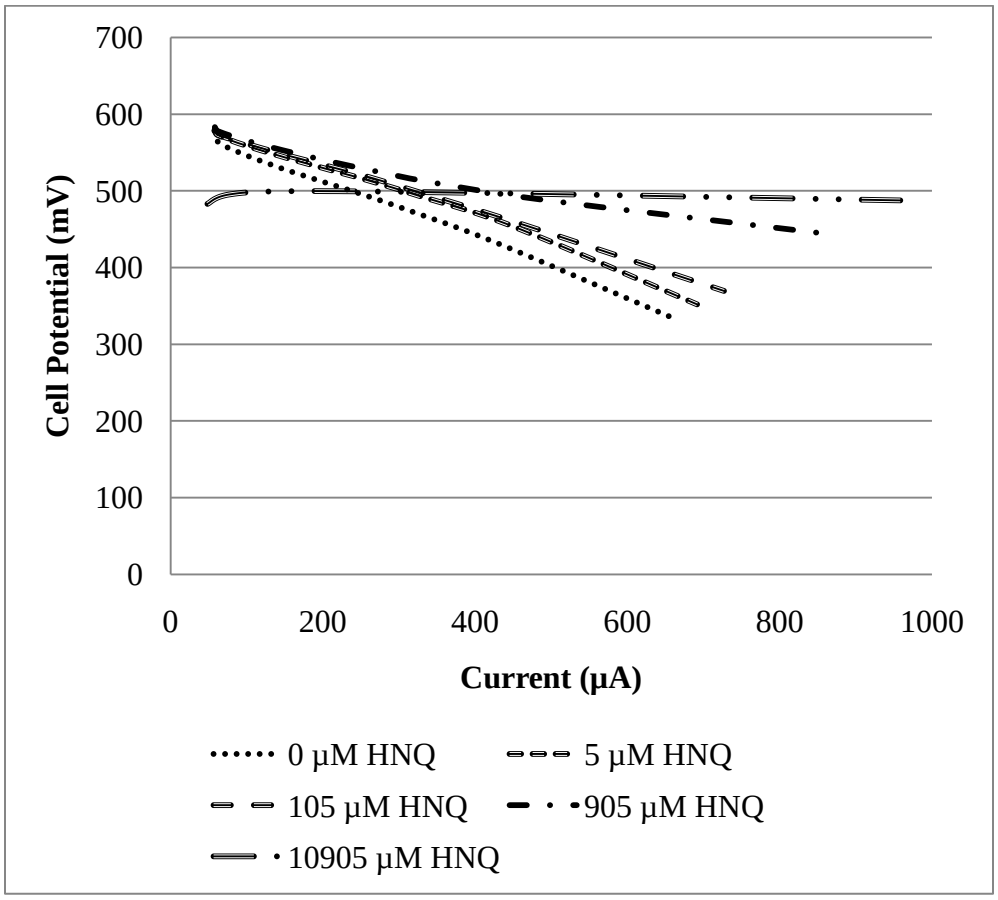


Figure 24. Effect of a Redox Mediator on the Activation and Polarization Losses in a Microbial Fuel Cell

Suggested Assessment

To assess the effectiveness of the implementation of microbial fuel cells into the chemical and biological engineering curriculum, one needs to implement an assessment mechanism by which the change can be judged. Fundamentally, the simplest evaluation mechanism would be the implementation of an examination question that addresses a fundamental principle associated with the microbial fuel cell experiment (mass transfer, transport phenomena, biological transport, etc.) that corresponds to the principle taught in the course.

To properly evaluate the change, some sort of control mechanism should be in place. For example, the instructor could choose write two different exam questions addressing the same principle; one question would be asked with regard to a microbial fuel cell and the other would not. The instructor could then distribute two versions of the exam and evaluate and compare the student performance on each individual exam questions. A statistically significant difference between student performance on the microbial fuel cell question and its counterpart question as compared to the performance of each cohort of students on the rest of the (same) exam, could indicate that the implementation of the hands-on experiment leads to different (and assumedly better) student learning, comprehension, and/or retention of materials.

Alternatively, the instructor could choose to offer pre- and post- tests for the evaluation of the hands-on experiment. Again, the evaluation of student learning due to the hands-on approach would need to be compared to student learning attributed to the course in general. As such, for the instructor to state that the implementation of the

hands-on experiment had a positive effect on student learning, the learning on this particular problem (as evaluated by graded pre- and post- exam problems) would have to be significantly (statistically) greater than on the other problems.

Moreover, for a complete assessment, an instructor might choose to collaborate with an instructor of a similar course at a different institution that has not yet implemented the hands-on experiment or collect data from a different cohort that has not had exposure to the hands-on experiment. Both cohorts would be given identical examination questions addressing the concepts related to or directly addressing the microbial fuel cell. An evaluation of the student performance on these questions normalized to their overall performance would give a clear indication of their learning due to their exposure to the hands-on experiment.

CONCLUSIONS

This dissertation is unique because it investigates two different aspects related to advancements associated with biological energy conversion: algal biofilm development and quantification of microbial fuel cell power generation. It also evaluates the implementation of a hands-on freshman laboratory in CHBE 100 at Montana State University and the evaluation of the implementation of a microbial fuel laboratory in this course. Finally, it presents a newly-designed microbial fuel cell laboratory appropriate for an upper-level chemical and/or biological engineering course.

The work done at Montana State and the Idaho National Laboratory laid a framework for a new vision of algal colonies: the biofilm. The scientific observation of algal surface attachment, detachment, and sloughing events are significant steps in classifying algal communities as biofilms. These phenomena were used, in part, to initially characterize bacterial biofilms.

There are parallels between the behavior of algal communities and bacterial biofilms. For instance, the observed algal sloughing event is reminiscent of the sloughing of an entire section of bacteria from a biofilm. The weak adherence on Teflon, a smooth, hydrophobic surface, is similar to weak bacterial biofilm adherence to smooth surfaces.

Further research into the structure, function, and phenotypic changes of the algae is needed for a complete characterization and classification, but these findings are a significant step in defining algal communities as biofilms.

Next, a simple technique was identified to identify the maximum sustainable power generated by microbial fuel cells. This technique can easily be used to report the

cell performance under a defined set of operational conditions. Moreover, experiments run at a constant resistance over longer periods of time (four hours or longer) verified the results of the technique. In these experiments, the cell potential and current decreased significantly when the applied resistance was lower than the resistance identified as yielding the sustainable power. The cell potential and current remained constant (neglecting the effect of power degradation) when the applied resistance was equal to or greater than this resistance.

This procedure was important in the microbial fuel cell community for a number of reasons. First, it addressed the inherent power limitations in the microbial fuel cells. Prior to this paper, most researchers studying MFCs make a point of estimating the power generated by these devices; some just measured the current by applying an arbitrarily selected external resistance and measuring instantaneous current and potential difference. Such instantaneous measurements estimate the power generated at the time of the measurement, but such data could not be used to determine the maximum sustainable power. Also, these previously-used approaches did not allow for the comparison of various devices and various operational procedures because the choice of the external resistor is arbitrary, and the power generated by the fuel cell is dependent upon that choice. Microbial fuel cells characterized by maximum sustainable power can be directly compared to each other.

The discussion of this procedure and its impact in the microbial fuel cell community now includes a more traditional voltage/current characterization of the microbial fuel as well as a discussion of the power limiting factors in a microbial fuel

cell. Specifically, the maximum theoretical number of electrons that could be liberated in a specific microbial fuel cell was calculated so the actual generated power could be compared to the maximum theoretical power.

The implementation of a hands-on style of teaching CHBE 100: Introduction to Chemical and Biological Engineering was initiated in 2003 with the hope that it would better meet the needs of the students by improving their learning in the course. Through hands-on laboratories and follow-up seminars, the students were also introduced to the breadth of traditional and non-traditional careers available to graduates in chemical engineering. A unique aspect of this laboratory based course is that most concepts are taught in an inductive learning format. Students were asked to predict experimental outcomes, perform the experiments, plot and analyze the data, and compare results to their predictions before being exposed to the underlying theory and predictive calculations.

The evaluation of the students' post-test of attitudes and self-reported skills indicates that the new course format has better prepared students for their education in chemical engineering than did the previous seminar style course. Students report that they feel actively engaged in the learning and feel comfortable in the department. Students also felt that their problem solving skills had improved over the course of the semester.

A hands-on microbial fuel cell laboratory experiment was designed and implemented in CHBE 100. Students identified this microbial fuel cell laboratory as providing significant learning. This experiment was updated to include both the assembly of a microbial fuel cell as well as the characterization of a microbial fuel cell. This

experiment has the potential to engage students in both chemical engineering and biological engineering. The experiment is designed with specific learning objectives and supporting activities that will engage a student's initial understanding of the material. This facilitates learning in a manner outlined in How People Learn: Brain, Mind, Experience, and School (Bransford and Brown 2000).

The experiment was also designed to allow an instructor to emphasize the area(s) of chemical and biological engineering of his/her choosing while introducing students to a novel research topic in a hands-on fashion.

APPENDICES

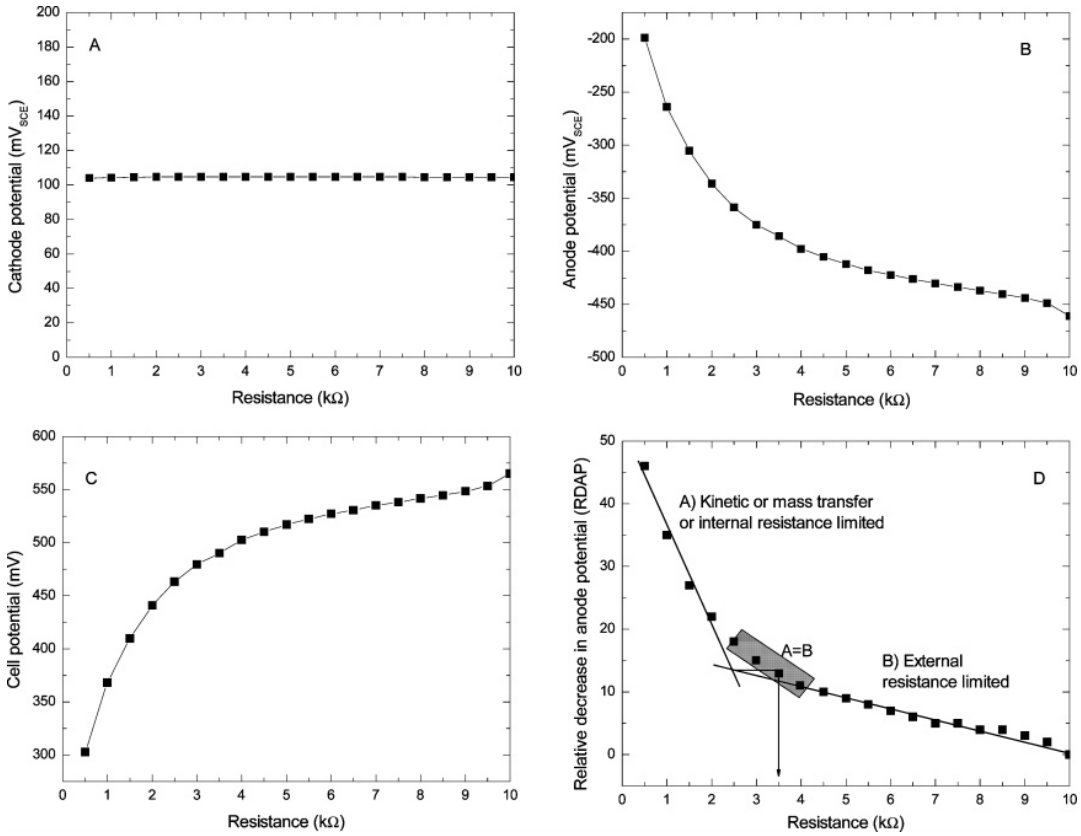
APPENDIX A

A GRAPHICAL ESTIMATION OF SUSTAINABLE CONDITIONS

Using the initial anodic potential ($E_{o,anodic}$) and anodic potentials at each applied external resistance, the relative decrease in anodic potential is defined by the authors as:

$$\text{relative decrease in anodic potential (\%)} = \frac{E_{0,anodic} - E_{anodic}}{E_{0,anodic}} X\ 100$$

The relative decrease in anode potential (RDAP) is used to estimate the maximum sustainable power graphically, as seen in the figure below. From the data in the figure, the potentials of the cathode (A), anode (B), and cell (C) and the relative decrease in anode potential (D) are plotted against the external resistance. The cathodic potential is constant (A) at each external resistance, showing that the current is limited by the anode (Sawyer, Heineman and Beebe 1984). The cell potential decreased significantly when a resistance less than 3 kΩ is applied (B and 15C). The standard deviations of the data points are very small relative to the cell potential. They ranged from a minimum of 0.11% of the cell potential at 7.5 kΩ to a maximum of 3% of the cell potential at 0.5 kΩ.



Effect of External Resistance on the (A) Cathodic, (B) Anodic, and (C) Cell Potentials. Anodic and Cathodic Potentials are Presented Against a Saturated Calomel Electrode (SCE). The Resistance Scan Rate was 500 Ω/60 s in All Experiments. (D) The Variation of Percent Deviation of Anodic Potential With Respect to Applied External Resistance. The Linear Fit at High External Resistances (Region B) Represents a Region in Which the External Resistance Controls the Power. The region at low external resistances (Region A) represents a region in which the power is limited by kinetics, mass transfer, or internal resistance.

The relative decrease in anodic potential (RDAP) is used to select the external resistor to measure the maximum sustainable power of this microbial fuel cell (D). When external resistance is high, the RDAP increases linearly with decreasing external resistance because the electron delivery to the cathode is limited by external resistance (region B in D). However, when a low external resistance is applied, the electron delivery to the cathode is limited by any one of the power limiting factors discussed in the

introduction (region A in D), and the RDAP increases with decreasing external resistance. However, the RDAP increases with decreased external resistance, with different slopes, for external resistance limited or internal resistance limited conditions. The conditions where external and internal resistance limitations are equal is somewhere between these two lines, which is presented as a shaded area (resistances between 2.5 and 4 kΩ). When line A and line B intersect, a horizontal line is drawn from the intersection to estimate the external resistor that allows for the measurement of sustainable power. Experiments showed that any external resistance between 2.5 and 4 kΩ provides very close power values (less than 20% difference between them, so that the selection of an external resistor between 2.5 and 4 kΩ does not produce a significant error in the estimation of sustainable power). However, the power remained constant for long period of time only when a 4 kΩ or larger resistor was used. Although this graphical procedure indicated that a 3.5 kΩ resistor should be used to obtain sustainable power, there is less than a 10% difference in the power generated when a 3.5 or 4 kΩ resistor was used. Therefore, a 4 kΩ resistor was used to measure power of this microbial fuel cell.

APPENDIX B

MICROBIAL FUEL CELL LAB: INTRODUCTION TO MICROBIAL FUEL CELLS

Today's laboratory is intended to serve as a hands-on introduction to microbial fuel cells. The biological fuel cell was described in 1969 as an "electrochemical energy converter" (Bockris & Srinivasan, 1969). In the 1990's, Allen and Bennetto described a microbial fuel cell as able to withdraw electrons from the oxidation of a carbohydrate (glucose) as electrical energy (Allen & Bennetto, 1996). A microbial fuel cell, like other electrochemical cells, has both an anode and a cathode, separated by a semi-permeable cation exchange membrane. Bacteria in the anodic compartment produce electrons through a microbial oxidation reaction. These electrons are consumed in the cathodic compartment for the reduction of another chemical species, often oxygen. The flow of electrons occurs through an external load in order to produce power. The flow of electrons is balanced with a flow of cations through the semi-permeable membrane. A microbial fuel cell is different from other fuel cells because the produced power is low compared to other conventional fuel cells. Either microbial or chemical, the power of the fuel cell is the most important parameter since it is used 1) to determine efficiency of the fuel cells, 2) to compare efficiency of the cells and, 3) used to compare different fuel cells.

The power of a microbial fuel can be measured by applying an external load (generally an external resistor) and measuring the potentials and the current.

The figure below shows a typical set-up of a microbial fuel cell. The cathodic side of the microbial fuel cell includes a saturated calomel electrode. This electrode is used to compare the potential in the microbial fuel cell to a known potential. The cathode itself is often a carbon (graphite) rod. The anodic side of the microbial fuel cell has living

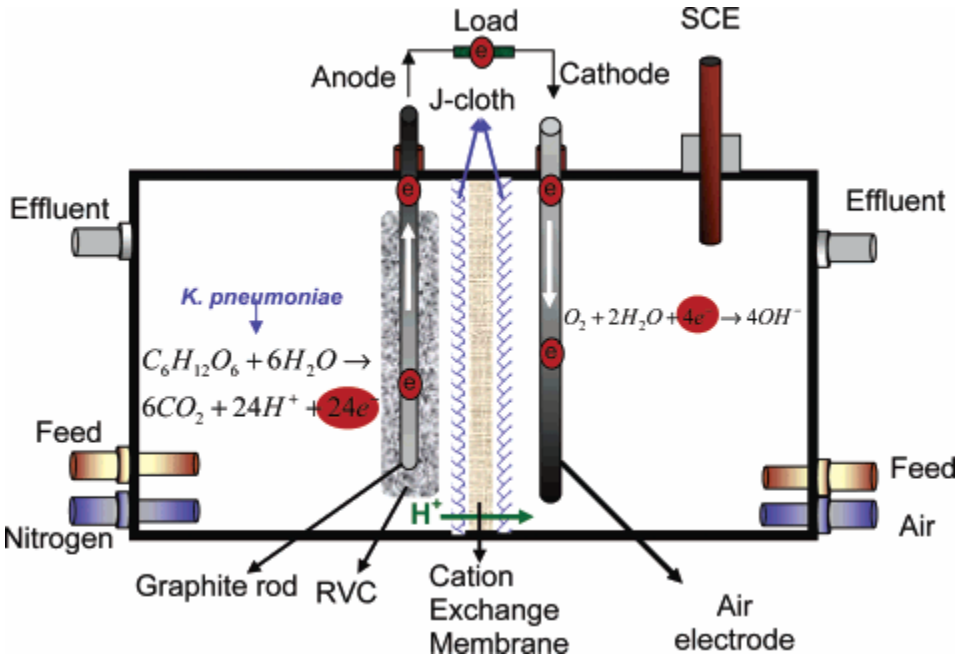
bacteria inside. These bacteria consume glucose and produce electrons. These electrons travel from inside the bacteria to the anode (often a graphite rod) and move to the cathode through a load. Examples of this load might be a light bulb, a resistor, or a chemical sensor. The flow of electrons from the anode to the cathode is called the current. The current is calculated using Ohm's Law:

$$V = I \cdot R$$

In this equation, V is the potential difference between the anode and the cathode (in Volts), I is the current (in Amps), and R is the resistance applied (in Ohms). The power in a microbial fuel cell is calculated using the following equation:

$$P = I^2 \cdot R$$

If calculated using Amps for current and Ohms for resistance, the resultant power has units of Watts.



The Microbial Fuel Cell

Station 1: Assembly and Disassembly Station

The idea behind this station is to be able to look at the components of a microbial fuel cell. Look closely at the assembled microbial fuel cell and the disassembled components of the microbial fuel cells. Take some time to assemble a microbial fuel cell using the already assembled cell as your guide.

Make a sketch of a microbial fuel cell and all of its components. Label all of the components. Include in your diagram a description of how the electrons flow in a microbial fuel cell.

Station 2: The Operational Microbial Fuel Cell

Look at the operational microbial fuel cell. Briefly identify any activity (bubbling, pumping, etc.) and when you have completed Station 1, include this information on your overall microbial fuel cell diagram. Use a multimeter to find the potential difference across the microbial fuel cell and use a 10 ohm resistor to find the current of the microbial fuel cell. Repeat these measurements using a "D-sized" battery.

Compare the potential of the microbial fuel cell to the potential of the battery. Explain the differences in terms of the maximum power that can be produced. Compare the current produced by the microbial fuel cell to that produced by the battery when the 10 ohm resistor is included in the circuit. Why does the current from the microbial fuel cell decrease with respect to time?

Calculate the current expected for the fuel cell and the battery using Ohm's Law. Use the potentials found in question 1 and the given resistor value. Compare these results to those found in question 2.

Station 3: Application of Microbial Fuel Cells I

These stations introduce a number of ways that microbial fuel cells may be further optimized in order to maximize their power. You will be asked a number of questions regarding these methods of optimization.

Carefully look at the column-type microbial fuel cell. Make a quick sketch and label each of the major components (anode, cathode, etc.).

Each stainless steel ball is an anode in the column type microbial fuel cell. There is only one cathode, at the top of the cell. Will there be differences in the current produced between each anode and the cathode? Why?

Station 4: Application of Microbial Fuel Cells II

Four "D-cell" batteries are connected in series in order to provide power to light a small bulb. Each switch increases the number of batteries involved in the powering of the light bulb. Switch 1 has only one battery, Switch 2 includes two batteries, and so on. Diagram the system and relate the number of batteries used to the brightness of the bulb. What is causing the change in brightness in the bulb?

The resistance of the light bulb is 0.7 ohm. Calculate the current passing through the light bulb and calculate the power used by the bulb for each of the four battery configurations. Explain how a series of microbial fuels might be different from a single microbial fuel cell.

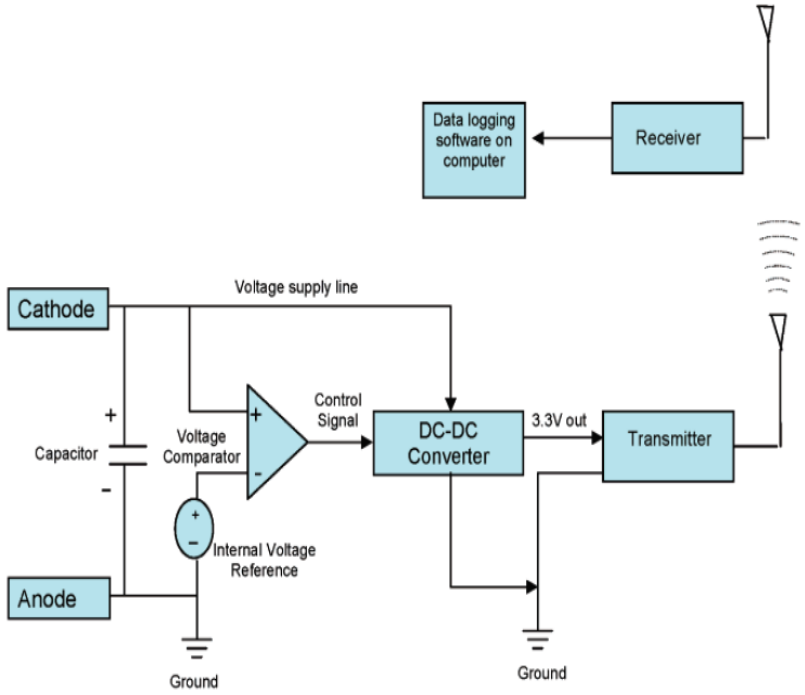
Putting It All Together: What Have You Learned?

A microbial fuel cell is different from other fuel cells because the produced power is low compared to other conventional fuel cells. However, an optimized microbial fuel

cell has been shown to be able to power a chemical sensor in order to monitor the concentration of heavy metals in natural bodies of water. How would you “optimize” a microbial fuel cell? What parameters can you control? What can’t you control?
The ability of microbial fuel cells to operate for very long periods of time makes them ideal for applications where power needs to be provided in small quantities but continuously. List five or six applications that you can think of that require very little power but have a practical use.

Microbial fuel cells also are thought to have greatest use when used in remote environments. List five or six remote environments that we, as scientists and engineers, have interest in.

A microbial fuel cell using the natural bacteria in a river was able to power a chemical sensor. The data collected was transmitted via satellite to a receiver and was accessible on the internet. A diagram of this process is shown in Shantaram, et al. (Shantaram, et al. 2005).

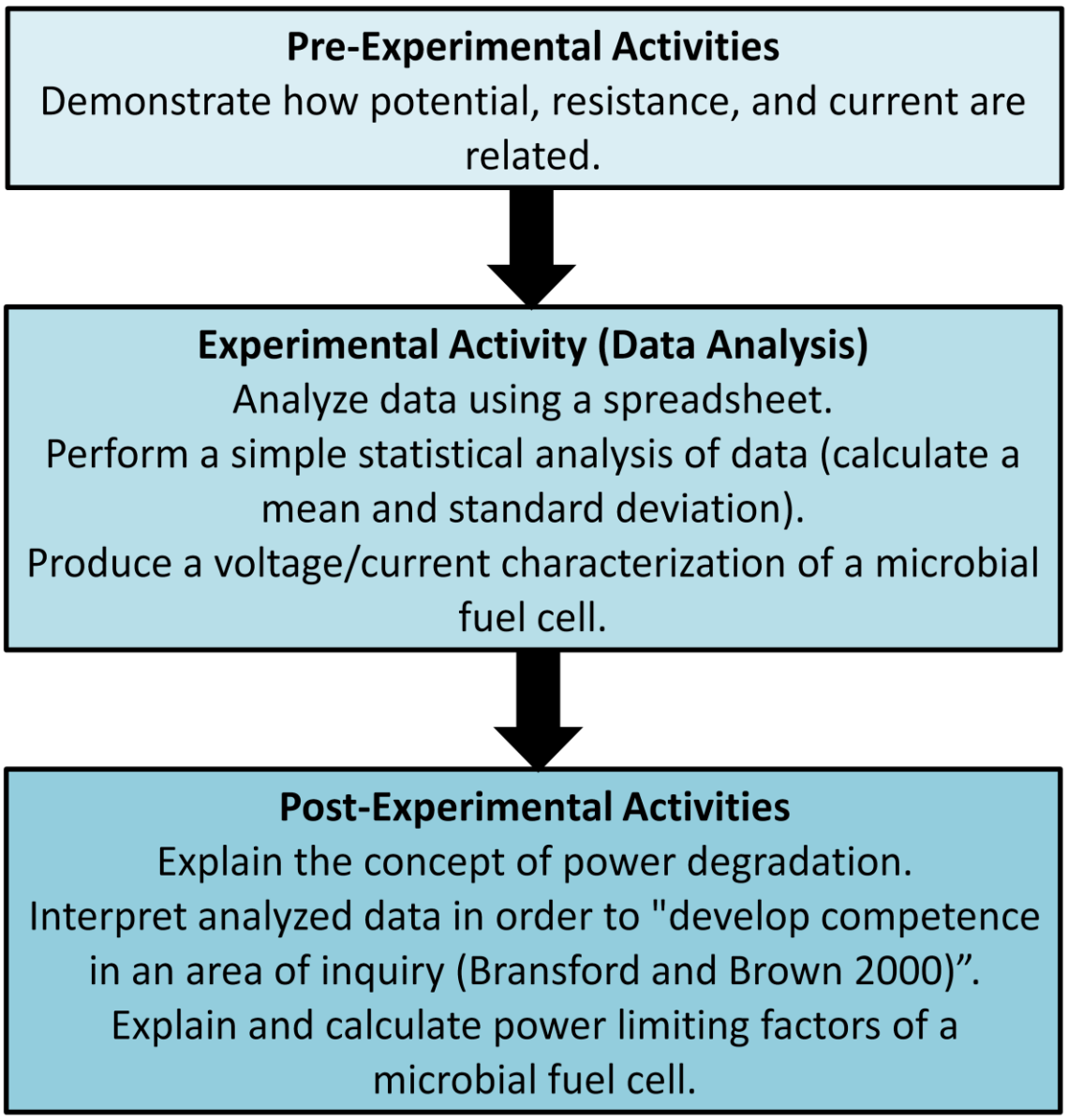


Block Diagram of the Telemetry System Powered by a Microbial Fuel Cell (Shantaram, et al. 2005). Used With Permission.

The ability to sit in a laboratory thousands of miles away from a body of water be able to monitor its condition is an example of how microbial fuel cells can be used in a remote environment. On the next page, do your best to sketch an application for a microbial fuel cell that is of your own design. Explain why you must be able to continuously provide power (sustainable power) using the microbial fuel cell as well as why a microbial fuel cell might be more appropriate than a traditional power source (battery, etc.) for this application.

APPENDIX C

IMPLEMENTATION OF LEARNING OBJECTIVES


Pre-Experimental Activities
Demonstrate how potential, resistance, and current are related.
Experimental Activity (Data Analysis)
Analyze data using a spreadsheet.
Perform a simple statistical analysis of data (calculate a mean and standard deviation).
Produce a voltage/current characterization of a microbial fuel cell.
Post-Experimental Activities
Explain the concept of power degradation.
Interpret analyzed data in order to "develop competence in an area of inquiry (Bransford and Brown 2000)".
Explain and calculate power limiting factors of a microbial fuel cell.

APPENDIX D

STEP-BY-STEP PROCEDURE FOR THE IMPLEMENTATION OF A MICROBIAL FUEL CELL EXPERIMENT INTO AN INTRODUCTORY CHEMICAL AND BIOLOGICAL ENGINEERING COURSE

Preparation of the Growth Media for the Anodic Compartment:

1. Place 10 g/L tryptone, 5 g/L yeast extract, 5 g/L sodium chloride, 9.12 g/L Na_2HPO_4, 1.75 g/L KH_2PO4, and 1 g/L glucose into an autoclavable vessel.
2. Add a magnetic stir bar and stir on a magnetic stir plate until fully dissolved.
3. Autoclave at 121^{o}C for at least 30 minutes.

Preparation of the Stock Culture:

1. Add 1 mL of a frozen culture of *K. pneumoniae* to 100 mL of growth media.
2. Place at room temperature on a rotary shaker (150 rpm) and allow growth overnight (18 h).

Preparation of the Phosphate Buffer:

1. Add 1.825 g/L of Na_2HPO_4 and 0.35 g/L of KH_2PO_4 to deionized water.
2. Adjust to a pH of 7.2.

Preparation of the Microbial Fuel Cell:

1. Soak the cation exchange membrane in 1 M NaCl for at least 24 hours.
2. Clean the microbial fuel cell thoroughly in deionized water.
3. Fill both compartments of the cell with deionized water.
4. Place a rubber stopper in the open port used to house the reference electrode.
5. Autoclave the MFC at 121^{o}C for at least 20 minutes.
6. Remove the rubber stopper and insert a saturated calomel electrode (SCE).
7. Drain the water in the anodic and cathodic compartments.
8. Pump growth medium into the anodic compartment of the cell until full, then cease pumping.
9. Pump phosphate buffer into the cathodic compartment of the fuel cell until full, then cease pumping.
10. Inoculate 60 mL of *K. pneumoniae* stock culture into the cell, replacing 60 mL of the sterile medium.
11. Allow the bacteria to grow in batch mode until the anodic potential stabilizes (~-450 mV_{SCE}). This will take up to five days.
12. Pump sterile medium into the anodic compartment at a flow rate of 0.3 mL/min for the duration of the experiments.

Preparation of Data Logger:

To Find the Potential Measurements (Menicucci, et al. 2006):

1. Connect the anode to potential channel 1 of the data logger.
2. Connect the cathode to potential channel 2 of the data logger.
3. Connect the SCE to the common terminal of the potential channels.

Preparation of Data Logger:

To Find the Current Measurement (Menicucci, et al. 2006):

1. Connect the anode and the cathode in series to the current channel through a variable resistor box.
2. Configure the data logger so it will collect potential and current measurements every ten seconds.
3. Start the data logger.
4. Set the variable resistance box so the total external resistance is equal to 10 kΩ
5. Start a stopwatch after making an electrical connection between the microbial fuel cell and the variable resistance box.
6. After 60 seconds, decrease the external resistance by 0.5 kΩ.
7. Repeat until the external resistance reaches 0.5 kΩ.
8. After 60 seconds, decrease the external resistance by 50 Ω.
9. Repeat until the external resistance reaches 50 Ω.
10. After 60 seconds, decrease the external resistance to 10 Ω.
11. Collect data for 10 minutes at 10 Ω.
12. Remove the connection between the microbial fuel cell and the variable resistance box.
13. Stop the data logger.
14. Export the data to Microsoft Excel (or a comparable spreadsheet).

Data Analysis:

1. Hide all columns except those that contain the measurement time, anodic potential, cathodic potential, and measured current.
2. Convert the potentials from units of V to units of mV.
3. Convert the current from units of A to units of μA.
4. Find the mean (and standard deviation) of each potential and current measurement at each external resistance.
5. Plot current vs. time.
6. Plot the cell potential vs. the current.

REFERENCES CITED

Aglan, H.A. and S.F. Ali. "Hands-On Experiences: An Integral Part of Engineering Curriculum Reform." *Journal of Engineering Education*, 1996: 327-330.

Akin, C., A. Maka, S. Patel, J. Conrad, and J. Benemann. *Removal of CO_2 from Flue Gases by Algae.* Chicago, IL: Institute of Gas Technology, 1993.

Allen, R.M., and H.P. Bennetto. "Microbial Fuel-Cells: Electricity Production from Carbohydrates." *Applied Biochemistry and Biotechnology* 39, no. 1 (1993): 27-40.

Amin, S.A., D.H. Green, M.C. Hart, F.C. Kupper, W.G. Sunda, and C.J. Carrano. "Photolysis of Iron–Siderophore Chelates Promotes Bacterial–Algal Mutualism." *Proceedings of the National Academy of Sciences of the United States of America* 106 (2009): 17071-17076.

Anderl, J.N, J. Zahller, F. Roe, and P.S. Stewart. "Role of Nutrient Limitation and Stationary-Phase Existence in *Klebsiella pneumoniae* Biofilm Resistance to Ampicillin and Ciprofloxacin." *Antimicrobial Agents and Chemotherapy* 47 (2003): 1251-1256.

Banerjee, A., R. Sharma, Y. Chisti, and U.C. Banerjee. "*Botryococcus braunii* : A Renewable Source of Hydrocarbons and Other Chemicals." *Critical Reviews in Biotechnology*, 2002: 245-279.

Bennetto, H.P., and G. Delaney. "Microbial Fuel-Cells." *Process Biochemistry* 18, no. 4 (1983): R17.

Bockris, John O'M, and Supramaniam Srinivasan. *Fuel Cells: Their Electrochemistry.* 1st Edition. New York: McGraw-Hill Book Company, 1969.

Bond, D.R., and D.R. Lovley. "Electricity Production by *Geobacter sulfurreducens* Attached to Electrodes." *Applied and Environmental Microbiology* 69, no. 3 (2003): 1548-1555.

Bransford, J.D., and A.L. Brown. *How People Learn: Brain, Mind, Experience, and School.* Washington, D.C.: National Academy Press, 2000.

Casadevall, E., D. Dif, C. Largeau, C. Gudin, D. Chaumont, and O. Dasanti. "Studies on Batch and Continuous Cultures of *Botryococcus braunii*: Hydrocarbon Production in Relation to Physiological State, Cell Ultrastructure, and Phosphate Nutrition." *Biotechnology and Bioengineering* 27 (1985): 286-295.

Chang, I. S., et al. "Continuous Determination of Biochemical Oxygen Demand Using Microbial Fuel Cell Type Biosensor." *Biosensors and Bioelectronics* 19, no. 6 (2004): 607-613.

Characklis, W.G., and K.C. Marshall. *Biofilms.* Edited by W.G. Characklis and K.C. Marshall. New York: John Wiley & Sons, 1990.

Chaudhuri, S.K., and D.R. Lovley. "Electricity Generation by Direct Oxidation of Glucose in Mediatorless Microbial Fuel Cells." *Nature Biotechnology* 19, no. 6 (2003): 1229-1232.

Chirac, C., E. Casadevall, C. Largeau, and P. Metzger. "Bacterial Influence Upon Growth and Hydrocarbon Production of the Green Alga Botryococcus braunii." *Journal of Phycology* 21 (1985): 380-387.

Choi, Y., E. Jung, H. Park, S. R. Paik, S. Jung, and S. Kim. "Construction of Microbial Fuel Cells Using Thermophilic Microorganisms, *Bacillus licheniformis* and *Bacillus thermoglucosidasius*." *Bulletin of the Korean Chemical Society* 25 (2004): 813-818.

Cobb, J. T., et al. "How Is the Marketplace Changing for Chemical Engineering?" *AIChE Centennial Book*, 2008: Chapter 24.

Crispim, C.A, P.M. Gaylarde, and C. C. Gaylarde. "Algal and Cyanobacterial Biofilms on Calcareous Historic Buildings." *Current Microbiology* 46 (2003): 79-82.

Dahm, K., and R.P. Hesketh. "Two Experiments for the Introductory Chemical Reaction Engineering Course." *Education for Chemical Engineers* 3, no. 1 (2007): e1-e5.

Dahm, K., R.P. Hesketh, and M.J. Savelski. "Micromixing Experiments in the Introductory Chemical Reaction Engineering Course." *Chemical Engineering Education*, 2005: 94-99.

Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J. W., and Greensberg. "The Involvement of Cell-to-Cell Signals in the Development of a Bacterial Biofilm." *Science* 280 (1998): 295-298.

Dewan, A., H. Beyenal, and Z. Lewandowski. "Intermittent Energy Harvesting Improves the Performance of Microbial Fuel Cells." *Environmental Science and Technology* 43 (2009): 4600-4605.

Dresselhaus, M.S., and I.L. Thomas. "Alternative Energy Technologies." *Nature* 414 (November 2001): 332-337.

Eberly, W.R. "Problems in the Laboratory Culture of Planktonic Blue-Green Algae." In *Environmental Requirements of Blue-Green Algae*, 7-34. Corvallis, OR: Federal Water Pollution Control Administration, 1966.

EG & G Services. *Fuel Cell Handbook.* 7th. Morgantown, WA: U.S. Dept. of Energy, Office of Fossil Energy, National Energy Technology Laboratory, 2004.

Farrell, S., and R.P. Hesketh. "An Introduction to Drug Delivery for Chemical Engineers." *Chemical Engineering Education* 36, no. 3 (2002): 198-215.

Felder, R.M., and R.W. Rosseau. *Elementary Principles of Chemical Processes.* New York: John Wiley and Sons, 2000.

Fernandes, H.L., F. Lupi, M.M. Tome, I. Sa-Correia, and J.M. Novais. "Rheological Behavior of the Culture Medium During Growth of the Microalga *Botryococcus braunii*." *Bioresource Technology* 38 (1991): 133-136.

Gilbert, P., A.J. McBain, and A.H. Rickard. "Formation of Microbial Biofiilm in Hygienic Situations: A Problem of Control." *International Biodeteriation and Biodegradation* 51 (2003): 245-248.

Gorby, Y.A., et al. "Electrically Conductive Bacterial Nanowires Produced by *Shewanella oneidensis* Strain MR-1 and Other Microorganisms." *Proceedings of the National Academy of Sciences of the United States of America* 103 (2006): 11358-11363.

Gzebyk, M., and G.P. Pozniak. "Microbial fuel cells (MFCs) with Interpolymer Cation Exchange Membranes." *Separation and Purification Technology* 41, no. 3 (2005): 321-328.

Hall-Stoodley, L., and P. Stoodley. "Developmental Regulation of Microbial Biofilms." *Current Opinion in Biotechnology* 13 (2002): 228-233.

Hamlen, R.P., and T.B. Atwater. *Handbook of Batteries.* New York: McGraw-Hill Professional, 2001.

Hartman, H., and M. Hartman. "Leaving Engineering: Lessons from Rowan University's College of Engineering." *Journal of Engineering Education,* 2006: 49-61.

He, Z., N. Wagner, S.D. Minteer, and L.T. Angenent. "An Upflow Microbial Fuel Cell with an Interior Cathode: Assessment of the Internal Resistance by Impedance Spectroscopy." *Environmental Science and Technology* 40 (2006): 5212-5217.

He, Z., S.D. Minteer, and L.T. Angenent. "Electricity Generation from Artificial Wastewater Using an Upflow Microbial Fuel Cell." *Environmental Science and Technology* 39, no. 14 (2005): 5262-5267.

Hesketh, R.P. "Wake-Up to Engineering." *Chemical Engineering Education* 30, no. 3 (1996).

Hesketh, R.P., C.S. Slater, S. Farrell, and M. Carney. "Fluidized Bed Polymer Coating Experiment." *Chemical Engineering Education* 36, no. 2 (2002): 138-143.

Hillen, L.W., G. Pollard, L.V. Wake, and N White. "Hydrocracking of the Oils of *Botryococcus Braunii* to Transport Fuels." *Biotechnology and Bioengineering* 24 (1982): 193-205.

Jahnke, J., and U.B. Priefer. "Phototrophic Biofilms of Restored Fields in the Rhenish Lignite Mining Area: Development of Soil Algal, Bacterial, and Fungal Biomasses." *Soil Biology and Biochemistry* 34 (2002): 1157-1165.

Jang, J. K., et al. "Construction and operation of a novel mediator and membrane-less microbial fuel cell." *Process Biochemistry* 39, no. 8 (2004): 1007-1012.

Jarvie, H.P., et al. "Phosphorus Uptake Into Algal Biofilms in a Lowland Chalk River." *The Science of the Total Environment* 282-283 (2002): 353-373.

Jayawickramarajah, Narendren. "Characterization of Surface Colonization by Microalgae Using *Botryococcus braunii* and *Dunaliella tertiolecta*." Bozeman, MT: Montana State University-Bozeman, 2003.

Kim, H.J., Park, H.S., H.S. Hyun, I.S. Chang, M. Kim, and B.H. Kim. "A Mediator-Less Microbial Fuel Cell Using a Metal Reducing Bacterium, *Shewanella putrefaciens*." *Enzyme and Microbial Technology* 30, no. 2 (2002): 145-152.

Kim, N., Y. Choi, S. Jung, and S. Kim. "Effect of Initial Carbon Sources on the Performance of Microbial Fuel Cells Containing *Proteus vulgaris*." *Biotechnology and Bioengineering* 70 (2000): 109-114.

Largeau, C., E. Casadevall, C. Berkaloff, and P. Dhamelincourt. "Sites of Accumulation and Composition of Hydrocarbons in *Botryococcus braunii*." *Physiochemistry*, 1980: 1043-1051.

Lee, S.A.L., Y. Choi, S. Jung, and S. Kim. "Effect of Inital Carbon Sources on the Electrochemical Detection of Glucose by *Gluconobacter oxydans*." *Bioelectrochemistry* 57, no. 2 (2002): 173-178.

Lefebvre, B.G., L. E. Connell, and K.D. Dahm. "Competition between student groups in the protein production challenge." *Education for Chemical Engineers*, 2009.

Liu, H., S. Cheng, and B. E. Logan. "Production of Electricity from Acetate or Butyrate Using a Single-Chamber Microbial Fuel Cell." *Environmental Science and Technology* 39 (2005): 658-662.

Liu, H., S. Cheng, and B.E. Logan. "Power Generation in Fed-Batch Microbial Fuel Cells as a Function of Ionic Strength, Temperature, and Reactor Configuration." *Environmental Science and Technology*, 2005: 5488-5493.

Logan, B., S. Cheng, V. Watson, and G. Estadt. "Graphite Fiber Brush Anodes for Increased Power Production in Air-Cathode Microbial Fuel Cells." *Environmental Science and Technology* 41 (2007): 3341–3346.

Man, B., S. Cheng, and B.E. Logan. "Electricity generation using membrane and salt bridge microbial fuel cells." *Water Research* 39 (2005): 1675-1686.

Menicucci, J.A., H. Beyenal, E. Veluchamy, R.R.A. Marsili, G. Demir, and Z. Lewandowski. "Procedure for Determining Maximum Sustainable Power Generated by Microbial Fuel Cells." *Environmental Science and Technology* 40, no. 3 (2006): 1062-1068.

Menicucci, Joseph, James Duffy, and Betsy Palmer. "Hands-On Introduction to Chemical and Biological Engineering." *American Society for Engineering Education Annual Conference & Exposition.* Honolulu, 2007.

Metzger, P., C. Berkaloff, E. Casadevall, and A. Coute. "Alkadiene- and Botryococcene-Producing Races of Wild Strains of *Botryococcus Braunii*." *Phytochemistry* 24 (1985): 2305-2312.

Morris, J. M., S. Jin, J. Q. Wang, C. Z. Zhu, and M. A. Urynowicz. "Lead Dioxide as an Alternative Catalyst to Platinum in Microbial Fuel Cells." *Electrochemistry Communications* 9 (2007): 1730-1734.

Mueller, R.F., W.G. Characklis, W.L. Jones, and J.T. Sears. "Characterization of Initial Events in Bacterial Surface Colonization by Two *Pseudomonas* Species Using Image Analysis." *Biotechnology and Bioengineering* 39 (1992): 1161-1170.

NSF. *New Formulas for America's Workforce: Girls in Science and Engineering, NSF 03-208.* Arlington, VA: NSF, 2003.

Oh, S. E., J. Kim, B. Min, and B. E. Logan. "Electricity Generation from Food and Animal Wastewaters Using Microbial Fuel Cells." *Abstracts of Papers of the American Chemical Society* . Washington D.C., 2005. U1691–U1692.

Paje, M.L.F, Kuhlicke, U., Winkler M., and T.R. Neu. "Inhibition of Iotic Biofilms by Diclofenac." *Applied Microbiology and Biotechnology* 59 (2002): 488-492.

Park, D. H., and J. G. Zeikus. "Electricity Generation in Microbial Fuels Using Neutral Red as an Electronophore." *Applied and Environmental Microbiology* 66 (2000): 1292-1297.

Park, D.H., and J.G. Zeikus. "Improved Fuel Cell and Electrode Designs for Producing Electricity from Microbial Degradation." *Biotechnology and Bioengineering* 81, no. 3 (2003): 348-355.

Park, H.S., et al. "A Novel Electrochemically Active and Fe(III)-reducing Bacterium Phylogentically Related to *Clostridium butyricum* Isolated from a Microbial Fuel Cell." *Anaerobe* 7, no. 6 (2001): 297-306.

Pham, T. H., J. K. Jang, I. S. Chang, and B. H. Kim. "Improvement of Cathode Reaction of a Mediatorless Microbial Fuel Cell." *Journal of Microbiology and Biotechnology* 14 (2004): 324-329.

Pizzariello, A., M. Stred'ansky, and S. Miertus. "A Glucose/Hydrogen Peroxide Biofuel Cell That Uses Oxidase and Peroxidase as Catalysts by Composite Bulk-Modified Bioelectrodes Based on a Solid Binding Matrix." *Bioelectrochemistry* 56, no. 1-2 (2002): 99-105.

Prasad, D., et al. "Direct Electron Transfer with Yeast Cells and Construction of a Mediatorless Microbial Fuel Cell." *Biosensors and Bioelectronics* 22 (2007): 2604-2610.

Prince, M. "Does Active Learning Work? A Review of the Research." *Journal of Engineering Education* 93 (2004): 223-231.

Pryfogle, P.A., J.A. Menicucci, N. Jayawickramarajah, J.T. Sears, and G.A. Bala. *Enhanced Conversion of CO_2 by Structured Microorganisms.* Idaho Falls, ID: INEEL, 2003.

Putt, R., N. Naimer, and B. Koretz. "Advanced Zinc-Air Primary Batteries." *The 39th Power Sources Conference.* Cherry Hill, NJ, 2000.

Rabaey, K, and W Verstraete. "Microbial fuel cells: Novel Biotechnology for Energy Generation." *Trends in Biotechnology*, 2005: 291-298.

Rabaey, K, N Boon, S.D. Siciliano, M. Verhaege, and W. Verstraete. "Biofuel Cells Select for Microbial Consortia That Self-Mediate Electron Transfer." *Applied and Environmental Microbiology* 70 (2004): 5373-5382.

Rabaey, K., P. Clauwaert, P. Aelterman, and W. Verstraete. "Tubular Microbial Fuel Cells for Efficient Electricity Generation." *Environmental Science and Technology* 39 (2005): 8077–8082.

Rabaey, K., W. Ossieur, M. Verhaege, and W. Verstraete. "Continuous Microbial Fuel Cells Convert Carbohydrates to Electricity." *Water Science and Technology* 23, no. 6 (2005): 515-523.

Reguera, G, et al. "Biofilm and Nanowire Production Leads to Increased Current in *Geobacter sulfurreducens* Fuel Cells." *Applied and Environmental Microbiology* 2006 (2006): 7345-7348.

Reimers, C. E., L. M. Tender, S. Fertig, and W. Wang. "Harvesting Energy from the Marine Sediment-Water Interface." *Environmental Science and Technology* 35 (2001): 192-195.

Rhoads, A., H. Beyenal, and Z. Lewandowski. "Microbial Fuel Cell using Anaerobic Respiration as an Anodic Reaction and Biomineralized Manganese as a Cathodic Reactant." *Environmental Science and Technology* 39, no. 12 (2005): 4666-4671.

Rice, A.R., M. A. Hamilton, and A.K. Camper. "Apparent Surface Associated Lag Time in Growth of Primary Biofilm Cells." *Microbial Ecology* 41 (2000): 8-15.

Romani, A.M., and S. Sabater. "Influence of Algal Biomass on Extracellular Enzyme Activity in River Biofilms." *Microbial Ecology* 41 (2000): 16-24.

Sawyer, D.T., W.R. Heineman, and J.M. Beebe. *Chemistry Experiments for Instrumental Methods.* New York: John Wiley and Sons, 1984.

Schaetzle, O., F. Barriere, and U. Schroder. "An Improved Microbial Fuel Cell with Laccase as the Oxygen Reduction Catalyst." *Energy & Environmental Science* 2 (2009): 96-99.

Schenk, Peer M., et al. "Second Generation Biofuels: High-Efficiency Algae for Biodiesel Production." *BioEnergy Research* 1, no. 1 (March 2008): 20-43.

Scheuerman, T.R., A.K. Camper, and M.A. Hamilton. "Effects of Substratum Topography on Bacterial Adhesion." *Journal of Colloid and Interface Science* 208 (1998): 23-33.

Schroder, U., and J. Niesen. "A Generation of Microbial Fuel Cells with Current Outputs Boosted by More Than One Order of Magnitude." *Angewandte Chemie, International Edition* 42, no. 25 (2003): 2880-2883.

Seymour, E.Y., and N.M. Hewitt. *Talking About Leaving: Why Undergraduates Leave the Sciences.* Colorado: Westview Press, 1997.

Seymour, J., S. Codd, J. Duffy, and B. Palmer. "Using MRI in an Introductory Chemical and Biological Engineering Course." *American Society for Engineering Education Annual Conference and Exposition.* Portland, Oregon, 2005.

Shantaram, A., H. Beyenal, R.R.A. Veluchamy, and Z. Lewandowski. "Wireless Sensors Powered by Microbial Fuel Cells." *Environomental Science and Technology* 39, no. 13 (2005): 5037-5042.

Simon, E., C.M. Halliwell, S.T. Chee, A.E.G Cass, and P.N. Bartlett. "Oxidation of NADH Produced by a Lactate Dehydrogenase Immobilised on Poly(aniline)-Poly(anion) Composite Films." *Journal of Electroanalytical Chemistry* 538-539 (2002): 253-259.

Stoodley, P., J.D. Boyle, I. Dodds, and H.M. Lappin-Scott. "Consensus Model of Biofilm Structure." In *Biofilms: Community Interactions and Control*, edited by J.W.T.

Wimpenney, P.S. Handley, P. Gilbert, H.M. Lappin-Scott and M. Jones, 1-9. Cambridge: Cambridge University Press, 1997.

Stoodley, P., S. Wilson, L. Hall-Stoodley, J.D. Boyle, H.M. Lappin-Scott, and J.W. Costerton. "Growth and Detachment of Cell Clusters from Mature Mixed-Species Biofilms." *Applied and Environmental Microbiology* 67 (2001): 5608-5613.

Wehr, J.D., and R.G. Sheath. *Freshwater Algae of North America.* Academic Press, 2003.

Wimpenny, J.W.T. "An Overview of Biofilms as Functional Communities." In *Community Structure and Co-operation in Biofilms*, by D.G. Allison, edited by D.G. Allison, P. Gilbert and H.M. Lappin-Scott, 1-24. Cambridge: Cambridge University Press, 2000.

Xu, K.D., M.J. Franklin, C. Park, G.A. McFeters, and P.S. Stewart. "Gene Expression and Protein Levels of the Stationary Phase Sigma Factor, RpoS, in Continuously-Fed *Pseudomonas aeruginosa* Biofilms." *FEMS Microbiology Letters* 199 (2001): 67-71.

Zhang, K., and E. Kojima. "Effect of Light Intensity on Colony Size of Microalga *Botryococcus braunii* in Bubble Column Photobioreactors." *Journal of Fermentation and Bioengineering* 86 (1998): 573-576.

Zhang, X.-C., and A. Halme. "Modelling of a Microbial Fuel Cell Process." *Biotechnology Letters* 17, no. 8 (1995): 809-814.