Energy and the Environment:

Electrochemistry of Electron Transport Pathways in Anode-Respiring Bacteria and Energy Technology and Climate Change in Science Textbooks

by

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ABSTRACT

The finite supply of current energy production materials has created opportunities for the investigation of alternative energy sources in many fields. One example is the use of microorganisms in bioenergy applications, such as microbial fuel cells. Present in many types of environments, microorganisms with the ability to respire solid electron acceptors have become of increasing relevance to alternative energy and wastewater treatment research. In this dissertation, several aspects of anode respiration are investigated, with the goal of increasing the limited understanding of the mechanisms of electron transport through the use of advanced electrochemical methods. Biofilms of *Geobacter sulfurreducens*, the model anode respiring organism, as well as its alkaliphilic relative, *Geoalkalibacter ferrihydriticus*, were investigated using chronoamperometry, electrochemical impedance spectroscopy, and cyclic voltammetry.

In *G. sulfurreducens*, two distinct pathways of electron transport were observed through the application of advanced electrochemical techniques on anode biofilms in microbial electrochemical cells. These pathways were found to be preferentially expressed, based on the poised anode potential (redox potential) of the electrode. In *Glk. ferrihydriticus*, four pathways for electron transport were found, showing an even greater diversity in electron transport pathway utilization as compared to *G. sulfurreducens*. These observations provide insights into the diversity of electron transport pathways present in anode-respiring bacteria and introduce the necessity of further characterization for pathway identification.

Essential to science today, communication of pressing scientific issues to the lay audience may present certain difficulties. This can be seen especially with the topics that are considered socio-scientific issues, those considered controversial in society but not for scientists. This dissertation explores the presentation of alternative and renewable energy technologies and climate change in undergraduate education. In introductory-level Biology, Chemistry, and Physics textbooks, the content and terminology presented were analyzed for individual textbooks and used to evaluate discipline-based trends. Additional extensions were made between teaching climate change with the active learning technique of citizen science using past research gains from studies of evolution. These observations reveal patterns in textbook content for energy technologies and climate change, as well as exploring new aspects of teaching techniques.

DEDICATION

To my parents:

Thank you.

Thank you for encouraging, supporting, and giving me the world.

With love,
Rachel

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ACRONYMS

Ag/AgCl	Silver/silver chloride (reference electrode)
ARB	Anode-respiring bacteria
COD	Chemical oxygen demand
CV	Cyclic voltammetry/voltammogram
ECM	Equivalent circuit model
EIS	Electrochemical impedance spectroscopy
GMOs	Genetically modified organisms
KK	Kramers-Kronig
SHE	Standard hydrogen electrode

PREFACE

Four years ago when searching for a graduate program, the Biodesign Institute at Arizona State University caught my eye for the types of interdisciplinary research focusing on scientifically and socially important research projects. I interviewed with the Biological Design Ph.D. program and the Swette Center for Environmental Biotechnology (SCEB). With my (separate) previous research experiences in microbial fuel cells and electrochemical impedance spectroscopy, SCEB and the lab of Dr. César Torres sounded like a good fit. In this dissertation, I present the results of my studies in microbial electrochemistry that have turned into a chapter of a published book and three articles for peer-reviewed journals.

My studies in microbial electrochemistry seek to understand more about the electron transport processes of microorganisms known as anode-respiring bacteria (ARB). The ARB colonize an anode in a microbial fuel cell (or microbial electrochemical system) and facilitate the breakdown of organic matter in wastewater, for example, and the production of energy. Although ARB and this unique ability to respire an extracellular electron acceptor (the process of moving electrons from the 'food source' to the 'sink' of the system during the metabolic process) have been known for at least a decade, surprisingly little about the actual mechanisms through which bacteria perform this unique function has been explored. In this work, I present the development of standardized laboratory techniques for a complicated technique called electrochemical impedance spectroscopy (EIS) during application in these microbial systems. From there, I utilize EIS and other electrochemical techniques to explore the electron transport pathways of various pure cultures of ARB, including the widely-considered model organism, *Geobacter sulfurreducens*, and its close relative thriving in alkaline conditions, *Geoalkalibacter ferrihydriticus*.

Building fundamental knowledge of the electron transport pathways in ARB expands our knowledge of how microorganisms utilize extracellular electron acceptors and has implications on the improvement of microbial fuel cell designs. These devices can be applied in wastewater treatment systems to offset the massive power costs and allow for greater flexibility in treatment procedures. Although these issues, wastewater treatment and energy, are grand challenges facing society today, I wanted to expand my horizons outside of the laboratory. Due to Dr. Torres's good humor, the open-mindedness of my committee, the freedom afforded by my National Science Foundation Graduate Research Fellowship, and the flexibility of the Biological Design Ph.D. program, I present a two-part dissertation. The first part, as discussed, focuses on microbial electrochemistry. The second part focuses on science education research. A comparatively recent field, science education research investigates the teaching and learning of science in a number of contexts, from the formality of a large university lecture hall to a museum display as an informal learning environment.

My serious interest in communication in science began when I presented my undergraduate research from Capital University at Posters on the Hill, a poster session for members of the United States Congress on Capitol Hill in Washington, D.C in 2010. This presentation brought together approximately 60 projects from across the United States and allowed the students and advisors to meet with Congressional leaders and staff. While in Washington, we advocated for funding for the sciences, and in particular, for undergraduate research while presenting and discussing our work. The power of conversation and the importance of being able to communicate technical work with many audiences became increasingly evident.

Combining my interests in environmental issues and communication in science, I developed a science education research project exploring the presentation of alternative/renewable energy technologies and climate change in the science classroom. The majority of this project focuses on an analysis of introductory-level university biology, chemistry, and physics textbooks. This information is augmented with interviews with university faculty and a few high school science teachers to gain a better understanding of the presentation and challenges faced by educators when discussing energy technologies and climate change in the classroom. For the education research, I developed partnerships with four major publishing companies in the United States, university faculty, and several school districts. The textbook analysis includes the results of a page-by-page analysis of 16 textbooks totaling over 15,000 pages. Overall, I can say that my proficiency in introductory-level biology, chemistry, and physics content has never been better! More importantly, however, my ability to critique specific aspects of presentation methods and discussion in textbooks proves an invaluable research skill. So far, my science education research project has turned into three papers for peer-reviewed journals and presentations at national and international conferences.

I have been interested in environmental issues and movements since I read a book about Rachel Carson as a little girl. Although intrigued by a scientist with whom I shared a name, I became interested in the specific environmental issues against which she so relentlessly campaigned. Reading *Silent Spring* and a Rachel Carson autobiography while in grade school opened my eyes to a new world of environmental research and activism. I was fascinated also with the communication of these topics and the difficulties faced by someone campaigning to preserve species and ecosystems.

The current environmental movement focuses on the most pressing issues facing the continued existence of human society as well as the persistence of countless other species. The geological epoch in which we are now living has been coined the Anthropocene. This time period is defined by the massive, nearly incomprehensible influence humans are having on the Earth. In many ecosystems across the world, we are collectively doing large-scale damage to our only home. However, not all of this damage is irreparable. With global climate change in the wake of COP21 and the groundbreaking global climate agreement, we see the pressing need to change the sources through which we obtain energy to power our ever-developing society. Alternative energy technologies, especially those considered to be renewable, and climate change are topics now encountered in everyday life – through mass media, in-person observations, and education. However, the use of fossil fuels and the global damage created continues. This is due in part to the reluctance of certain powerful groups, especially those with vested interests in the political system in the United States, to deny categorically the massive evidence of climate change currently occurring, the causes, and the projection for the future. This denial is manifested also in the general population of the United States. As a result of what appears to be a campaign against science, many people believe that the climate change we are currently experiencing is due to the natural cycling of the Earth or the result of an outright conspiracy. The power of humans in the Anthropocene has pushed much farther than the natural cycling of the Earth.

With the categorical denial of pressing environmental issues, I observe a two-fold difficulty: first, as a species we must jump to action in implementing renewable energy sources and attempt to mitigate previously caused damage; and second, we must incite a concern in individuals to make these changes. This creates a vicious cycle. An individual cannot be forced to have empathy or demonstrate concern. In the educational process, students are exposed to countless ideas in the process of becoming (hopefully) a productive member of society. In the current K-20 educational system, courses and disciplines are largely compartmentalized and specializations are created. The issue comes when considering the largely interdisciplinary topics of climate change and energy technologies. Many fields, even outside of the sciences, must be considered and understood in order to evaluate data and certain scenarios. The presentation of these interdisciplinary topics is poorly understood in the sciences, especially with respect to educational materials such as textbooks.

In my opinion, the two parts of this dissertation – microbial electrochemistry and energy technologies/climate change in science education – encompass some of the most important topics facing society today. I hope you find some of the ideas and findings as compelling as I do.

CHAPTER 1

BACKGROUND AND INTRODUCTION TO MICROBIAL ELECTROCHEMICAL SYSTEMS RESEARCH

1.1 Microbial Electrochemical Systems

Over the last decade, research in the field of microbial electrochemistry has boomed, particularly in the areas of practical application. With possibilities for wastewater treatment and the removal of certain environmental contaminants, microbial electrochemical cells present a unique combination of fundamental and engineering research questions. Microbial electrochemical systems utilize microorganisms to catalyze reactions, often breaking down organic matter in waste streams (such as wastewater or industrial effluents) through a set of redox reactions to generate energy or other chemical products. Despite its relevance in environmental cycling and biotechnological applications, the processes involved are poorly understood (Bird et al, 2011; Lovley 1991; Lovley 2006; Bird et al, 2011; Yoho et al, 2014; Yoho et al, 2015).

The unique phenomenon of direct transformation of chemical energy to usable electrical energy by microorganisms can be investigated in the microbial electrochemical system with biofilms of the microorganisms growing on the electrode surface. Many studies, including those presented in this dissertation and others reviewed by Logan et al (2006; 2008a), Logan and Rabaey (2012), and Rabaey and Verstrate (2005), investigate this energy transformation catalyzed by microorganisms. These microorganisms, called "anode-respiring bacteria" (or ARB) (Torres et al, 2009) have created a revolutionary approach to offsetting the enormous costs associated with wastewater treatment (Oh et al, 2010) through the utilization of an anode as a final electron acceptor (Torres et al, 2009), but much research is needed before widespread implementation can become a reality. Several possible configurations exist in this type of flexible system, including the direct generation of electricity and the production of useful products. Figure 1.1 shows a typical representation of these systems.

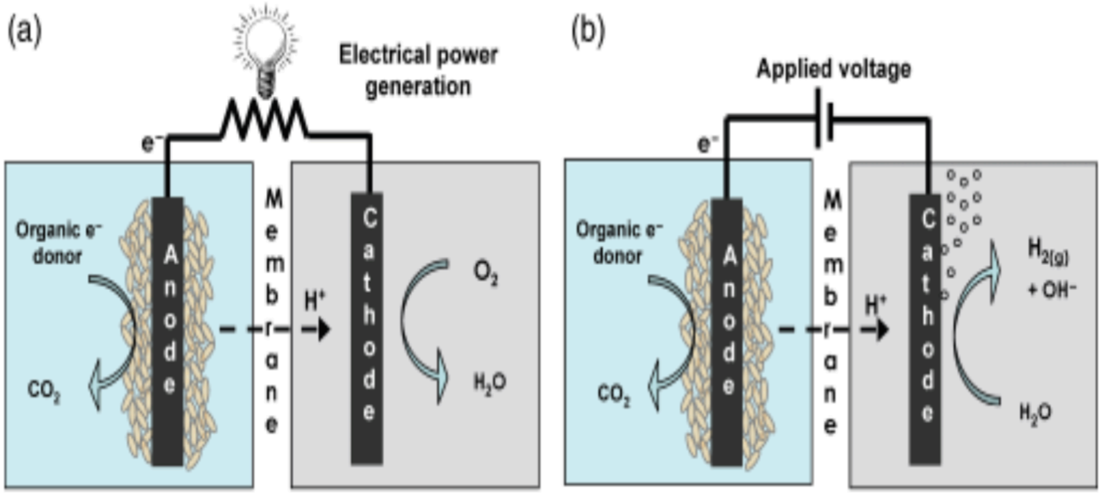


Figure 1.1. (a) A microbial electrochemical system shown transforming the energy stored in an organic material into CO_2 in an anode biofilm. The electrons are shown to move to the cathode through a circuit, where oxygen is reduced to water. Protons generated at the anode pass through a proton exchange membrane to reach the cathode in this system. (b) A microbial electrochemical system similarly utilizing a biofilm on the anode to catalyze the breakdown of an organic material to CO_2. In this system, a potential is applied and the reaction at the cathode instead is H_2O becoming hydrogen gas and OH^-. This figure is reproduced from Torres et al (2010), FEMS Microbiology Reviews, 2010, 34, 1, 3-17, by permission of Oxford University Press.

Figure 1.1a shows representations of the microbial fuel cell (MFC), with applications in low power demanding devices (Tender et al, 2002) and wastewater treatment (Oh et al, 2010). Figure 1.1b represents a microbial electrolysis cell (MEC) for hydrogen gas production (Liu et al, 2005; Logan et al, 2008b). Although many reactor designs exist and are continuing to be developed, these two schematics represent the most simplified conceptual scenarios. Adding to the MFC and MEC, Torres (2014) provides a concise summary of the other ways in which microbial electrochemical systems have been exploited for useful gains. In general, these additional configurations of the microbial system are similar to the MEC, but include desalination (MDC) (Cao et al, 2009), biocathode bioremediation (Aulenta et al, 2010; Tandukar et al, 2009), product synthesis (Nevin et al, 2011; Rabaey and Rozendal, 2010), fermentations (Dennis et al, 2013), and in applications with plants (Deng et al, 2012).

For bioanodes in each of these widely varying configurations and applications, the ARB biofilms have the ability to transport electrons over long distances to respire the anode (Bond et al, 2012; Malvankar et al, 2011; Snider et al, 2012; Strycharz-Glaven and Tender, 2012) and can be visible, as seen on anodes in Figure 1.2 and as scanning electron microscope images in Figure 1.3.

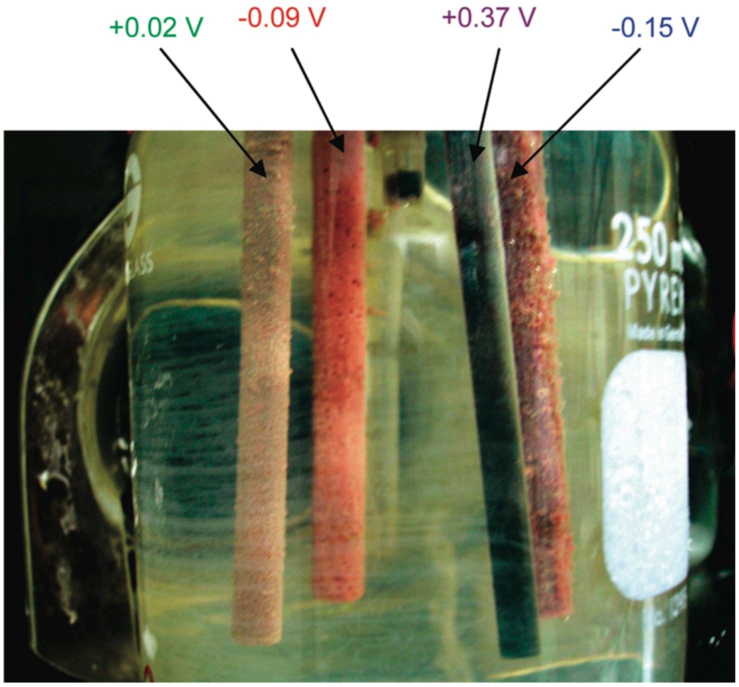


Figure 1.2. Biofilms dominated by *G. sulfurreducens* on anodes poised at different potentials and inoculated from anaerobic digester sludge. Reprinted with permission from Torres CI, Krajmalnik-Brown R, Parameswaran P, Marcus AK, Wanger G, Gorby YA, Rittmann BE, (2009), Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization, Environmental Science and Technology 42:9519-9524. Copyright 2009 American Chemical Society.

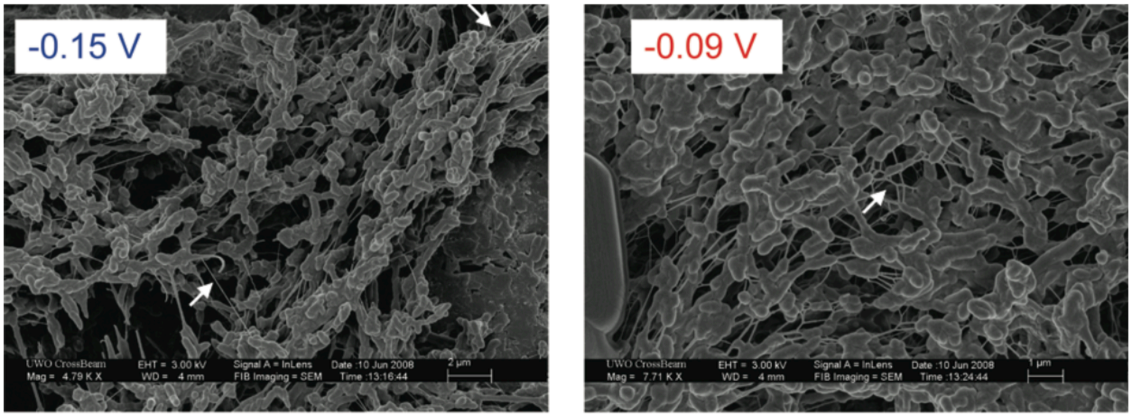


Figure 1.3. Two scanning electron microscope images of two of the biofilms grown at different anode potentials and shown in Figure 1.2 with white arrows indicating putative nanowires. Reprinted (adapted) with permission from Torres CI, Krajmalnik-Brown R, Parameswaran P, Marcus AK, Wanger G, Gorby YA, Rittmann BE, (2009), Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization, Environmental Science and Technology 42:9519-9524. Copyright 2009 American Chemical Society.

1.2 Electron Transport

A key piece in the biogeochemical cycling of carbon and metals, the abilities of dissimilatory metal-reducing and ARB are globally important and prevalent especially in aquatic environments (Lovley, 1991). These organisms can break down a wide variety of electron donors, starting with the most fundamental, acetate or hydrogen (Bond and Lovely, 2003) and can be useful for even complex wastewater (Oh et al, 2010). In Chapters 2-5, I present the data resulting from the utilization of acetate by microbial biofilms of *Geobacter sulfurreducens* and *Geoalkalibacter ferrihydriticus* under multiple environmental and reactor conditions.

Among researchers investigating microbial electrochemical systems, two methods to describe the phenomenon of extracellular electron transport can be utilized, the physical structure creating the connections of the biofilm and the electron transport process. Three physical scenarios are now widely described and accepted in the field, including direct electron transfer (where the individual cell is in direct contact with the anode), electron shuttles (a shuttle produced by the cell is used to transport electrons), and the solid conductive matrix (an extracellular matrix produced by the microorganisms) (Torres et al, 2010). These three types of transport are seen in Figure 1.4.

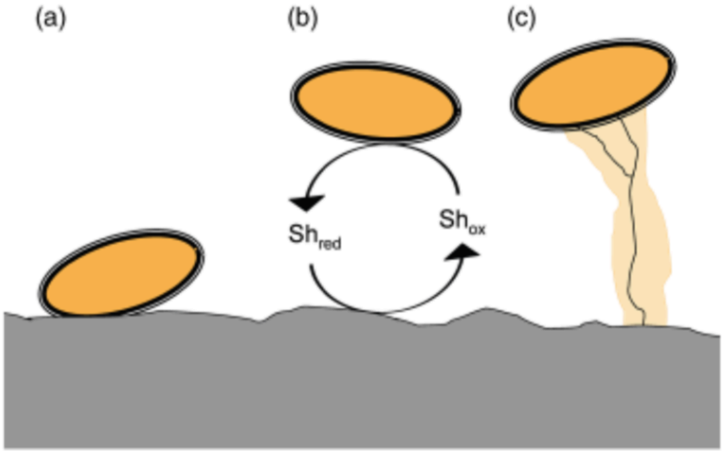
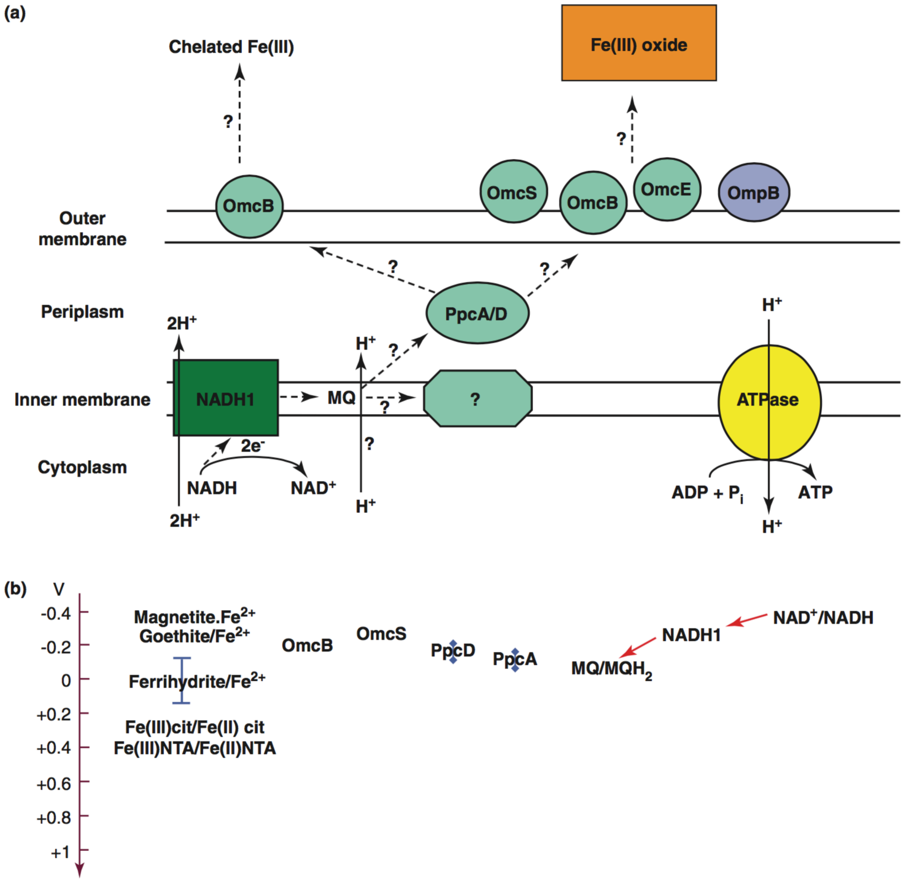


Figure 1.4. Three representatives of bacterial cells performing anode respiration are shown with **(a)** the direct electron transfer method when the cell is in physical contact with the surface (shaded in gray), **(b)** the use of electron shuttles produced by the cell, indicating the cycling of oxidized and reduced states of the shuttle, and **(c)** a solid conductive matrix allowing the cell to respire the surface at relative large distances. This figure is reproduced from Torres et al (2010), FEMS Microbiology Reviews, 2010, 34, 1, 3-17, by permission of the Oxford University Press.

The extracellular matrix is a structure made of a mixture of cellular products including pili with cytochromes and polysaccharides surrounding and encasing the cells within the biofilm (Bond et al, 2012; Malvankar et al, 2011; Snider et al, 2012; Strycharz-Glaven and Tender, 2012). In contrast to the physical setup of the microorganism in its biofilm for electron transport, the mechanism or mechanisms through which the transport of electrons outside of the cell occurs is hotly debated in the field (Torres, 2014). The debate involves the method through which ARB move electrons from the inside of their cell to the extracellular electron donor. One side argues that the ARB have electrically-conductive pili (Malvankar et al, 2011) and the other presents a cytochrome-hopping method (Strycharz-Glaven et al, 2011). I choose here to not become further involved in this spirited debate by providing additional commentary. What I provide instead is a schematic from five years ago of the speculated electron transport pathways of *Geobacter sulfurreducens* (Bird et al, 2011) in Figure 1.5.



TRENDS in Microbiology

Figure 1.5. The proposed pathways for respiration by *G. sulfurreducens* to an extracellular iron electron acceptor. This figure, from Bird et al (2011) is reproduced with permission and reprinted from Trends in Microbiology, 19(7), Lina J. Bird, Violaine Bonnefoy, and Dianne K. Newman, Bioenergetic challenges of microbial iron metabolisms, 330-340, 2011, with permission from Elsevier.

Although it is important to understand the complexities of extracellular electron transport, the contributing internal processes have additional implications on fundamental studies of electron transport in ARB and later applications in biotechnology. In Figure 1.5, many question marks can be found. This uncertainty in the mechanisms of extracellular respiration in *G. sulfurreducens* remained largely unchanged over several years. Only recently have studies (Levar et al, 2014; Yoho et al, 2014; Zacharoff et al, 2016), including those presented in this dissertation, explored the processes behind these question marks in greater depth.

1.3 Studying Biofilms of Pure Cultures

Since first being shown to grow and produce electricity while attached to electrodes (Bond and Lovley, 2003), the knowledge of which particular microorganisms are able to respire an anode has increased enormously. Although the model organism, *Geobacter sulfurreducens*, a member of the *Geobacteraceae,* was first to this illustrious title (Bond and Lovley, 2003), others such as *Shewanella oneidensis* have risen to prominence in the research community (Gorby et al, 2006; Marsili et al, 2008). Although many known ARB thrive under standard environmental conditions (i.e., pH and temperature), new developments in the area of "extremophiles" within the ARB community have recently come into prominence. Such examples include *Geoalkalibacter ferrihydriticus* (Badalamenti et al, 2013; Zavarzina et al, 2006) and *Geoalkalibacter subterraneus* (Greene et al, 2009; Badalamenti et al, 2013), both close relatives to *G. sulfurreducens* (all members of δ-Proteobacteria). Beyond the extremophilic ARB related to *G. sulfurreducens*, the ability to respire an anode in the microbial electrochemical cell has been found in Gram positive organisms as well, notably *Thermincola ferriacetica*, a member of the Class Firmicutes (Marshall and May 2009; Parameswaran et al, 2013; Zavarzina et al, 2007).

Studying biofilms of pure culture organisms in the microbial electrochemical system is of particular importance to understanding the system as a whole during the application process. Although optimizing design/operational conditions and microbial community ecology are important for the development of microbial electrochemical systems as sustainable methods of wastewater treatment (Oh et al, 2010), we must understand the fundamental mechanisms through which these organisms respire an extracellular electron acceptor. Electrochemical and other analyses of biofilms of pure culture ARB are essential for learning about the abilities through which these organisms transport electrons to respire an extracellular electron acceptor. Complementary analyses to biofilm electrochemistry include genetic identification of key participants in the electron transport pathways and their up- and down-regulation with changing environmental conditions, especially utilizing the "-omics" approaches (for example, transcriptomics and proteomics).

1.3.1 *Geobacter sulfurreducens*

First described by Caccavo et al (1994), *G. sulfurreducens* has become a widely investigated, yet poorly understood, organism in the field of microbial electrochemistry over the past two decades. Originally isolated as strain PCA from a hydrocarbon-contaminated soil sample from Norman, Oklahoma, this microorganism is capable of dissimilatory metal- and sulfur-reduction (Caccavo et al, 1994). As mentioned, this organism is a member of the δ-Proteobacteria.

Capable of growing utilizing a number of extracellular electron acceptors (i.e., iron oxides, elemental sulfur, fumarate, malate, among others), *G. sulfurreducens* has become a commonly considered “model organism” for anode respiration. A Web of Science search (Thomson Reuters, 2016) using the keywords, “*Geobacter sulfurreducens*,” returns a staggering 1,054 papers since its discovery (result current to January 5, 2016). In this vast quantity of papers is a wealth of knowledge of appearance of this organism, applications, environmental/operating conditions, and microscopy/laboratory analyses. Members of the *Geobacteraceae* have been found to perform many useful tasks for environmental engineers, for example uranium reduction in contaminated groundwater (Gregory and Lovley, 2005; Holmes et al, 2002). Although these studies are informative, especially from the applications perspective, a fundamental understanding of the actual mechanisms through which these ARB, especially *G. sulfurreducens*, transfers electrons from the electron transport chain out of the cell and to the metal (or anode) is greatly lacking.

1.3.2 *Geoalkalibacter ferrihydriticus*

Another member of the δ-Proteobacteria, *Geoalkalibacter ferrihydriticus*, has been introduced only recently into study in the field of microbial electrochemistry. Originally isolated and characterized from the sediments of an alkaline soda lake in Russia (Zavarzina et al, 2006), this organism spans two main research interests in the community. Those interested in fundamental studies are targeting this organism as a representative ARB with electron transport pathways suited for alkaliphilic conditions. Application-focused researchers see a possibility for the eventual optimization of operating conditions for treating alkaline waste streams.

In the Swette Center for Environmental Biotechnology at Arizona State University, Dr. Joseph Miceli III isolated the *Glk. ferrihydriticus* organism from a sample of wastewater treatment plant sludge and Dr. Jonathan Badalamenti provided the first electrochemical characterization (Badalamenti et al, 2013). In this characterization, Badalamenti and collaborators (2013) found that both *Glk. ferrihydriticus* and its close relative preferring saline conditions, *Geoalkalibacter subterraneus*, both produce comparable current densities to those generated by other ARB in the laboratory. These current densities fall in the range of 5.0 to 8.3 A m^{-2} in both pure and mixed cultures (Badalamenti et al, 2013; Carmona-Martinez et al, 2013; Pierra et al, 2015). Very few studies have been performed on *Glk. ferrihydriticus* specifically (Badalamenti et al, 2013; Zavarzina et al, 2006; Zavarzina et al, 2011), although it has the possibility of becoming the model ARB for alkaline environments. In Chapters 4 and 5 of this work, I provide information on the electron transport pathways of *Glk. ferrihydriticus* as a comparison to those uncovered in the model organism, *G. sulfurreducens*.

1.4 Electrochemical Techniques

With advancements in microbial electrochemical systems, the need for biofilm characterization becomes increasingly important to learn about the fundamental microbial processes. Electrochemical techniques are useful for interpreting the physical characteristics of the biofilm, but structured methods are required to produce reliable data available for interpretation. In these experiments, a potentiostat is used to implement the electrochemical techniques of chronoamperometry, cyclic voltammetry, and electrochemical impedance spectroscopy.

1.4.1 Potentiostat

In these experiments, the potentiostat is an invaluable tool for performing complex electrochemical experiments and monitoring biofilm performance in real-time.

The potentiostat is the controller used for implementing the electrochemical technique, often applying a potential and measuring the current response of the biofilm. A computer is used to manage the potentiostat and record the data generated. In these experiments, I utilize two different types of potentiostats, a single channel Ivium CompactStat (Ivium Technologies, Eindhoven, Netherlands) and a multi-channel Bio-Logic VMP3 (Bio-Logic, Grenoble, France). Both have the capabilities to perform multiple types of electrochemical experiments described here, particularly chronoamperometry, cyclic voltammetry, and electrochemical impedance spectroscopy. Key experimental parameters relating to the use of the potentiostat are discussed in detail in Chapter 2.2.2, especially as they relate to the application of electrochemical impedance spectroscopy, a technique introduced in Chapter 1.4.4 and studied in great detail in Chapter 2.

The most fundamental technique used in these experiments with the potentiostat is chronoamperometry. It is used primarily to apply a fixed potential to the electrode and monitor the current density response of the biofilm. This technique can be useful for applying fixed potential steps and monitoring respiration rate changes as is presented in Chapter 3. Cyclic voltammetry utilizes a similar applied potential technique in a time-dependent manner.

1.4.2 Cyclic Voltammetry

The technique of cyclic voltammetry and the resulting cyclic voltammogram (CVs) are transient and dynamic electrochemical measurements with an applied potential swept at a constant rate and the resulting current density recorded. During the application of this technique, the respiration of the biofilm changes as a result of the differences in energy available (LaBelle and Bond, 2008; Marsili et al, 2008; Marsili et al, 2010; Torres et al, 2008). Although the rates of scanning (in mV s^{-1}) can vary widely, the CV does not allow for the investigation of steady-state responses of the biofilm.

With acetate, a common electron donor, the response generated from a CV is seen as a sigmoid (Marsili et al, 2008; Marsili et al, 2010; Strycharz-Glaven and Tender, 2012). I present many CVs with biofilms utilizing acetate in Chapters 2, 3, 4, and 5 (Yoho et al, 2014; Yoho et al, 2015). In the presence of the electron donor, acetate, these are called “turnover” conditions, and when the electron donor is not present or removed from the system, they are “non-turnover” (LaBelle and Bond 2008). I present also non-turnover CVs for two ARB, *G. sulfurreducens* (in Figure 3.2) and *Glk. ferrihydriticus* (in Figure 4.10).

1.4.3 Nernst-Monod Equation

Since the first modeling attempt for microbial electrochemical systems (Kato Marcus et al, 2007), several endeavors have been made to model accurately the electrochemical responses of microbial biofilms. The Nernst-Monod model was shown first to fit the sigmoidal shape of the curve resulting from a CV (Kato Marcus et al, 2007). Overall, the response of the biofilm is observed to be that of an irreversible enzymatic reaction (Kato Marcus et al, 2007; Yoho et al, 2014). To date, most of the modeling techniques used center on the idea that there is a single limiting process, a redox enzyme, controlling the process (Yoho et al, 2014). These models include the Nernst-Monod (Kato Marcus et al, 2007; Torres et al, 2008; Strycharz et al, 2010), Butler-Volmer-Monod (Hamelers et al, 2011), and the Nernst-Ping-Pong model (Peng et al, 2013). Essentially, Butler-Volmer-Monod is an extension of the Nernst-Monod while setting zero value for the equilibrium potential and a Monod constant dependent on the poised anode potential (Hamelers et al, 2011). The Nernst-Ping-Pong relies on a separate set of assumptions including a non-rate limiting electrochemical step and a constant substrate (electron donor) concentration (Peng et al, 2013). These modeling methods are mentioned again in Chapter 3, especially as they apply to studies of pure culture anode biofilms.

The Nernst-Monod model describes the current density (j) generated as a response to the sweeping potential applied to the working electrode (E), as seen in Equation 1.

$$j = j_{max}\left(\frac{1}{1+\exp\left[-\frac{nF}{RT}(E-E_{\mathrm{KA}})\right]}\right) \tag{1}$$

As described by Kato-Marcus et al (2007), the additional components in Equation 1 are j_{max}, the maximum current density obtained by the biofilm, E_{KA}, the midpoint potential of the limiting process occurring, n, the number of electron transported during this process (typically assumed n=1), F, Faraday's constant, R, the ideal gas constant (R=8.3145 J mol^{-1} K^{-1}), and T, the temperature (in degrees Kelvin). However, the Nernst-Monod equation does not always fit the data (Hamelers et al, 2011; Yoho et al, 2014). In Chapter 3, I provide additional commentary on and data supporting the lack of suitability of the Nernst-Monod equation to describe the dynamic processes occurring in the anode biofilms. The lack of "fitting" found in the data is addressed in one other paper, Snider et al (2012), by using an additional parameter to include the inherent difficulty of modeling multiple redox co-factors, as opposed to a single dominant process. Although a suitable method for adapting the Nernst-Monod equation to improve the alignment with experimental data, the fundamental learning gains on the actual electron transfer processes occurring within the biofilm are small. In contrast, the data presented in Chapters 3 and 4 build upon the shortcomings of the original Nernst-Monod as a learning experience.

Since the time the experiments described in this dissertation began, considerable knowledge has been developed to augment the assumptions of the single rate-limiting step. As I will describe in Chapter 3, the research community is now aware of more than one dominant electron transport process in the model organism, *G. sulfurreducens*. This changes the discussion of the modeling techniques and calls for new methods beyond the addition of another adjustment parameter (as seen in Snider et al, 2010). This is followed by additional complexity in *Glk. ferrihydriticus* displaying at least four dominant pathways, as described in Chapters 4 and 5.

The complexity is accentuated when describing the midpoint potential of the CV (E_{KA}). The definition provided by Kato Marcus et al (2007) involves the potential when the electron acceptor concentration is equal to the half-max-rate of electron acceptor concentration. As mentioned, when the assumption of a single rate-limiting process is utilized, the E_{KA} becomes the midpoint potential of the rate-limiting process (often assumed to be a single enzyme). The presence of more than one dominant electron transport pathway necessitates a change in the typical conversation to be the E_{KA} of the overall biofilm response. This paradigm shift in the "typical" discussion is continued in Chapter 5.

1.4.4 Electrochemical Impedance Spectroscopy

Only recently utilized in microbial electrochemistry, potentiostatic electrochemical impedance spectroscopy (EIS) utilizes the electrodes to investigate the dynamics of a living microbial biofilm by applying a sinusoidal perturbation in potential. In this work, I employ a three-electrode setup for EIS work to investigate the electron transport pathways in pure culture biofilms.

Several recent studies are now using EIS in a wide variety of applications in the study of microbial biofilms on electrodes. These include for example exploring biofilm development (Martin et al, 2013) and community growth (Sanchez-Herrera et al, 2014), mediator use (Hosseini and Ahadzadeh, 2013), reactor and operational design (Sevda et al, 2015), and various environmental or substrate conditions (Hidalgo et al, 2015; Rosseau et al, 2015; ter Heijne et al, 2015; Yin et al, 2013). These studies represent a selection of the newest developments in the expanding area of microbial electrochemistry utilizing EIS to explore the dynamics of electrochemical reactors, biofilms, and electron transport.

Before the work contained in this dissertation began (pre-2013), only few studies employing relatively inconsistent EIS methods can be found in the literature. However, the increasing trend in publications over time can be seen in Figure 1.6. This figure is part of a comprehensive review by Dominguez-Benetton et al (2012) investigating the “accurate” use of EIS and its interpretation in the field of microbial electrochemistry. The study by Dominguez-Benetton et al (2012) and the work contained in this dissertation represent a paradigm shift in the implementation of EIS for microbial systems. Dominguez-Benetton et al (2012) begins the call for a greater understanding and uniform implementation and evaluation of EIS data.

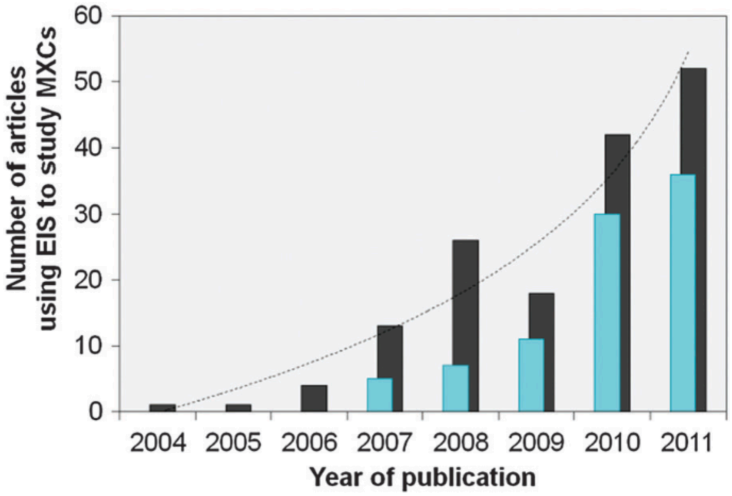


Figure 1.6. Publications in the time period 2004-2011 using the EIS technique in microbial electrochemical systems. Black bars represent total number of articles known at the time, while the blue bars are those in the Science Direct repository. Reproduced in part from Dominguez-Benetton et al (2012) with permission from The Royal Society of Chemistry.

This work develops standardized methods for the use of EIS in microbial electrochemical systems in order to resolve the issues in consistency of application and establish guidelines for generating reliable, reproducible results. Laboratory experiments are presented in Chapter 2, alongside extensive background on the technique presented in a manner designed for the diverse scientific trainings of individuals in the field. The goal of this background is to transform the EIS technique from realm of technical electrochemistry into the toolbox of the working microbial electrochemist. The discussion and procedural guidelines can be found in Chapter 2.

1.4.5 The Microbial Electrochemistry Setup

As shown in Figure 1.7, the majority of the experiments in this dissertation are in reactors made from a modified 100 mL bottle (VWR, Radnor, PA, USA). This design is useful for fundamental studies, as it has an electrochemistry anode through the side, as shown in detail in the inset of Figure 1.7.

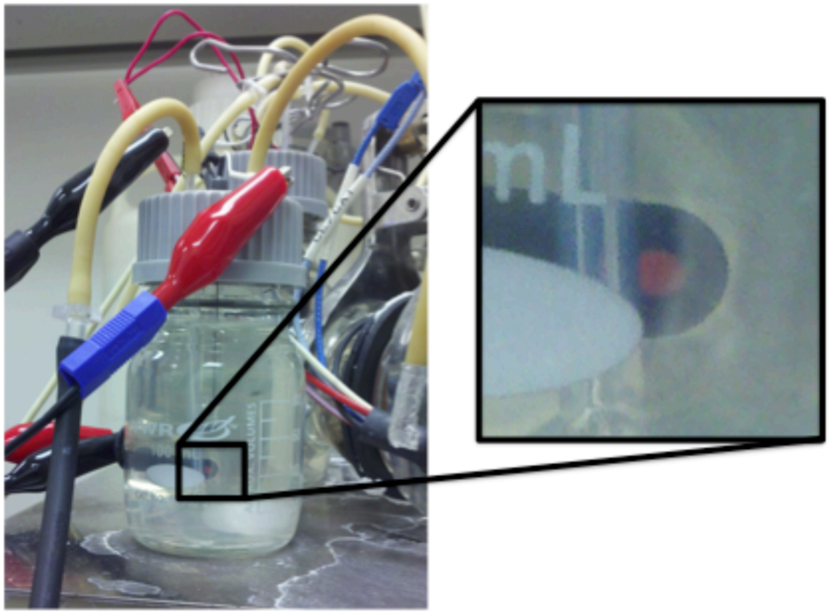


Figure 1.7. An image of an electrochemical cell showing a visible orange biofilm of *G. sulfurreducens* (Yoho et al, 2014). This image is reproduced with permission from John Wiley and Sons, Wiley Online Library © 2014 Wiley-VCH Verlag CmbH & Co. KgaA, Winheim.

This electrochemistry anode (shown as a flat 3.14 mm^2 gold surface, BASi, West Lafayette, IN) is of use for these experiments as its small size presents minimal potential gradients in the two dimensions. A schematic detailing the component parts is presented in Figure 1.8.

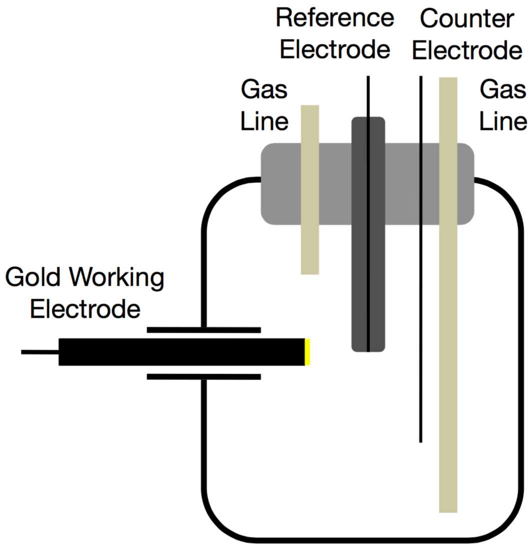


Figure 1.8. A schematic of the microbial electrochemical cells (MXCs) used in this study (Yoho et al, 2014). This image is reproduced with permission from John Wiley and Sons, Wiley Online Library © 2014 Wiley-VCH Verlag CmbH & Co. KgaA, Winheim.

These systems are used to grow ARB biofilms on the anode and implement electrochemistry experiments to investigate the phenomenon of extracellular electron transport. In Figure 1.8, the working electrode is seen through the side, while the remaining component parts are inserted through a rubber stopper in the top of the reactor. The gas inflow and outflow lines are used to fill the reactor with media anaerobically in a sterile environment, and the reference and counter electrodes are used for three-electrode electrochemistry experiments (discussed in Chapter 2.2.1).

1.5 Dissertation Objectives and Outline

In this dissertation, I present data providing an electrochemical characterization of the electron transport pathways involved in extracellular electron transport in ARB, especially the model organism, *Geobacter sulfurreducens*, through electrochemical methods. In order to do so, Volume 1 describes the results of validating an established method, electrochemical impedance spectroscopy (EIS), for use to analyze anode biofilms of microbial electrochemical systems. Five chapters following this build from the method to applications exploring electron transport pathway utilization in *G. sulfurreducens* and *Glk. ferrihydriticus*.

In Chapter 2, I present the background and experimental development of EIS in microbial electrochemical systems. Chapter 3 utilizes the EIS technique alongside other dynamic electrochemical methods (including chronoamperometry and cyclic voltammetry) to elucidate the two dominant electron transport pathways in the model ARB, *G. sulfurreducens*. Chapter 4 presents data supporting the existence of four dominant electron transport processes in *Glk. ferrihydriticus*. This work favors the use of cyclic voltammetry and increases the interpretation of this method through the analysis of the first derivatives of the catalytic curve found in the CVs. Building upon this work, Chapter 5 expands on the knowledge of *Glk. ferrihydriticus* by exploring the utilization of the four established pathways for anode respiration under several pH conditions. Chapter 6 summarizes the results of the work in microbial electrochemistry and suggests future directions before this dissertation transitions to Volume 2, investigating the presentation of energy technologies and climate change in science education.

CHAPTER 2

ESTABLISHING STANDARDIZED METHODS FOR APPLYING ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY ON MICROBIAL ELECTROCHEMICAL SYSTEMS

This chapter was published in collaboration with Sudeep C. Popat, Francisco Fabregat-Santiago, Sixto Giménez, Annemiek ter Heijne, and César I. Torres as Chapter 8 of Electrochemically active biofilms in microbial fuel cells and bioelectrochemical systems: from laboratory practice to data interpretation. Eds: Beyenal & Babuta. John Wiley & Sons. Hoboken, New Jersey. 2015. Copyright © 2015 by John Wiley & Sons, Inc. All rights reserved.

2.1. Introduction

Microbial electrochemical cells (MXCs) represent a new class of technologies, within the realms of wastewater treatment, bioremediation, and bioenergy generation (Logan et al. 2006; Logan et al. 2008; Rabaey and Verstraete, 2005). As mentioned in Chapter 1, MXCs rely on the unique capability of certain bacteria to use electrodes in their metabolism, either as electron acceptors or electron donors (Logan, 2009; Lovley, 2006; Lovley, 2010). However, MXCs represent one of the most challenging types of bioreactors and electrochemical reactors to scale up. Unlike typical bioreactors, MXCs include electrochemical components that need to be considered and optimized. For example, scaling up MXCs in volume requires that very small distances be maintained between the anode and the cathode to minimize Ohmic losses from transport of ions between the two electrodes; this presents some restrictions on using traditionally scalable bioreactor designs. At the same time, MXCs differ from other electrochemical cells, such as the proton exchange membrane (PEM) fuel cell, because of the environmental conditions that are required to grow microorganisms (e.g., nutrient availability, water content, neutral pH, etc.). In general, we want to achieve high efficiency in an MXC, which translates to large energy recovery in a microbial fuel cell (MFC) or small energy input in a microbial electrolysis cell (MEC). Unlike in typical bioreactors, where we would consider only the extents and kinetics of a given microbially-catalyzed conversion in this regard, in MXCs, just like in electrochemical cells, the overpotentials at the electrodes, as well as those due to transport of ions and electrons, are also important in determining performance.

The characterization of overpotentials in MXCs is significantly different than in other electrochemical cells as well. In MFCs, for example, anode-respiring bacteria catalyze the oxidation of organic compounds, while oxygen is reduced at the cathode, usually on metal catalysts. Thus, classical activation and concentration overpotentials occur at the cathode, while complex metabolic and electron transport overpotentials occur at the anode. The fact that many microorganisms form biofilms on electrodes places the complication of a series of distinct intracellular cell reactions connected to the electrodes as a porous film through a series of distinct extracellular reactions, which represents a more complex system than reactions on one or more catalyst sites on a planar or porous electrode in typical fuel cells. Recently, interest has increased in new types of MXCs in which bacteria accept electrons from the cathode (referred to as biocathodes), and reduce an electron acceptor of interest (e.g., CO_2, nitrate, perchlorate, Cr (VI) etc.) (He and Angenent, 2006; Rabaey and Rozendal, 2010). The most attractive application of biocathodes is the synthesis of precursors to liquid fuel from CO_2, using electrical current derived from renewable sources (Lovley and Nevin, 2013). The electrochemical characterization of biocathodes is even more complicated than that of bioanodes, as we know virtually nothing yet about the mechanisms of electron transport to microorganisms from a cathode (Rosenbaum et al. 2011).

The most common electrochemical techniques used to study overpotentials in MXCs are those that measure a change in current as the potential is changed. Cyclic voltammetry (CV) is the most popular technique, while others include linear sweep voltammetry, chronoamperometry, potential step-voltammetry etc. (Fricke et al. 2008). CV involves changing the electrode potential (*V*) within a pre-specified range of potentials at a defined linear rate, and measuring the resulting electrical current (*i*). The result of a CV is an i-V relationship that often shows the total overpotential for the whole cell, or for a working electrode, when performed with respect to a reference electrode. However, CV does not specifically help identify the individual contribution of different phenomena that occur in an MXC to the overall i-V relationship. For example, the Ohmic loss that occurs between the reference electrode and the working electrode while performing CV measurements is inherently not distinguishable in the i-V response collected. When applied to studying electron transfer at the microbial anode, CV does not specifically help identify the contribution of different processes (e.g. microbial kinetics of substrate oxidation vs. extracellular electron transfer vs. reactant/product transport) to the total overpotential. These processes are lumped together in the response of the system studied as a whole. Additionally, other techniques like chronoamperometry and potential-step voltammetry have these same limitations.

2.1.1 Background on Electrochemical Impedance Spectroscopy

There are however electrochemical techniques available that are able to, theoretically, identify and quantify the individual contribution of various simultaneous processes to the overall overpotential in electrochemical cells. This chapter focuses on the use of electrochemical impedance spectroscopy (EIS), which has only recently found application in studies on MXCs (He and Mansfield, 2009). Unlike direct current (DC) techniques such as CV, EIS is an alternating current (AC) method. Potentiostatic EIS involves applying small sinusoidal amplitude over a fixed voltage value at a range of frequencies, and measuring the resulting magnitude and phase shift in current (Figure

2.1). Because different processes govern the *i*-*V* response at different frequencies, it is thus theoretically possible to quantify individual contributions of different processes to the overall overpotential provided that these processes have different characteristic time constants. While uses of EIS in research applications in many distinct fields are various, as reviewed elsewhere (Amirudin and Thieny, 1995; Katz and Willner, 2003; Lisdat and Schäfer, 2008; Pejcic and De Marco, 2006), it has also been used extensively in PEM fuel cell research to identify and quantify individual resistances, and thus overpotentials (Wu et al. 2008; Yuan et al. 2997).

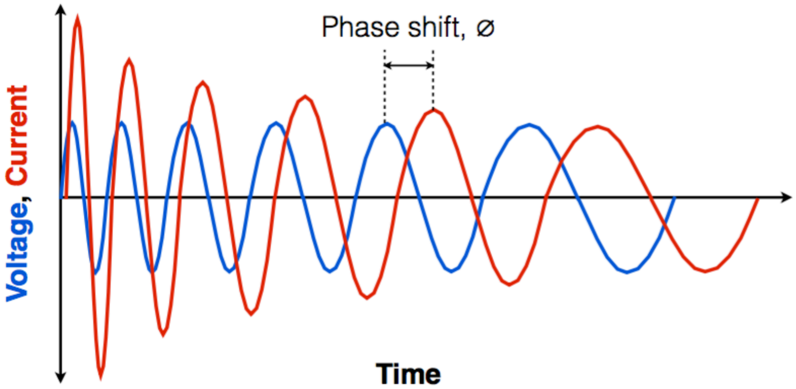


Figure 2.1. Sinusoidal current response to the sinusoidal voltage change made during potentiostatic EIS measurements. Each cycle represents a different frequency. A wide range of frequencies is usually used in EIS measurements.

Although the details of the theory behind EIS are available in literature (Macdonald and Johnson, 2005), I will review the concept of impedance in relatively simple terms, before discussing special requirements in experimental design, parameter selection, and protocol, in its application to MXCs, as well as highlighting standard and alternative methods of data analysis.

Impedance is similar to resistance, except it also includes a time domain linked to frequency of the sinusoidal amplitude applied on the system under study. Using Ohm's law, impedance (*Z*) can be represented as:

$$Z = \frac{E(t)}{i(t)} \tag{Equation 2.1}$$

Here, *E(t)* and *i(t)* are the potential and current as a function of time *t*. Since we apply a sinusoidal amplitude on the potential as a function of frequency, *E(t)* can be represented as:

$$E(t) = \Delta E \sin(\omega t) \tag{Equation 2.2}$$

Here, w is the radial frequency (equivalent to ***2πf***, where *f* is the frequency in Hz) at time t, and *DE* is the amplitude in voltage.

An ideal resistor, following Ohm's law (Eq. 1), would show constant impedance over time at the various frequencies, and thus the use of EIS would reduce to the measurement of a single point repeatedly over the entire frequency range. However, many processes that we want to study do not only act as resistances to electron flow, but also as capacitors, giving them the ability to store an electrical charge. A good example of a capacitor in a fuel cell is the electrical double layer at the interface of the electrode, where the charge transfer reaction occurs. As you can imagine, microbial cells, or even enzymes, have capacitance as well, due to their ability to store electrons in reaction centers or in redox compounds. Diffusion limitations also lead to a similar behavior. Now, since the electrons do not only follow Ohm's law, but also are capable of charging (or discharging) the capacitor there is a phase shift, *f*, between the voltage *E(t)* and the current *i(t)*, that can be represented as:

$$i(t) = \Delta i \sin(\omega t + \emptyset) \tag{Equation 2.3}$$

Thus, the impedance can then be represented as:

$$Z = \frac{\Delta E \sin(\omega t)}{\Delta i \sin(\omega t + \emptyset)} = |Z| \frac{\sin(\omega t)}{\sin(\omega t + \emptyset)} \qquad \text{(Equation 2.4)}$$

Here, $|Z|$ is the absolute magnitude of the impedance. What allows us to separate different electrochemical processes using EIS is the fact that capacitors have a frequency-dependent ability to be charged or discharged, a property called capacitive reactance. As a consequence, different capacitances within the system will respond at different frequencies, allowing us to temporally separate the electrochemical processes in EIS experiments based on their capacitive reactance. Similarly, diffusion limitations also usually have a characteristic low frequency where they predominantly govern the system response, and thus are easily distinguishable.

The information provided by EIS can be plotted in different graphical representations. Figure 2.2 shows one common representation of EIS data, called a Bode plot. In a Bode plot, the absolute magnitude of the impedance (Figure 2.2a) and the phase shift (Figure 2.2b), both of which are experimentally measured, are plotted against the frequency. In this example, two different processes can be identified, which are ascribed to different charge transfer events. More specifically, three clear regions are visible in Figure 2.2b. First, the phase shift is zero at high frequencies; the high frequencies do not allow electrons to interact with any capacitor, and thus only Ohmic resistance is measured. Then two phase-shift peaks are apparent as the frequency is decreased, both corresponding to individual charge transfer processes (shown on the figure as Ct,1 and Ct,2). The phase shift *f* occurs due to the existence of a capacitive process in the charge-transfer mechanism. Thus, from the Bode plot, we can conclude that there are in this example, at least, two charge transfer processes in the system studied. It is important to note that the presence of two phase shifts does not always necessarily indicate the presence of two charge transfer processes, but can also indicate diffusion limitations, especially if noticed at lower frequencies. I elaborate further on how to identify the nature of the processes in Section 4 of this chapter.

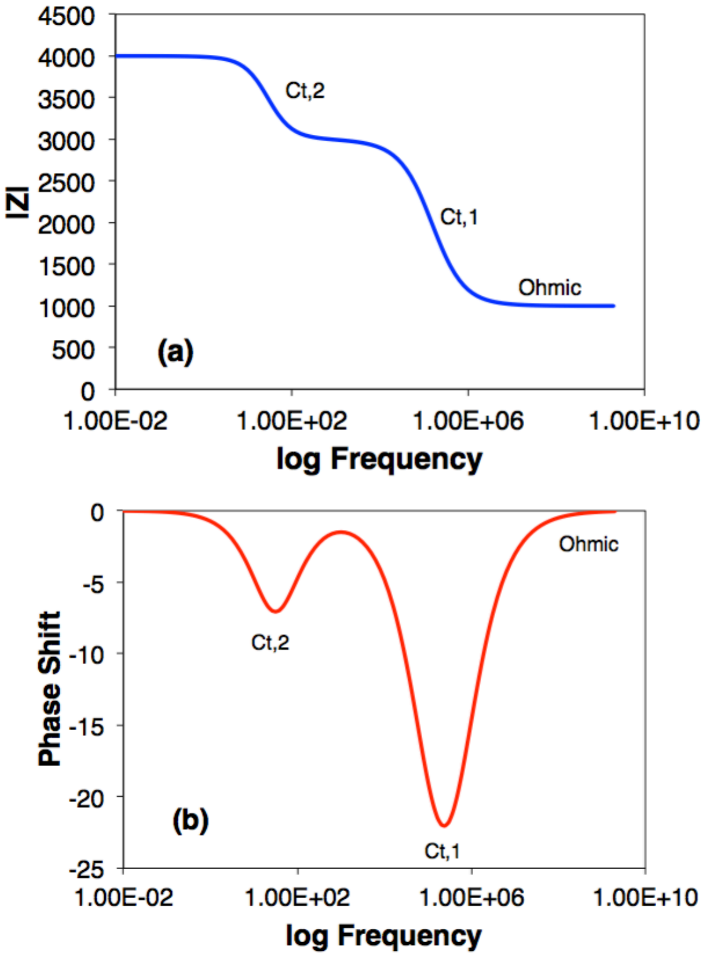


Figure 2.2. A representative Bode plot, showing: (a) absolute impedance (|Z|) versus frequency and (b) phase shift versus frequency. $C_{t,1}$, $C_{t,2}$, and Ohmic represent three major processes contributing to the overall impedance.

While it is sometimes difficult to get quantitative information about overpotentials from individual processes out of the Bode plots, they do provide a very good opportunity to identify important processes that govern the electrochemical response. This is especially helpful in selecting an appropriate bio-physico-chemical model for the system being studied (discussed in Section 4) that can be applied to such methods of data analysis as the equivalent circuits fitting described later. Additionally, the Bode plot helps to understand more fundamentally the physical response of the system with respect to the perturbation in amplitude as a function of frequency.

2.1.2. Data Representation through the Nyquist Plot

The impedance, as shown in Eq. 2.4, can also be represented as a complex function as in Eq. 2.6, derived using the correlation of a sine function to complex number theory (Eq. 2.55):

$$\sin x = \frac{e^{jx} - e^{-jx}}{2j} \qquad \text{(Equation 2.5)}$$

$$Z = |Z| \frac{e^{j\omega t} - e^{-j\omega t}}{e^{j(\omega t + \emptyset)} - e^{-j(\omega t + \emptyset)}} = |Z| e^{j\emptyset} \qquad \text{(Equation 2.6)}$$

Here j equals the square root of -1. Impedance can be further represented as in Eq. 2.8, derived using Euler's formula (Eq. 2.7):

$$e^{jx} = \cos x + j \sin x \qquad \text{(Equation 2.7)}$$

$$Z = |Z|(\cos \emptyset + j \sin \emptyset) \qquad \text{(Equation 2.8)}$$

This relationship of the impedance as a complex function, shown in Eq. 2.8, is often why MXC researchers sometimes look at EIS as a complicated, mysterious technique. I believe that this possibly is a contributing factor to the sparse and incomplete application of EIS in MXC studies. The principles of EIS have foundations in basic mathematics of complex numbers. Since we apply sinusoidal amplitude on voltage, both the voltage and the current with time have to be represented as a sine function, and it is through Euler's formula that these and the resulting impedance can be represented as complex functions.

In Cartesian coordinates, the impedance can then also be represented as,

$$Z = Z' - jZ'' \quad \text{(Equation 2.9)}$$

Here, *Z'* and *Z"* (also often represented as *Re(Z)* and *–Im(Z)* respectively) represent the real and imaginary part of the impedance determined as below.

$$Z' = |Z|\cos\emptyset \quad \text{(Equation 2.10)}$$

$$Z'' = -|Z|\sin\emptyset \quad \text{(Equation 2.11)}$$

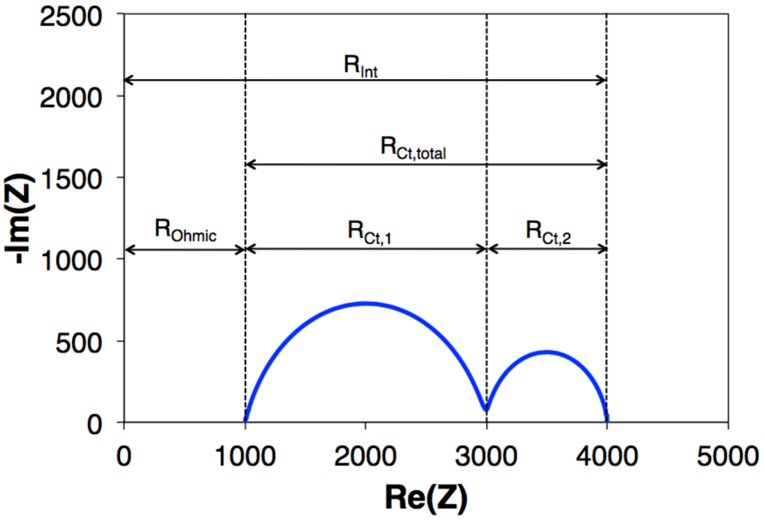


Figure 2.3. A representative Nyquist plot. R_{int} represents the total internal resistance of the cell, while R_{ohmic} representsthe Ohmic resistance, and R_{ct} the charge transfer resistance.

The relationship of the impedance as a complex function is also what leads to the most common data representation in EIS, known as the Nyquist plot (Figure 2.3). In the Nyquist plot, *–Im(Z)* is plotted vs. *Re(Z)* over the entire frequency range of the EIS measurement. One of the main shortcomings of the Nyquist plot is that the frequency is not shown; there is only an implicit understanding that high frequencies are at the lower *Re(Z)* values and decrease in the positive X-direction. In the example provided in Figure

2.3, which is the same as the system represented in the Bode plot in Figure 2.2, three separate resistances are apparent as intercepts on the X-axis. Such a response is often observed in fuel cells where reactants and products are supplied in excess, and the only resistances governing potential losses are the Ohmic resistance and the activation losses at the anode and the cathode.

In the above example, Ohmic resistance is the first intercept on the X-axis (*Re(Z)* = 1000). Usually Ohmic resistance is measured at a high frequency, and results in a zero phase shift. The other two resistances can be interpreted as charge transfer resistances, and they show a characteristic semicircular shape on the Nyquist plot. This results from the capacitive nature of the interface at which the electrochemical reactions occur, and is an indication of a characteristic phase shift distribution over a range of frequencies. As previously mentioned, low frequency semicircles can sometimes be related to diffusion limitations. The Nyquist plot is used popularly because it is easy to obtain a quantitative value of the various resistances and capacitances by fitting the data to an equivalent circuit model (described in detail in Section 4). Despite its advantages in quantitation, the Nyquist plot also has several limitations. For example, often times two charge transfer processes have characteristic frequencies so close to each other in the Nyquist plot that it is almost impossible to distinguish them, perform equivalent circuit fitting, and obtain accurate resistances. Sometimes, it even leads to selection of incorrect or incomplete equivalent circuits. In this regard, the Bode plot could help identify these processes better. But even so, if the two characteristic frequencies are too close to each other, usually less than a decade apart, the EIS results would only measure the combined effect of the two charge-transfer processes. This is one of the main limitations of using EIS in complex systems, such as MXCs. The selection of an adequate experimental setup and EIS parameters can help minimize these limitations for fundamental EIS studies.

2.2. Experimental Designs and Parameters for Application of EIS

Before discussing the control experiments to perform to ensure that valid data is obtained through EIS measurements, I first discuss the various ways in which EIS has been and can be applied in the study of MXCs. The kind of information that can be obtained from EIS measurements depends largely on two important aspects of the experimental plan: the cell design, and the measurement parameters. Below I discuss both of these in detail, to help researchers in the MXC field use EIS to its maximum potential.

2.2.1. Two-Electrode vs. Three-Electrode Setups

The electrochemical investigation of all energy conversion devices in general must be carried out in two-electrode configuration, in order to properly account for the total losses in the system. However, when the subject of interest is centered on the response of either anode or cathode of the device, three-electrode measurements provide more accurate results, since the overpotential at the counter electrode can be neglected. Additionally, reference electrodes provide an absolute reference for the electrochemical events and therefore, energy diagrams can be constructed based on this characterization.

The earliest application of EIS in MXCs was indeed with two-electrode setups (He et al. 2006). In such a setup (Figure 2.4a), one of the electrodes is used as the working electrode, while the other as the counter as well as the reference electrode. When performing EIS measurements in this configuration, the overall impedance of the cell is obtained. In fuel cells, this is usually referred to as the internal resistance. This resistance includes the overpotentials at both the anode and the cathode, resulting from both activation and concentration overpotentials at the individual electrodes, as well as the Ohmic resistance between the two electrodes. Ideally, EIS measurements should be done at a range of cell potentials, to recreate the entire polarization curve. Using an applicable bio-physico-chemical model, translated into an equivalent circuit, and fitting the data to this model, can help deduce the contribution of each individual process to the overall response of the system, as long as each of them have different characteristic time constants.

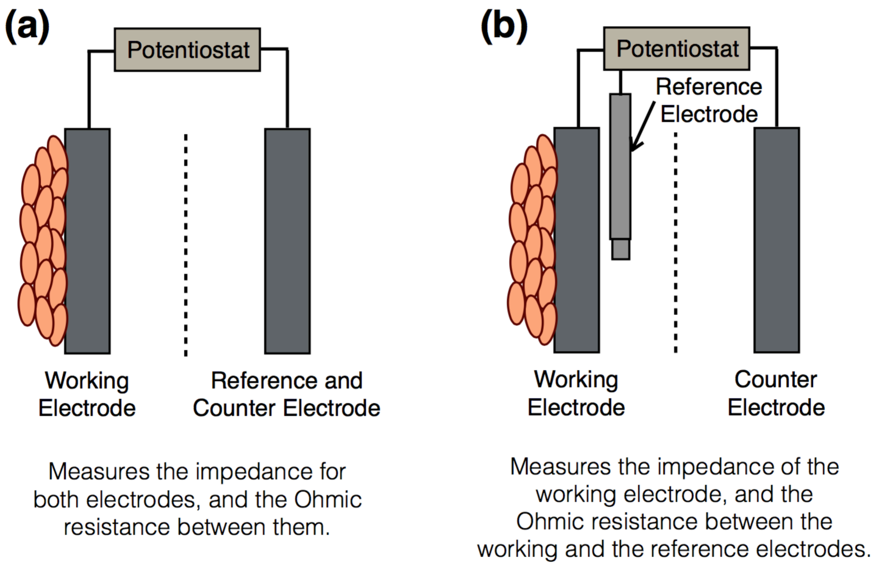


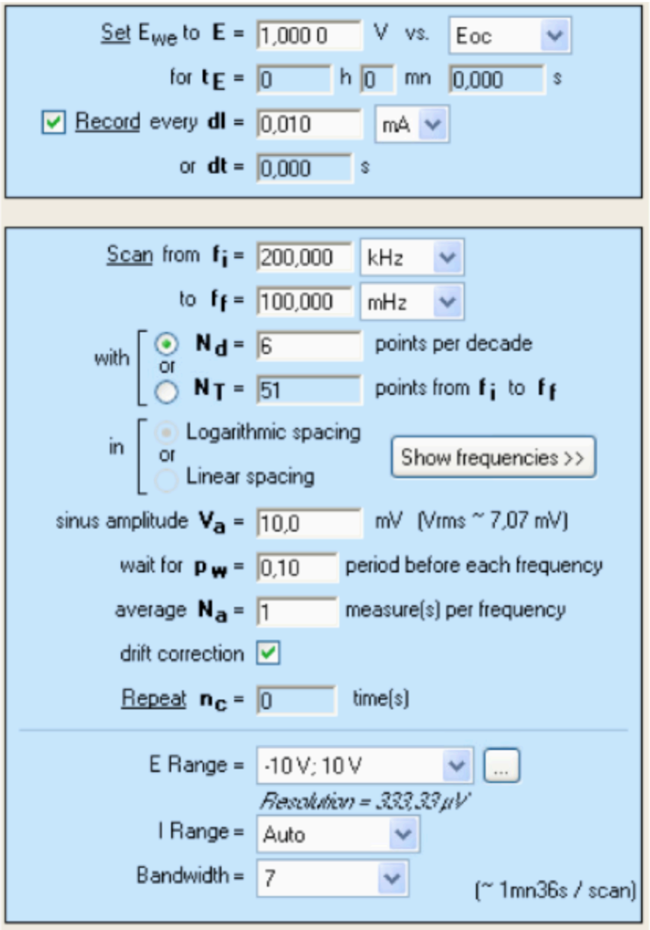
Figure 2.4. Cell configurations to use for EIS measurements: (a) two-electrode setup that measures impedance for both working and counter electrodes, and the Ohmic resistance between them and (b) three-electrode setup that measures the impedance of the working electrode, and the Ohmic resistance between the working and the reference electrodes.

However, sometimes it is difficult to identify each electrode process through EIS measurements with a two-electrode setup, and thus it is important to use the three-electrode setup (Figure 2.4b) to verify their identity and/or their magnitude (Borole et al. 2010). In a three-electrode setup, a separate reference electrode is used, and the EIS measurements are performed for the working electrode with respect to the reference electrode. For example, to measure resistances from all anode processes, the anode would be selected as the working electrode, the reference electrode would be placed close to the anode, and the cathode will be used as the counter electrode. In this case, the impedance measured includes the resistance from all processes occurring at the anode, as well as the Ohmic loss that occurs between the anode and the reference electrode. The latter can also be used to perform *i-R* correction for CV measurements, which is an important step to accurately interpret CV data, especially when these resistances are large. Similarly, to obtain the cathode resistances, the cathode would be selected as the working electrode. While the three-electrode setup for EIS measurements has been used primarily to support data from two-electrode setups in MXC research, recently there have been a few studies published where the three-electrode setup is used to understand microbial electron-transfer processes, both at the anode and the cathode, at a fundamental level, in further detail (Jung et al. 2011; Ter Heijne et al. 2011). I believe that this represents one of the most promising applications of EIS. It is the three-electrode setup, as mentioned in Chapter 1, that this work and subsequent chapters utilize to investigate anode biofilms in microbial electrochemical cells.

I do, however, want to point out an application of the two-electrode setup that has not been used extensively yet in MXC research. I believe that EIS can be a simple tool to rapidly quantify the resistances from various membranes that are used in MXCs. Since MXCs involve ionic solutions apart from the membrane, unlike in PEM fuel cells, traditional methods for measuring membrane conductivity with interdigitated electrodes cannot be directly applied (Lefebvre et al. 1999). There is a simple method to measure membrane conductivity in relevant solutions to compare various membranes. In this method, two electrodes (typically Pt) can be placed on either side of the membrane in ionic solutions of interest. EIS, at high frequencies, can be performed to obtain Ohmic resistance between the two electrodes with and without the membrane separating them. When not using the membrane, the Ohmic resistance is from the resistance to ion transport in solution, while when using the membrane it is from the resistance of both the solution and the membrane. The membrane resistance, and thus conductivity, can then be determined by subtraction. One important consideration while using this method however is to ensure that the electrodes are placed as close to the membrane as possible, in order to minimize the solution resistance. This is especially required to ensure that the membrane resistance is not low enough to be indistinguishable from the uncertainty in the solution resistance. Alternatively, the projected membrane areas can be selected to be very small, so that it is possible to increase the Ohmic resistance from them significantly above the uncertainty in the solution resistance. I provide more detail about this experiment in an example procedure described in Section 5.

2.2.2. Key Experimental Parameters

Shown in Figure 2.5 is a typical parameter selection window in a potentiostat. Most of the parameter selection options are similar across all well-known potentiostats used by MXC researchers. I discuss below in detail the rationale behind selecting some of these parameters.



Set E_{we} to **E** = 1,000 0 V vs. Eoc

for **t$_E$** = 0 h 0 mn 0,000 s

☑ Record every **dI** = 0,010 mA

or **dt** = 0,000 s

Scan from **f$_i$** = 200,000 kHz

to **f$_f$** = 100,000 mHz

with ● **N$_d$** = 6 points per decade

or ○ **N$_T$** = 51 points from **f$_i$** to **f$_f$**

in ● Logarithmic spacing or ○ Linear spacing

sinus amplitude **V$_a$** = 10,0 mV (Vrms ~ 7,07 mV)

wait for **p$_w$** = 0,10 period before each frequency

average **N$_a$** = 1 measure(s) per frequency

drift correction ☑

Repeat **n$_c$** = 0 time(s)

E Range = -10 V; 10 V

Resolution = 333,33 µV

I Range = Auto

Bandwidth = 7

(~ 1mn36s / scan)

Figure 2.5. A dialog box for EIS parameter selection in EC-Lab software by Bio-Logic Instruments. Some of the main selecting parameters are working electrode potential (E_{we}), cell equilibration time (t_E), range of frequency from f_i to f_f, numbers of points per decade (N_D), and the sinus amplitude (V_s).

2.2.2.1. Working Electrode Potential

Sometimes, EIS has been used only at open circuit conditions, in MXC research (Malvankar et al. 2012a; Malvankar et al. 2012b; Manohar et al. 2008). I believe that this form of measurement rarely gives data of any direct relevance to the system being studied in the context of characterizing individual resistances or overpotentials in the systems being studied, other than the Ohmic resistance. This is because no reactions are occurring. The Ohmic resistance measurements however can be valuable for quickly evaluating the efficacy of the membrane being used, or the effect of the distance between electrodes. It can also be used to distinguish between electronic and ionic conductivity (Malvankar et al. 2012b). Also, any measurements made of charge transfer resistances at open circuit can be a strong function of the amplitude applied, especially since most bacterial electrocatalysis kinetics characterized so far follows Nernstian behavior. In other electrochemical cells, such as photo-electrochemical cells, open circuit measurements are often used to measure the capacitance of the electrodes. However, it is well known that the largest fraction of capacitance in MXCs comes from biological redox cofactors that store charge (Busalmen et al. 2008; Malvankar et al. 2012b). The capacitance of MXC electrodes thus changes significantly with electrode potential, thus also reducing the relevance of open circuit measurements. I believe that EIS measurements, when done in three-electrode setups, should be performed over a range of working electrode potentials that are known to result in an electrochemical response, as also predetermined through other electrochemical analyses such as chronoamperometry and/or CV. I direct the readers to two excellent studies, by Jung et al. and ter Heijne et al. (Jung et al. 2011; Ter Heijne et al. 2011), where EIS measurements were performed over a range of potentials for an anode biofilm of *Geobacter sulfurreducens* and a biocathode, respectively. When performing EIS measurements with a two-electrode setup, a wide range of potentials that cover the entire polarization curve should be used.

2.2.2.2. Frequency Range

Most modern potentiostats allow scanning the frequency between the ranges of tens of MHz to tens of mHz. Frequency ranges used in MXC research have also varied significantly. Generally, the EIS measurements should be carried out starting from high frequencies and finishing at low frequencies. It should be noted that at very high frequencies, the effects of inductance can cause the data to be not particularly useful, while at very low frequencies measurements are often small enough that they are masked by noise. In our experience, 200-500 kHz to 10-50 mHz, is enough to capture all the important processes that occur in an MXC.

2.2.2.3. Number of Points per Decade

An important parameter that is rarely ever reported in studies on EIS measurements in MXCs is the number of points per decade. This represents the number of frequencies that the measurements are made between ranges of an order of magnitude. For example, if we select 10 points per decade, then between the frequencies of 10 and 100 Hz, measurements will be made at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Hz, while if we select 5 points per decade, the measurements will be made at 10, 32.5, 55, 77.5 and 100 Hz. The number of points per decade has a direct effect on the number of points available for data fitting in the Nyquist plot. Thus, to obtain better fits, it is obvious to select a large number for points per decade. However, the more the points per decade, the longer the experimental measurement duration is, especially if scanning down to very low frequencies. While this may not necessarily be a problem in certain kinds of electrochemical cells, it is difficult to maintain absolutely steady conditions in MXCs for long durations due to changes in microbial processes. For reference, a scan from 200 kHz to 1 mHz, with 20 points per decade, will take approximately three hours. I have tested several different numbers of points per decade, and suggest using 10 points per decade to have enough points to perform a reasonable data fitting, while ensuring that the experimental duration is a few (<10) minutes for scanning down to 10-20 mHz.

2.2.2.4. Perturbation Amplitude

The perturbation amplitude applied is one of the key parameters that govern the eventual validity of the data from EIS measurements. In general, amplitudes of 10-20 mV have been used in MXC research. I believe these amplitudes represent a good range to perform experiments in, but it is important to verify this through some of the control experiments described later.

In addition to the above parameters, another important parameter is the equilibration time at the selected working electrode potential prior to beginning the EIS measurements. This too, like the amplitude, has implications on the validity of the data, and thus I discuss in further detail below. Many studies do not report on the selection of this parameter, but as I show later, it is important to select an appropriate equilibration time. Generally, the equilibration time can be chosen as the inverse of the lowest frequency selected for the analysis, in order to guarantee that the system is relaxed when the next perturbation is applied.

2.3. Experimental Protocol to Ensure Data Validity

To be able to use the data generated during EIS measurements for any type of data analysis, it is important to consider that EIS measurements are only valid when the following four conditions are met:

1. Linearity: One of the first, fundamental assumptions of EIS measurements is that the system behaves linearly in change in current in response to change in potential. This is illustrated in Equations (1) and (4) above. Of course, no electrochemical or biological system is perfectly linear, but the amplitude of the applied perturbation can be selected such that the system can be approximated to be linear. Usually, this amplitude is thus selected to be small, but it should be

considered that the amplitude be large enough to distinguish real measurements over noise.

2. Stability: EIS measurements usually take anywhere from a minute to tens of minutes, depending on the range of frequencies selected. A key requirement of the experimental setup is that the system stays stable over the course of the experimental duration, i.e. there are no inherent temporal changes in the system due to factors other than the applied perturbation in voltage. This can be particularly important with biological systems that can be affected by growth, transport of reactants/products, and cellular adaptations to the selected potential.
3. Causality: The system studied by EIS should not be affected by external perturbations other than the perturbation applied. Often times, causality is a result of noise in measurements due to internal potentiostat controls, interfering instruments, or bad wire lead connections. When the noise in current is significant with respect to the signal obtained due to change in potential, the data quality can be considered poor.
4. Finiteness: One of the assumptions from Equation (4) is that the change in current as a function of change in potential not be zero. Thus the value of impedance has to be finite at all frequencies applied. If there is no current response due to infinite impedance, the data analysis cannot be performed, as there is no signal response.

In this section, I discuss in detail how selection of various experimental parameters affects each of these conditions. One of the first studies on EIS measurements in MXC applications, by Strik et al. (2008) covers some of these conditions very well, but I provide an expanded explanation here. While all these conditions are especially difficult to fulfill in a typical electrochemical cell, the conditions used in MXCs exacerbate the problem further. For example, it is known that polarization curves for microbial anodes exhibit non-linear, Nernstian responses (Torres et al. 2008). Thus, there are regions in the polarization curve where the system may not behave linearly even when small amplitudes are applied. The irreversibility of the enzymatic responses also leads to regions where finiteness is not met (Figure 2.6). These cases would also run into difficulties in terms of the signal-to-noise ratio when small amplitudes are applied at potentials on the saturation region of the polarization curve. Similarly, since MXCs are biological reactors, and can have changes in microbial responses due to small perturbations outside the control of researchers, conditions of both stability and causality are difficult to fulfill.

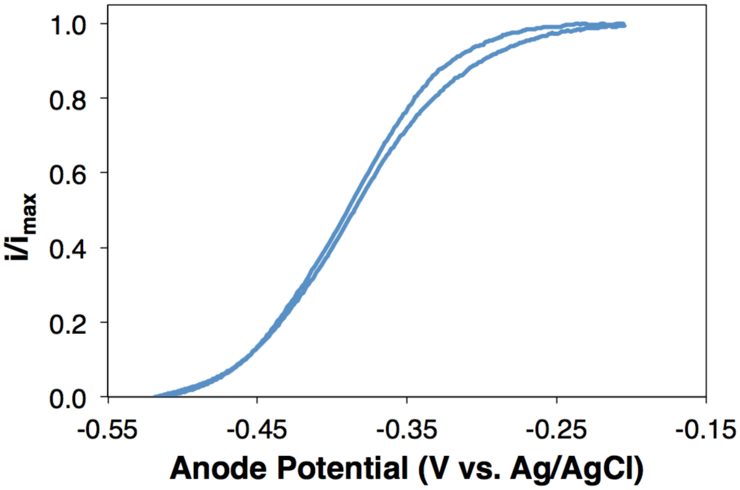


Figure 2.6. A typical cyclic voltammogram of a *Geobacter sulfurreducens* biofilm. The current (i) is shown normalized to the maximum current (i_{max}).

I have investigated various experimental procedures to ensure the accurate application of EIS to MXCs. In the following sections, I discuss EIS experiments performed with biofilms of *G. sulfurreducens* grown on gold or graphite anodes of various geometries in three-electrode setups. These experiments were used to investigate several parameters, including perturbation amplitudes, working electrode potentials, equilibration times, and electrode materials and sizes to use to ensure obtaining accurate EIS data. Wherever needed, I compare experimentally collected data to the Kramer-Kronig relationship, a relationship that ensures that the data collected meet the four condition requirements of EIS (Boukamp, 1995; Macdonald, 1985). More details about the K-K relationship are available in literature cited above. I used two different potentiostats, from Ivium (Compactstat) and Bio-Logic (VMP3) for the studies described here; the potentiostat used for the data collection is understood to be the Ivium Compactstat unless otherwise noted. While the data shown here was collected from three-electrode setups i.e. performing EIS specifically to understand anode phenomena, the same guidelines and principles outlined would apply for two- and four-electrode setups as well.

2.3.1. Linearity and Finiteness

There are two important parameters that affect the condition of linearity and finiteness required for accurate application of EIS. I discuss below how to ensure the appropriate selection of both the parameters.

2.3.1.1. Perturbation Amplitude

The perturbation amplitudes that can be used during EIS can be both theoretically and experimentally evaluated. In theory, the practical operating perturbation amplitude should be less than the product of the Boltzmann constant ($K_b=8.617*10^{-2}$ mV/K) and the temperature at which the experiment is performed. This requirement was shown clearly by Barbero et al. in their study on EIS measurements on electrolytic cells (Barbero et al. 2005). At 30 °C, a common temperature in MXC operation, the amplitude should thus be less than ~26 mV. While this theoretical guideline is generally valid, in case of MXCs, the amplitude selected also needs to take into consideration the potential of the working electrode. For example, amplitudes much smaller than 26 mV would be required if performing EIS on the anode in the regions that transition to or from the linear response around the midpoint potential (-0.4 vs. Ag/AgCl in Figure 2.6).

In general, there are two considerations for the amplitude that can be used. Larger amplitude can be advantageous because it provides a larger signal-to-noise ratio. However, smaller amplitudes may allow the system to fulfill the condition of linearity better, especially in the potential regions mentioned above. This can be seen in Figure 2.7 a, showing the Nyquist plot at open circuit potential (-0.5 V vs. Ag/AgCl), which will be in the activation region of the Nernstian response. It appears that the 5 mV and 10 mV amplitudes provide similar results; however as the amplitude is increased to 50 mV and 100 mV, progressively lower impedance responses as a result nonlinear behavior are apparent. For most setups that I have tested in MXC anodes, I have observed that at open circuit < 20 mV is the optimum amplitude to use. I note here that such amplitude validation tests should be done with electrodes that already have the biofilms of interest developed on them, as bare electrodes may not necessarily follow the same kinetic behavior in relation to an applied overpotential.

Close to the midpoint potential, as determined from Figure 2.6, the system shows essentially linear behavior, and there is less dependence of the impedance response on the perturbation amplitude used (Figure 2.7b). At this potential, it seems that it would be acceptable to perturb the potentials in the linear region with almost any of amplitudes selected. However, the data collection should remain consistent with the amplitudes that meet the requirements towards the extremities of the appropriate potentials to investigate, as well as the theoretical limit of amplitude (using the k_BT relationship).

2.3.1.2. Potentials

Figure 2.8 shows a comparison of the Nyquist plots of the experimental data collected on the Bio-Logic (VMP3) potentiostat with amplitude of 10 mV at three different working electrode potentials. The figure also includes the Kramer-Kronig relationship fits for each condition. The Kramer-Kronig relationship does not fit the actual data at the higher potential (-0.3 V vs. Ag/AgCl, Figure 2.8a), suggesting that the system is not compliant to one of the conditions required for obtaining valid EIS data. In this case however, I believe this condition is finiteness, and not linearity. As seen in the CV shown in Figure 2.6, the system under consideration can be considered infinite with respect to the impedance, i.e. in either direction of -0.3 V vs. Ag/AgCl, for a 10 mV amplitude, there is no change in steady-state current, or it is smaller than the noise in the system.

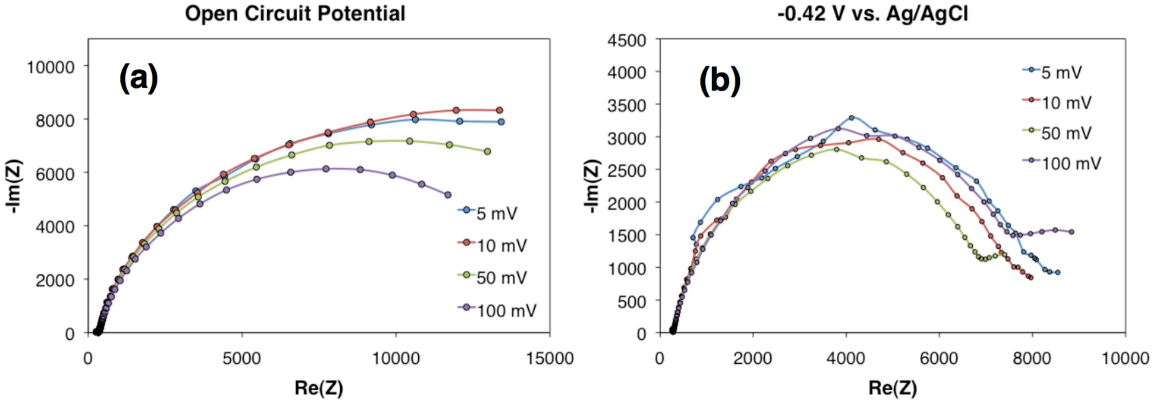


Figure 2.7. The effect of the voltage amplitude during EIS measurements on a *Geobacter sulfurreducens* biofilm on the Nyquist plots obtained under: (a) open-circuit conditions and (b) with the anode poised at -0.41 V versus Ag/AgCl, which is close to the midpoint potential in the *i*-V relationship.

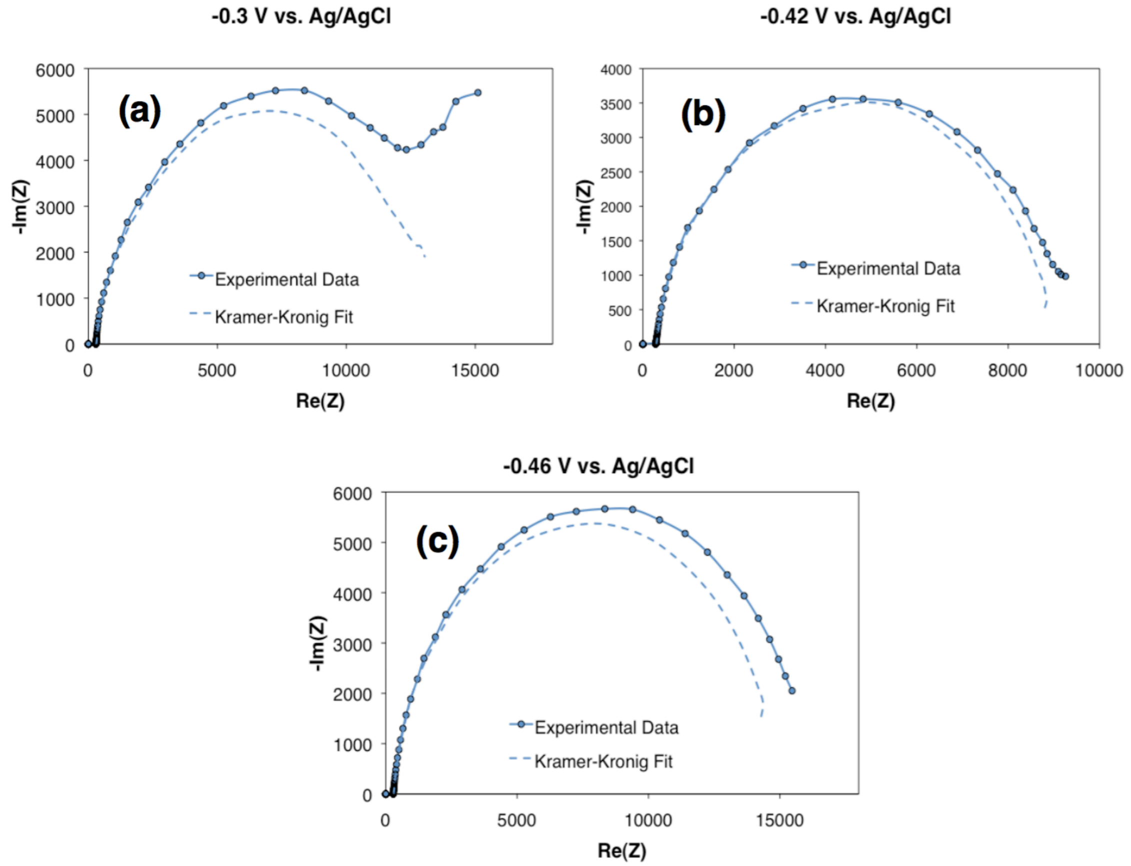


Figure 2.8. Nyquist plots form EIS measurements on a *Geobacter sulfurreducens* biofilm at three different potentials: -0.3 V versus Ag/AgCl, which is close to saturation potential in the *i*-V relationship, (b) -0.42 V versus Ag/AgCl, which is close to the midpoint potential in the *i*-V relationship, and (c) -0.46 V versus Ag/AgCl, which is close to open-circuit anode potential. The dotted lines show Kramers-Kronig fits.

Close to the midpoint potential (Figure 2.8b), the Kramer-Kronig fit appears to validate the EIS data. At this potential, the system can be considered both linear and finite at the amplitude used. Towards the more negative end of the CV (-0.46 V vs. Ag/AgCl, Figure 2.8c), the Kramer-Kronig fit again begins to deviate from the data collected. In this activation region, again the linearity is in question, and the data becomes less reliable, at least at the amplitude selected.

In summary, due to the Nernstian response of bacteria to the working electrode potential, it is important to perform several control studies, as described here, to select the optimum conditions under which both requirements of linearity and finiteness are met. Through the use other electrochemical techniques such as CV, and also the use of data validation methods such as the Kramer-Kronig fitting, the appropriate perturbation amplitudes and working electrode potentials for EIS can be determined for the bacterium and reactor configuration under study.

2.3.2. Stability

A time delay for equilibration of the system to the selected working electrode potential is required before performing an EIS measurement. This equilibration time allows the system to adjust to the specified condition and achieve a pseudo-steady state. It is of the utmost importance that the system has a stable current during the EIS measurement, since EIS measures small changes in current as a result of the AC voltage perturbation. Biological systems are difficult to get to a pseudo-steady state because of the dynamic nature of bacteria. Unlike electrochemical cells, MXCs can take several minutes to reach an equilibrium state while the cells adapt to the potential of the electrode by changing cellular processes such as intracellular conditions and protein expression. Additionally, the presumably short time at which the system is at open circuit when changing between potentials for different scans allows charge to be built up in the system.

For example, while the method parameters are being changed, during which the system will be at open circuit, the heme groups of extra cytoplasmic cytochromes that are known to provide a location for electron storage due to continued bacterial respiration might build up charge (Bonanni et al. 2012). The re-polarization of the electrode creates a quickly decreasing higher current as compared to the steady state. In addition to these, there are a number of cellular processes, including changing electron donor concentrations, cell growth, mixing patterns, among others that can affect the stability of the system during EIS. These changes in bioanode or biocathode performance in time (as a result of growth for example), are an important aspect to take into account in EIS measurements, especially when the measurements take place over a longer period of time. In addition to the EIS measurements, it is important to provide information of the stability of the bioanode or biocathode performance. This can be done by recording the changes in current density as a function of time (at constant potential), and/or regular recording of polarization curves before and after the EIS measurement.

On Figure 2.9a, I show the equilibration time before an EIS experiment of a *G. sulfurreducens* biofilm as a result of an adjustment to the potential of the electrode (from -0.3 V vs. Ag/AgCl during chronoamperometry to -0.42 V vs. Ag/AgCl to perform EIS). The maximum in the transient current shown immediately after the polarization of the electrode is a result of the re-oxidation of cytochromes that had been previously reduced during the interruption due to built-up charge. Thereafter, the current reaches a minimum, presumably because the bacteria had changed their metabolic state during open circuit conditions. Approximately, 10 minutes were required in this example to achieve a stable current density that is appropriate for EIS measurements. I stress that it is important to perform such equilibration time measurement before performing EIS measurements to ensure that the system is steady prior to beginning the EIS measurements.

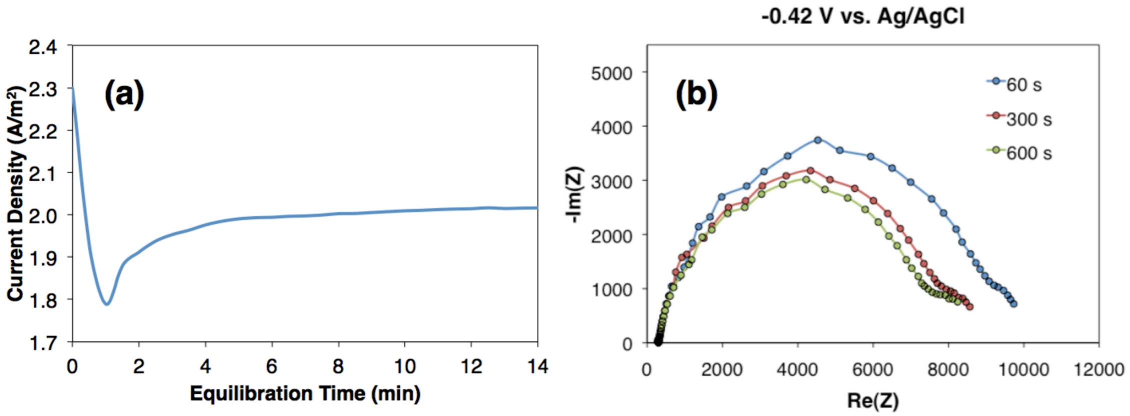


Figure 2.9. The effect of equilibration time on EIS measurements on a *Geobacter sulfurreducens* biofilm: (a) shows the time it takes to reach a steady current after a brief period at open circuit while the EIS method is input and (b) shows the effect of different equilibration times on the Nyquist plot obtained.

Figure 2.9b shows the Nyquist plot resulting from performing EIS measurements at different equilibration times. For each of these, the chronoamperometry was interrupted, allowing the cell to reach open circuit, before starting the equilibration time measurement at the selected working electrode potential and performing EIS measurements thereafter. The equilibration time selected affects the total impedance and capacitance of the system, and can be seen in the changes in the Nyquist plot. Here, if I waited only one minute before beginning EIS measurements, I was essentially conducting EIS at the lower current density observed in Figure 2.9a, which is an artifact of the transient open circuit induced while changing the method on the potentiostat. Usually, the size of the electrode and the thickness of the biofilm affect the amount of charge collected when the system is not poised, thus affecting the equilibration time required. Thus, it is important to perform a stability analysis (as shown in Figure 2.9a for the MXC under study, before performing EIS measurements.

Overall, the stability principle applies to not only how stable the system appears before EIS collection, but during a sweep as well. For more complicated systems, such as continuous flow cells, the flow rates must be kept constant during the scan to ensure a relatively constant concentration of electron donor and other media constituents. In general, every effort should be made to evaluate the experimental conditions and ensure that the system does not change prior to or during data collection.

2.3.3. Causality

The principle of causality for EIS measurements is related to many different sources that can cause interferences and noise in the data. For example, too many instruments running in a particular area can cause interferences. Orazem (2011) recommends that particular attention be paid to avoiding frequencies including 60 Hz, 120 Hz, 50 Hz, and 100 Hz (all +/- 5 Hz), as at these frequencies other instruments in the surrounding can affect the data collected.

2.3.3.1. Magnetic Stirring

Although magnetic stirring is a common way to ensure proper mixing of media in a bioreactor and to minimize diffusion of species within the electrolyte, the magnetic field created may cause problems with data collection for certain reactor geometries. I have observed that there are certain cases where magnetic stirring does not interfere with the measurements, while some in which the noise in EIS measurement is large due to magnetic stirring. Whether or not magnetic stirring is acceptable during EIS data collection should be determined for each setup. One way to monitor noise of the system is the chronoamperometry method, which monitors the current output over time. In our experience, the noise in current should be <5% of the signal, to be able to perform EIS measurements at most working electrode potentials.

2.3.3.2. Faraday Cage

In theory, a Faraday cage can be used to house the reactor and shield sensitive electrical connections from outside interferences. I have performed several measurements with and without a Faraday cage to determine its effect. In every situation I tested, the data suggested that the particular reactor setup I employed was not sensitive to the effects of external electromagnetic noise. However, other environments may have significant interferences and would require the use of a Faraday cage in order to obtain accurate EIS measurements. Outside interferences can increase the noise in the current measurements, making it more difficult to separate individual impedances from the results.

2.3.3.3. Electrode Size

The size or type of electrode can alter the measurement performed with EIS. Larger and porous electrodes have higher capacitances at the electrode surface that can interfere with other measurements. In larger working electrodes, potential gradients can also lead to variable conditions depending of the location; thus, the recorded EIS measurement is a composite response of a range of potentials within the electrode. Larger electrodes are also more susceptible to variable fluid dynamics and substrate concentrations, which can affect stability of the system.

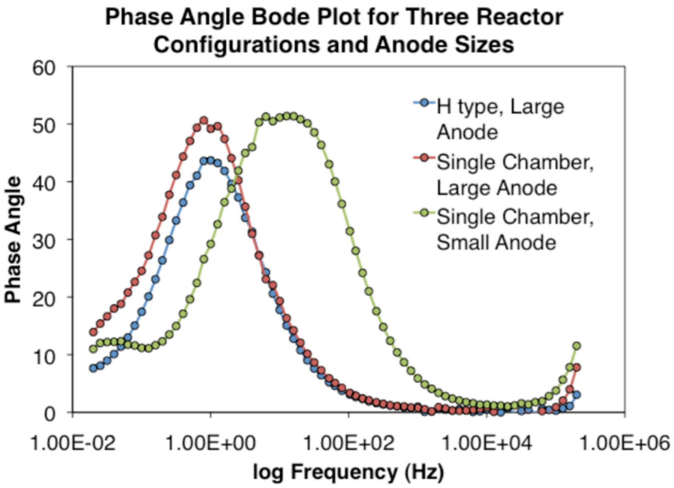


Figure 2.10. Bode plot for *Geobacter sulfurreducens* biofilms at the same anode potential, but with different cell configurations/anode sizes.

Figure 2.10 shows the Bode phase angle plots for EIS measurements conducted for a working electrode potential of -0.36 V vs. Ag/AgCl with an amplitude of 10 mV for three reactor configurations (an H-type MXC with 4.2 cm^2 cylindrical graphite working electrode, a single-chamber MXC with 4.2 cm^2 cylindrical graphite working electrode, and a single-chamber MXC with 3.14 mm^2 disc-shaped gold working electrode). It is important to compare the Bode plots here, because the Nyquist plots for the different set-ups show no significant differences (except obviously for the magnitude). Figure 2.10 however suggests that processes can be manifested at different frequencies, depending on the size of electrode, or type of cell configuration used. In the high frequency region of the figure, the Ohmic effects appear to be elongated for the larger sized electrodes. Additionally, a final process is visible for the smaller electrode at the lowest frequencies (below 1 Hz), suggesting that the two processes may be merged on the larger size electrodes, and thus are inseparable. Although the size of the electrodes to be used may depend on the intended application, our results suggest that smaller electrodes may be most effective in analyzing distinct bacterial processes using EIS.

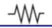
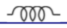
2.4. Data Analysis

2.4.1 Equivalent Circuit Modeling

In order to correctly interpret the experimental information provided by EIS, conveyed either in Nyquist or Bode plots, the use of a sound physical model describing the relevant bio-physico-chemical processes taking place in the system is essential. A simple strategy to deal with the experimental information involves the implementation of the model into an equivalent circuit, which contains all the information of charge transport. In the equivalent circuit, the resistances and capacitances describe the charge loss and accumulation mechanisms that can take place in the system. Below I first describe the most common circuit elements used in EIS data analysis, followed by the most common equivalent circuits used to describe typical electrochemical cells.

A resistor, along with a constant phase element (signifying a non-ideal capacitor) in parallel, is usually used to describe a charge transfer process. Diffusion is usually accounted by a Warburg element, which contains information about the diffusion coefficient and diffusion length of the carriers. More complex situations take place when there is coupling between diffusion and resistive losses, and consequently distributed elements, such as the Gerischer element, can be introduced in the equivalent circuit to account for these complex phenomena. This is especially the case when porous electrodes or conducting polymers are studied. An extensive development of the theory of diffusion and recombination on thin layers has been carried out by Bisquert, which I refer the readers to (Bisquert, 2002). Table 2.1 summarizes the basic elements that are conventionally employed to describe simple physico-chemical processes and the impedance associated to these elements.

Table 2.1. List of common circuit elements used in equivalent circuit modeling for Nyquist plots generated from electrochemical impedance spectroscopy.

Denomination	Symbol	Scheme	Impedance
Resistance	R		R
Capacitance	C		$\frac{1}{i\omega C}$
Inductor	L		$i\omega L$
Constant phase element (CPE)	Q		$\frac{1}{(i\omega)^n Q_n}$
Warburg (conducting boundary)	W_S		$R_D \frac{\tanh\left[(i\omega/\omega_d)^{1/2}\right]}{(i\omega/\omega_d)^{1/2}}$
Warburg (blocking boundary)	W_O		$R_D \frac{\coth\left[(i\omega/\omega_d)^{1/2}\right]}{(i\omega/\omega_d)^{1/2}}$
Gerischer	G		$\frac{R_G}{(1+i\omega/\omega_{rec})^{1/2}}$

The most common approach to describe the physico-chemical processes taking place within an MXC entails the use of equivalent circuits as those listed in *Table 2*. I also show in the table the corresponding Nyquist and Bode plots for each circuit. I describe some of these circuits in brief here. For example, circuit 1 is a simple circuit representing the Ohmic resistance in a system. This is a linear resistor, for which the Bode plot shows a straight line at a single impedance value throughout the range of frequencies.

A more relevant example is related to the charge transfer at an electrode, which is in contact with a liquid solution. This situation is best described by a parallel combination of a resistance and a capacitance (circuit 2), where the resistance conveys the charge transfer kinetics at the electrode/liquid interface and the capacitance reflects the accumulation of charge at the Helmholtz layer (Helmholtz capacitance). A series resistance (denoted here by R_2) associated to this RC combination reflects the Ohmic losses. This equivalent circuit is termed Randles' circuit. On the other hand, if the relevant capacitive processes identified in the electrochemical response of the system are not ideal, which is often the case for porous electrodes, constant phase elements (Q) can replace the capacitances (circuit 3). The parameter accounting for the non-ideality of the system (n) must be comprised between 0.8 and 1 (to model a non-ideal capacitance). Otherwise, for n <0.8, the CPE can be related to a different process, which is not essentially capacitive. It is this circuit that is most commonly used to describe charge transfer from electrode-attached biofilms to the electrode. While researchers have used this circuit successfully to obtain relevant data, recently it was proposed that the capacitance due to the double layer at the electrode, and due to the redox cofactors produced by bacteria, be modeled separately (Dominguez-Benetton and Sevda, 2012).

A more complex situation in MXCs involves the presence of diffusive limitations of various relevant species especially at the biofilm/liquid interface or within the biofilms. In this case, a Warburg element can be introduced into the equivalent circuit to take into account this behavior. Depending on the nature of the interface (blocking or permeable), an open-circuit or short-circuit Warburg element can be employed (circuits 4 and 5, respectively). Charge transfer and diffusion limitations can coexist in some systems and in order to account for this situation, a simple approach is including a Warburg element in a Randles' circuit (see circuit 6). This approach has been successfully employed to model the electrochemical response of a O_2 reducing biocathode (Ter Heijne et al. 2011).

Table 2.2. List of common equivalent circuits used for modeling Nyquist plots generated from electrochemical impedance spectroscopy.

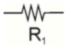
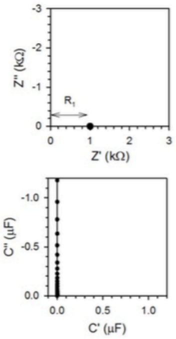
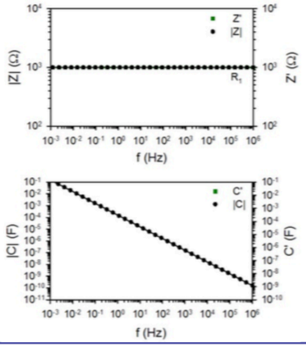
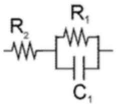
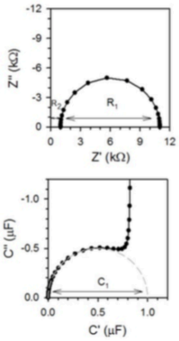
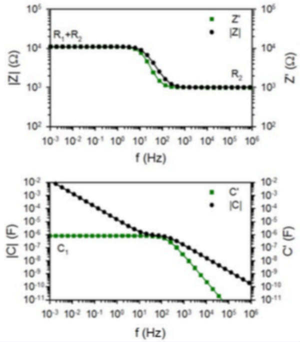
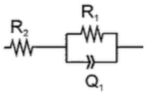
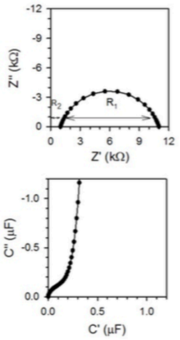
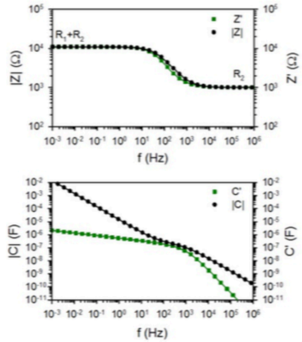
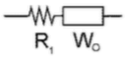
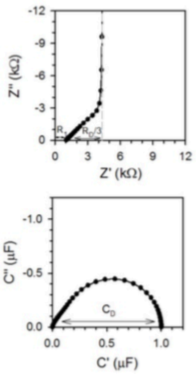
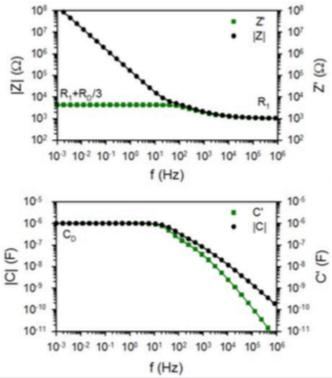
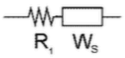
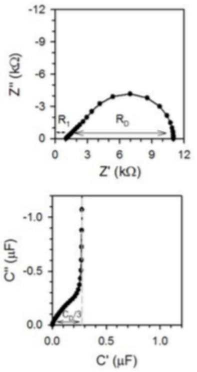
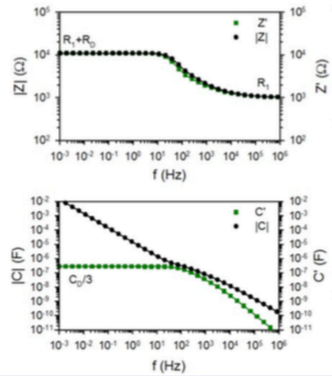
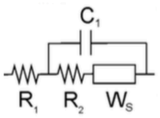
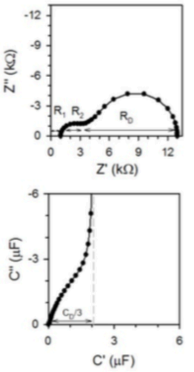
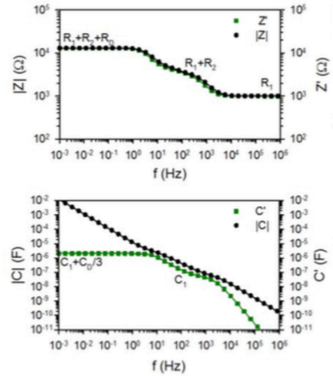
	Equivalent circuit	Nyquist	Bode
1	 R1 = 1 kΩ		
2	 $R_2 = 1$ kΩ $R_1 = 10$ kΩ $C_1 = 1$ μF·		
3	 $R_2 = 1$ kΩ $R_1 = 10$ kΩ $Q_1 = 1$ μF·s^{1-n} n = 0.8		

Table 2.2. (continued)

4	 $R_1 = 1$ kΩ $R_D = 10$ kΩ $w_D = 100$ s^{-1} $C_D = 1/(R_D \cdot w_D)$		
5	 $R_1 = 1$ kΩ $R_D = 10$ kΩ $w_D = 100$ s^{-1} $C_D = a^2/(R_D \cdot w_D)$ $a = R_D/(R_1 + R_D)$		
6	 $R_1 = 1$ kΩ $R_2 = 2$ kΩ $C_1 = 0.1$ μF $R_D = 10$ kΩ $w_D = 10$ s^{-1} $C_D = a^2/(R_D \cdot w_D)$ $a = R_D/(R_1 + R_2 + R_D)$		

2.4.1.1. The Importance of Experimental Verification

A good numerical agreement between the experimental data and the model fit does not necessarily mean that the chosen equivalent circuit is valid. Different models may give similar results in terms of numerical fit. The physical interpretation of the different parameters must be carefully taken into account in order to arrive at a valid interpretation of the EIS data. To verify the validity of the equivalent circuit to which the EIS results are compared, systematic changes in experimental conditions are of importance. For example, in the EIS work performed on biocathodes that catalyze oxygen reduction, the Nyquist plots showed a single arc (Ter Heijne et al. 2011). In general, this single arc is interpreted as a single charge transfer resistance. Changes in the experimental conditions, like the linear flow velocity, however, revealed that besides charge transfer, also diffusion resistance influenced the width of the arc. Analysis with the standard Randles circuit that includes both charge transfer and diffusion resistance (circuit 6 in Table 2), gave consistent results: with increasing linear flow velocity (improved mass transfer of oxygen), a decrease in diffusion resistance was observed. At the same time, as expected, charge transfer resistance remained unaffected at constant cathode potential. This showed that the standard interpretation of the single arc in the Nyquist plot, representing a charge transfer resistance, was not valid for the system under study. Similarly, measurements at different electrode potentials can be used to reveal the contribution of charge transfer resistances to the total impedance. I provide further details about this experiment in Section 5.

Another example of the use of experimental verification of a selected equivalent circuit includes the study by Ramasamy et al. (Ramasamy et al. 2009) who performed EIS measurements on MFCs containing *Shewanella oneidensis* at the anode. Since *Shewanella* spp. secrete flavins as soluble electron mediators between the cells and the anode (Marsili et al. 2008), the authors included a charge transfer resistance in the equivalent circuit for flavins, based on their initial experiments. However, they also confirmed the selected of this circuit further by adding additional flavins, which resulted in a lower charge transfer resistance. Such experiments are vital in ensuring that equivalent circuits used are appropriate, rather than just an abstract mathematical formulation.

2.4.1.2. The Importance of Capacitance

The identification of charge storage mechanisms is of paramount importance in order to understand charge transfer in MXCs. The information on charge storage is conveyed by the capacitance and monitoring the evolution of this parameter with applied voltage can easily identify the origin of a capacitive response. Indeed, the different processes taking place simultaneously in the system can be resolved by the identification of their capacitive response. Additionally, the capacitance provides a means to verify the validity of the model. For example, the double layer capacitance is not expected to change considerably during the experiment, and should be independent of changes in the experimental conditions. So, if changes in double layer capacitance are found between different types of experiments, one might need to reconsider the chosen model that represents the system under study. On the other hand, electrodes with a high capacitance may complicate the interpretation of EIS data, particularly the distinction between the different processes.

2.4.2. Other Methods

Often times, it is difficult to develop a first approximation of an equivalent circuit upon which to test various experimental conditions. This is because more than one process can have characteristic frequency so close to each other that it is almost impossible to distinguish the two in Nyquist or Bode plots. To overcome this limitation, researchers in the field of solid oxide fuel cells have developed alternative techniques that allow for a better estimation of equivalent circuit to use to fit the data. Two examples of these are the distribution of relaxation times (DRT) method and the analysis of difference in impedance spectrum (ADIS). While these have never been applied to MXC studies, I believe these could provide new information of processes that researchers may not have been able to identify before. I direct the readers to references where these methods are discussed in more detail (Jensen et al. 2007; Schichlein et al. 2002; Sonn et al. 2008). In addition to these two methods, Dominguez-Benetton recently reviewed other alternative methods of data analysis that could be applied to MXCs, that I strongly suggest the readers to also investigate (Dominguez-Benetton and Sevda, 2012).

2.5. Examples of EIS Applications in MXCs

I provide here a brief guide for performing EIS on MXCs to gain specific types of information, using three examples. It is difficult to review all the applications of EIS, but I provide here the three that I think cover a significant breadth of information that can be obtained through EIS measurements. These examples focus on: (i) characterization of Ohmic resistance of membranes of interest, (ii) revealing factors affecting the kinetics of electrode reactions catalyzed by bacteria, and (iii) characterization of a complete MXC system.

2.5.1. Characterization of Ohmic Resistance

In this first example, I describe a simple method to easily and rapidly characterize the Ohmic resistance for a membrane used in an MXC. This method can be used to compare and select from various membranes, as I have reported recently (Ter Heijne et al. 2010). A simple H-type MXC, with an electrode fixed in each chamber, is required for this measurement. The idea is to measure Ohmic resistance between the two electrodes in the presence and absence of the membrane. By subtraction the resistance due to the membrane can then be calculated. I do want to caution here that the effective distance between the electrodes that is occupied by the solution should remain the same with or without the membrane. This is often difficult to ensure, since when a membrane is placed between two fixed electrodes, it occupies a small volume that the solution initially occupied. The effect this will have on the measurement depends on the relative magnitudes of the solution or membrane resistance.

It is important to optimize the surface area of the membrane and the distance between the two electrodes to get good measurements. We want the Ohmic resistance of the membrane to not be masked by noise and uncertainty, and thus want it to be significantly higher than the Ohmic resistance of the solution. The best way to assure this is to use very small distances (<1 cm) between the two electrodes, but also very small cross-sectional area of the membrane for ion flow. Since resistance is inversely proportional to the cross-sectional area, ensuring a smaller area will result in a higher resistance. Note that for the electrodes, any conductive material is fine, as we are not looking specifically for any particular reactions on the electrodes. For the solution between the electrodes, it is recommended to perform the EIS experiment several times in solutions of different conductivities, as membrane resistance will change as the solution conductivity changes. In any case, the measurement should at least be done in the solution of interest to be used for further applications.

Once the electrochemical cell is ready, it needs to be connected to an EIS-capable potentiostat/channel in a two-electrode setup. This means one electrode is connected as the working electrode, while the other as the counter and the reference electrode. Most potentiostats include a specific EIS method for measurement of Ohmic resistances. For reference, for the Bio-Logic potentiostats with EC-Lab software for channels with EIS capability, the method can be found under “Ohmic Drop Determination” as “IR compensation (PEIS) – ZIR”. Since Ohmic resistance is measured at high frequencies, the concept of the measurement is to apply amplitude of 10-20 mV on the cell at open circuit, at high frequency (usually >100 kHz, but should be confirmed through measurements at multiple frequencies), and measure the resulting ratio of change in current to the change in voltage. I recommend doing at least 10 measurements and taking an average. The procedure must be repeated with and without the membrane of interest separating the two electrodes. It is absolutely essential to not disturb the location of the electrodes while doing so, and thus I recommend having the electrodes fixed in place in the chambers.

For potentiostats that do not include a specific method for Ohmic drop determination, a full EIS scan over a range of frequencies from high to low can be used. In the resulting Nyquist plot, the first intercept on the X-axis is the Ohmic resistance. In case this is not directly apparent, an equivalent circuit including an Ohmic resistor can be used to fit the data, and obtain the Ohmic resistance. Once the resistance is determined, it should be reported in the form of area specific resistance (ASR), as ohm cm^2. Thus the area of the membrane used should be multiplied with the subtracted value from the measurement with and without membrane.

2.5.2. Characterization of Electrode Processes

For the second example, I provide an overview of the study published by ter Heijne et al. (2011) on identifying the importance of charge and mass transfer resistances in an oxygen-reducing biocathode. In this study, EIS measurements were done on a three-electrode setup with the biocathode selected as the working electrode. This, as was discussed earlier, helps elucidate the resistances from all the processes occurring at the biocathode. I do want to point out here that this study was a follow-up to an earlier study (Ter Heijne et al. 2010) where the authors used chronoamperometry and CV to identify important phenomena that govern the performance of the biocathode. This highlights how EIS can be used in concert with, or to support, other techniques. This is a very important consideration when performing EIS measurements to understand electrode processes. Before performing the EIS measurements, it is important to have a good idea about what parameters affect performance and thus could affect the resistances that can be measured by EIS. For example, the initial study clearly laid out how cathode potential and oxygen availability affected biocathode performance, thus providing the conditions to change for each EIS measurements.

In this case, EIS measurements were thus performed at different cathode potentials, and with different oxygen flow rates. The authors had hypothesized from the initial study that the cathode potential would affect the charge transfer resistance, and the oxygen flow rate the mass transfer resistance. For each EIS measurement done at a different cathode potential or oxygen flow rate from a frequency of 100 kHz down to 1 mHz, the perturbation amplitude (10 mV) was applied after 300 seconds at the condition selected. This should be done to ensure that the biofilm adapts to the new fixed potential or oxygen flow rate. I showed earlier how this equilibration time should be selected through a series of control studies. EIS measurements were done at five different cathode potentials that were selected from earlier j-V curves and CV scans obtained for the biofilms that showed the range within which catalytic current as observed for the biofilm. This stresses the importance of using voltammetric scans to determine exactly what range of potentials are important to obtain EIS measurements at. Similarly, seven different oxygen flow rates were used.

The Nyquist plots obtained were fit to multiple equivalent circuits to determine what model works the best. Since the authors had already determined earlier the importance of cathode potential and oxygen availability, it could easily be anticipated that the equivalent circuit should include at least one charge transfer and one mass transfer resistance, in addition to the Ohmic resistance between the working and the reference electrodes. Even so, changing the cathode potential and oxygen flow rates clearly confirmed which circuit should be used. I suggest that any time EIS measurements are done to understand electrode processes, such studies be performed to confirm the validity of the equivalent circuit used to model the data.

This study is also a good example of how EIS provides additional data to what can be obtained from chronoamperometry or cyclic voltammetry. Since the data can be fit to a circuit, parameters like charge transfer resistance and diffusion lengths can be obtained from the fitting. In this case, charge transfer resistance was used to validate the Nernstian-based model used to fit the j-V relationship. Also, Dominguez-Benetton and Sevda (2012) used the data later to extract important information related to the capacitance of the biofilm and how it could relate to biofilm thickness. Similarly, the experiments with different oxygen flow rates helped determine the diffusion lengths in each case, which can now help develop physico-chemical models that not just include biofilm kinetics but also substrate transport.

A similar approach was also used by Jung et al. (2011) where they looked at, through EIS measurements, the effect of anode potential and pH on anodic biofilms, which were both found to have an effect on the performance in chronoamperometric and voltammetric measurements. I encourage the readers to go through that study too to learn more about performing EIS measurements to understand electrode processes.

2.5.3. Characterization of Complete MXC System

For the final example, I select the study by Borole et al. (2010), which is one of the first reports on the use of EIS to completely characterize an air-cathode MFC over time, using both two-electrode and three-electrode configurations. In this study, the MFC was characterized through EIS measurements at the cell voltage that resulted in the highest power density. A more expanded characterization would also include doing the measurements throughout the entire range of cell voltages in the polarization curve. However, the focus of this study was to do the characterization over time of operation. For the two-electrode configuration, the anode was used as the counter and the reference electrode, while the cathode as the working electrode. The same measurement could also be done with the anode as the working electrode, and the cathode as the counter and the reference electrode. While not necessary, it is advisable for whole cell measurements to verify that the results of the measurements made through both of these configurations are similar. For the three-electrode configuration, a reference electrode was used, and either the anode, or the cathode, was used as working electrode and the other as the counter electrode. All EIS measurements here were done with amplitude of 1 mV and between the frequency ranges of 100 kHz to 100 mHz. While most resistances appeared to be captured through the equivalent circuit modeling of the data obtained from scanning down to 100 mHz, it is important to note that scans to even lower frequencies could reveal resistances not detected.

The authors used an equivalent circuit model for the whole MXC that included an Ohmic resistance and two charge transfer resistances – one from the anode and one from the cathode. While this was an empirical selection during the time the study was published, Dominguez-Benetton and Sevda (2012) showed later how certain graphical methods could be used to determine the number and types of circuit elements to use in the equivalent circuit. While this does not mean there are only two governing resistances. It is possible that several of that several processes are merged on the Nyquist plot due to overlapping time constants. Doing three-electrode measurements could help reveal if this is really the case. Irrespective, one of the important findings from this study was that over long-term operation, cathode appears to limit the power densities that can be achieved in MFCs. This has since been confirmed through other studies using other techniques, such as that by Popat et al. (2012) which identified a large concentration overpotential related to pH being the most dominant factor. Recent studies done on air-cathodes for MFCs that include EIS measurements show a large diffusion resistance (Huchinson et al. 2011; Lanas et al. 2011), corroborating this limitation.

2.6. Summary

In order to develop efficient MXC applications, it is imperative that we understand and predict overpotentials in these systems. The complexity of MXC processes requires that researchers use many techniques to achieve this goal. The use of EIS has been extremely helpful for other fuel cell applications to measure and characterize overpotentials; as such, EIS is also helpful in MXC research. Nevertheless, one must be aware that the interpretation of EIS data requires previous knowledge of the processes involved in order to build an adequate equivalent circuit. The validation of such equivalent circuit can in part occur through multiple EIS experiments in which external parameters are varied and impedances are measured as a function of these parameters. The use of alternate analytical techniques can also be used in along EIS to build a better understanding of the electron-transport processes. I am hopeful that this chapter will provide a background and guidelines suited for MXC researchers to use EIS as part of their analytical toolbox.

CHAPTER 3

CHARACTERIZING MULTIPLE ELECTRON TRANSPORT PATHWAYS IN *GEOBACTER SULFURREDUCENS*

This paper was published in a modified format in *ChemSusChem* in collaboration with César Torres and Sudeep Popat (Yoho et al, 2014).

3.1 Introduction

Microbial respiration to solid electron acceptors, such as metals or electrodes, is not completely understood despite its relevance in the environment and biotechnology applications (Bird et al, 2011; Lovley, 1991; Lovley, 2006). Electrochemical techniques applied in microbial electrochemical cells (MXCs) provide a great opportunity to study these bacteria (henceforth referred to as anode-respiring bacteria, or ARB), with many possibilities for investigating their bioenergetics and kinetics (Fricke et al, 2008; Marsili et al, 2008a; Marsili et al, 2010; Torres et al, 2008a). As mentioned in Chapter 1, MXCs are also touted as a promising bioenergy technology, since they can be used to convert the chemical energy in organic compounds directly to electrical energy (Logan et al, 2006; Logan et al, 2008; Logan and Rabaey, 2012; Rabaey and Verstrate, 2005). The performance of MXCs is evaluated not just in the context of rates of microbial conversions but also overpotentials, and thus understanding the response of ARB to the electrode potential is critical for practical applications (Rozendal et al, 2008; Torres, 2014). Herein, I utilize electrochemical techniques, including EIS (as investigated in Chapter 2), on *Geobacter sulfurreducens* biofilms to elucidate important details of their current-potential (*j*-*V*) response.

Cyclic voltammetry (CV) is the most widely used technique for this purpose since it consists of measuring the current response to a potential sweep, wherein the energy available to the bacteria through respiration is changed (Fricke et al, 2008; Harnisch and Freguia, 2012; LaBelle and Bond, 2008). As seen in Chapter 1, CV has been applied for the characterization of model ARB in several studies to gain information on their energy metabolism, and its relation to the performance of MXCs (Carmona-Martinez et al, 2011; Carmona-Martinez et al, 2013; Marsili et al, 2008a; Marsili et al, 2008b; Marsili et al, 2010; Richter et al, 2009; Strycharz et al, 2011; Torres et al 2008a). *G. sulfurreducens* is an ARB of interest since it forms biofilms on electrodes, wherein long-distance electron transport is achieved through an extracellular matrix that is known to contain pili, cytochromes and polysaccharides (Bond et al, 2012; Malvankar et al, 2011; Snider et al, 2012; Strycharz-Glaven and Tender, 2012). The exact mechanisms of this long-distance electron transport however, to this date, remain highly debated (Malvankar et al, 2012; Strycharz-Glaven and Tender, 2012; Torres, 2014). In this work, I explore how a combination of electrochemical techniques can reveal new information on the *j-V* response of *G. sulfurreducens* biofilms that could have a potential implication on the understanding of mechanisms of electron transport outside the cells.

The CVs for *G. sulfurreducens* biofilms performed in the presence of the electron donor, acetate, show a sigmoidal shape for the *j-V* relationship, as shown in Figure 3.1 and previously in Figure 2.6, and by other studies (Marsili et al, 2008a, Strycharz-Glaven and Tender, 2012; Torres et al, 2008a). Considerable efforts have been spent in modelling this sigmoidal response (Hamelers et al, 2011; Peng et al, 2013; Strycharz et al, 2011; Torres et al, 2008a), which is consistent with that of an irreversible enzymatic reaction, as reviewed for observations for several enzymes immobilized on electrodes (Marcus et al, 2007). Thus, most models assume that this sigmoidal response is governed by one limiting redox enzyme along the electron transport pathway of *G. sulfurreducens*.

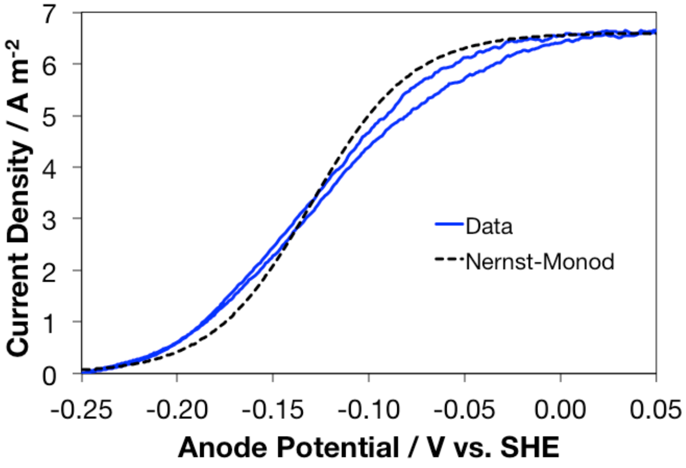


Figure 3.1. An example of a CV for a fully developed *G. sulfurreducens* biofilm grown with an anode potential of -0.02 V, showing a sigmoidal *j*-*V* relationship that deviates from the Nernst-Monod equation.

As described in Chapter 1, the Nernst-Monod equation (Equation 1) that was confirmed to accurately fit the CVs of *G. sulfurreducens* (Fricke et al, 2008; Torres et al, 2008a; Torres, 2014). In Equation 3.1, the current density produced by the biofilm (*j*) plotted against the anode potential (*V*) is a function of the maximum current density (j_{max}) and the mid-point potential of the limiting redox process (E_{KA}). Also, n is the number of electrons transferred, and this is assumed to be 1 for the rate-limiting redox enzyme. Since then, other models have been developed, as it was found that the Nernst-Monod equation does not always predict or fit the data (Hamelers et al, 2011). This is highlighted in Figure 3.1 where deviations in the top and bottom of the sigmoidal response between the data and the model are evident.

$$j = j_{max}\left(\frac{1}{1+\exp\left(-\frac{nF}{RT}(E-E_{KA})\right)}\right) \qquad \text{(Equation 3.1)}$$

Other models, such as the Butler-Volmer-Monod model and the Nernst-Ping-Pong model, have included new concepts while still adhering to the assumption of a single limiting and/or governing enzymatic or redox reaction along the electron transport pathway (Hamelers et al, 2011; Peng et al, 2013). The only model that goes beyond this assumption is that put forth by Snider et al. (2012), where they introduce an empirical parameter "*g*" to describe the presence of multiple redox cofactors governing electron transfer outside the cells. Even so, a greater fundamental understanding of the respiratory metabolism of *G. sulfurreducens* is needed to interpret the electrochemical responses that lead to deviation from most current models. Several researchers have also obtained CVs in the absence of acetate that show the involvement of multiple redox processes (Fricke et al, 2008; LaBelle and Bond, 2008; Strycharz-Glaven and Tender, 2012); I show an example in Figure 3.2. Here, processes could refer to a single redox enzyme or an enzymatic pathway with a characteristic mid-point potential; so far, this distinction has been difficult to make with the techniques used.

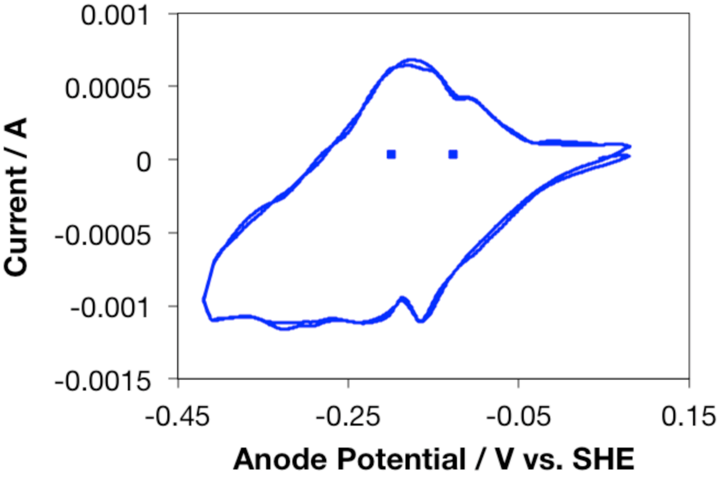


Figure 3.2 An example non-turnover cyclic voltammogram (CV, 3 mV s^{-1}) of a *G. sulfurreducens* biofilm grown with an anode potential of -0.02 V.

The results summarized so far show an incomplete understanding of the electrochemical response of *G. sulfurreducens*. The discrepancy between experimental data and theoretical calculations suggests a complex electrochemical behaviour, involving multiple redox processes that cannot be explained with our current mechanistic models. Our inability to represent experimental data connotes our knowledge gap related to electron transport pathways in anode respiration and impedes our ability to predict ARB behaviour in MXCs. A better understanding of electron transfer kinetics by ARB and the relationship with the anode potential, presents possibilities of optimizing operating conditions of MXCs in various biotechnology applications and predicting the response of ARB though advanced modelling techniques. In this study, I aimed to evaluate the contribution of multiple governing redox processes to the turnover CVs of *G. sulfurreducens* biofilms. Since CV represents a transient electrochemical method, I used it in conjunction with other electrochemical methods that provide more detailed, pseudo steady-state information to highlight the involvement of multiple governing redox processes shaping the overall *j-V* relationship. I pay special attention to the use of chronoamperometry where time dependent changes as a function of the anode potential can be observed and EIS (utilizing the techniques demonstrated in Chapter 2) performed at different anode potentials to evaluate charge transfer resistances. Our results suggest a complex electrochemical response of *G. sulfurreducens* that has not been characterized in detail before, in which at least two major electron transport pathways contribute to current production. The potential-dependent response of *G. sulfurreducens* biofilms I show here may fundamentally change our understanding of their metabolism and the interpretation of all previously collected and future datasets.

3.2 Materials and Methods

3.2.1 Bacterial Strain and Culture Media

I sub-cultured *G. sulfurreducens* from a stock of microorganisms isolated in our laboratory and determined to be genetically similar to previously isolated *G.*

sulfurreducens (Badalamenti et al, 2013). The culture was grown in an anaerobic medium containing per 1 L: $NaHCO_3$ 2.50 g (30 mM), NH_4Cl 1.50 g (20 mM), NaH_2PO_4 0.60 g (4 mM), KCl 0.10 g (1 mM), sodium acetate trihydrate 1.36 g (10 mM), vitamin mix 10 mL, trace minerals 10 mL. The trace minerals solution contained per 1 L: Nitrilotriacetic acid, trisodium salt 1.5 g (5.5 mM), $MgSO_4{\cdot}7H_2O$ 3.0 g (12 mM), $MnSO_4{\cdot}H_2O$ 0.5 g (2.9 mM), NaCl 1.0 g (17 mM), $FeSO_4{\cdot}7H_2O$ 0.1 g (0.36 mM), $CaCl_2{\cdot}2H_2O$ 0.1 g (0.68 mM), $CoCl_2{\cdot}6H_2O$ 0.1 g (0.42 mM), $ZnCl_2$ 0.13 g (0.95 mM), $CuSO_4{\cdot}5H_2O$ 10 mg (0.4 mM), $AlK(SO_4)_2{\cdot}12H_2O$ 10 mg (0.2 mM), H_3BO_4 10 mg (0.16 mM), $Na_2MoO_4{\cdot}H_2O$ 2.5 mg (0.01 mM), $NiCl_2{\cdot}6H_2O$ 2.4 mg (0.01 mM), $Na_2WO_4{\cdot}2H_2O$ 0.25 mg (8.5 μm). I provided acetate as the electron donor at 30 mM. I bubbled all media with N_2/CO_2 (80/20 v/v) to remove oxygen before autoclaving.

3.2.2 Microbial Electrochemical Cells

I constructed three-electrode MXCs (Figures 1.2 and 1.3) from 100 mL VWR bottles that were modified to have an opening on one side for the working electrode. I constructed rubber stoppers for the gas inflow and outflow, reference electrode, and nickel wire counter electrodes. I used gold electrodes of a 2 mm diameter (3.14 mm^2 area, BASi, West Lafayette, IN), and silver/silver chloride (Ag/AgCl) reference electrodes (+0.28 V vs. SHE in the medium used as determined through the procedure described earlier (Parameswaran et al, 2013), BASi, West Lafayette, IN), as the working and reference electrodes respectively. All potentials reported are vs. SHE. The MXCs were magnetically stirred at 250 rpm and continuously flushed with humidified N_2/CO_2 gas (80/20 v/v). I sterilized the MXCs by autoclaving prior to filling media through sterile Norprene tubing by using filtered N_2 gas (0.22 mm pore size), in a biosafety cabinet. I inoculated the MXCs with approximately 2 mL volume inoculum from suspension of larger H-type MXCs, set up as described before (Torres et al, 2008b).

3.2.3 Electrochemical Measurements

I performed all electrochemical measurements using a VMP3 digital potentiostat (Bio-Logic USA, Knoxville, TN). I used chronoamperometry to poise the anode potential at different fixed values (see text in Results and Discussion for details). I performed CV by scanning the anode potential forward and reverse twice starting from the open circuit potential at a scan rate of 1 mVs^{-1} or 5 mVs^{-1} over the potential window usually of -0.25 V to +0.05 V. I used the second scans for all data analysis. I corrected the CVs for i-R loss as described earlier (Popat et al, 2012).

I performed EIS mesurements at different anode potentials (depending on experiment, see text in Results and Discussion) following an equilibration time at the set potential (of a minimum of 10 minutes) to ensure steady-state conditions for the experiment. I used an amplitude of 10 mV and used a frequency range of 200 kHz to 10 mHz, with 10 measurements per decade. I fit the EIS measuremnt data in the form of the Nyquist plots to an equivalent circuit model (ECM) using the EC-Lab software. The circuit for the ECM used is presented as an inset in Figure 3.4d. I performed Kramers-Kronig (KK) test on EIS data to ensure data validity, using the KK-test software (Rittmann and McCarty, 2003). Within the range of the anode potentials tested, all data conformed to the KK tests with residuals of <3%. These parameters and the KK-test are described in detail in Chapter 2.

3.3 Results and Discussion

I investigated first if the deviations in CVs of *G. sulfurreducens* biofilms from the Nernst-Monod equation, as shown in Figure 3.1, manifest with the maturity of the biofilms. In our previous analyses, researchers in the Swette Center for Environmental Biotechnology at Arizona State University have discussed that a deviation in the CV at high current densities could be the consequence of a potential gradient along the biofilm (Torres et al, 2008a). This deviation results in a slope less steep at anode potentials above the E_{KA}. I performed CV on *G. sulfurreducens* biofilms grown at an anode potential of -0.02 V, as a function of biofilm growth. I show in Figure 3a the CVs for one biofilm from measurements made when the current densities reached 0.5, 1, 3, 5, and 6 A m^{-2}. I repeated these measurements with four different biofilms, and observed the same trend (data not shown). It is difficult to observe any major changes in the sigmoidal shape of the CVs through this representation. However, it is evident that the onset potential, where positive current can first be measured, decreases with increasing biofilm growth. I show in Figure 3.3c Nernst-Monod equation fits for each of the CVs shown in Figure 3.3a. Figure 3.3c suggests that the deviation from the Nernst-Monod equation starts occurring when the current density is >3 A m^{-2}.

To observe the pattern of changes in the CVs shown in Figure 3a over time not directly apparent, I normalized each CV to the maximum current density for each curve, j_{max}, as shown in Figure 3b. This figure suggests that with increasing growth, there is a change in the sigmoidal shape with (i) the mid-point decreasing to more negative values, and (ii) a decrease in the overall slope. This change is more pronounced in the region of 0.2 j/j_{max}, showing a 25 mV shift in the anode potential from -0.152 V to -0.177 V. Interestingly, the normalized CVs mostly overlap at higher potentials, except for a small peak visible in the early growth stages (<1 A m^{-2}) at -0.075 V. This peak is observed in other studies as well (Strycharz-Glaven and Tender, 2012), and its origin has remained unknown. Nonetheless, this is where earlier I discussed that a potential gradient would most change the slope of the CVs (Torres et al, 2008a). Thus, the shift I observed here cannot be attributed to a strong potential gradient along the biofilm.

Other studies focused on electrochemical analyses of *G. sulfurreducens* biofilms also suggest such changes in the CVs with biofilm growth. For example, Marsili and colleagues also showed similar changes (Marsili et al, 2010), except they showed derivative CVs instead of normalized CVs as I do here. Strycharz-Glaven et al. also performed CVs as a function of biofilm growth, wherein changes in the CVs are apparently only in the low current region ($<0.15\, j/j_{max}$) (Strycharz-Glaven and Tender, 2012).

The normalized CVs suggest that there are features in the respiratory metabolism of *G. sulfurreducens* that are not captured by an assumption of one governing redox process (represented with n=1). In fact, these suggest an adaptation to lower potentials within the biofilm, with more current produced at lower anode potential. This too refutes the possibility of a directly potential-gradient related shift in the shapes of the CVs, as one would expect the potential gradient to decrease the normalized current produced at a given anode potential, not increase it. The shift also supports our observation of the deviation of the CV from the Nernst-Monod relationship on fully developed biofilms, such as that shown in Figure 3.1.

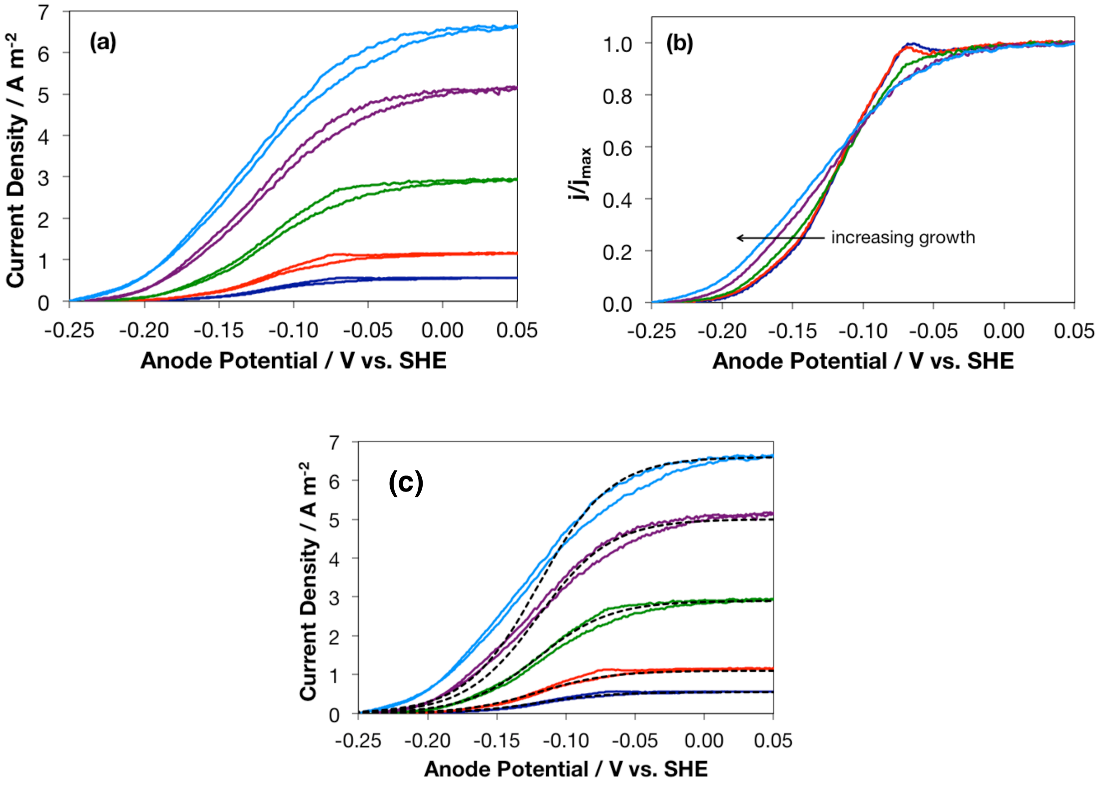


Figure 3.3. (a) CVs at various stages of growth for a *G. sulfurreducens* biofilm grown with an anode potential at -0.02 V. (b) CVs from (a) normalized to the maximum current density at each stage of biofilm growth, showing shifts in the slope of the sigmoidal *j*-*V* relationship. (c) CVs at various stages of growth for a *G. sulfurreducens* biofilm grown with an anode potential of -0.02 V (Figure 3.3a), showing the Nernst-Monod equation fits (E_{KA}=-0.02 V for all fits). Deviations from the single E_{KA} assumption are evident at current densities higher than 3 A m^{-2}.

3.3.1 EIS Measurements

To elaborate further on this observation, I performed EIS measurements on a fully developed *G. sulfurreducens* biofilm grown at -0.02 V vs. SHE, over a range of discrete anode potentials. Since CV is a transient method that may not allow obtaining a pseudo steady-state *j*-*V* response, I used EIS to get a relationship of charge transfer resistance (R_{ct}, Ohm cm^2) to the anode potential. Unlike direct current techniques such as CV, EIS is an alternating current method. I used potentiostatic EIS, which involves applying small sinusoidal amplitude over a fixed anode potential at a range of frequencies, and measuring the resulting magnitude and phase shift in the current. Because different processes govern the response in current to change in potential at different frequencies, it is thus theoretically possible to quantify individual contributions of different processes to the overall resistance.

The Nernst-Monod equation can be modified to fit the R_{ct}-V response. R_{ct} is related to j through Ohm's law ($R_{ct}=dV/dj$), and so the sigmoidal shape for a j-V relationship, changes to a U-shape for R_{ct}-V relationship, similar to the derivative of a CV. Since EIS is performed at a single anode potential at a time, it should provide pseudo steady-state responses for the R_{ct}-V relationship.

Three examples of Nyquist plots taken at different poised anode potentials can be seen in Figure 3.4. An example experimental dataset of the Nyquist plot obtained through EIS measurements showing the equivalent circuit model (ECM) used (figure inset) can be seen in Figure 3.4d.

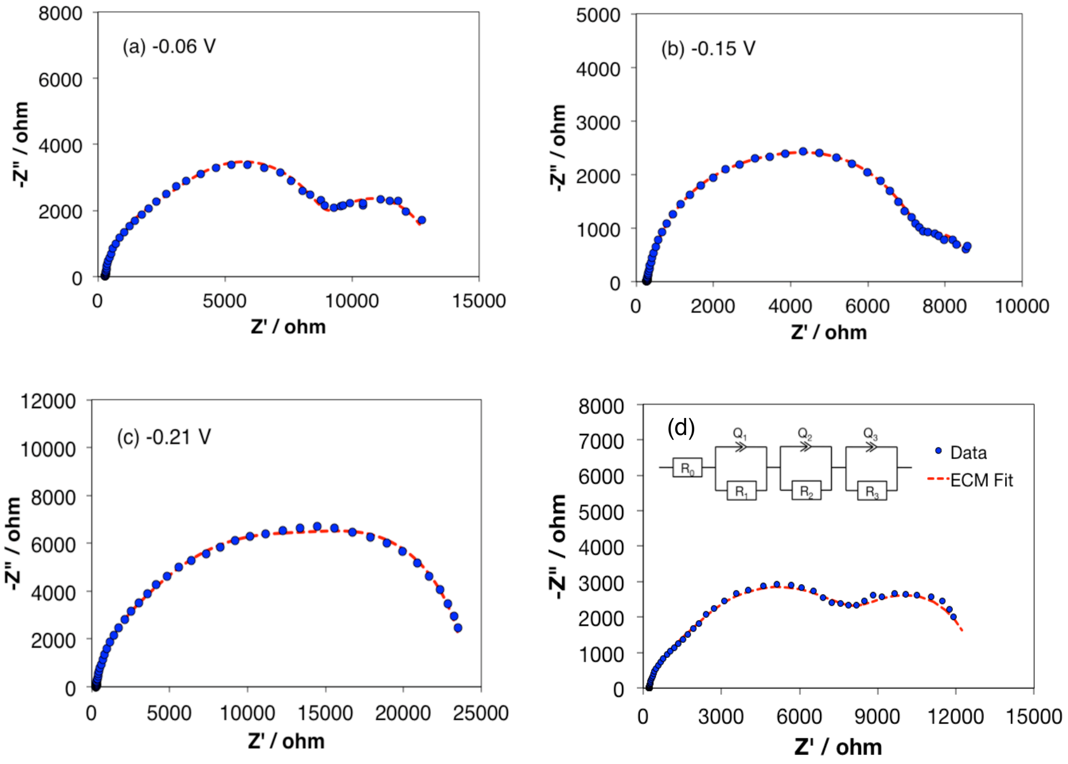


Figure 3.4. Example of the Nyquist plots from EIS measurement on a *G. sulfurreducens* biofilm grown at an anode potential of -0.02 V. Conditions used for this example are: anode potential of (a) -0.06 V, (b) -0.15 V, and (c) -0.21 V, amplitude of 10 mV, and frequencies ranging from 200 kHz to mHz. The red dotted line represents the best equivalent circuit model fit. (d) An example of the Nyquist plot from EIS measurement on a *G. sulfurreducens* biofilm grown at an anode potential of -0.02 V. Conditions used for this example were: anode potential of -0.12 V, amplitude of 10 mV, and frequencies ranging from 200 kHz to 10 mHz. The ECM is shown as the inset to the Figure.

Fitting the Nyquist plot to an ECM is a generally accepted method to obtain values for particular resistances within an electrochemical system, provided that the model accurately depicts the physico-chemical-biological phenomena occurring. I selected a circuit that contains three charge transfer resistances. This selection was made on the basis of the Bode plots from several EIS measurements that showed three peaks in the phase shift. Since I anticipate these responses to come from the electron transport chain, I used the ECM with series resistances. When fitting to the Nernst-Monod equation, I used the sum of the values for all the three resistances, R_1, R_2, and R_3, since it models the overall response of the cells, not just an individual component.

In Figure 3.5a, I show the total R_{ct} ($R_1 + R_2 + R_3$) versus the anode potential for a *G. sulfurreducens* biofilm grown at -0.02 V vs. SHE. I repeated these measurements on four different biofilms two times each, and obtained the same trends as shown here. I show three additional plots in Figure 3.6.

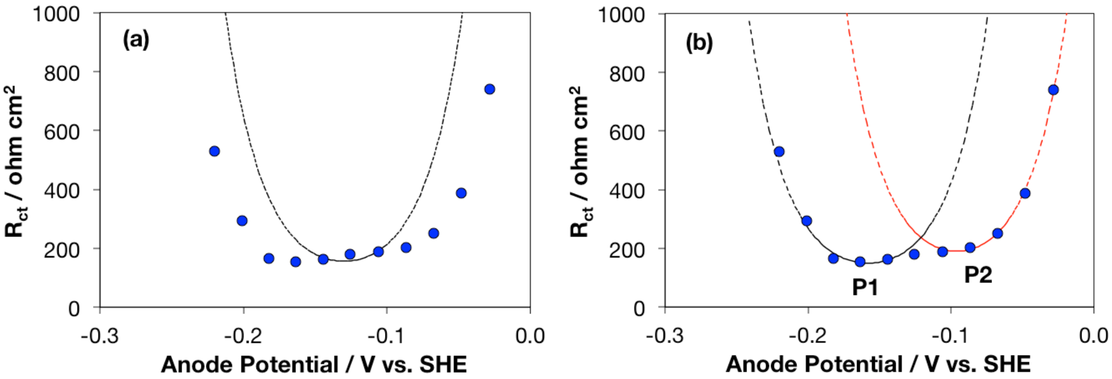


Figure 3.5. Charge transfer resistance (R_{ct}) as a function of the anode potential for a fully developed *G. sulfurreducens* biofilm grown with an anode potential of -0.02 V. The experimental data is shown in blue dots, where R_{ct} is the sum of three charge-transfer resistances. The dashed lines show best fits with the Nernst-Monod equation considering (a) a single pathway with the same parameters used to fit the CV in Figure 3.1, and (b) two pathways, P1 and P2, with j_{max} = 7 and 5.5 A m^{-2} respectively and E_{KA} = -0.157 and -0.097 V respectively. Parameters were obtained through a best fit by minimizing the sum of least squares using Excel Solver.

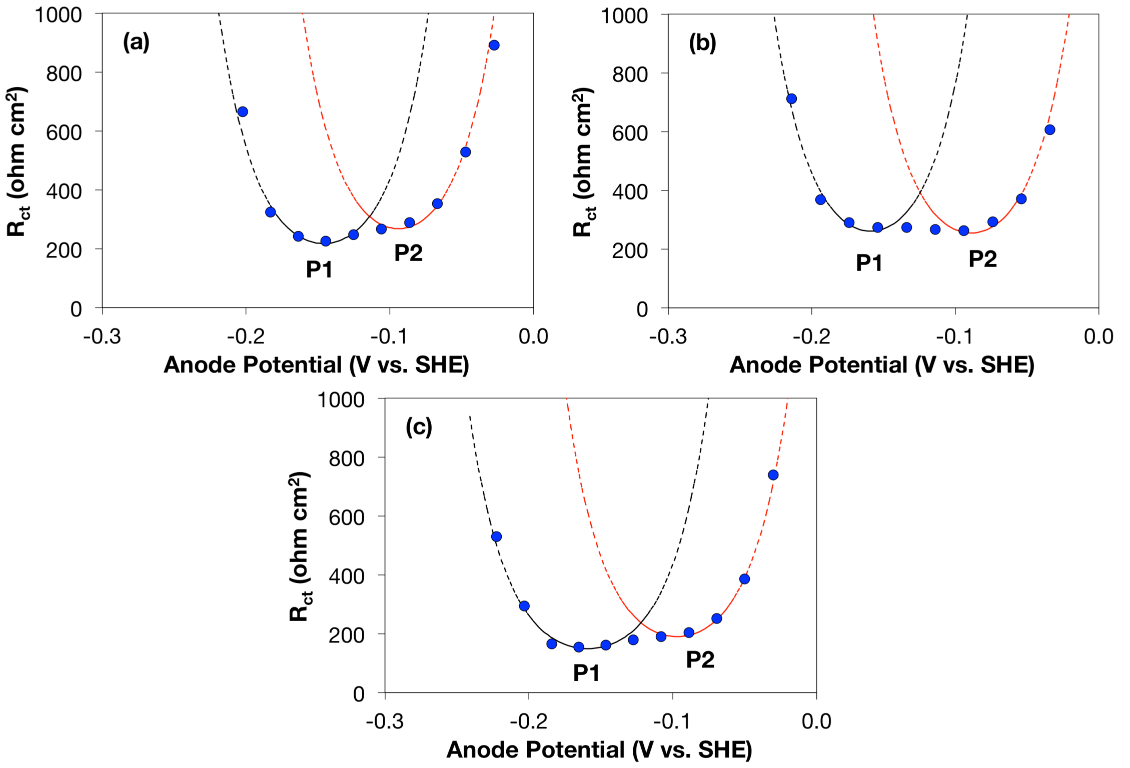


Figure 3.6. Replicate datasets for Figure 3.5 shown as charge transfer resistances (R_{ct}) as a function of the anode potential for a fully developed *G. sulfurreducens* biofilm grown with an anode potential of -0.02 V. (a) For a biofilm that produced a maximum current density of 5.4 A m^{-2} during the measurements, the Nernst-Monod equation fits provide E_{KA1} = -0.145 V, E_{KA2} = -0.095 V, j_{max1} = 4.8 A m^{-2}, j_{max2} = 3.9 A m^{-2}, (b) for a biofilm that produced a maximum current density of 5.5 A m^{-2} during the measurements, the Nernst-Monod equation fits provide E_{KA1} = -0.158 V, E_{KA2} = -0.09 V, j_{max1} = 4.1 A m^{-2}, j_{max2} = 4 A m^{-2}, and (c) for a biofilm that produced a maximum current density of 7.1 A m^{-2} during the measurements, the Nernst-Monod equation fits provide E_{KA1} = -0.158 V, E_{KA2} = -0.098 V, j_{max1} = 7.1 A m^{-2}, j_{max2} = 5.4 A m^{-2}.

As I mentioned earlier, this plot can be considered as the derivative of the CV ($R = dV/dj$ according to Ohm's Law). Additionally in Figure 3.5a, I overlay a black dotted line, which represents the best fit of the Nernst-Monod equation modified to show dV/dj. The curve shows a minimum at the E_{KA} with an R_{ct} value at this potential that is determined by j_{max}. It also shows two slopes (one at each side of the E_{KA}), which should be symmetrical and are associated with the n value (n=1 in Figure 3.5a).

From Figure 3.5, I observe that the Nernst-Monod equation does not accurately describe the R_{ct}-V relationship as determined through EIS measurements. Similar to the CV shown in Figure 3.1, the Nernst-Monod equation does not fit the data. However, while the apparent deviations in experimental and modelling results are small for the CV in Figure 3.1, those in the R_{ct}-V relationship are more glaring (as seen in Figure 3.5a). In particular, I observe that the width of the U-shape is significantly underestimated by the Nernst-Monod equation. In fact, the experimental data suggest that there are two minima in the R_{ct}, which can be modelled by two separate Nernst-Monod equations with different E_{KA} and j_{max} values, each with n=1, shown in the black and red dotted lines in Figure 3.5b. This suggests that there are two major electron transport processes, henceforth-denoted P1 and P2, each with different characteristic mid-point potentials.

I can estimate the maximum current density, j_{max} for each of the processes, as 7 and 5.5 A m^{-2} from the model fits performed by minimizing the sum least squares between the data and the Nernst-Monod equation modified to represent the R_{ct}-V response. Additionally, the two processes appear to have different E_{KA} values for governing redox cofactors of -0.157 V for P1 and -0.097 V for P2, while the often reported single E_{KA} is -0.14 V vs. SHE (Fricke et al, 2008; Marsili et al, 2008a; Marsili et al, 2008b; Marsili et al, 2010; Torres et al, 2008a). Averaging all the replicate measurements, even at different current densities, values of E_{KA} of -0.155 (±0.005) and -0.095 (±0.003) V vs. SHE for P1 and P2 can be seen. Interestingly, the two processes do not appear to be additive, as the overall j_{max} (6.9 A m^{-2} during the EIS measurements) is significantly lower that the sum of the j_{max} for both processes. This suggests that the measured processes are the result of a shift from one pathway to another, as a function of the anode potential. Thus, from now on, I will refer to them as electron transport pathways.

3.3.2 Low Anode Potential Biofilm Experiments

To further investigate whether a pathway shift is responsible for the observed response of *G. sulfurreducens*, I grew biofilms at a relatively lower anode potential (-0.145 V) in an effort to direct the active expression of only P1. As shown in Figure 3.5, the selected potential is close to the E_{KA} of P1, while it gives a very large R_{ct} for P2.

I performed chronoamperometric *j*-*V* curves in which I stepped the anode potential through a defined range, allowing sufficient time (at least 10 minutes) for the biofilm to equilibrate at each potential. This should allow a shift from one pathway to the other, even if it implies the expression of new proteins. Figure 3.7a shows the results of this experiment, which resemble a slow-scan simulated CV. The results suggest that there is a decrease in current production as the pathway shifts from the one with the lower midpoint potential to the one with the higher midpoint potential. This further confirms why a simple additive model for the two pathways does not explain the R_{ct}-*V* data shown in Figure 3.5.

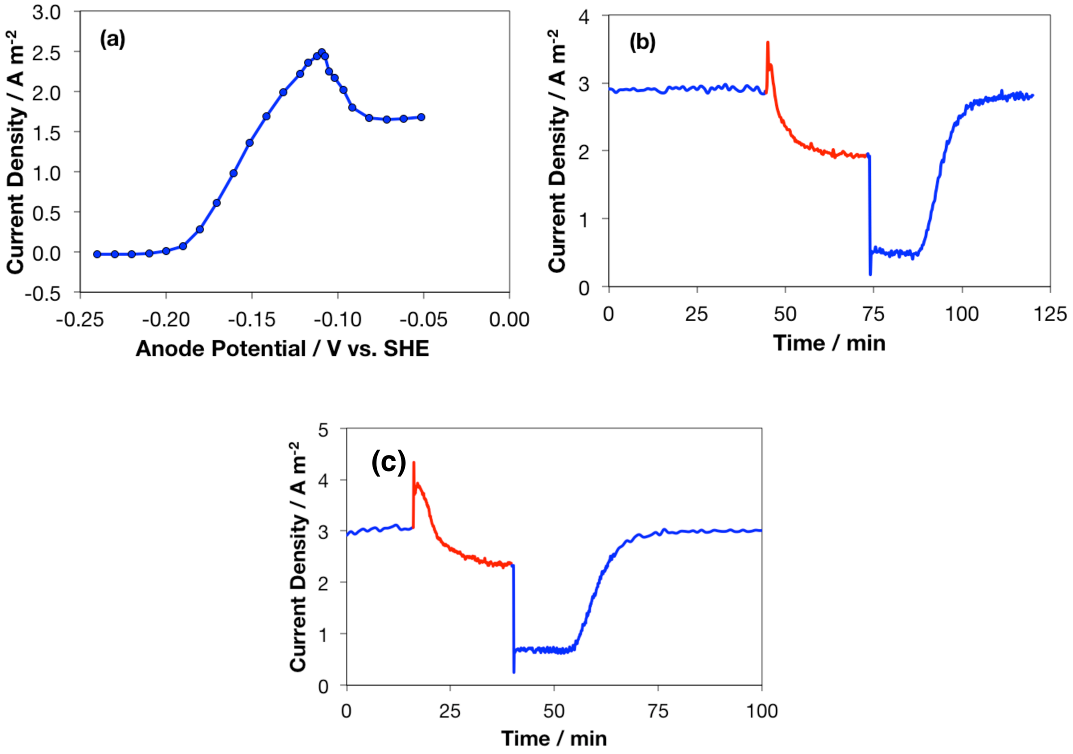


Figure 3.7. (a) *j*-*V* curve for a fully developed *G. sulfurreducens* biofilm grown with an anode potential -0.145 V. (b) Chronoamperometric measurements on a fully developed *G. sulfurreducens* biofilm grown with an anode potential of -0.145 V, showing shifts in the current density when the anode potential is shifted from -0.145 V (blue) to -0.02 V (red). Note that (a) and (b) are from the same biofilm but at different time points. (c) Replicate dataset for Figure 3.7(b).

I also performed a potential step experiment on a biofilm grown at -0.145 V to track the time it takes to change from one pathway to the other. In this experiment, the potential was changed from the poised growth potential (-0.145V, shown in blue) to -0.02 V (shown in red) for approximately 30 minutes and then changed back to the original growth potential (shown in blue). I show the data in Figure 3.7b, with a replicate dataset also shown in Figure 3.7c. In this experiment, when the anode potential was stepped up to -0.02 V, the biofilm responded by increasing current production, as expected by the Nernst-Monod equation. However, this increase only lasted approximately 2 minutes, and then the current started to decrease, finally equilibrating to a lower value, a behavior similar to that shown in Figure 3.7a. I must point out that the observed response is that of a biofilm grown at the relatively lower potential; a biofilm grown at -0.02 V would be able to produce > 6 A m^{-2} as shown in Figure 3.3a. A similar but opposite response is observed when the potential is stepped back down to -0.145 V; a lower current is observed for approximately 20 minutes and is then followed by an increase, leading to the original steady-state current. These results suggest that the shift in pathways is also associated with a change in the current density, which is an indirect measurement of *G. sulfurreducens* respiration rate. It also suggests that the pathway shifts occur in a manner of minutes.

To confirm further that there is a shift in electron transport pathways of *G. sulfurreducens*, I performed the CVs shown in Figure 3.9. A replicate is also shown in Figure S6. In this experiment, CVs were performed on a biofilm grown at -0.145 V at a scan rate of 5 mV s^{-1}, for a total scan time of 80 seconds, which is lower than the time observed in Figure 3.7b for the pathway change. After a CV was performed at -0.145 V, the potential was changed to -0.02 V for 1 hour, allowing for the shift in the electron transport pathway. Then, I performed a second CV at 5 mV s^{-1}, which shows a clear shift in the midpoint potential. This was also accompanied by a lower current density (j_{max} = 5.7 A m^{-2} at -0.145 V vs. j_{max} = 4.8 A m^{-2} at -0.02 V). These experiments confirm that there is a shift between the two distinct pathways that have governing redox cofactors with different characteristic mid-point potentials. The derivative of the CVs show that a minimum is observed at -0.105 V and -0.159 V, values that are similar with those observed in EIS measurements (Figure 3.5). While the derivatives show primarily only one pathway for each CV scan, the lack of symmetry in both curves suggests that the other pathway was not completely eliminated in each case, but only suppressed it so that the signature of the major pathway can be observed. While similar responses have been observed in mixed culture biofilms, where pathways from different microbial species or strains are selected based on the anode potential (Commault et al, 2013; Torres et al, 2009; Zhu et al, 2014), this pathway shift had not been observed on the same biofilm of a pure culture ARB until now.

From these results, it is evident that *G. sulfurreducens* biofilms can change electron transport pathways dynamically as a response to the anode potential. I hypothesize that the most likely reason for this pathway change is due to an energy optimization in their respiration, and thus is probably similar to how facultative anaerobic bacteria shift between oxygen and lower potential electron acceptors to optimize their growth and yield (Rittmann and McCarty, 2003). If this is true for *G. sulfurreducens*, the shift in pathways would occur inside the cells and be associated to a proton-pumping protein in the electron transport chain, thus increasing the proton-motive force (PMF) and ATP production. If our hypothesis is correct, *G. sulfurreducens* should be able to grow at a faster rate at the higher potentials, as ATP can be produced at a faster rate. In fact, I estimated the doubling times for the two different potentials studied based on the exponential increase in current density after inoculation (see Figure 3.8).

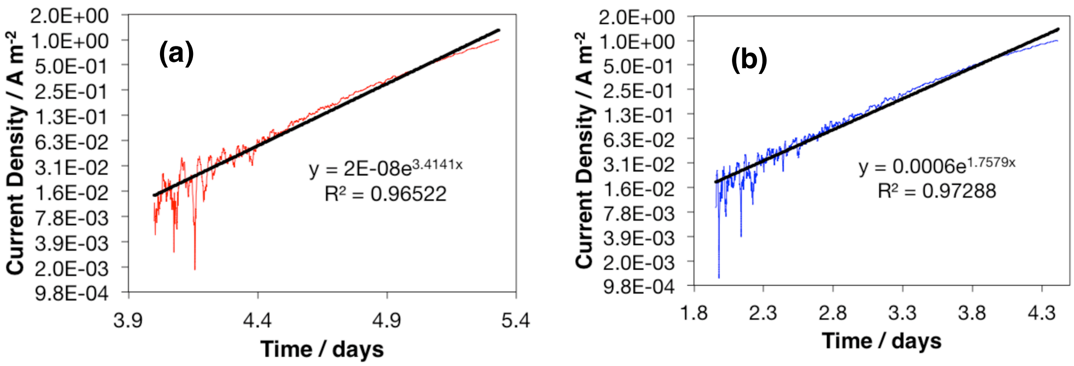


Figure 3.8. (a) Doubling time calculation for a biofilm grown with an anode potential of -0.02 V. (b) Doubling time calculation for a biofilm grown with an anode potential of -0.145 V.

Based on these, I calculated the doubling time of approximately 7 hours at -0.02 V, while it was approximately 14 hours at -0.145 V. It is known that the doubling times on different electron acceptors differ for *G. sulfurreducens* (Esteve-Nunez et al, 2005), and I show here that this difference could arise even from a fairly small increase in redox potential from -0.145 V to -0.02 V.

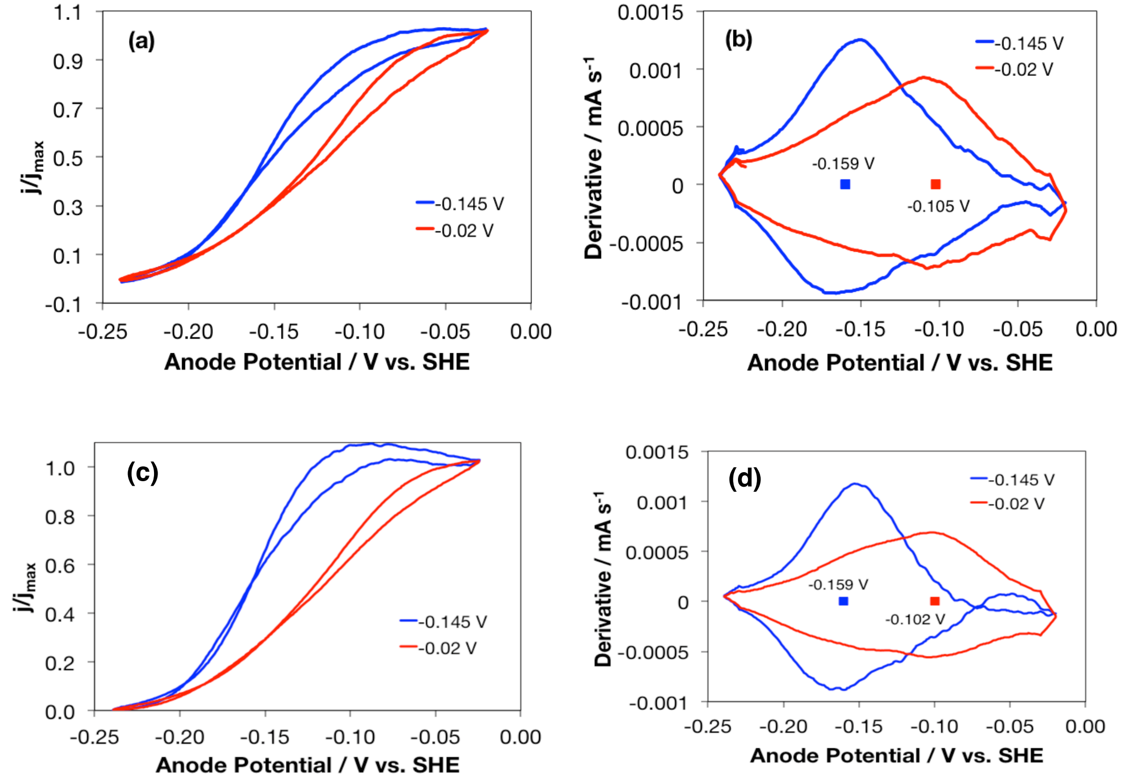


Figure 3.9. (a) A comparison of the CVs obtained for a fully developed *G. sulfurreducens* biofilm grown at an anode potential of -0.145 V (blue) and the same biofilm 1 hour later after it has been adapted to an anode potential of -0.02 V (red). (b) Derivatives of the CVs in (a). (c) Replicate dataset for Figure 3.9a and (d) derivatives of CVs from (c).

The hypothesis of energy optimization would also explain the change in current density observed in Fig. 5 and 6 as the potential is changed. As the cells obtain more PMF per electron respired at higher potentials, the cells reaches a maximum ATP production rate with a lower respiration rate. This would manifest as a lower current density at the higher potential pathway. While our data support the mentioned hypothesis, it is rather difficult to confirm that the pathway change is associated to a shift in proteins within the electron transport chain. To date, researchers in the field of microbial electrochemistry do not have a full understanding of the electron transport chain in *G. sulfurreducens*, which contains over 100 cytochromes that could be involved in different respiratory pathways (Methé et al, 2003), including the ones characterized here.

The dynamic pathway shift observed, in the order of minutes, can explain many observations related to the electrochemical responses of *G. sulfurreducens*. Non-turnover CVs already show the presence of two redox peaks, which I now suggest could be related to two separate pathways instead of different redox proteins within a single pathway, as previously hypothesized (Fricke et al, 2008). Under turnover conditions, CVs often show a higher current at lower potentials (such as that shown in Figure 3.7a); this observation has been attributed to negative faradaic resistances (Matsuda et al, 2011), and can now be explained more mechanistically through the pathway shift. The shifts in CVs as a function of growth, such as the ones shown in Figure 3.3, suggest an adaptation of the biofilm through a pathway optimization as the biofilm matures. Thus, the dynamic pathway shift characterized in this work has inadvertently affected the interpretation of most electrochemical data collected on *G. sulfurreducens* so far.

3.4 Conclusions

From the experimental data using several electrochemical techniques, I observe that the biofilms of *G. sulfurreducens* exhibit at least two major pathways for electron transport during anode respiration. I have observed also that there can be a shift between these pathways on a time scale of minutes. The quick response to a potential change suggests that there is an efficient capability in *G. sulfurreducens* to sense this potential. This sensing mechanism has not yet been discovered for *G. sulfurreducens*, and is most likely used for respiring to solid metal oxides that have different redox potentials in nature. The dynamic shift in pathways is also associated with a change in respiratory rates, as observed by a change in current density. The Nernst-Monod equation was useful in determining the mid-point of the governing redox cofactor in each pathway, but as of yet does not model the overall *j-V* response, especially as changes in pathway are on the order of minutes, which is similar to that of a low scan rate CV. I have confirmed that the deviations from theoretical Nernst-Monod calculations observed in the CV of *G. sulfurreducens* (Figure 3.1) are the result of transient changes in pathways as the CV is performed.

I propose that the manifested changes are inside of the cell as part of their electron transport chain, especially because of the differences in doubling times observed when grown under conditions that result in preferable expression of only one pathway. Additionally, the rapidity of the expression changes and equilibration to different potentials on a time scale of minutes (seen in Figure 3.7b) suggest that the changes are inside the cells. Changes outside of the cell would be more energetically “expensive” changes and would, likely, be slower than what have been observed.

While changes in the slope of the CVs observed in Figure 3.3a as a function of biofilm growth can now primarily be attributed to the two pathways, and not necessarily potential gradients, I should acknowledge that the use of a second pathway could be an implication of the potential gradient. Several studies have shown that the anode biofilms become more reduced on the outside (Esteve-Nunez et al, 2005), which could trigger the use of P1 instead of P2 in biofilms grown at a relatively higher anode potential. Even so, since I anticipate that pH would also play a major role in the energetics of a given pathway, pH gradients which have already been shown to occur in these biofilms (Torres et al, 2008b), could also influence changes in the shape of the CVs, and thus should be a topic of studies focused on gradients.

The expression of different pathways depending on the anode potential for *G. sulfurreducens* has far-reaching implications on the interpretation of all previously collected and future datasets. At present, I cannot determine whether these results are the work of multiple proteins or a single protein with multiple "exit pathways." These results and conclusions open up more research questions as to the mechanisms and the identity of these pathways for the electron transport chain in *G. sulfurreducens*.

CHAPTER 4

GEOALKALIBACTER FERRIHYDRITICUS ANODE BIOFILMS EXHIBIT SIGNATURES OF MULTIPLE ELECTRON TRANSPORT PATHWAYS

This chapter was published in *Langmuir* in a modified format with the collaboration of César Torres, Sudeep Popat, Laura Rago, and Albert Guisasola.

4.1 Introduction

Thriving under alkaliphilic conditions, *Geoalkalibacter ferrihydriticus* (*Glk. ferrihydriticus*), provides a possibility for applications treating alkaline waste streams as well as a possible new model organism for microbial electrochemistry. Despite significant research efforts in the past decade, the pathways and the mechanisms through which bacteria perform extracellular respiration to a metal or an electrode remain vaguely understood (Lovley, 1991; Lovley, 2006; Bird et al, 2011). Many studies, including those found in Chapter 3, have shown the advantages of using electrochemical techniques to investigate biofilms of anode-respiring bacteria (ARB) towards the goal of understanding the mechanistic aspects of this unique biological phenomenon (Marsili et al, 2008; Marsili et al, 2008b; Torres et al, 2008; LaBelle and Bond, 2008; Richter et al, 2009; Carmona-Martinez, et al, 2011; Malvankar et al, 2011; Strycharz et al, 2011; Harnisch and Freguia, 2012; Strycharz-Glaven and Tender, 2012; Snider et al, 2012; Bond et al, 2012; Carmona-Martinez et al, 2013; Carmona-Martinez et al, 2013b; Yoho et al,, 2014). In Chapter 3, I presented the use of advanced electrochemical techniques to reveal that biofilms of *G. sulfurreducens*, a model bacterium for metal reduction and anode respiration, exhibit more than one pathway of electron transfer to an anode, depending on the poised anode potential (Yoho et al, 2014). With more than one pathway, the organisms are able to maximize their respiration under a variety of conditions, similar to the utilization of multiple extracellular acceptors in nature. To date, one of these pathways that first revealed through applying advanced electrochemical techniques was suggested to include an inner membrane cytochrome, ImcH (Levar et al, 2014). This cytochrome is responsible for allowing the respiration to electron acceptors, including electrodes, at a higher redox potential.

Other ARB, such as *Geoalkalibacter* spp. have been shown to produce high current densities in microbial electrochemical cells (Badalamenti et al, 2013). These range from 5.0 to 8.3 A m^{-2}, but under alkaline or saline conditions for pure cultures (Badalamenti et al, 2013; Carmona-Martinez et al, 2013) or ARB-dominated mixed cultures (Pierra et al, 2015). Both *Glk. subterraneus* and *Glk. ferrihydriticus* have been confirmed to perform long-range electron transfer through a mechanism associated with the solid biofilm matrix, as opposed to soluble shuttles (Badalamenti et al, 2013). Even so, for both organisms only a few studies have been performed. Studies on *Glk. ferrihydriticus* have shown high current densities and Coulombic efficiencies during the utilization of both ethanol and acetate as substrates (Badalamenti et al, 2013; Greene et al, 2009; Zavarzina et al, 2006; Zavarzina et al, 2011), but no further metabolic or electrochemical characterization has been performed. In comparison to *G. sulfurreducens*, *Glk. ferrihydriticus* represents an alternative set of conditions for investigating electron transport and distinct practical applications in alkaline environments.

A Nernstian relationship, typical of the electrokinetic behavior of enzymes, is often used to describe the current/potential response of several different ARB (Hamelers et al, 2011; Peng et al, 2013; Strycharz et al, 2011; Torres et al, 2008). This response is usually observed as a sigmoidal shape that is obtained through using cyclic voltammetry (CV) at slow scan rates (Carmona-Martinez et al, 2011; Carmona-Martinez et al, 2013;

Marsili et al, 2008; Marsili et al, 2008b; Marsili et al, 2010; Richter et al, 2009; Strycharz et al, 2011; Torres et al, 2008; Yoho et al, 2014) and shown in Chapters 2 and 3. For biofilms of *Glk. ferrihydriticus*, CV measurements showed a characteristic sigmoidal shape under turnover conditions, and under non-turnover conditions the appearance of multiple peaks suggesting the involvement of more than one redox protein in electron transport (Badalamenti et al, 2013).

Glk. ferrihydriticus, only recently studied among ARB, produces high current densities (Badalamenti et al, 2013) and has a limited number of cytochromes (Badalamenti et al, 2015). This organism may be applied in electrochemical systems for the treatment of high pH wastewaters, thereby changing the proton transport and the influence of pH gradients. I hypothesized that since *Glk. ferrihydriticus* is phylogenetically closely related to *G. sulfurreducens*, there also may be more than one pathway for electron transport in *Glk. ferrihydriticus*. Previous turnover CVs revealed that the midpoint potential for *Glk. ferrihydriticus* is lower than that for *G. sulfurreducens* (Badalamenti et al, 2013). As *Glk. ferrihydriticus* is alkaliphilic, the difference in environmental pH may require different pathways for electron transport optimized for lower redox potentials. In this chapter, I present results demonstrating further implications that there may be more than two pathways in *Glk. ferrihydriticus*.

5.2 Materials and Methods

In this work, I subcultured *Geoalkalibacter ferrihydriticus* from a culture stock of DSM 17813 from the German Collection of Microorganisms and Cell Cultures (DSMZ) stored in the Swette Center for Environmental Biotechnology laboratory. The culture was grown in anaerobic medium (pH 9.3) containing (per 1 L), as described by Badalamenti et al, (2013):

0.2 g KCl, 0.1 g $MgCl_2 \bullet 6H_2O$, 0.2 g KH_2PO_4, 1 g NaCl, 1.36 g sodium acetate trihydrate (as electron donor), 1 mL trace elements solution, 0.5 mL selenite-tungstate solution (Widdel and Bak, 1992). After sparging with N_2 gas and autoclaving, 20 mL 1 M Na_2CO_3, 111 mL 1 M $NaHCO_3$, 10 mL 1 M NH_4Cl, and 1 mL vitamin solution (from laboratory stock, based on the Wolfe's vitamin solution) were added.

I constructed three-electrode microbial electrochemical cells, as described in Chapter 3 (Yoho et al, 2014) using modified 100 mL VWR bottles with a gold working electrode (3.14 mm^2 area, BASi, West Lafayette, IN), nickel wire counter electrode (Sigma Aldrich), and a silver/silver chloride (Ag/AgCl) reference electrode (BASi, West Lafayette, IN). All potentials reported are vs. standard hydrogen electrode (SHE), using the conversion factor for Ag/AgCl reference electrode of +0.27 V vs. SHE for the medium used (Badalamenti et al, 2013). Media bottles were mixed and re-flushed with filtered N_2 gas prior to use. I inoculated the electrochemical cell with approximately 2 mL inoculum from a media tube or another electrochemical cell.

I performed all electrochemical measurements as described in Chapter 3 (Yoho et al, 2014) using a VMP3 digital potentiostat (Bio-Logic USA, Knoxville, TN). Chronoamperometry was used to monitor the current production by biofilms over time, and also in combination with cyclic voltammetry (CV) to investigate the utilization and contribution of multiple electron transport pathways for current production after poising the biofilm at different potentials. I performed CVs at scan rates of 1 mV/s for at least two cycles, depending on the experiment (see text) over a potential range of +0.07 V vs. SHE to -0.38 V vs. SHE, using the final cycle for data analysis. Multiple biofilms were grown for each of the reported potentials. Additional data can be found in the Supporting Information. I performed EIS measurements at a range of anode potentials (depending on the experiment) following the method as described previously (Yoho et al, 2014). These data were fit using equivalent circuit models (ECMs) using the EC-Lab software on the potentiostat. I performed Kramers-Kronig (KK) tests on EIS data to ensure data validity, using the KK-test software (EC-Lab, BioLogic USA, Knoxville, TN). For more information on the KK-test, see Chapter 2.

4.3 Results and Discussion

A *Glk. ferrihydriticus* biofilm was grown at +0.07 V, an anode potential oxidizing enough to promote high current densities (Badalamenti et al, 2013). The plot of current density over time can be seen in Figure 4.1, presented alongside the same for another biofilm grown at a more reducing potential, -0.13 V.

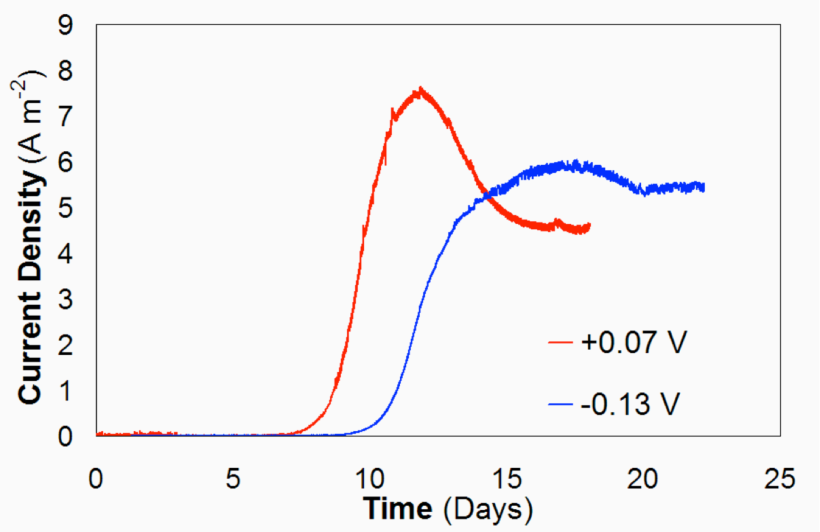


Figure 4.1. In red, the growth curve for the *Glk. ferrihydriticus* biofilm shown in the CVs in Figure 4.3, grown at +0.07 V vs. SHE and in blue, the growth curve for a *Glk. ferrihydriticus* biofilm grown at -0.13 V vs. SHE.

The lag time in current production before the exponential growth phase in Figure 4.1 indicates that either the inoculum used was of a sufficiently small volume or the cells took significant time to adapt to the electrode, so the current produced can be correlated with the growth rate (Badalamenti et al, 2013; Marsili et al, 2010). The difference in the set anode potential between the two growth curves does not appear to result in different current production in the long term, as both biofilms equilibrate at similar current densities. However, Figure 4.1 indicates that the biofilms grown at different anode potentials may have some unknown differences in cell or biofilm structure or function, which manifest as different growth rates (see Figure 4.2) and maximum current density produced. Again, I observe that the growth rate and energy conservation are correlated, aligning with results seen previously in Chapter 3 for *G. sulfurreducens* (Yoho et al, 2014).

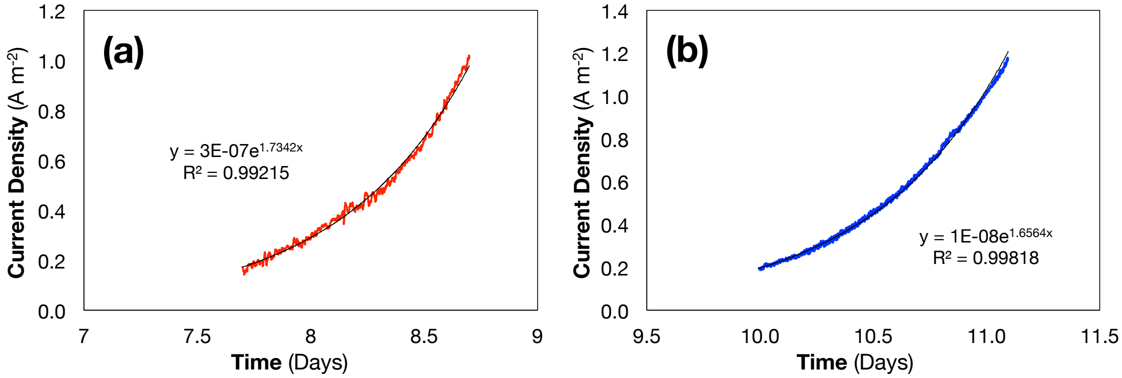


Figure 4.2. Growth curves for *Glk. ferrihydriticus* biofilms grown at +0.07 V and (b) -0.13 V from the biofilms presented in Figure 4.1. The estimated growth rates from the data in Figure 4.1 differ, with 1.18 d^{-1} for the biofilm grown at +0.07 V and 1.75 d^{-1} for the biofilm grown at -0.14 V. This indicates that the biofilm grew at a faster rate, but with a longer lag time at the lower potential.

The curves in Figure 4.1 suggest an interesting behavior of the biofilms in which the current density at which the biofilm stabilizes is noticeably lower than the maximum current density. The peak current density is higher and more pronounced in biofilms grown at the higher anode potential. This behavior, reported here and shown previously in pure cultures of *Geoalkalibacter* spp. (Carmona-Martinez et al, 2013; Badalamenti et al, 2013) and *Geoalkalibacter*-dominated mixed cultures (Pierra et al, 2015), suggests that there is likely an optimum biofilm thickness for maximum current density production, leading to a hypothesis that the biofilm may be mass transport-limited. Although one common limitation is substrate (acetate) concentration (Renslow et al, 2013), the acetate concentration (30 mM) used is much higher than the half-saturation concentrations observed in ARB biofilms (Lee et al, 2009). Overall, these growth curves indicate that the maximum current density produced by the biofilm depends strongly on the applied anode potential, while the stable current density does not.

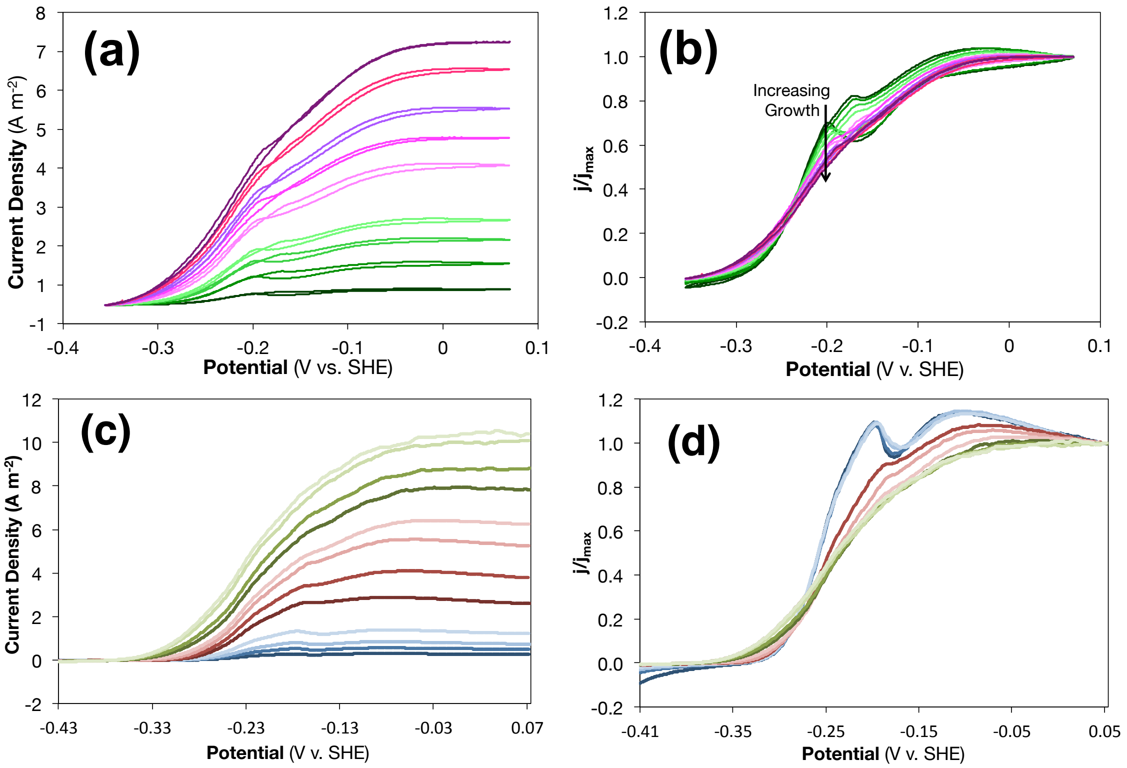


Figure 4.3. **(a)** CVs as a function of growth for a *Glk. ferrihydriticus* biofilm grown at +0.07 V vs. SHE. CVs were performed at a rate of 1 mV s^{-1} when the biofilm had reached the following current densities: 0.4 A m^{-2}, 1.1 A m^{-2}, 1.6 A m^{-2}, 2.2 A m^{-2}, 3.5 A m^{-2}, 4.0 A m^{-2}, 5.0 A m^{-2}, 6.1 A m^{-2}, and 7.0 A m^{-2}. **(b)** The same CVs from for the *Glk. ferrihydriticus* biofilm normalized to the maximum current density at each growth stage. **(c)** CVs as a function of growth for a biofilm grown at -0.13 V. **(d)** The CVs from (c) normalized to the maximum current density.

I performed CVs as a function of growth at discrete current densities, shown in Figure 4.3a. These CVs do not show an obvious shift in the sigmoidal shape as a function of growth, which I have previously shown in *G. sulfurreducens* in Chapter 3 to be an indication of there being a different pathway used for electron transport as the biofilms mature (Yoho et al, 2014). However, upon normalizing these CVs to the maximum current density at each stage as shown in Figure 4.3b, a peak near the midpoint can be observed that changes with biofilm maturity. As compared to the CVs as a function of growth previously observed in Chapter 2 for *G. sulfurreducens* (Yoho et al, 2014), the CVs in Figure 4.3 show a similar response, but with different magnitudes of change. With *G. sulfurreducens*, I observed a more pronounced shift towards negative potentials (Yoho et al, 2014), while in both microorganisms I observe a decrease in the peak height with biofilm maturity. I hypothesize that this decrease in peak height indicates a different type of shift between possible pathways - a difference in the contribution of pathways to overall current - as the biofilm matures.

Next, I fit a CV performed when the current density of a biofilm grown at -0.13 V had saturated to the Nernst-Monod equation, shown below as Equation 4.1. As described also in Chapter 3, two important parameters in the equation are the maximum current density (j_{max}), and the midpoint potential of the rate-limiting step (E_{ka}). In addition, n represents the number of electrons transferred in the rate-limiting step, which is set to n=1 for Figure 4.4. The remaining parameters consist of the Faraday constant (F), the gas constant (R), and temperature (T).

$$j = j_{max}\left(\frac{1}{1+\exp\left[-\frac{nF}{RT}(E-E_{KA})\right]}\right) \qquad \text{(Equation 4.1)}$$

In Figure 4.4, significant deviations can be seen between the experimental data and the Nernst-Monod fit. In this case, the experimental data is not well described by an n=1 (one electron transfer) reaction, even with the selection of an appropriate j_{max} and E_{KA}. This is consistent with previous observations for *G. sulfurreducens* biofilms from Chapter 3 (Yoho et al, 2014) and hints at the existence of more than one pathway for electron transport out of the cells.

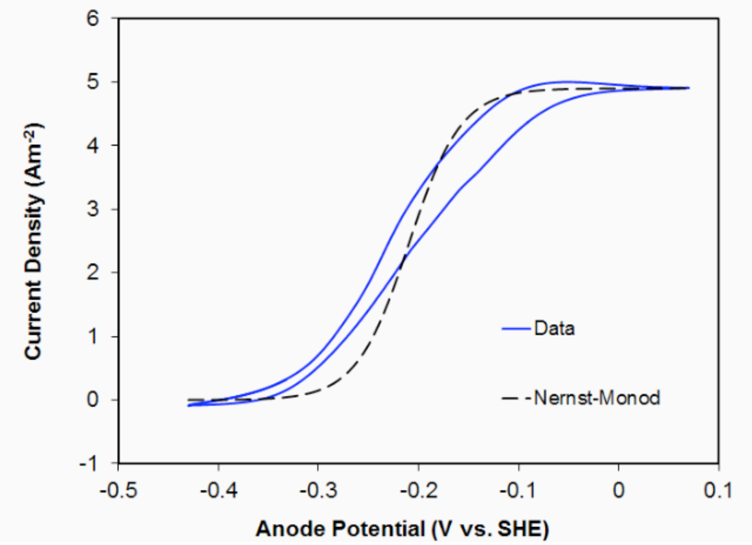
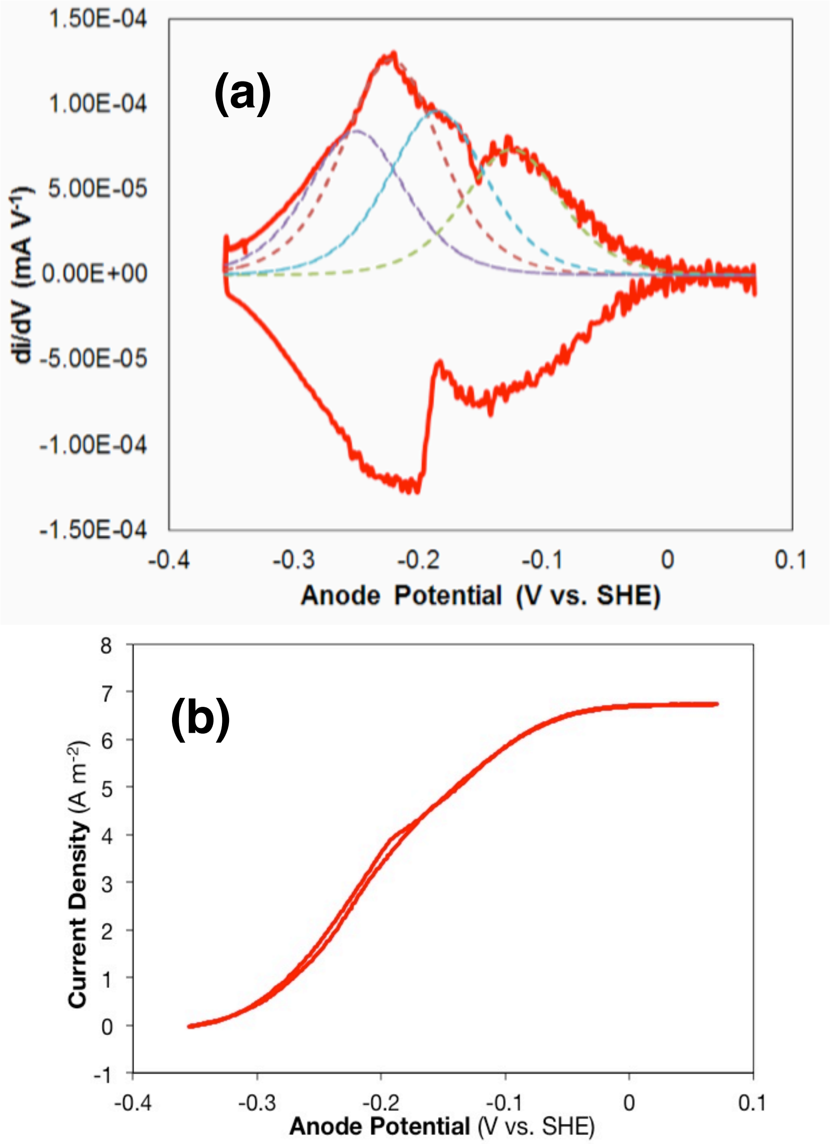


Figure 4.4. A CV poorly fit with an n=1 (one electron transfer) Nernst-Monod equation for a mature *Glk. ferrihydriticus* biofilm grown at -0.13 V scanned at 1 mV s^{-1}.

The derivatives of the CVs can be a useful investigational tool to determine the number of electron transport pathways that are manifested at different mid-point potentials and any shifts in those as a function of the poised anode potential (LaBelle and Bond, 2008). I investigated the derivative CVs for biofilms grown at +0.07 V and -0.13 V. In the past, high current densities for *Glk. ferrihydriticus* biofilms grown at the higher potential (+0.07 V) have been shown (Badalamenti et al, 2013). As this potential is in the saturation region of the CV, I decided to investigate also a relatively lower, more reducing potential for growth (-0.13 V), closer to the apparent midpoint potential. I fit the forward scan portion of each of the derivative CVs using multiple Nernst-Monod equations. Just as the derivative for the data can be taken, I take the derivative for the Nernst-Monod equation and adjust the parameters to fit the data. I have used such a method to model EIS data in Chapter 3 (Yoho et al, 2014).



Biofilm grown and poised at +0.07 V vs. SHE		
Pathway	E_{ka} (V vs. SHE)	Peak Height (mA V^{-1})
P1	-0.25	8.38×10^{-5}
P2	-0.222	1.26×10^{-4}
P3	-0.185	9.58×10^{-5}
P4	-0.125	7.36×10^{-5}

Figure 4.5. **(a)** Derivative of a CV performed at 1 mV s^{-1} from a *Glk. ferrihydriticus* biofilm grown at +0.07 V vs. SHE when the current density was approximately 7 A m^{-2}. Experimental data shown in red, Nernst-Monod fit data shown in dashed lines and color-coded in the table. **(b)** The original experimental CV.

In Figure 4.5a, I show a derivative CV for the biofilm grown at +0.07 V when the highest current density was observed (7 A m^{-2}). The derivative CV reveals at least four peaks, which could be associated with four different electron transport pathways. This data can be fit using four separate Nernst-Monod equations for an n=1 pathways based on the visible peaks observed. When considering the derivative CVs, the peak heights of the four pathways demonstrate the relative contribution of each proposed pathway. For example, an increase in the peak height indicates an increase in the contribution of that pathway, producing a higher current density. From the shape, I assume there to be four pathways, although they are not clearly delineated as individuals, but rather appear to be not clearly separated in the electrochemical response. However, the pathway I call P4, the highest potential pathways is the most clearly defined and demonstrates a slope corresponding to an n=1 process. The separation of this pathway and the fit of the slope support the assumption for the presence and apparent merging of the other pathways.

I used the derivative of the forward CV scan for fitting, as this direction more clearly showed the individual peaks. The reverse scans demonstrate a rapid shift between the pathways, further supporting the hypothesis of the involvement of multiple contributing pathways to the overall response of the biofilm. The Nernst-Monod fitting to the experimental data provided values for the midpoint potentials and maximum current density for each pathway. The curves in Figure 4.5 are color-coded for ease of comparison between figures (purple, red, teal, and green). In the table in Figure 4.5, I report, along with the mid-point potentials, the peak height for the derivative CVs that was used for fitting the data. This peak height is directly related to the maximum current density possible for each pathway. I provide figures illustrating proposed pathway utilizations, both additive and transient, in Figures 4.6 and 4.7.

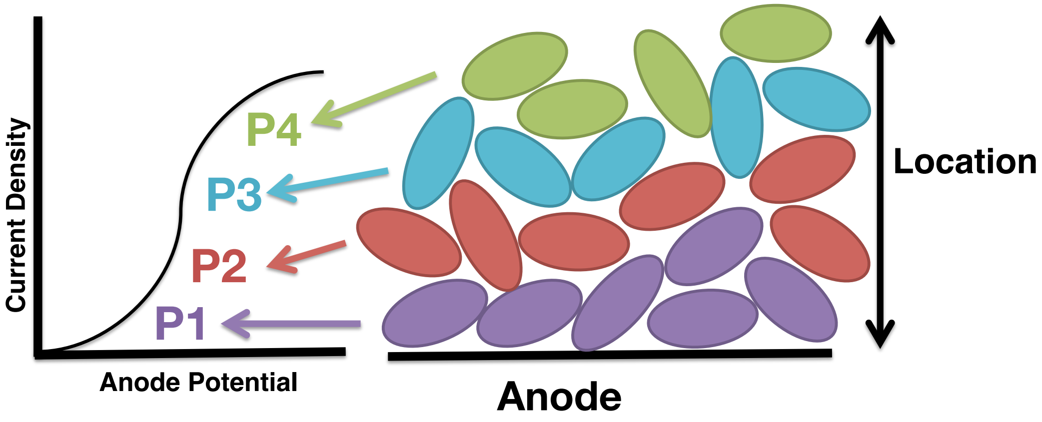


Figure 4.6. A simple schematic to illustrate the possibility of additive processes, with different cells within the biofilm expressing the four dominant pathways observed. Each pathway would contribute to the overall current as the scan approaches its midpoint potential. The biofilm would adapt to the new potential as the scan progresses and the poised potential changes.

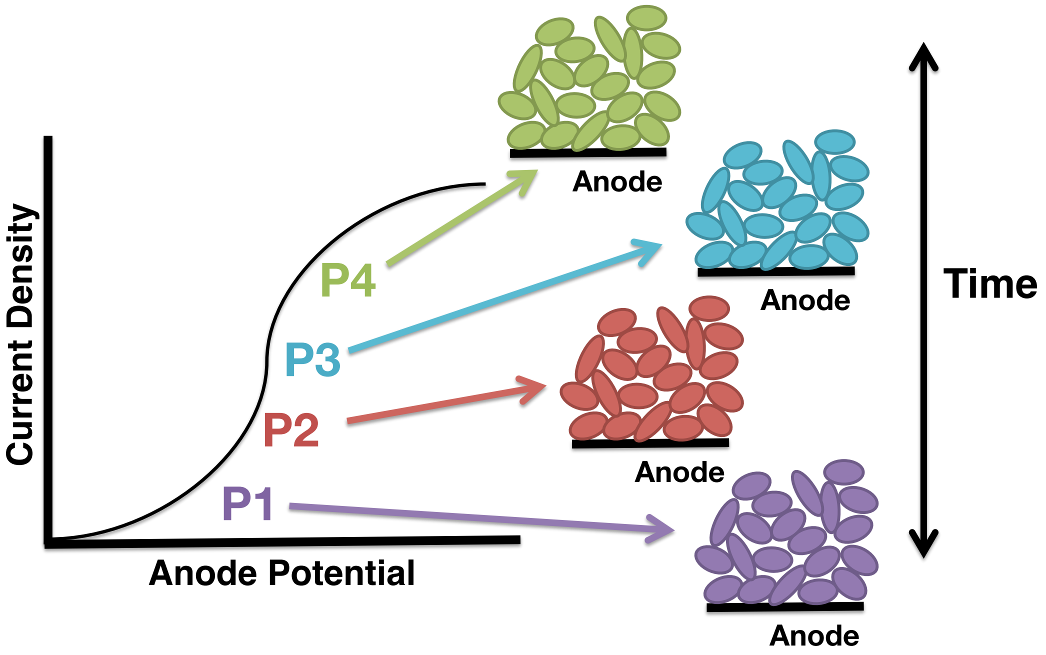
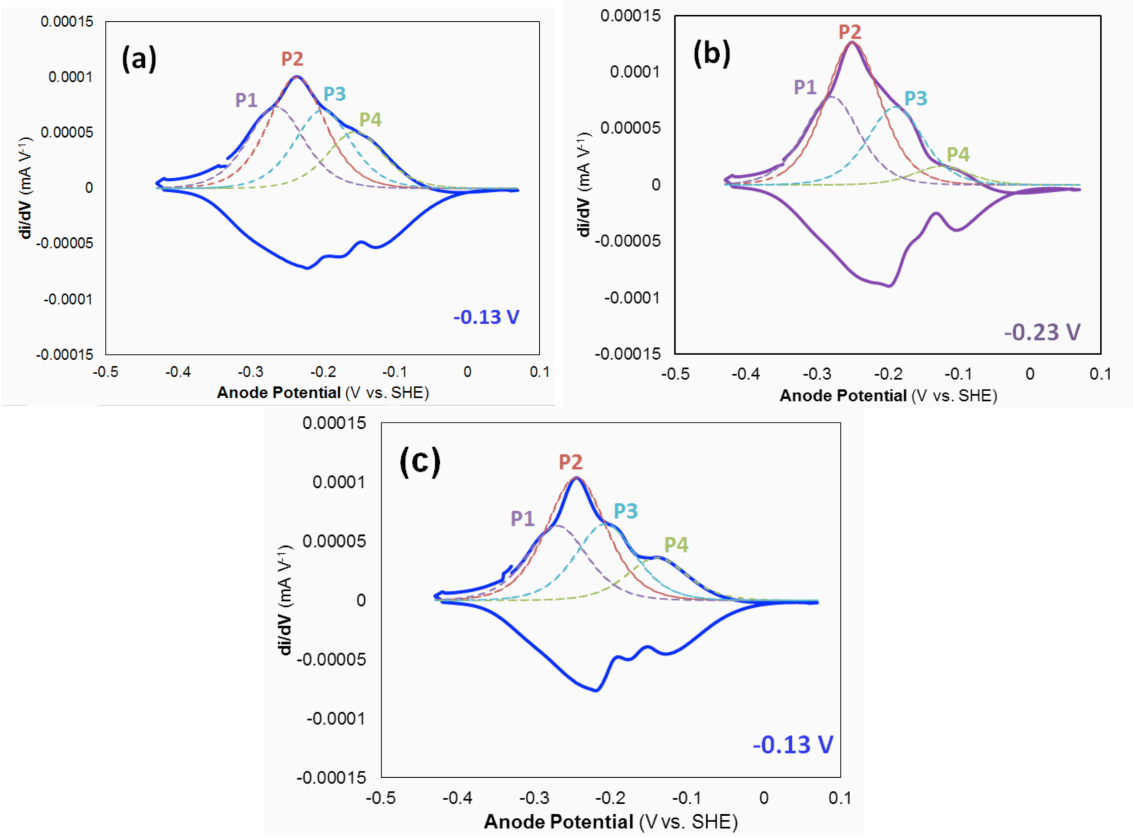


Figure 4.7. A representative schematic illustrating the utilization of transient pathways, with cells within the biofilm utilizing the four dominant pathways depending on the applied potential with time.

Following the observation of at least four pathways in the biofilm grown at +0.07 V vs. SHE, I grew a biofilm at a relatively more reducing potential of -0.13 V vs. SHE (growth curve shown previously in Figure 4.1). In this work, I used potentials more adequate to pathways on the saturation region of the CV (+0.07 V) and then used a potential change experiment for those around the midpoint potential (-0.13 V and -0.23 V). I designed a set of experiments to investigate changes in the contribution of each pathway to overall current product in a single *Glk. ferrihydriticus* biofilm. I show in Figure 4.8 the derivative CVs for an experiment where one CV performed after growing the biofilm at -0.13 V (Figure 4.8a), then the potential was lowered to -0.23 V and held for 24 hours followed by a second CV (Figure 4.8b). After this, the potential is returned to -0.13 V for 24 hours and a third CV is performed (Figure 4.8c). Additional datasets are presented in Figure 4.10. The corresponding experimental CVs to Figures 4.10 and 4.10 are presented in Figures 4.9 and 4.10d.

As in Figure 4.5, the forward direction of the derivative CVs can be fit using four Nernst-Monod equations shown as color-coded dashed lines in Figure 4.8. For the duration of this experiment, I assume that the 1 mV s^{-1} scan rate creates a pseudo-steady state condition. To this end, it is unlikely that the peaks shown in these figures, resulting from inflections in the CV, are a product of a discharge of capacitance (LaBelle and Bond, 2008). Commonly, capacitance is more influential under non-turnover conditions, where lower current densities are observed. I show a non-turnover CV in Figure 4.11 for comparison.



	Biofilm poised at -0.13 V vs. SHE (a)		**Biofilm poised at -0.23 V vs. SHE (b)**		**Biofilm poised at -0.13 V vs. SHE (c)**	
Pathway	E_{ka} (V vs. SHE)	Peak Height (mA V^{-1})	E_{ka} (V vs. SHE)	Peak Height (mA V^{-1})	E_{ka} (V vs. SHE)	Peak Height (mA V^{-1})
P1	-0.265	7.35x10^{-5}	-0.28	7.76x10^{-5}	-0.27	6.31x10^{-5}
P2	-0.236	1.00x10^{-4}	-0.25	1.26x10^{-4}	-0.245	1.04x10^{-4}
P3	-0.2	7.04e^{-5}	-0.19	6.87x10^{-5}	-0.207	6.44x10^{-5}
P4	-0.155	5.10x10^{-5}	-0.125	1.69x10^{-5}	-0.14	3.61x10^{-5}

Figure 4.8. **(a)** Derivative of a CV performed on a *Glk. ferrihydriticus* biofilm grown and poised at -0.13 V vs. SHE. Experimental data shown in blue. **(b)** Derivative of a CV performed on the same *Glk. ferrihydriticus* biofilm after the potential was changed to -0.23 V vs. SHE and held for 24 hours. Experimental data is shown in purple. **(c)** Derivative of a CV performed on the same *Glk. ferrihydriticus* biofilm after the potential was changed back to -0.13 V vs. SHE and held for 24 hours. Experimental data is shown in blue and Nernst-Monod fit data is shown color-coded in the included table. An additional data set is shown in Figure 4.10.

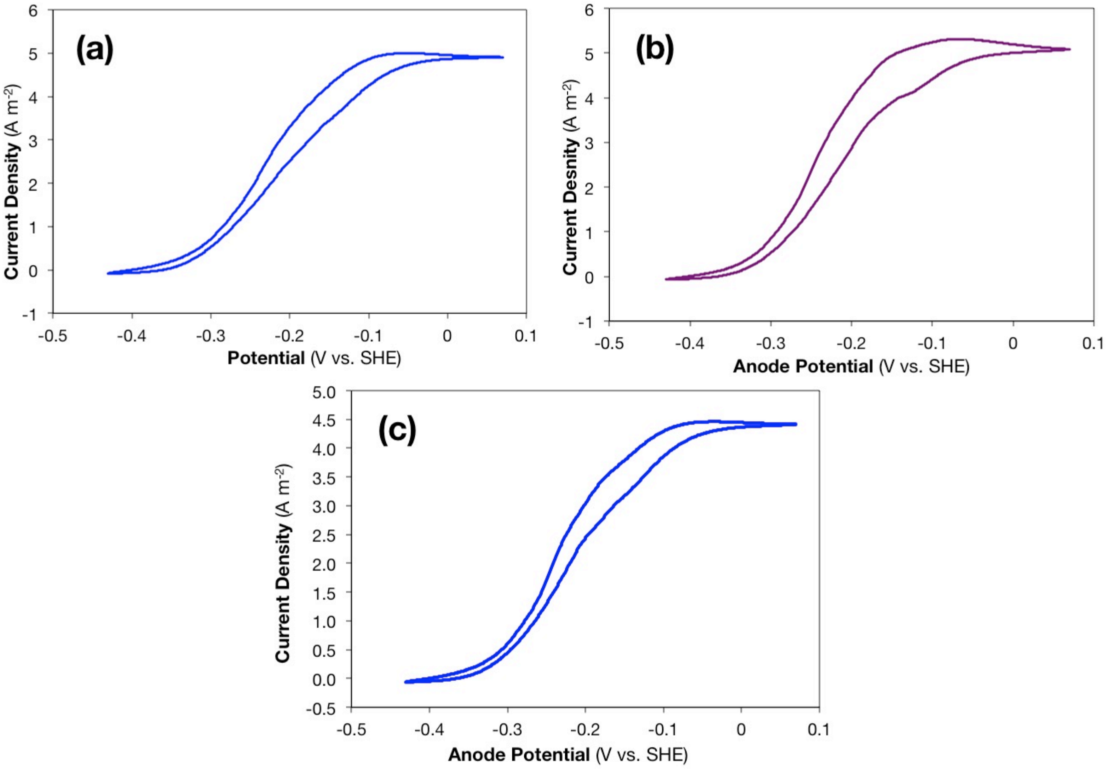


Figure 4.9. **(a)** Corresponding sigmoidal CV from Figure 4.8a. **(b)** Corresponding sigmoidal CV from Figure 4.8b. **(c)** Corresponding sigmoidal CV from Figure 4.8c.

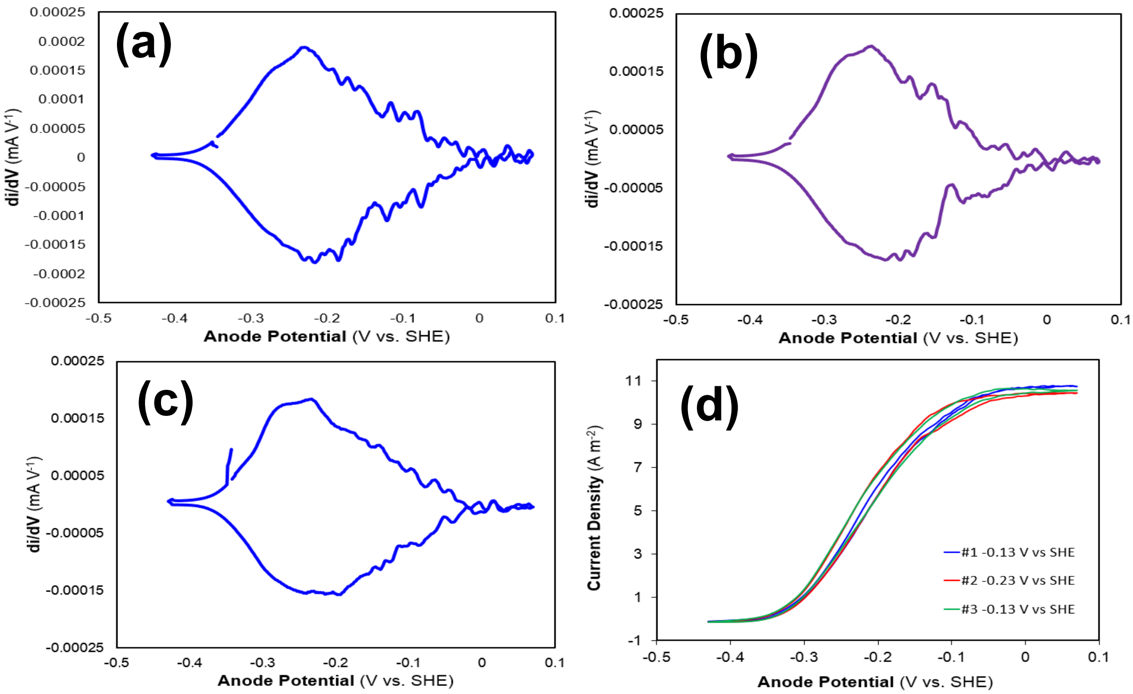


Figure 4.10. Duplicate experiment from Figure 4.8 **(a, b, c)** and **(d)** the corresponding experimental CVs.

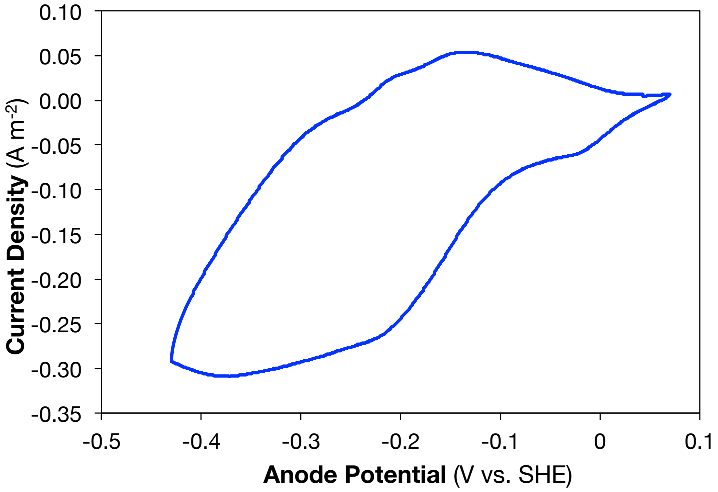


Figure 4.11. Non-turnover CV grown at -0.13 V vs. SHE performed at 1 mV s^{-1}.

In Figure 4.8, I show the midpoint potentials and the peak heights for each pathway. The derivative CVs from the experiment in Figures 4.8 and 4.10 when the poised potential of the biofilm is changed and returned to its original potential further supports the existence of four pathways and provides their respective mid-point potentials. The changes when the potential was lowered to -0.23 V vs. SHE suggest that small shifts in the midpoint potentials of the pathways and their utilization (resulting current) are reversible when returning to the original poised potential. The corresponding experimental CV data for Figure 4.8 can be found in Figure 4.9.

In Figure 4.8, a change in the contribution of the individual pathways to overall current when the poised potential of the biofilm was changed is apparent. The pathway manifested at the highest potential, named P4, has a decreased contribution at the lower poised potential (-0.23 V), but its contribution is restored when the biofilm is returned to the original growth potential. In contrast, contribution of P2 increases at the lower poised potential. Experiments in which I shifted potentials over one hour (instead of 24 hours shown in Figure 4.8) did not show a significant change (data not shown), suggesting that the time to adapt to the new potentials is longer than that observed with *G. sulfurreducens* in Chapter 3 (Yoho et al, 2014), in which I observed changes to occur in as little as 30-60 minutes. Additionally, I note that the -0.23 V was a poised potential, not the one at which the biofilm was grown. The decrease in P4 does not indicate necessarily that the biofilm would not utilize P4 for development at that potential, but suggests that it its contribution appears to decrease during current production at -0.23 V. The potential and time dependency of the utilization of these pathways strongly suggests that these are separate electron transfer pathways in *Glk. ferrihydriticus*. Overall, the four pathways exhibit consistency between the two applied anode potentials, with visible changes in the peak heights between the two potentials. Additionally, the lower portions of the derivative CVs show a change in current roughly similar to that seen in Figure 4.5.

4.3.1 Additive versus Transient Utilization of Pathways

For *G. sulfurreducens* in Chapter 3, I found that the contribution of the two pathways discovered was not additive (*i.e.* simultaneously expressed), but that they were differentially used over time depending on the poised anode potential. Figures 4.6 and 4.7 provide possible representations of a biofilm utilizing additive or transient pathways, respectively. If the response observed in *Glk. ferrihydriticus* is the combination of four pathways simultaneously utilized, then I should be able to fit the derivative CVs by adding four individual Nernst-Monod equations that are different in j_{max} as compared to what I used for the fittings in Figure 4.5 and 4.8. This represents an additive model, as a transient process cannot be modeled effectively. The sum of the four pathways (orange dashed line) was overlaid on the derivative CV, shown in Figure 4.12. It appears that the midpoint potentials of the four pathways are too close to allow the separate utilization (current) to fit the derivative CV. Additionally, I fitted the forward scan of the derivative CV, shown in Figures 4.12b and 4.12d, by altering the j_{max} and E_{KA} of the four pathways added. The fitted line closely models that of the experimental data, suggesting that the four pathways could be utilized simultaneously within the *Glk. ferrihydriticus* biofilm.

Figure 4.12 suggests that there are two possibilities for the nature of the pathways observed. This information is provided as an exercise, not to present final information about the utilization of pathway, but to begin the exploration in this area. Figure 4.12 builds on the idea of the additive pathways and suggests that the responses observed are not transient. In this work, I was unable to document a clear shift between two pathways, as I showed in Chapter 3 for *G. sulfurreducens* (Yoho et al, 2014), likely because of the existence of four pathways instead of just the two *in G. sulfurreducens* and the complexity in deciphering the electrochemical response through a simple chronoamperometric measurement. The model fitting performed in Figure 4.12b and

4.12d suggests that the four pathways are most likely additive, or utilized simultaneously at different locations in the biofilm. However, the addition of the four pathways would create a smooth curve, as shown by the fitted line, but the CV derivatives show sharp inflections in between the peaks. These inflections are even more pronounced on the reverse scan (negative values in the derivative). These inflections likely are only possible if there is a shift between pathways, not an additive process. However, I am not able to model the reverse scan, again suggesting that these are not straightforward additive pathways. Therefore, I present evidence for both possibilities, but further studies would be required to provide additional information. I provide the information in Figure 4.12 and 4.13 to introduce the conversation of how the pathways are expressed, whether simultaneously or differentially. Supporting information for the physiological aspects of this discussion is introduced in Figures 4.6 and 4.7.

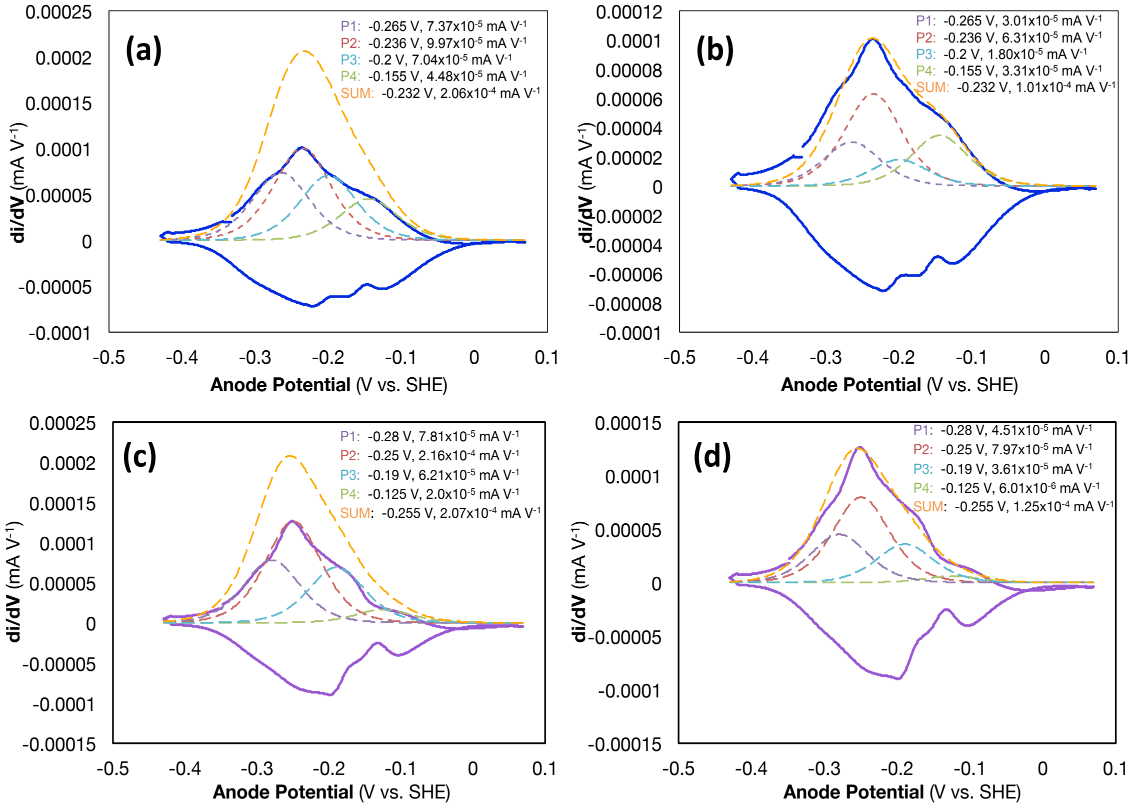


Figure 4.12. **(a)** The sum of the pathways (orange dashed line) shown in Figure 4.8a in the text, demonstrating that these may not be "additive." **(b)** The same figure, with alternative fitting for the pathways to make the sum more closely fit the experimental data. The adjustments are seen in the inset of the figure. **(c)** An example of a similar figure to 4.12a, but with a biofilm equilibrated to -0.23 V. **(d)** Adjustments to the pathways from 4.12c to make the sum more closely fit the experimental data. Additional figures shown in 4.13.

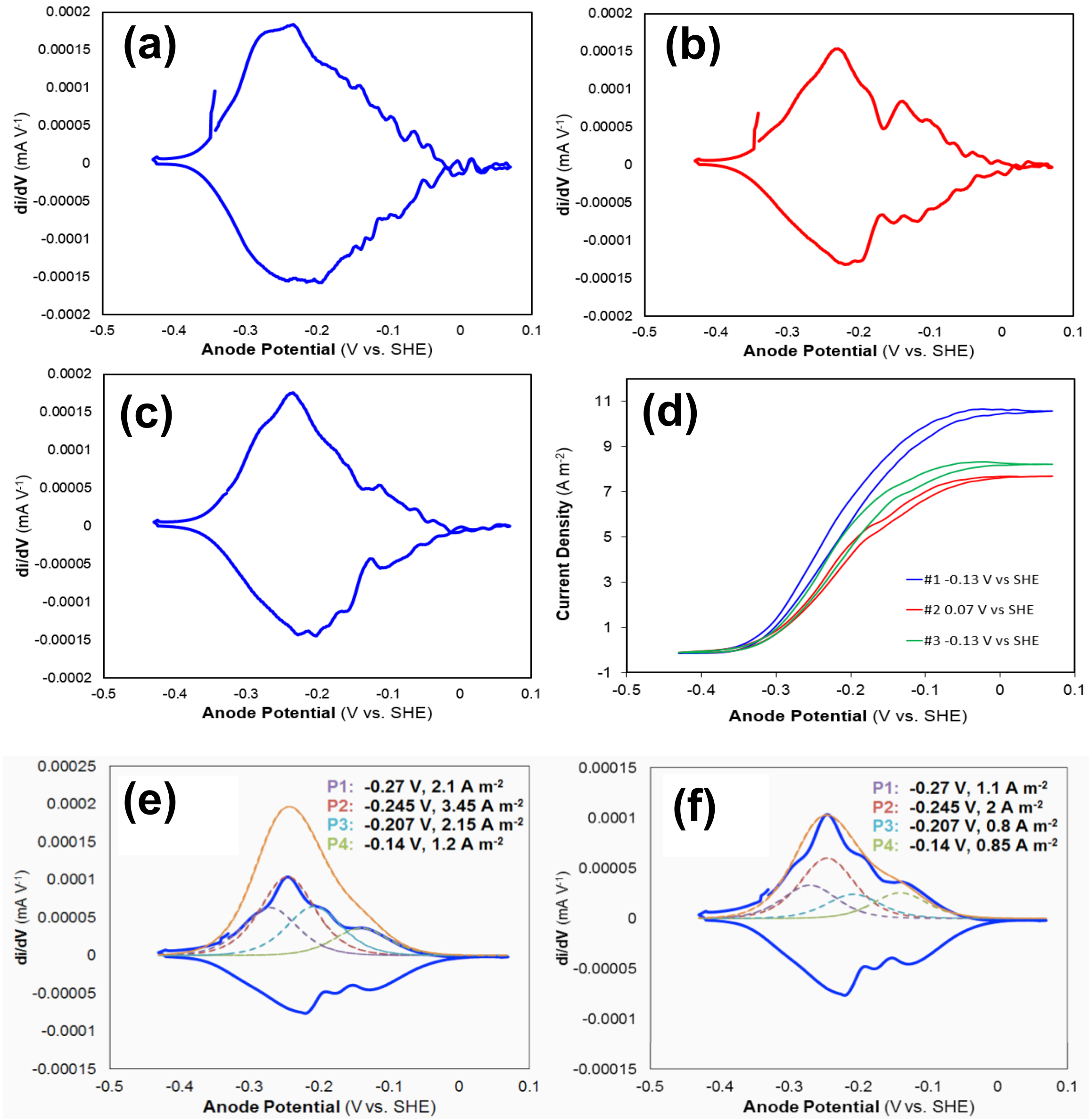


Figure 4.13. A potential change experiment performed on the same biofilm as shown in S8 and S9, with 24 hour equilibrations **(a)** from -0.13 V **(b)** to +0.07 V **(c)** to -0.13 V and **(d)** the corresponding experimental CVs. **(e)** and the adjustments to model the experimental data **(f)** upon the return of the biofilm to equilibration at -0.13 V.

4.3.2 Electrochemical Impedance Spectroscopy

In the analysis of *G. sulfurreducens* shown in Chapter 3, I employed EIS to investigate the two pathways differentially observed. As a final confirmation of the existence of multiple pathways for electron transport in *Glk. ferrihydriticus*, I used EIS on the biofilms when the current density reached an equilibrated saturation point. I used EIS was over a range of poised potentials to determine the charge transfer resistance (R_{ct}), which is plotted against the anode potential in Figure 4.14. The EIS experiments were performed utilizing the techniques established in Chapter 2. Additional datasets are included here as Figure 4.15.

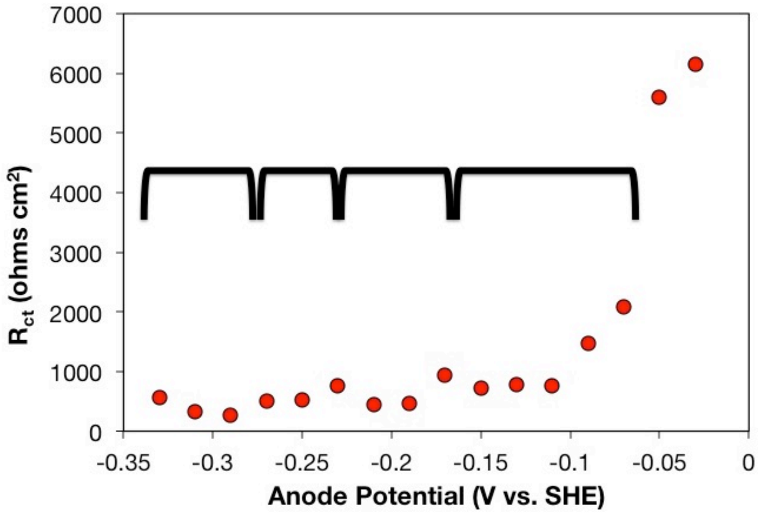


Figure 4.14. Charge transport resistance from EIS data fitting using an equivalent circuit model with a fixed ohmic resistance of 158 ohms. Brackets indicate hypothesized pathways observed.

There are four minima observed in the R_{ct}. versus anode potential data (Figure 4.14), suggestive of the four pathways identified with CVs. For *G. sulfurreducens* biofilms shown in Chapter 3 (Yoho et al, 2014), I was able to fit this type of curve using individual Nernst-Monod equations, but given that in this case two of the mid-point potentials identified in the derivative CVs are < 30 mV apart, the resolution of the multiple EIS measurements was not adequate. Nonetheless, the width of each U-shape around the four minima is consistent with multiple Nernstian pathways being used for electron transport in *Glk. ferrihydriticus*.

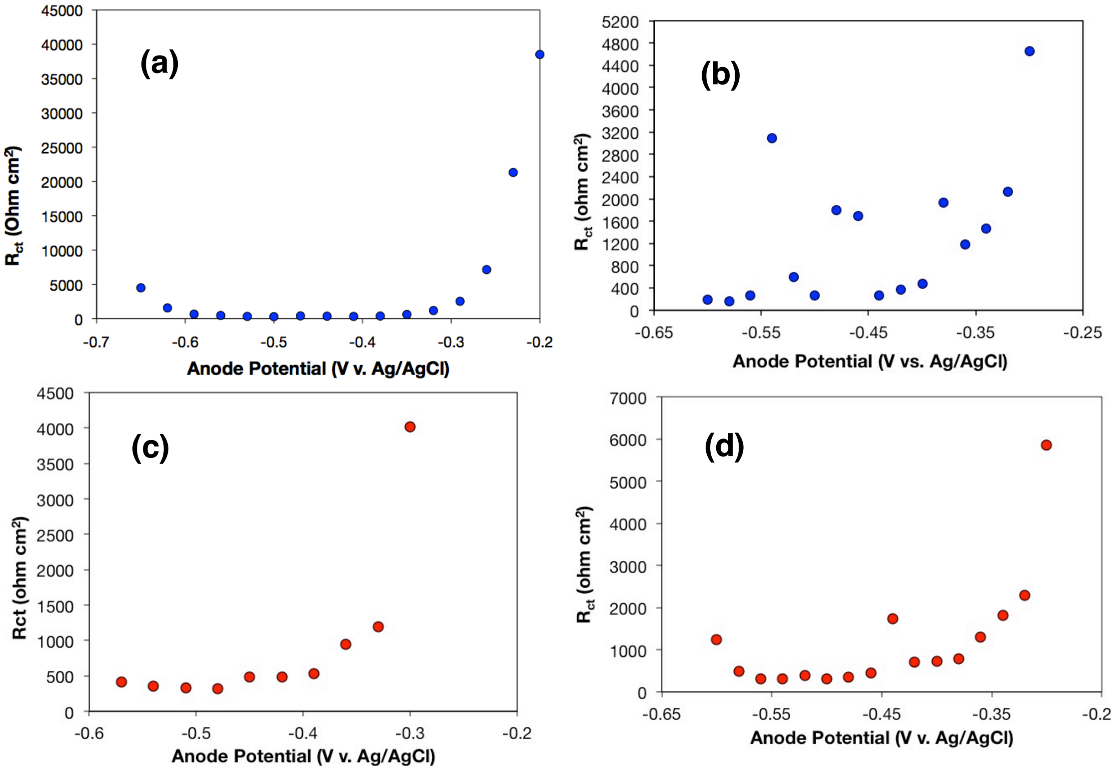


Figure 4.15. R_{ct} data from EIS on four *Glk. ferrihydriticus* biofilms grown at either -0.13 V (blue) as seen in **(a)** and **(b)** or +0.07 V (red) shown in **(c)** and **(d)**.

In summary, similar to the biofilms of *Geobacter sulfurreducens* from Chapter 3, the biofilms of *Glk. ferrihydricus* show a dynamic response to the changes in anode potential. The sensing mechanism for redox potential has not been discovered yet for this organism. As shown in Chapter 3 with *G. sulfurreducens* (Yoho et al, 2014), the manifested pathway changes are likely in the part of the electron transport chain found inside of the cell, due to the rapidity of equilibration between the anode potentials. Nothing further can be determined from these experiments as to whether the results indicate a single protein with four pathways or the involvement of multiple proteins (or combinations thereof). However, these observations create many possibilities for future studies. Additional investigations are necessary to identify the sensing mechanisms for *Glk. ferrihydriticus* biofilms and to identify the pathways manifested at the potentials identified in this work. Further information is required to understand more completely the pathways of electron transport from substrate utilization to final acceptor and the multiple pathways observed in *Glk. ferrihydriticus* biofilms. Future studies on the newly released genome of *Glk. ferrihydriticus* (Badalamenti et al, 2015) will be beneficial to identify key cytochromes of the pathways, similar to those investigated in *G. sulfurreducens* (Levar et al, 2014).

4.4 Conclusions

Herein, I used a combination of electrochemical techniques on biofilms of *Glk. ferrihydriticus* to investigate their dynamic responses to anode potential. I observed there to be at least four dominant pathways expressed during anode respiration by *Glk. ferrihydriticus* within the anode potential range studied. The utilization of each pathway appears to vary depending on the set anode potential to which the biofilm is equilibrated. These pathways appear to be manifested at approximately -0.14 V, -0.2 V, -0.24 V, and -0.27 V. I provide only approximate manifestations, as the midpoints are determined from fittings of CVs performed at slightly different currents, resulting in small variations in the derivative CVs. These numbers provide approximations based on the Nernst-Monod fitting model used and the implications on pathway utilization are still under experimental investigation.

I observed the utilization of pathways to depend on the applied anode potential, but not on the potential at which the biofilm was grown. Changes in the use of the four pathways were seen upon equilibration from the growth potential to another. Additionally, the utilization of pathways (including both the midpoint potential and the contribution of the overall current produced) appear to be reversible when the biofilm was changed to another potential and back. The pathways involved in the anode respiration mechanisms of *Glk. ferrihydriticus* follow a similar, but more complicated, pattern as to those observed previously in Chapter 3 for the model organism, *G. sulfurreducens* (Yoho et al, 2014). This implies an even greater diversity of extracellular electron transport pathways in the extremophilic ARB, as in this case for *Glk. ferrihydriticus*. As compared to *G. sulfurreducens*, *Glk. ferrihydriticus* has fewer cytochromes (Badalamenti et al, 2015), but this data suggests that it uses more pathways for anode respiration under the potential window studied. Further studies of these cytochromes may advance the fundamental understanding of extracellular electron transport in microorganisms and the function of cytochromes.

CHAPTER 5

INVESTIGATING ELECTRON TRANSPORT PATHWAYS *GEOALKALIBACTER FERRIHYDRITICUS* UNDER VARYING ENVIRONMENTAL CONDITIONS

This chapter has been submitted for publication in a modified format with the collaboration of César Torres, Sudeep Popat, and John Pennington.

5.1 Introduction

5.1.1 Overview

The mechanisms through which anode-respiring bacteria (ARB) transfer electrons from oxidation of an electron donor to a solid electrode, resulting in electrical current production, are only minimally understood. Among the few ARB investigated, *Geoalkalibacter ferrihydriticus* (*Glk. ferrihydriticus*), a Gram-negative alkaliphilic bacterium, utilizes multiple electron transport pathways for respiration to an anode. I investigated the electrochemical response of anode biofilms of *Glk. ferrihydriticus* as a function of pH in the bulk medium in microbial electrochemical cells. In the four previously discovered electron transport pathways, I observe a pH dependence of the midpoint potential of each pathway, but no effect on the contribution of each pathway to overall respiration rates.

In addition to having applications in high pH wastewater treatment, *Glk. ferrihydriticus* may become a useful microorganism for describing anode respiration under alkaliphilic conditions in the laboratory. The new insights I provide here on the effect of pH on electron transport pathways in *Glk. ferrihydriticus* shed light on the ability of this microorganism to optimize metabolic processes to varying environmental pH conditions. I hypothesize that this versatility is to take advantage of the energetics that change with pH.

5.1.2 Background

The mechanisms through which certain microorganisms perform extracellular electron transport (EET) to solid electron acceptors remain poorly understood (Bird et al, 2011; Lovley, 1991; Lovley, 2006), despite recent increased research efforts in the field. Model microorganisms provide a means to study these mechanisms, especially with recent efforts on understanding EET in *Shewanella oneidensis* (Pirbadian et al, 2014) and *Geobacter sulfurreducens* (Levar et al, 2014; Yoho et al, 2014; Zacharoff et al, 2016). More recently other microorganisms capable of extracellular respiration under a more diverse range of conditions (e.g. pH, salinity and temperature), have been discovered and are useful for further studying electron transport mechanisms and pathways. One example is *Glk. ferrihydriticus*, an alkaliphilic bacterium capable of iron reduction with a pH range of approximately 7.7-9.8 (Zavarzina et al, 2006).

Bacteria capable of EET also are well known to have the ability to respire to anodes in microbial electrochemical systems. In the Swette Center for Environmental Biotechnology at Arizona State University, we have studied several microorganisms capable of anode respiration in microbial electrochemical cells (and thus are referred to as anode-respiring bacteria, or ARB) and uncovered the existence of more than one pathway for electron transport in *Geobacter sulfurreducens* (Yoho et al, 2014) and *Glk. ferrihydriticus* (Yoho et al, 2015). These experiments are detailed in Chapters 3 and 4. I hypothesize that these multiple pathways likely developed for preferentially utilizing different electron acceptors and environmental conditions in nature.

While many studies are now investigating the multiple electron transport pathways in *G. sulfurreducens* (Commault et al, 2015; Levar et al, 2014; Rimboud et al, 2015; Yoho et al, 2014; Zacharoff et al, 2016), similar studies with other members of *Geobacteraceae* are sparse. Beyond the initial characterization for *Glk. ferrihydriticus* and *Glk. subterraneus* (Greene et al, 2009; Zavarzina et al, 2006), only a few studies exist that include fundamental microbiology for co-culture and selection studies (Pierra et al, 2015; Zavarzina et al, 2011) and applied microbiology including initial characterizations in MXCs (Badalamenti et al, 2013; Carmona-Martinez et al, 2013). Studies from our laboratory in the Swette Center for Environmental Biotechnology at Arizona State University have shown the signatures of multiple electron transport pathways in *Glk. ferrihydriticus* that manifest as a result of variations in anode potential (Badalamenti et al, 2013; Yoho et al, 2015). Similar characterization has been performed with *G. sulfurreducens* to show anode potential dependencies of the *G. sulfurreducens* biofilm (Levar et al, 2014; Yoho et al, 2014; Zacharoff et al, 2016); however there are also likely pH dependencies (Katuri et al, 2010).

In microbial electrochemical cells, the energy available for the microorganisms is represented as the difference between the redox potential of the electron donor and the anode potential. I have observed that changes in the anode potential result in changes in the utilization of electron transport pathways in *G. sulfurreducens* (Yoho et al, 2014), likely due to different energetics. However, the potential of the electron donor is also dynamic, depending on the concentration of reactants and products. One of the products is protons (H^+); in Equation 1, the acetate oxidation reaction can be seen, which is a common electron donor for ARB, leads to a 67 mV per pH unit shift at STP, based on the Nernstian equilibrium.

$$CH_3COO^- + 4H_2O \leftrightarrow 2HCO_3^- + 9H^+ + 8e^- \qquad \text{(Equation 5.1)}$$

I hypothesize that the pathways for electron transport may depend on conditions other than the anode potential as well. Based on the Nernst equation and the likelihood that electron transfer is proton-coupled, I propose that pH (both within the biofilm and the bulk medium) may influence the manifestation of the midpoint potentials and utilization of each electron transport pathway in *Glk. ferrihydriticus*.

In this study, I investigated the effects of medium pH on the metabolic versatility of the *Glk. ferrihydriticus* biofilms with respect to the four electron transport pathways. I utilize cyclic voltammetry/voltammograms (CVs) to measure the current produced by the biofilm as a function of the energetics available to the biofilm based on a linear sweep in the poised anode potential (Marsili et al, 2008; Marsili et al, 2010; Torres et al, 2008; Yoho et al, 2014; Yoho et al, 2015). I especially focus on the first derivative of the CV, which has provided valuable information on the existence of multiple electron transport pathways that are indicated by several local maxima in the curve, as I have shown previously for *Glk. ferrihydriticus* in Chapter 4 (Yoho et al, 2015).

I hypothesized in this study that the CVs would reflect the metabolic versatility of the biofilm under distinct pH conditions, particularly through shifts in potentials at which the pathways are manifested. Accordingly, the relative contributions of the electron transport pathways of *Glk. ferrihydriticus* to overall respiration rates would indicate likely contributory optimization to the overall electrochemical response of the biofilms.

5.2 Materials and Methods

I subcultured *Geoalkalibacter ferrihydriticus* from the DSM 171813 strain stored in the laboratory as described by Yoho et al. (2015) and grown in an anaerobic medium with a target final pH of 9.2 presented by Badalamenti et al. (2013) with the following per 1 L: 0.2 g KCl, 0.1 g $MgCl_2•6H_2O$, 0.2 g KH_2PO_4, 1 g NaCl, 1.36 g sodium acetate trihydrate (as electron donor), 1 mL trace elements solution, 0.5 mL selenite-tungstate solution. After sparging with N_2 gas and autoclaving, 20 mL 1 M Na_2CO_3, 111 mL 1 M $NaHCO_3$, 10 mL 1 M NH_4Cl, and 1 mL vitamin solution were added.

According to previous work (Yoho et al, 2014; Yoho et al, 2015), I constructed three-electrode microbial electrochemical cells modified to have a gold working electrode (3.14 mm^2 surface area) through the side of the 100 mL VWR bottle. A silver/silver chloride (Ag/AgCl) reference electrode (BASi, West Lafayette, IN) and nickel wire counter electrode (Sigma Aldrich or Malin Co., Brookpark, OH) were used in these systems. I report anode potentials against the standard hydrogen electrode (SHE), as calculated to have a conversion factor of +0.27 V vs. SHE in this medium (Badalamenti et al, 2013). The electrochemical cells were inoculated using approximately 2 mL of medium containing cells from a tube or another electrochemical cell. This amount was determined in other studies to be a comparatively small amount used to observe the correlation between the growth rate and the current density generated (Badalamenti et al, 2013; Yoho et al, 2014; Yoho et al, 2015).

Electrochemical measurements were performed as described previously in Chapters 3 and 4 (Yoho et al, 2014; Yoho et al, 2015) using chronoamperometry to poise the anode potential and monitor the current density of the biofilm over time. Cyclic voltammetry (CV) was used to investigate the electrochemical signatures of electron transport pathways at a scan rate of 1 mV/s for two cycles over a potential range of +0.07 V to -0.38 V. The second cycle was used for data analysis. In this experiment, multiple biofilms were grown at the original media pH (9.2) to maturity, as determined by chronoamperometry.

pH adjustments were performed using a media replacement method that involves pumping previously adjusted pH medium at a rate of 17 mL s^{-1} through autoclaved tubing. Exiting medium in the adjusted bottle is offset with nitrogen from a Tedlar® gas bag

(Sigma-Aldrich). The pumping of the medium at this rate minimized any disturbances to the biofilm. I present data from three different biofilms that were subject to medium replacements using the method described for discrete pH in the range between 8.65 and 9.7. The electrochemical cells were equilibrated to the adjusted pH for a minimum of 1 hour before CVs were performed for six pH adjustments. For additional information and comparison, three pH adjustments reflect data generated by allowing the current density to equilibrate for approximately 24-48 hours.

5.3 Results and Discussion

Glk. ferrihydriticus biofilms were grown at -0.13 V, an anode potential demonstrated to facilitate the development of mature biofilms generating high current densities (Badalamenti et al, 2013; Yoho et al, 2015). CVs of these biofilms at the growth pH (9.2) demonstrate similar manifested characteristics as those previously observed in the Swette Center for Environmental Biotechnology laboratory (Badalamenti et al, 2013; Yoho et al, 2015). The original medium pH in these experiments (pH = 9.2), falls within the reported optimal range of pH 9.0 – 9.2 from DSMZ, the German Culture Collection of Microorganisms and Cell Cultures to facilitate growth (DSMZ, 2007). Once developed into a mature biofilm as determined by an apparent stabilization in the current density, I used the medium replacement method to flow medium of a pre-adjusted pH through the reactor. In Figure 5.1a, I show a representative set of CVs for three distinct bulk media pH from a single biofilm (9.2, 9.6, and 8.65).

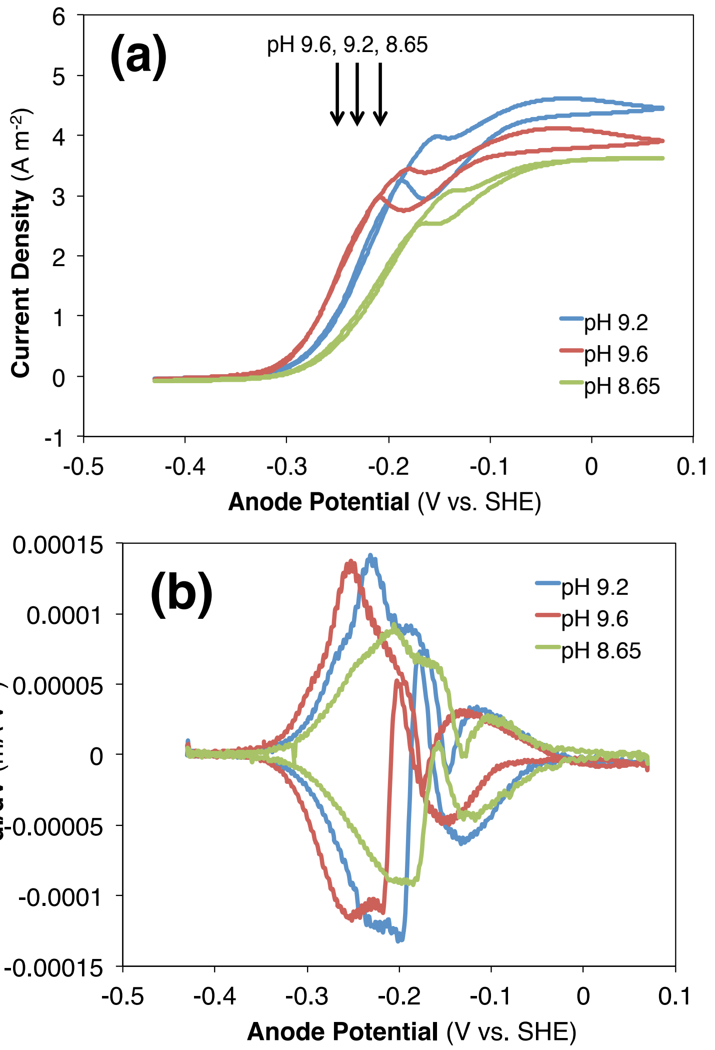


Figure 5.1. **(a)** CVs of an individual *Glk. ferrihydriticus* biofilm grown at pH 9.2 and adjusted to pH 9.6 and 8.65 with arrows indicating approximate midpoints of each CV and **(b)** the derivatives of the CVs presented in (a).

Beyond the differences in the maximum current density, j_{max}, the CVs in Figure 5.1a suggest a previously undocumented shift in the overall midpoint potential (indicated by black arrows) of the biofilm as a result of changes in pH. In this figure, the entire catalytic curve appears to shift to the more positive potentials as the pH decreases. The derivative CVs, as shown in Figure 5.1b, demonstrate this shift more clearly. Figure 5.1b supports the presence of the four dominant electron transport pathways proposed previously in Chapter 4 (Yoho et al, 2015), with examples of data fitting for the four dominant pathways shown in a single derivative CV from another biofilm in Figure 5.4a. In this work, I present both the individual and aggregate results of the effect of the bulk medium pH on a total of three different *Glk. ferrihydriticus* biofilms.

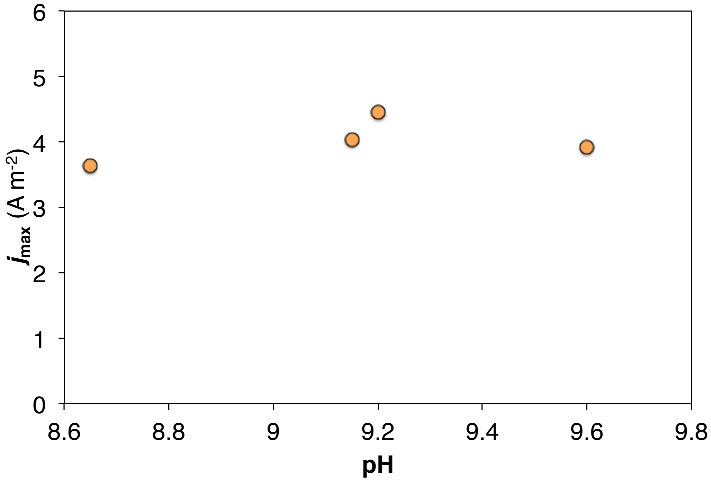


Figure 5.2. Current density as a function of pH for one anode biofilm of *Glk. ferrihydriticus*. Two additional biofilms can be seen in Figure 5.8 (in the appendix of Chapter 5).

Figure 5.2 suggests no significant pH dependence on the maximum current density, j_{max}, produced by the biofilm. However, these experiments were performed within only one pH unit span over the original medium (pH = 9.2) and within the general tolerance range of the biofilm. This alkaliphilic organism cannot be grown at neutral pH, for example, and large changes in pH can affect the biofilm and overall current density produced, as can be observed in some experiments (Figure 5.3). Therefore, these results (along with Figure 5.8 in the appendix of Chapter 5) suggest only a slight dependence of the current density on the pH, within the typical tolerance range of the organism.

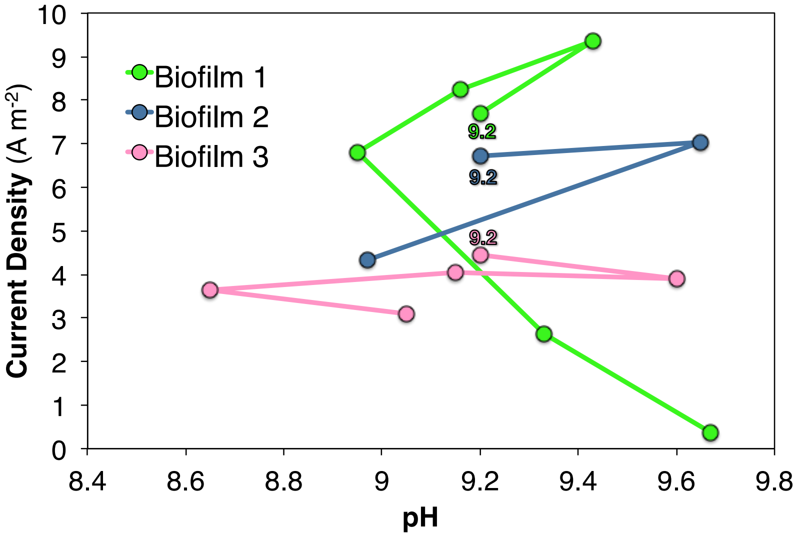
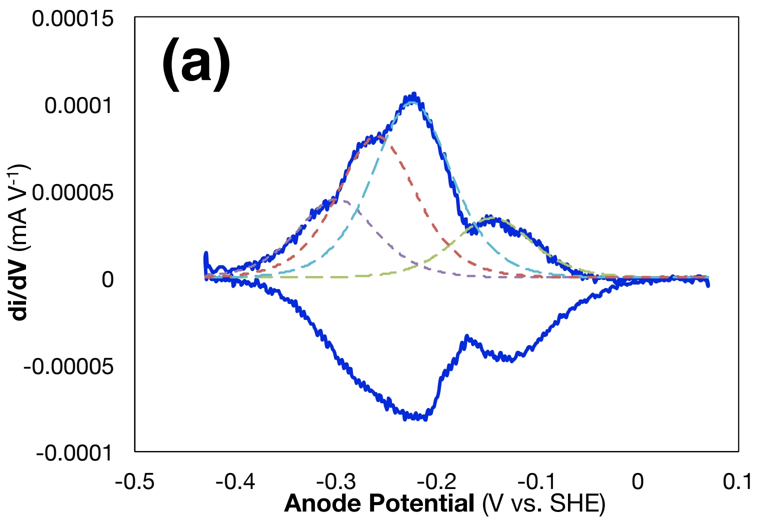


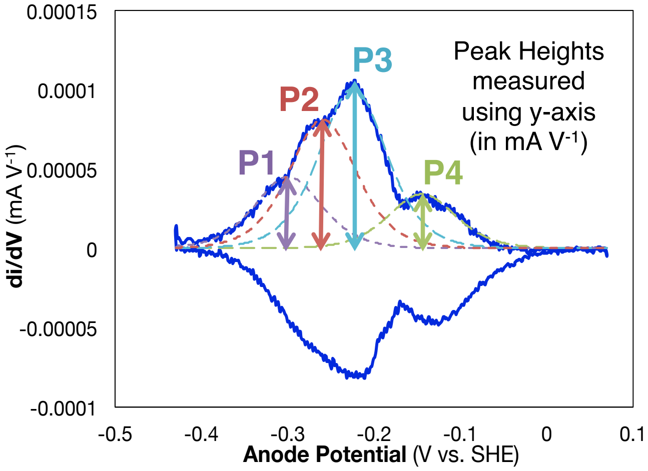
Figure 5.3. Sequential pH changes for each of three biofilms, with 9.2 indicating the starting point of the experiments.

However, Figure 5.2 and Figure 5.3 also indirectly supports the pattern indicated in Figure 5.1a, for example, of an apparent difficulty for the maximum current density, j_{max}, to adjust to later changes in the bulk media pH through the maintenance of similarly high values. Figure 5.3 shows a general decrease in the current density for later pH adjustments, suggesting a negative effect of multiple pH changes on the biofilm. Overall, the results indicate a difficulty encountered by the biofilm to readjust to a similarly high current density when returned to a previous pH once it has been changed to an unfavorable pH.

The results of the discrete pH investigated (Figure 5.1b) indicate a general trend in the relative contributions of each individual pathway. I show the fits for individual pathways in Figure 5.4a.



(b) Peak Height as Indicative of Pathway Utilization



Example Percent Contribution for P1:

$$\left(\frac{P1}{P1+P2+P3+P4}\right)\bullet(100)$$

Uses peak heights calculated indicated by arrows in above figure

Figure 5.4. (a) A *Glk. ferrihydriticus* biofilm grown at pH 9.2 and adjusted to pH 9. Four dominant electron transport pathways are demonstrated (dashed lines) as P1 through P4 in purple, red, teal, and green. **(b)** The same figure as in (a) while illustrating the calculations for peak heights.

In this work, I consider the peak height to be indicative of the contribution of the pathway to overall rates. These calculations translate into an average percent contribution, based on the peak height in the derivative CVs relative to the sum of the four peak heights. An illustrated example is provided in Figure 5.4b. The relative contributions show a significant difference between each of the four dominant electron transport pathways. All biofilms were grown and maintained at a poised anode potential of -0.13 V, closest to the midpoint potential of pathway 4 (P4 in the Figure 5.4a), the pathway manifested at the most positive potential as observed in these experiments and previously (Yoho et al, 2015). In Figure 5.5, I present the average contributions of the four dominant pathways for each of the pH conditions utilized in this experiment, as determined from measurements with multiple biofilms. These aggregate results (*n*=14) indicate a distinct contribution of each of the pathways across the environmental conditions within the general tolerance range of this organism. Additional information on the individual data contributing to the averages in Figure 5.5 can be found in Figure 5.6.

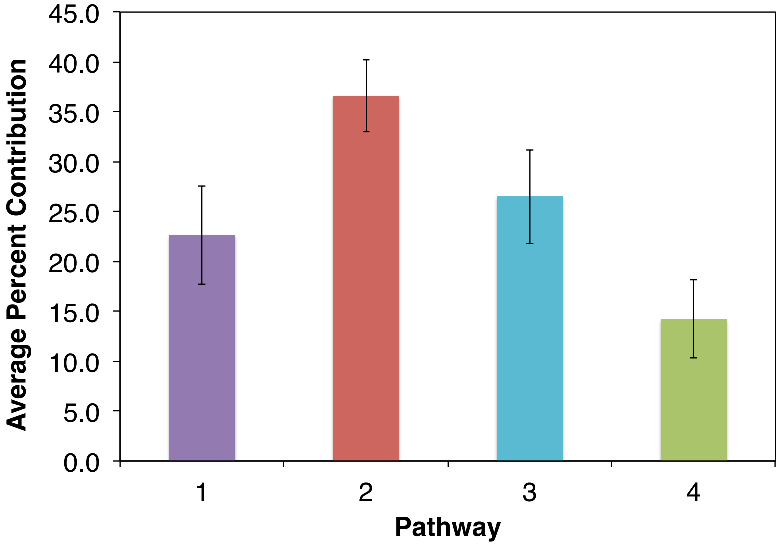


Figure 5.5. Average percent contributions for each of the four dominant electron transport pathways observed for all pH conditions investigated (*n*=14). The percent contributions are calculated from the peak height of one pathway divided by the sum of the four observed. All biofilms were grown and maintained at a poised anode potential of -0.13 V, closest to the midpoint potential of pathway 4, the pathway manifested at the most positive potential.

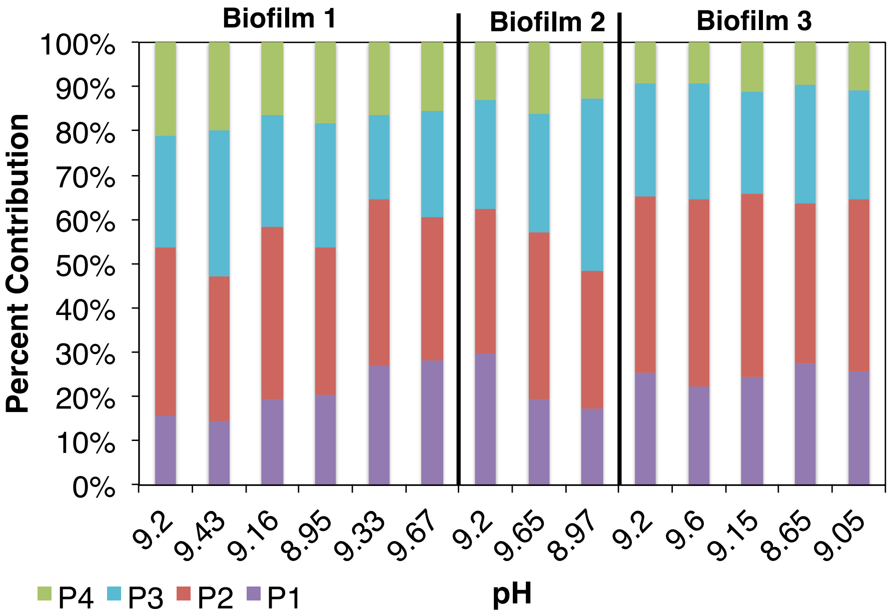


Figure 5.6. Individual trends in contribution of each pathway per pH utilized in order of experimentation, displayed for each of three distinct biofilms, including those returned to the original pH (9.2).

Beyond the contributions of each pathway to overall respiration rates as determined from the peak heights, the midpoint potentials of the four individual pathways indicate a general shift towards more negative potentials as pH increases in Figure 5.7. The results for two additional biofilms can be seen in Figure 5.9 (in the appendix of Chapter 5). This data support the general trend indicated in Figure 5.1a of the slight shift in the entire catalytic curve in the negative direction as the bulk media pH increases.

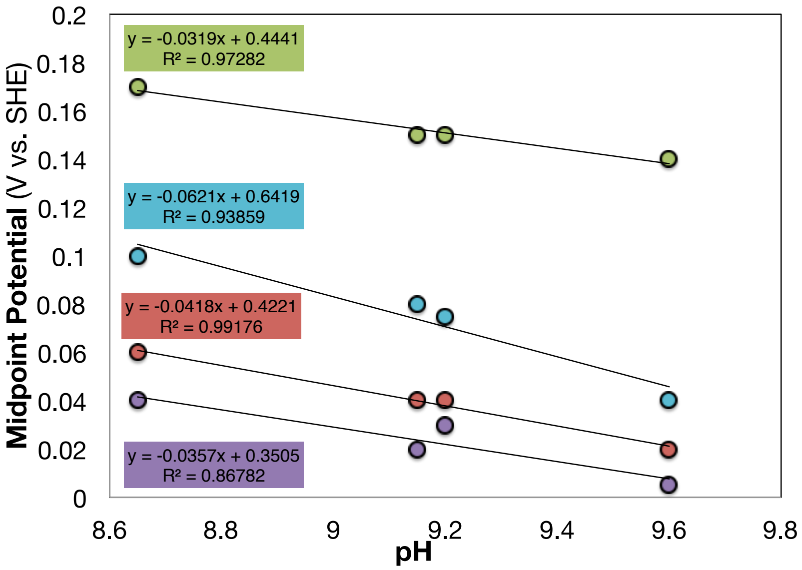


Figure 5.7. Midpoint potentials of each of the four pathways observed as a function of pH, known as P1 through P4 in purple, red, teal, and green, respectively. Data from additional biofilms can be found in Figure 5.9 (in the appendix of Chapter 5).

When considering Figures 5.5 and 5.6, I observe the shifts in manifested potential to be largely independent of the relative contributions of the individual pathways to the total. This suggests that the contributions of the pathways largely are conserved during the slight shifts in manifested potential. Utilizing three biofilms, the changes in each of the four pathways as the pH increases show decreases estimated at 0.022, 0.028, 0.085, and 0.050 V pH^{-1}, respectively. Overall, the four pathways demonstrate an average decrease in the midpoint potential of approximately 50 mV per pH unit increase.

Based on the one electron transport (n=1) Nernstian model utilized for fitting the pathways contributing to the overall response in the CV, the stoichiometry discussed indicates that these are electron transport responses. In theory, a proton-coupled mechanism would result in a shift of 0.067 V pH^{-1}, a larger value than I observe in this experimental data. However, the presence of additional pH gradients within the biofilm may distort the experimental shifts in midpoint potential as a function of pH. Due to these gradients and as the pH shifts one unit, the results indicate an aggregate shift of the entire biofilm. A one unit pH shift in the bulk does not imply that the pH has changed one unit throughout the entire biofilm because of the pH gradient that exists. Nonetheless, I observe the pH dependency to affect the total energetics of the *Glk. ferrihydriticus* biofilms.

5.4 Conclusions

Based on the applications of multiple pH conditions of the media on biofilms of *Glk. ferrihydriticus*, I observe that pH within the range investigated does not appear to be a major driving factor governing the contribution of each electron transport pathway to the overall respiration rates in *Glk. ferrihydriticus* biofilms. Additionally, I find that multiple changes in the pH of the bulk media cause the current density of the biofilm to decrease. In these experiments, I observe slight shifts in the manifested potential of the four dominant electron transport pathways as a function of pH. The relative contributions of each of the pathways observed remains distinct and appear consistent over the experiments performed.

From these experiments, I observe that the overall energy available to the biofilm is changing as the pH changes. However, this does not shift the relative utilization of these pathways on average. The individual midpoint potentials of the four dominant contributing pathways shift slightly with bulk pH within this narrow range, and the individual contributions of each appears to be conserved. Further studies expanding the pH range investigated and the maintenance of high current density outputs will be necessary to evaluate larger trends in electron transport pathway shifts.

5.5 Appendix

Here in the appendix to Chapter 5, I present two sets of additional figures supporting the main text, especially Figures 5.2 and 5.7, respectively. These figures are the results of two additional biofilms.

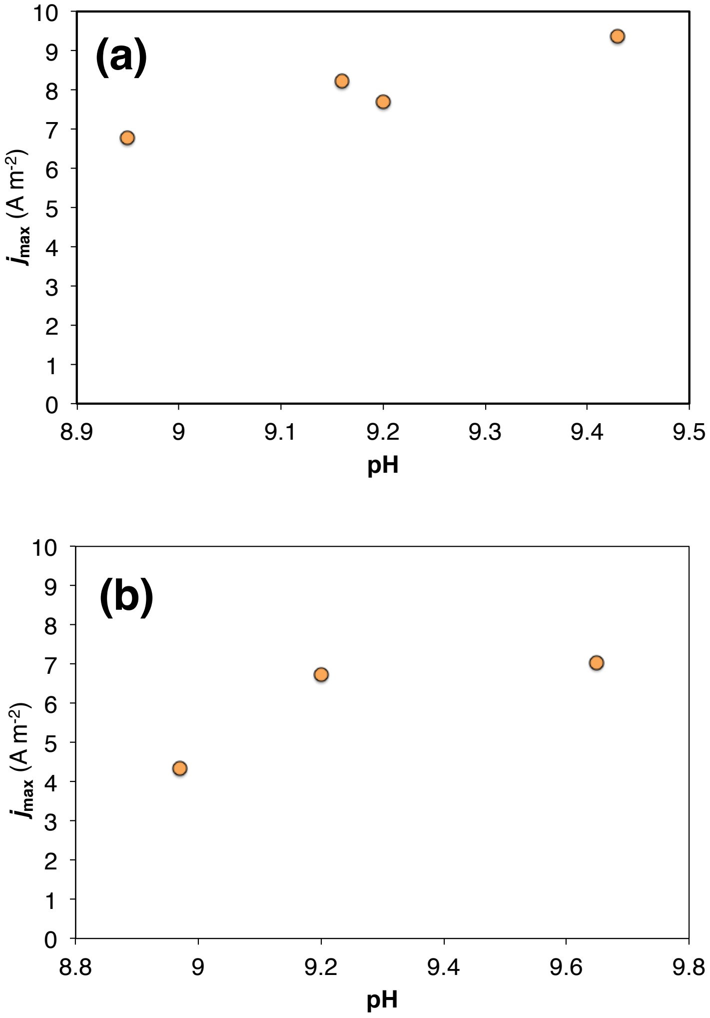


Figure 5.8. Current density as a function of pH for two additional biofilms.

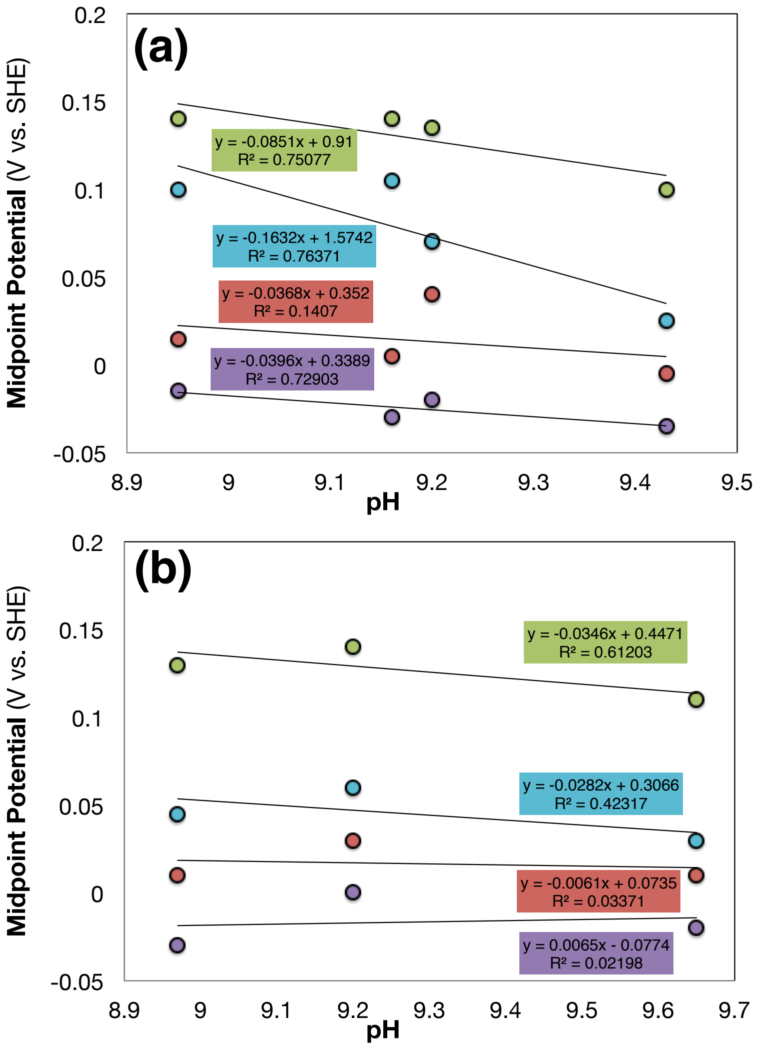


Figure 5.9. Individual trends in midpoint potentials for each of the four dominant electron transport pathway as a function of pH for two additional *Glk. ferrihydriticus* biofilms.

CHAPTER 6

MICROBIAL ELECTROCHEMISTRY SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

6.1 Summary of Research

The work in microbial electrochemistry applies several electrochemical techniques to living systems. In particular, Chapter 2 takes a technique, electrochemical impedance spectroscopy (EIS), and explores its applications by developing a sound methodology. This technique, alongside other electrochemical methods, forms the basis for investigation of electron transport pathways in anode-respiring bacteria (ARB). In Chapter 2, I explored the technique of EIS and created a set of guidelines for generating reliable, repeatable data from its application. The laboratory component formed the basis of comparison for cell geometries (i.e., single-chamber, H-type) and electrode material, while providing also a discussion of the technique in an accessible manner for researchers of many backgrounds. Standard guidelines and procedures were set forth that allow users of different potentiostats to choose parameters for experimentation to fit their needs (for example, frequency range, points per decade, and perturbation amplitude). The goal of this portion of the work was to provide a useful guide for researchers in the field and add another method to their growing toolbox of techniques, particularly for investigating microbial systems. The studies on EIS form the foundation for the later studies investigating the electron transport pathways of *G. sulfurreducens* and *Glk. ferrihydriticus*.

The results presented in Chapter 3 detail the existence and manifestations of two previously unexplored electron transport pathways in *G. sulfurreducens*. Utilizing only the electrochemical techniques of chronoamperometry, CV, and EIS, I identify the signatures of at least two dominant pathways and the ability of the organism to change pathway utilization in a matter of minutes. This observation indicates the existence of a previously undocumented sensing mechanism in its ability to adapt to changing environmental conditions, particularly the applied anode potential. This work came about as a result of an inability to properly fit a CV using a single n=1 Nernst-Monod curve for a *G. sulfurreducens* biofilm. In this electrochemical cell geometry, deviations between the experimental results and the model were observed over significant portions of the curve. With the increased understanding of the existence of multiple electron transport pathways, the research community now has the possibility of improving the theoretical models in the future.

Several extensions were made from the work on *G. sulfurreducens* presented in Chapter 3. Based on the experimental evidence indicating rapid changes between pathways, I proposed that the changes in expression may be inside of the cell. The alternative, part of the extracellular matrix, could require additional time or energy investments by the cell that are not directly supported by the experimental observations. The observations include the doubling times of the organism under conditions supporting an individual pathway and the rapidity of switching between pathways depending on the poised anode potential. By happening inside the cell, the organism would not have to generate an additional pathway and export it physically to the extracellular matrix.

The observations with *G. sulfurreducens* from Chapter 3 led to the speculation that other ARB may have more than one electron transport pathway. *Glk. ferrihydriticus*, a close phyologenetic relative of *G. sulfurreducens*, was chosen as a candidate for investigation because of its ability to respire anodes in alkaline conditions. The discussion of Chapter 4 explores the much more complex results of *Glk.*

ferrihydriticus. This organism appears to display the electrochemical signatures of at least four dominant electron transport pathways. This work, although supported by EIS and the techniques established in Chapter 2, became increasingly complex in its interpretation. In this work, I introduced a new method of exploring and fitting the first derivative of the CV with multiple n=1 Nernst-Monod fitting curves. With this fitting method, I was able to provide estimates for the potentials at which the electron transport pathways are manifested under the experimental conditions utilized.

Following the presentation of at least four dominant electron transport pathways for *Glk. ferrihydriticus* in Chapter 4, I began exploring other experimental conditions. As this organism is alkaliphilic, pH was a natural choice for investigating pathway utilization. In these experiments, pH conditions covering a one pH unit span in the alkaline tolerance region of the organism were applied. Overall, the available energy for the biofilm changes as a function of pH. Within the range investigated, the manifested midpoint potentials of the four contributing processes introduced in Chapter 4 shift in the negative direction as the pH increases, as expected from the Nernst equation. Additionally, the results indicate that the pH dependency for survival does not necessarily indicate a great ability to modify electron transport pathways during anode respiration. Building upon the first derivative of the CV interpretation introduced in Chapter 4, I employed a method establishing the “utilization” of those pathways based on peak height in the derivative of the CV. On average, the peak heights were largely conserved over the pH range investigated. The subsequent interpretation reveals that the four electron transport pathways have both distinct potential manifestations (E_{KA}) and utilization, easily suggesting their individual nature. Although a limited versatility in electron transport pathway utilization was an unexpected observation for *Glk.*

ferrihydriticus, investigation of a larger pH range may provide more evidence in the future.

6.2 Recommendations for Future Research

The results provide a basis for utilizing electrochemical methods as a comparatively simple method for exploring electron transport pathways in ARB. These are available as established, useful techniques in the repertoire of microbial electrochemical researchers and can be performed in real time using a potentiostat. These electrochemical techniques can be utilized on pure culture biofilms of anodic and cathodic ARB (although only anodic methods were explored in this dissertation) to provide microbial electrochemical information. The information gained can be used to guide genetic studies and assist in data interpretation.

While bioprospecting for ARB, I believe that these electrochemical techniques, particularly EIS and the interpretations of the first derivative of the CV, can be employed for initial characterizations of organisms. These characterizations can help the community to build a greater understanding of the pathways utilized by ARB and build a comparative model, such as a "phylogenetic tree" of possibly related electron transport pathways in the known ARB. Overall, electrochemical techniques can be used to provide early experimental evidence and support later genetic investigations of ARB.

6.2.1 Further Characterization of *G. sulfurreducens*

Although *G. sulfurreducens* has been a prominent organism in the ARB community for some years, our knowledge of the structure and function of the electron transport pathway has been lacking until recently. My work in Chapter 3, along with the work of Levar et al (2014) and Zacharoff et al (2016), both publications from the lab of

Dr. Daniel Bond at the University of Minnesota, have opened the door to new understandings of the pathways that showed so many question marks in Figure 1.5 (Bird et al, 2011). With that being said, however, there is much yet to learn. In order to understand the organism and optimize the conditions for operation in a microbial electrochemical cell, as a research community we must explore the overall utilization of those pathways and the proteins involved. I propose a two-sided approach: first, explore those pathways under different environmental conditions from an electrochemical perspective, and second, employ advanced genetic techniques, including those commonly known as the "-omics" approaches, to explain them.

6.2.1.1 Pathway Utilization with Environmental Condition

As established, electrochemical methods provide a useful introduction to investigating the utilization of electrochemical pathways in ARB. In the Swette Center for Environmental Biotechnology at Arizona State University, we are utilizing the techniques applied in this dissertation to investigate the electron transport pathways of ARB biofilms. The current research direction with *G. sulfurreducens* PCA is the investigation of the two dominant electron transport pathways under varying environmental conditions. For example, I am working with an undergraduate student, Denton Holzer, from Barrett, the Honors College at Arizona State University, to explore the effects of pH, bicarbonate buffer concentration, and acetate concentration on the relative utilization of electron transport pathways. We have preliminary results investigating the cyclic voltammograms (CVs) and pathways P1 and P2 described in Chapter 3 as a function of pH, buffer, and acetate concentration. I present a preliminary figure depicting the change in CVs as a function of bicarbonate buffer concentration in Figure 6.1.

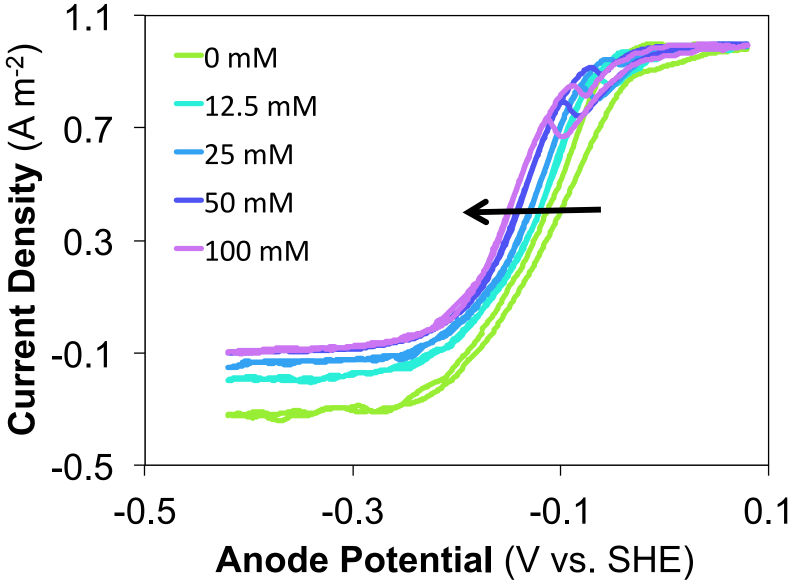


Figure 6.1. Normalized CVs performed at a scan rate of 5 mV s^{-1} for a series of five concentrations of bicarbonate buffer from 0 to 100 mM. The resulting pH were found to be: 6.7, 7.3, 7.5, 7.7, 7.9, for each concentration respectively while increasing from 0 to 100 mM bicarbonate buffer. Black arrow indicates shift observed in the entire catalytic curve as a result of increasing bicarbonate concentrations.

Figure 6.1 demonstrates a shift in the entire catalytic curve as a function of increasing bicarbonate buffer concentration as it is increased from 0 to 100 mM. As a result of the changes in buffer in the system during the experiment, the pH increases from 6.7 to 7.9. Beyond the shift to more negative potentials as a function of increasing bicarbonate concentrations, other changes in features can be observed. For example, there is a feature manifested in the positive region of the curve that appears to increase in its noticeability as the buffer concentration increases. As with the rest of the curve, it also seems to shift in the negative direction as the experiment progresses. This feature is reminiscent of that demonstrated by *Glk. ferrihydriticus* in Chapter 4 (see Figure 4.3), however, that was manifested closer to the midpoint potential and decreased with biofilm maturity. In the case of Figure 6.1, the biofilm was fully mature throughout the duration of the experiment. Additional electrochemical techniques, especially the exploration of the first derivative of the CV, likely will provide additional useful information on the features shown and electron transport pathways of *G. sulfurreducens*.

I anticipate a substantial increase in the understanding of the utilization of two dominant electron transport pathways as a function of various environmental conditions. Currently we are assessing the data generated from experiments with pH and buffer while employing methods similar to those described in Chapter 5. We are exploring the effect of acetate concentration as well, however, the experimental procedure has not yet produced nonturnover conditions within the time frame anticipated by the experimental procedure. In Figure 6.2, an example from the media replacement process for the acetate concentration experiment can be seen.

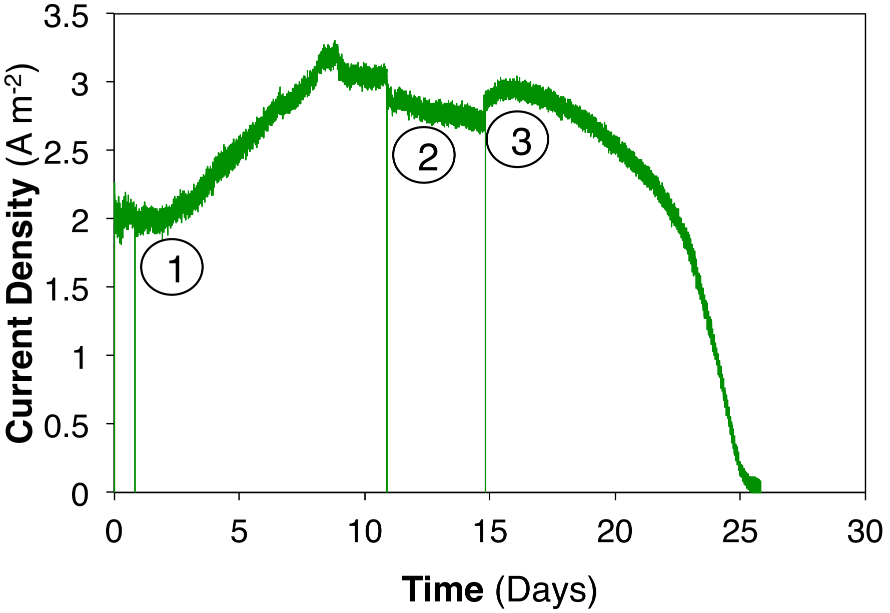


Figure 6.2. Chronoamperometry after replacement with acetate-deficient medium. Figure indicates (1) sampling for chemical oxygen demand (COD), (2) beginning to continuously bubble the reactor with 80:20 N_2/CO_2, and (3) halting the flow of N_2/CO_2.

Following the media replacement, the current density remained at a relatively stable, positive current density for more than two weeks. This result, although unexpected, appears to be repeatable with the biofilm in this reactor geometry. From Figure 6.2, it appears that there may be a supply of electron donor to the biofilm beyond the acetate provided. Several hypotheses were made in this instance, for example hydrogen cycling, SMP/EPS (soluble microbial products and extracellular polymeric substances produced by the biofilm and found in the extracellular matrix) utilization as stored energy, and an incomplete media replacement. The hydrogen cycling hypothesis was investigated by reinstating the flow of 80:20 N_2/CO_2 through the electrochemical cell. The time period shown between the numbers 2 and 3 in Figure 6.2 emphasizes the continuous bubbling of the system. No significant changes were observed in the current density output of the biofilm based on the continuous bubbling of N_2/CO_2.

The SMP/EPS hypothesis presented a greater challenge for investigating as compared to the hydrogen cycling. As a preliminary test, the COD (chemical oxygen demand) of the media was investigated and found to be approximately 155 mg L^{-1} for the media in the reactor and approximately 304 mg L^{-1} for the acetate-free media. At the time of this experiment, the current density was stable at approximately 2 A m^{-2}. This test was performed at the marker with a number 1 in the figure. More investigation into the role of SMP/EPS in the small anode biofilms (3.14 mm^2 anode surface) in these electrochemistry reactors is needed.

As the occurrence of incomplete non-turnover conditions with the media replacement method occurred with more than one biofilm, the hypothesis of lack of complete media replacement was investigated. For another biofilm investigated, approximately 500 mL of acetate-deficient media was passed through the electrochemical cell (internal volume approximately 100 mL). This biofilm performed similarly to the one shown in Figure 6.2 in that it held a sustained nonzero current density for many days. Based on these observations, it is unlikely that the media replacement method is causing electron donor to remain in the solution and have the biofilm continue operating in turnover conditions. Further experimentation is necessary with this method to determine the optimal method for investigating the electron transport pathways of *G. sulfurreducens* PCA with respect to electron donor (acetate) concentration.

Based on the experiments outlined in this section, I think that the most effective route to understanding the utilization of the electron transport pathways in *G. sulfurreducens* is investigating environmental conditions. Likely, these pathways were developed to allow the organism a measure of versatility in extracellular electron acceptor in its natural environment. The genetic knockout work done by Levar et al (2014) and Zacharoff et al (2016) are invaluable contributions to the knowledge in this area. Levar et al (2014) revealed the role of the inner membrane cytochrome, ImcH, likely in the lower potential pathway I describe electrochemically in Chapter 3. Similarly, Zacharoff et al (2016) revealed the necessity of CbcL, another key component of a low potential pathway. Necessary future work to continue this line of investigation is the quantification of the contributions of the relevant pathways. Beyond a fundamental understanding, increased knowledge of these pathways may lead to more effective selections in the design of microbial electrochemical cells for applications in biotechnology-related fields.

6.2.1.2 The Need for "-omics"

The primary limitation with the electrochemical analysis approach is the lack of identification of the key participants, likely cytochromes, in those pathways. With a transcriptomics analysis, a study of the genetic transcripts, biofilms grown at a potential selective to a single pathway can be used to study the specific genes transcribed with respect to the environmental conditions.

The primary limitation to the transcriptomics analysis at this point is the geometry of the electrochemical cell. It is necessary to immobilize the biofilm rapidly to halt and prevent the continued utilization of pathways, as this can skew the results of the target conditions in a transcriptomics analysis. The geometry shown in Figures 1.2 and 1.3 will not be easily translated to these experiments, as the modified working electrode through the side does not facilitate rapid removal of the biofilm.

Supporting information to the transcriptomics analysis, provided by proteomics, can be used to improve our collective understanding. The proteomics analysis, a study of the proteins, will provide greater detail on the participating proteins (beyond simply the genes transcribed), especially during comparisons with differing environmental conditions. More detailed transcriptomic and proteomic approaches based on the location within the biofilm or the conditions to which the cells are exposed, for example, may provide information relevant to improving operating conditions and the understanding of electron transport pathways in ARB. As with most of the studies on electron transport pathways in ARB, goals of this research would be to generate fundamental knowledge that is also useful for applications in microbial electrochemical systems.

6.2.2 Identification of Pathway Components for *Glk. ferrihydriticus*

Similar to the studies introduced by Levar et al (2014) and Zacharoff et al (2016) for *G. sulfurreducens*, genetic studies are necessary for identifying participatory proteins in the electron transport pathways for *Glk. ferrihydriticus*. Unfortunately, this organism presents unknown additional complications with the existence of at least four dominant electron transport pathways. Genetic studies become increasingly more complex with this organism. The ability to perform selective transcriptomics and proteomics analyses as described previously for *G. sulfurreducens* is limited as well. Additionally, the results observed for *Glk. ferrihydriticus* may complicate the ability of selective knockout studies to elucidate the participants in each of these pathways. The four pathways described in Chapter 4 are manifested at close potentials and, as such, it would be difficult to grow or poise the biofilm at a potential to select for the utilization of a single pathway. Likely, specialized approaches and studies with multiple knockouts will need to be applied for this complex alkaliphilic organism. However, these efforts show great promise in increasing our collective understanding of electron transport in ARB outside of the neutral environmental conditions. These fundamental learning gains may have applications in the design of industrial waste treatment streams. The development of specialized microbial electrochemical cells supporting *Glk. ferrihydriticus* as a dominant community member may result from additional electrochemical and genetic studies.

6.2.3 Caution with Data Interpretation

Here, I feel compelled to provide a note of caution. I believe these techniques are most useful when applied on ARB in pure cultures. I have seen a number of researchers recently attempt to utilize similar electrochemical techniques and interpretations on mixed cultures and point out the existence of "multiple pathways." Although citing my work in Chapter 3 as supporting evidence is flattering, I believe the proposition is inherently flawed. In my opinion, the majority of the value from the experimental work in Chapter 3 is the electrochemical techniques being applied to a pure culture. It is this culture that would be able to selectively utilize an electron transport pathway depending on the environmental condition (i.e., poised anode potential). A mixed culture would have an unknown distribution of community members and possibly multiple ARB respiring the electrode in concert. The “multiple pathways” observed in the electrochemical techniques may be as simple as the increased or decreased respiration of particular organisms depending on the anode potential or other experimental condition. More complex explanations involve the added discussion of gradients of substrate and products, competing species, and diversion of electrons to alternative products (not the electrode) that can occur in the mixed culture environment. In this discussion, I do not wish to state that these techniques are completely without value for mixed cultures. Their utility lies in the understanding biofilm adaptation to anode potentials and the utilization of certain unknown respiratory pathways. The general indication of respiratory pathways (i.e., high potential or low potential) can influence the selection of experimental parameters for biofilm production and function. Electrochemical techniques are useful for characterization of these mixed culture systems, however the results must be interpreted with caution in order to maintain a reliable physical interpretation of the system investigated.

CHAPTER 7

BACKGROUND AND INTRODUCTION TO SCIENCE EDUCATION RESEARCH

7.1 The Educational System and Reform

Education throughout human history has been defined by a lecturer and apprentice model based on initial assumptions or memorization, beginning with classical educators such as Confucius and Socrates (Henson, 2003). The lack of change for several thousand years can be attributed to the idea that the teacher is the source of knowledge the student lacks and can acquire (Elton, 2001). Even today, the educational disciplines are compartmentalized into fields such as social studies, science, mathematics, and language arts; these tend to facilitate pathways towards the development of specializations. Especially in the sciences, individual fields and courses offered provide this version of compartmentalized and mentored education. Introduced comparatively recently in the 17th century is the idea of learning by doing in experiential education (Henson, 2003). Advanced study in most fields involves expertise of a small body of knowledge, generally within a single discipline. This expertise is developed as a combination of coursework and often highly mentored research. At the university level, a number of casual links in causation can be drawn between good research and good teaching (Elton, 2001), although primary research as scholarship is unique to this educational level.

7.1.1 Active Learning as Educational Reform

Within higher education, a revolution is occurring now in the educational model. Beginning slowly from the early calls in the 1980s (Bonwell and Eison, 1991; Chickering and Gamson, 1987; Michael, 2006), changes are seen now in the movement away from the long-standing lecturer and apprentice system. For example, active learning strategies abandon the single speaker and chalkboard approach and allow students to participate in the educational process (Elton, 2001). Active learning is often intuitively defined (Bonwell and Eison, 1991) as opposed to a single working strategy. These approaches center on activities in reading, writing, discussion, and problem solving in order to facilitate higher order thinking (Bonwell and Eison, 1991) and promote conceptual understanding [often defined with the ability to transfer skills (Holme et al, 2015)]. In contrast to the historical learner-centered educational model that has roots in both philosophy and psychology (Henson 2003), the role of educators is now changing from being a lecturer to a facilitator. This represents a paradigm shift from delivering to facilitating knowledge development.

National calls have been made for educational reform in the K-12 environment, especially those outlined in the *Next Generation Science Standards* (National Research Council, 2012). Reform movements have increased in popularity since the early calls, for example *Science Teaching Reconsidered* (Moore et al, 1997). Decreasing rote memorization and increasing emphasis on promoting "deep understanding" are prominent in the K-12 (Singer et al, 2010) and university learning environments. Many of the messages in the structured K-12 curriculum are similar to those at the more loosely defined standards for the undergraduate level. At the undergraduate level in the Biological Sciences for example, the *Vision and Change in Undergraduate Biology* (American Association for the Advancement of Science, 2013) movement and *A New Biology for the 21st Century* (National Research Council, 2009) have continued the messages for promoting higher order thinking and adapted them to the discipline.

It is the "higher order" thinking cited in the early calls for educational reform that was designed to help the United States remain globally competitive in academic achievement (Gardner et al, 1983). In all, active learning techniques counter the positivist approach of direct transfer of knowledge and “uncritical absorption” (as defined by O’Loughlin, 1992) in the educational system. Active learning strategies encompass many engaging classroom techniques including activities in flipped classrooms (video lectures at home with in-class active learning activities), collaborative projects (Smith et al, 2005; Zoller, 1993), debates, inquiry-based or problem-based learning (Healey, 2007; Smith et al, 2005), and hands-on projects. Teaching techniques commonly include many activities such as think-pair-share, clicker questions, journal writing, reflective activities, and participation in authentic scientific research.

Active learning techniques are designed to develop critical thinking skills, which include the “mental processes, strategies, and representations people use to solve problems, make decisions and learn new concepts” (Zoller, 1993). Perhaps the largest question of recent years in educational reform movements is whether active learning is effective for educational gains. In a meta-analysis, a comparison of active learning and traditional lecturing reveals overall increases in student performance and decreased failure rates while using active learning (Freeman et al, 2014) and it is viewed as widely effective (Michael, 2006).

As the calls for educational reform continue, especially for the increased integration of active learning in the classroom, the resulting research has been shown to produce positive overall gains in the classroom (Freeman et al, 2014). The extension of the first question presents new ideas: for whom is active learning effective? Studies have indicated that these strategies are useful particularly for decreasing achievement gaps and promoting interaction with course material (i.e., completing assigned reading or increased time studying) (Eddy and Hogan, 2014). Increasing the time spent on course material, for example textbook reading, may promote additional learning gains through interactions with multiple facets of information delivery systems.

7.1.2 Sustainability Education

The sciences are compartmentalized in two similar manners: by teaching methods (content delivery) and disciplinary lines. Alongside the compartmentalization in the teaching and learning of science, difficulties are found in particular for the translation of sustainability education for students. This type of education breaks the pattern of teaching to “know” to teaching to “think” in interdisciplinary and transdisciplinary education (Zoller, 2015). The broadly defined aspects of sustainability education draw upon interdisciplinary (between the disciplines) and transdisciplinary (across the disciplines) content knowledge and techniques to create approaches for improving technologies and quality of life, while reducing negative environmental impacts.

This reversal in educational methodology reflects in the sociocultural approach of contextualized learning, based on the individual experiences and perspectives of the student (O’Loughlin, 1992). The educational goals then become the development of responsible citizens, often through activities developing higher order cognitive skills during the educational process (Zoller, 2015), again exemplifying the ideals of active learning. The development of these higher order cognitive skills is essential for decision-making and other critical reasoning scenarios (Crowe et al, 2008; Zoller, 1993). In place of simply the passage of knowledge, the goals of science education are now often a working skill set (Crowe et al, 2008; Zoller, 1993), especially useful when combined with reasoning abilities for science issues in everyday life. As Bunten and Dawson (2014) stated, we “all are, or will become, citizens who will need throughout their life to be able to reach judgments on claims purporting to be based on science.”

As global citizens, collectively we are responsible for addressing many science-related issues. As defined by Zoller (1993), critical thinking is “rational, logical, and consequential *evaluative thinking* in terms of what to accept (or reject) and what to believe in, followed by a decision [what to do (or not to do) about it], followed by an accordingly responsible action.” However, there is a distinction and many variations in the meanings of “knowledge” and “belief” (Southerland et al, 2001) and as such, I include Zoller’s (1993) definition with the caveat that “belief” should be indicative of thoughts and motivations for action that is firmly grounded in established knowledge and data. Presenting scientific concepts as a question of “belief” provides the opportunity for students to interpret a scientific theory as “susceptible to faith” (Bunten and Dawson, 2014).

As such, an understanding of the nature of science, including how science works, is particularly important as citizens interact with science issues in the policy, legal, and funding spheres (McComas et al, 2002). This creates a major component of overall science literacy and the general public’s grasp of science, especially when placed in the form of broad goals (DeBoer 2000). The nature of science is an essential concept in science education because “in order to understand scientific issues, you have to have a clear picture of what science is and what scientists do” (Ben-Ari 2005, page ix). Along the lines of the dramatic shifts in educational standards towards the “spirit of science” (Singer et al, 2010), it is the conceptual understanding of science that requires piecing together knowledge into a greater functional ability. The complex issues in sustainability education, including energy technologies and climate change, exemplify the depth and breadth of content knowledge and the nature of science required for later decision-making. Active learning, especially within the context of developing higher order cognitive skills, resonates strongly in sustainability education while developing a skill set from which informed choices can be made.

7.1.3 Course Content and Materials

Textbooks are a long-standing symbol in the classroom as an educational resource. They have been the fundamental delivery method for information in higher education since the printing press entered into widespread use (McFall, 2005). The poor understanding of science in the general population can be attributed to the way in which science teaching and textbooks emphasize facts and memorization over knowledge development (McComas et al, 2002), among other possible reasons. Teachers and textbooks define some of the "traditional" classroom methods, while alternative methods are a direct reaction to this widespread reliance and may follow a constructivist format (Taşdelen and Köseoğlu, 2008). Along the lines of the active learning context, the constructivist format places more responsibility on the student as the learner with the teacher as a supporter in the educational process (Taşdelen and Köseoğlu, 2008).

Although remaining a fundamental educational tool, the role of the textbook may be decreasing (McFall, 2005) and subject to increased variability in use during the transition to an active learning classroom. As mentioned previously in Section 7.1.2 however, active learning strategies may influence and promote the amount of time students spend interacting with course materials (Eddy and Hogan, 2014). These materials would include the textbook, if offered as an accompanying resource to the course.

The role of the textbook in the science classroom is largely at the discretion of the instructor at the university level. Curricula for science courses have no common guidelines, especially with respect to specific topics and concepts (Lederman, 1992), at the university level, although commonalities and trends are observed easily. As opposed to the rigorously structured K-12 curriculum, textbooks exemplify perhaps one aspect of individual-level academic freedom. Within the K-12 environment, the representation of the nature of science in a textbook is a complex negotiation between the author, editor, and publisher (DiGiuseppe, 2014). By extension, most topics in textbooks at any level would still have a measure of cooperative agreement between the stakeholders in its development.

A wide variety of textbooks with many foci can be found in introductory-level science courses, providing flexibility for faculty and departments to find options to suit their needs. These textbooks are found in printed form or online “ebooks,” or a combination of the two. Although newer, ebooks are suggested to result in similar learning and usage as compared to the printed counterpart (McFall, 2005). In this work, I make no distinction between the two delivery methods of textbook material.

7.1.4 Compartmentalization of Education

The compartmentalization of instruction in the sciences provides a context for studying the presentation of contemporary, cross-disciplinary issues, especially those related to sustainability. Textbooks are considered generally to be synonymous with learning. Beyond the determination of content for lessons, textbooks also determine teaching procedures (Devetac and Vogrinc, 2014). The design of the textbook can affect how students absorb the content and adapt it to their preexisting knowledge (Feser et al, 2013). Even with the contemporary changes to the role of the printed volume (Shapiro, 2012), the importance of textbooks in the classroom environment cannot be underestimated. The interdisciplinary nature of understanding the use of energy, fossil fuels, and alternative technologies provide complications in the presentation of material in single subject textbooks. As with other aspects of sustainability education, these topics are vastly interdisciplinary and require the development of higher order decision-making skills. This can be a challenge at the introductory level or for general education credits in the sciences, as required at many universities.

7.2 Controversial Topics in Science Education

Science education addresses some of the most socially controversial topics today including genetically modified organisms (GMOs), vaccines, evolution, the Big Bang, and climate change. These controversies may "become the new normal" (Berkman and Plutzer, 2012). The non-scientific opinions may be based on cognitive predispositions of the human mind (Blancke et al, 2015) or conscious choice (i.e., religious preference) (Berkman and Plutzer, 2012). With GMOs for example, the controversy includes an understanding of how genetic material is exchanged between levels of organisms (Carolan, 2008), a basic Biology topic that appears to be poorly understood in the population.

In 1983, the National Committee on Excellence in Education described the need that "citizens must be able to reach some common understanding on complex issues, often on such notice and on the basis of conflicting or incomplete evidence" and must be assisted by education (Gardner et al, 1983). Often, alternative viewpoints countering those with scientific evidence are presented under the guise of academic freedom (Matzke, 2015). By common definition, academic freedom is the instructor's or researcher's freedom to teach or investigate with immunity from excessive repercussions. The First Amendment protects it, however a discrepancy is observed in "academic freedom" as "a non-legal term referring to the liberties claimed by professors through professional channels against administrative or political interference with research, teaching, and governance" (Byrne, 1989). The difference exists with "constitutional academic freedom" being that present in the legal spheres and may refer to the collective level (university) rather than the individual faculty member (Byrne, 1989).

The legal history of science controversy related to evolution in the United States encompasses many trials, far more than just the Scopes "monkey" trial in 1925 (Armenta and Lane, 2010). In addition to the judicial system, the legislative system has targeted many similar issues with science, particularly at the state level. To date with evolution, there are 71 bills that have been proposed in approximately 30% of the states in the U.S. (Matzke, 2015). Interestingly, accompanying the anti-evolution legislation are often general policies emphasizing "critical analysis," ultimately targeting human cloning and global warming in the classroom as well (Matzke, 2015). The lineage of bills related to evolution has been traced; with approximately 12 bills in a particular related line addressing evolution introduced, two have passed in Louisiana and Tennessee to date (Matzke, 2015). In addition to sweeping legislation, disclaimers appear in certain textbooks to indicate that items are "just a theory" as a result of particular agendas, causing confusion and degrading standards in science education (French, 2006). The selective use of the word "theory" in science textbooks indirectly influences students' interpretations of the uncertainty in the scientific process of developing knowledge (French, 2006).

7.3 Climate Change and Energy Technologies

Among an ever-growing body of scientific literature, climate change exists as a veritable reality for studies across the disciplines. Yet, the American public continues to demonstrate widespread skepticism, with 35% of Americans disagreeing with the existence of solid evidence for average global temperature increases (Motel, 2014). Worldwide, a recent study from the United Nations revealed that 70% of participants consider climate change to be a global responsibility and 79% would like their country to decrease emissions of greenhouse gases regardless of the choices made by other countries (United Nations Framework Convention on Climate Change, 2015). Through this study of 10,000 citizens in 97 countries, 97% of participants self-reported as concerned about the effects of climate change (United Nations Framework Convention on Climate Change, 2015).

The communication of climate change and, by extension, the energy technologies and strategies to mitigate its impacts, forms one of the most strongly contested issues today facing both science and society (National Research Council, 2011). Whether it is called global warming or climate change, this topic has made a seemingly irreversible jump from the scientific forum to the political world. It has become what can be called a socio-scientific issue. Although the topics are firmly rooted in science, the discussion and in this case debate, are social issues. Bornmann et al (preprint) finds that 1.2% of political policy papers cite specific scientific research papers in climate change policy. Of the papers in the 1.2% mentioned, 92% were included only one or two times through the policy study (Bornmann et al, preprint). This exemplifies both the large disconnect between science and society, and the nature of the socio-scientific issue regarding the perception of data in the political climate change discussion.

Individual experiences, such as local weather, are commonly discussed as anti-global warming evidence in both daily life and governmental proceedings, as well as studied in the scientific literature (Egan and Mullin, 2012; Schuldt and Roh, 2014). With climate change in particular, several factors may contribute to doubt in the adult population including partisan politics (Bohr, 2014; Hamilton, 2011; Hmielowski et al, 2013; Schuldt and Roh, 2014), framing in the media (Schafer and Schlichting, 2014; Schuldt and Roh, 2014), and worldview (*i.e.*, Stevenson et al, 2014). Although these may not be insurmountable factors, they present many difficulties in communication. Additionally, the communication of climate change issues and literacy education have been shown to be ineffective in the adult populations, but have more promise among adolescents (Stevenson et al, 2014).

In this dissertation, I focus on the fundamental education of individuals during the period of worldview development at the university level. Trends continue to show that our post-industrial society has been shifting towards higher demand for skilled, university-level educated employees (Otani, 2015), resulting in higher enrollments and an ever-increasing percentage of the population in some form of postsecondary education. With more students enrolling in university courses, the percentage of individuals with exposure to introductory-level science education likely will increase. The “new norm” of a college education is creating an opportunity in science education to study the exposure of both the general education and science students to topics at the introductory levels. As stated by Bunten and Dawson (2014), “climate change is inter-disciplinary, requiring an understanding of: physics – especially thermodynamics; chemistry – particularly the carbon cycle; biology – the carbon cycle and the impacts of climate change on the biosphere; and of course geology.”

The teaching of controversial issues permeates the educational levels. School-age students have been shown to have a low conceptual understanding of energy topics, including resources acquisition, generation, consumption, conservation, and impacts of energy development and technology implementations on society and the environment (Bodzin et al, 2012). Although critically important, energy concepts are considered “one of the most difficult topics of secondary school science” (Stylianidou et al, 2002).

Conceptual understanding, only recently defined in the literature, has underpinnings in the depth of knowledge and abilities to transfer, predict, translate, and solve problems (Holme et al, 2015).

As a general concept, energy has been established for use as a tool for multidisciplinary analysis (Jin and Anderson, 2012). This overall understanding of energy is essential for grasping many aspects related to energy technologies (as the application of energy) and climate change (resulting from the application of selected technologies). As a form of analysis, a critical preparation area for the future workforce includes a subset of knowledge outside of a primary discipline and the ability to work with interdisciplinary collaborators to address contemporary challenges (Feser et al, 2013). The concepts related to energy and its application to nearly all aspects of life exemplify this interdisciplinary understanding. Alongside perhaps geography (Healey, 2007), climate change and energy technologies are one of the few vastly interdisciplinary topics that can be investigated within an educational context.

Various aspects of the complex interactions of energy technologies and education have been investigated including opinions, knowledge, attitudes (Holden and Barrow, 1984; Saschewski et al, 2014), and concept inventories/literacy surveys (DeWaters and Powers, 2008). With respect to worldview, the representation of science by textbooks is of the utmost importance because it shapes how individuals view the discipline as a result of readings as a student (Souque, 1987) and are a primary way to introduce the public to science (Shapiro, 2012).

7.4 Dissertation Objectives and Outline

In this volume of my dissertation, I utilize climate change and energy technologies as indicators of interdisciplinary learning in the sciences. As such, in

Chapter 8, I display the presentation of these topics across undergraduate introductory-level textbooks in Biology, Chemistry, and Physics. These three subjects were chosen based on their prominence at the undergraduate level and the interdisciplinary nature of climate change and energy technologies. This survey includes a page-by-page analysis of over 15,000 textbook pages. These pages come from 16 U.S. textbooks obtained through partnerships I developed with four major publishing companies for the purposes of this research. Several trends in overall presentation and by discipline are discussed.

Building upon the analysis for climate change and energy technologies, Chapter 9 discusses the role of nuclear energy in the introductory level university textbook. The content from this analysis comes from the same textbooks as the previous study, but the topic forms a unique outlier in the climate change and energy technology discussion. Nuclear energy is an "alternative" to fossil fuels, but not a "renewable" technology, as were the primary foci of Chapter 8.

The technical analyses of Chapters 8 and 9 are followed by Chapter 10, a theoretical perspective describing a possible linkage to strengthen the teaching methodology for issues of socio-scientific controversy in the classroom. The chapter links the teaching of climate change to past educational gains in evolution utilizing citizen science as an educational tool. This tool is chosen to exemplify the ability of science to include participants at all levels in the research process, particularly non-scientists. Finally, Chapter 11 summarizes the results of the science education studies and suggests future directions in research. This chapter utilizes preliminary results from additional studies including structured interviews in the university and K-12 educational environment to place the learning gains into context within the scope of socio-scientific issues in science education.

CHAPTER 8

ESTABLISHING A BASELINE PRESENTATION OF ENERGY TECHNOLOGIES AND CLIMATE CHANGE IN THE UNDERGRADUATE EDUCATIONAL SYSTEM THROUGH TEXTBOOK ANALYSIS

This chapter is being prepared for publication in collaboration with Bina Vanmali.

8.1 Introduction

8.1.1 Overview

As seen in the scientific literature and mass media, global climate change and the implementation of renewable energy technologies form one of the most pressing issues facing society today, with related content spanning the science disciplines. Through an analysis of introductory-level university textbooks from four major publishers in Biology, Chemistry, and Physics, I present trends in terminology use and content presentation. “Global warming” and “climate change” are used nearly equally across the disciplines as the overarching term to describe the situation. Related content is presented on average on less than 4% of pages with high amounts of variability between individual textbooks. The location of the content follows a general pattern of minimal presentation through the book as divided into thirds (i.e., less than 10 pages per section) for Biology and Physics, with Chemistry showing an increase on average in the final third. Presentation of these interdisciplinary topics has implications on public perception of science and informed citizenship.

8.1.2 Background

As a major driving force, climate change and energy technologies realize extremes from a major global threat to a commonplace topic of discussion in everyday life. In the wake of the global climate agreement from COP21, the meeting of the United Nations Conference on Climate Change, an increasing number of statements from religious leaders (McNutt, 2014; Forum on Religion and Ecology at Yale, 2015) and presence in the media, these topics have become a staple in the media and political discussion. Climate change exists as a bona fide reality for studies across the disciplines (Oreskes, 2004; Maibach et al, 2014; Leiserowitz et al, 2014). Yet, the American public continues to demonstrate skepticism, with approximately half of the population expressing some level of concern (Yale Project on Climate Change Communication, 2014) and 35% of Americans disagreeing with there being solid evidence for average global temperature increases (Motel, 2014). Whether the terminology used is “global warming” or “climate change,” this topic has made a seemingly irreversible jump from scientific discourse to politicized debate. Additionally, confusion between climate and weather are present in government (Bump, 2015) and studied in scientific literature (Schuldt et al, 2011; Egan and Mullin, 2012; Schuldt and Roh, 2014).

Energy technologies and climate change together form one of the most strongly polarized issues in science and society (National Research Council, 2011). In higher education for example, sustainable energy sources form one of the four main challenges presented in the driving force redefining life sciences education, *A New Biology for the 21st Century* (National Research Council, 2009). However, no single discipline can tackle this alone (Feser et al, 2013). According to the U.S. Department of State (2010), the far-reaching global impacts require changes in “individual attitudes, societal norms, and government policy.” However, the media popularization and political debates

(Boykoff, 2007) may be impeding the educational process (National Research Council, 2011). The disciplinary compartmentalization of science education provides an effective means to investigate the presentation of climate change and energy technologies to introductory-level undergraduate students. These topics define the paradigm-shifting issues facing science policy, education, and research today and serve as an indicator of interdisciplinary learning. Beyond determining lesson content, the design of a textbook can affect how students absorb the content and adapt it to their pre-existing knowledge (Feser et al, 2013). Even with the contemporary changes to the role of the printed volume, the importance remains for textbooks as resources for a readership population who will not pursue careers in science (Shapiro, 2012). To do so, I explore introductory-level undergraduate textbooks in biology, chemistry, and physics.

8.2 Materials and Methods

Introductory-level university textbooks in biology, chemistry, and physics were obtained through partnerships developed with prominent publishing companies for the purpose of this research. The publishers provided specific books, allowed selection, or provided open access to their entire collections. This choice was determined entirely by the publishing company. The publishers were assured of anonymity for their materials and the presentation of results, as the authors do not seek to promote or discourage the use of any particular textbook over another.

The textbooks utilized a page-by-page combing approach to evaluate the main text content for topics relevant to the research project. These topics included current energy technologies (especially fossil fuels) and their impacts, climate change/global warming (including impacts and projections), and renewable energy technologies (especially development, implementation, and advantages/disadvantages). I include also environmental topics commonly associated with fossil fuels and human impacts on the atmosphere such as acid rain and ozone layer depletion. These are included here as they relate to fossil fuels, energy production/consumption, or the common erroneous association of ozone depletion with global warming/climate change (Shepardson et al, 2011).

For this study, 16 current-edition textbooks with publication dates between 2013 and 2015 were used. The following editions were used for the Biology, Chemistry, and Physics textbooks: 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 10th, 12th. The average edition number was greater than 6, and textbooks with higher edition numbers were chosen preferentially. Textbooks with many editions typically have a larger distribution in use and show subsequent updates in versions. The quantity of textbooks for each discipline represents the diversity in the selection available from these partnerships. For example, the Biology books showed the greatest number of possible books and largest diversity in terms of scope and focus.

A focused rubric was developed for agreement comparison as both the first author and a research volunteer explored the content in depth. Any pages lacking agreement were re-assessed. The first author evaluated all 16 and the research volunteer assessed 6 textbooks. The reviewers found a high level of agreement, with those books reviewed by the second author increasing in page count by less than 1% of the total for each book on average. The main text was evaluated (including footnotes, in-chapter "quick" questions, and examples). The authors excluded end-of-chapter materials (reviews and assessments), figures, and figure captions (with the exception of the Keeling Curve).

Following the page-by-page analysis, the data were analyzed by several numerical assessments and conceptual coding schemes. Page counts and averages were utilized for several main points in topics distribution and main concepts and themes were determined by thematic framing. The topics were used then to compare distributions in content between the textbooks.

8.3 Results and Discussion

Utilizing a page-by-page combing approach, I evaluated seven Biology, five Chemistry, and four Physics textbooks with publication dates between 2013 and 2015 obtained through partnerships with four major publishing companies. The research topics include global warming/climate change, renewable and sustainable energy technologies, as well as current fossil fuel methods (specifically excluding nuclear technologies and energy as a non-renewable, non-fossil fuel). Here, I present aggregate results along and specific anonymous quotes or examples so as to refrain from endorsements.

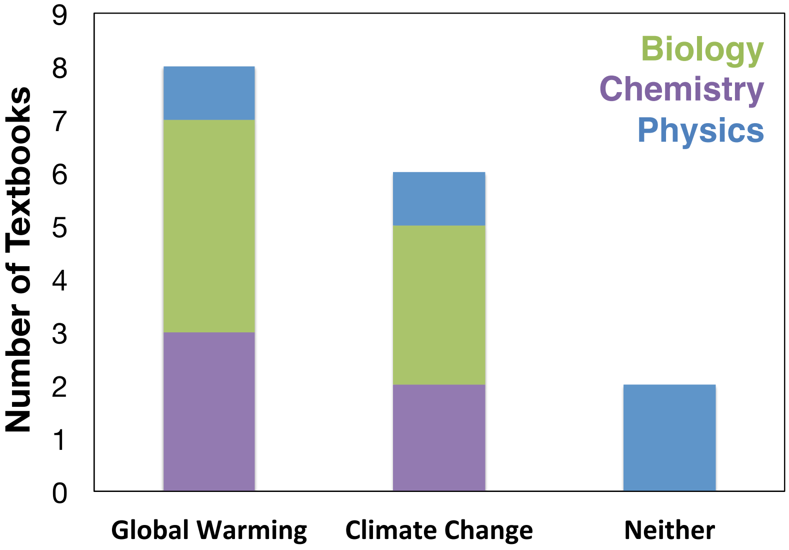


Figure 8.1. The terminology, either “global warming” or “climate change,” used for labeling the overarching issue. I assess the usage from the in-text discussion and “neither” refers to the books that described without naming.

Here, I present example supporting definitions illustrating the categorizations for the definitions of global warming and climate change:

- Biology: “Increasing concentrations of greenhouse gases have been linked to global climate change, of which one major aspect is global warming.” (emphasis removed)
- Biology: “Climate change is primarily due to an imbalance in the chemical cycling of the element carbon. The increased amount of carbon dioxide (and other gases) in the atmosphere is causing a rise in temperature called global warming.” (emphasis removed)
- Biology: “Ecologists are concerned that human activities are increasing the greenhouse effect and causing global warming (also called global climate change), a gradual elevation of the Earth’s temperature.”
- Physics: “the phenomenon is called global warming or its result – climate change”

As terminology plays a key role in the public reception of and response to this global issue (Schuldt et al, 2011; Schuldt and Roh, 2014), I assessed the 16 textbooks for the use of ‘global warming’ or ‘climate change’ in the text. In Figure 8.1, the results appear to slightly favor the use of ‘global warming.’ This split in terminology suggests a discrepancy in undergraduate science educational materials. The definitions of ‘global warming’ and ‘climate change’ vary and often include references to greenhouse gases. The terms are often linked, and when both are present, global warming is used to define the overarching term, climate change. For example, “the phenomenon is called global warming or its result – climate change” (Physics). I present additional information and exemplifying quotes in the supplemental material. I show that two books (Physics) did not provide a specific name for the phenomenon of global warming/climate change. However, these books are not excluded from the remainder of the study as they discuss energy technologies and include statements such as “geologists are concerned with…the gradual warming of ice expanses in the Arctic and Antarctic” and “the evidence is increasingly clear that humans are altering the Earth's climate and causing other global changes."

While the remainder of the study is limited to analysis of text, I assess the presentation of the Mauna Loa CO_2 chart ("Keeling Curve"). This iconic chart of atmospheric CO_2 data or a corresponding chart including extrapolated data from ice cores is often a foundation of climate change lessons. Of the textbooks investigated, 6 of 7 Biology, 4 of 5 Chemistry, and 1 of 4 Physics books presented the Mauna Loa figure or similar. In Biology, the remaining textbook instead describes the increase in atmospheric CO_2 levels in the text. Importantly, the Keeling Curve incites alarm in scientists, but to lay audiences indicates that climate change is not an urgent issue (Center for Research on Environmental Decisions, 2009). To non-scientists, the chart suggests that the CO_2 accumulation has been occurring over a long time (Center for Research on Environmental Decisions, 2009). The effect on and responses of undergraduate students, especially in introductory-level classes with a mixture of declared science and non-science majors, is unknown.

To expand on the use of terminology, I quantify the distribution of information related to the topics of global warming/climate change (henceforth for clarity I use 'climate change') and energy technologies through the textbook. The results of generalized page counts for the three disciplines and the corresponding standard deviations can be seen in Figure 8.2. No statistically significant differences can be seen between Biology, Chemistry, and Physics in terms of page counts. Across the disciplines, the averages are less than five percent of pages. By comparison, an average chapter in Biology is 2.7 ± 1.1%, in Chemistry 4.5 ± 1.1%, and in Physics 2.7 ± 1.4% of the total book (see Figure 8.2).

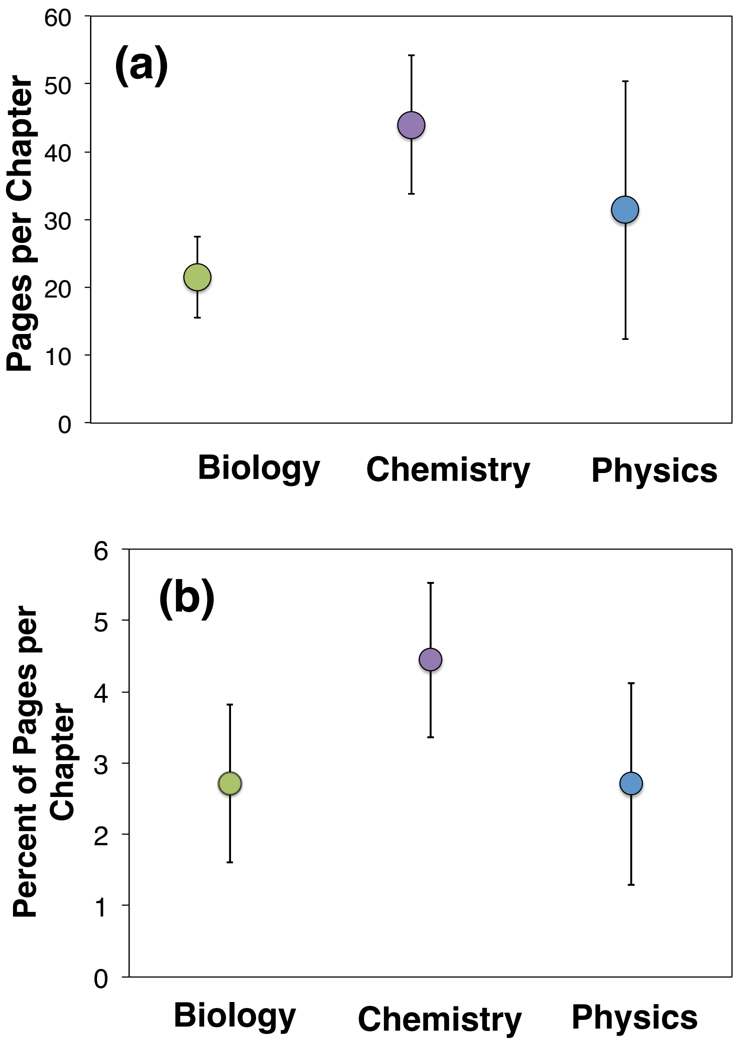


Figure 8.2. A comparison of chapter length by discipline. (**a**) Average chapter length in pages and one standard deviation displayed by discipline for the textbooks investigated. Biology and chemistry show a difference in the number of pages per chapter, while Physics chapters show the most variability. (**b**) The percentage of pages present in one chapter by discipline. The percentage of pages per chapter indicates difference in the average, but no significant difference overall based on the standard deviations. Overall, the Biology textbooks appear to be the shortest number of pages and smallest percent per chapter of the total.

With a comparison of percentage of pages by discipline, the topics related to fossil fuels, climate change, and renewable energy technologies account for approximately one chapter each of the three disciplines.

Breaking down the percentage of pages with study topics presented in Figure 8.3a into the climate change and renewable energy components, I find significant differences between biology and physics textbooks, but not between either discipline and chemistry (for more information, see Figure 8.4).

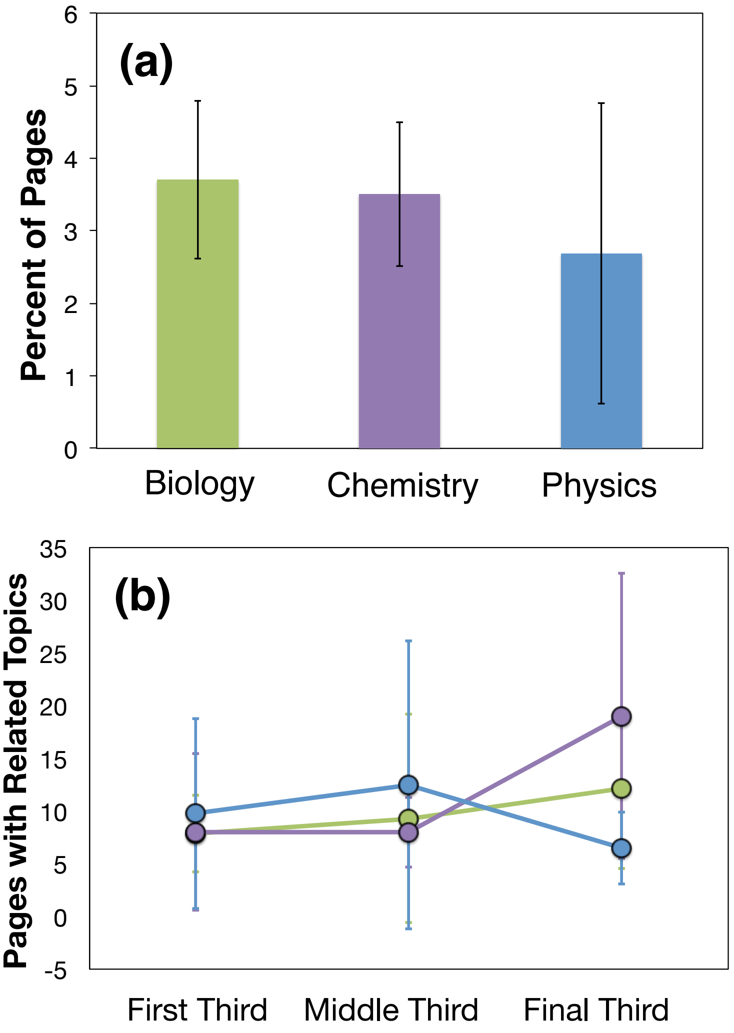


Figure 8.3. Climate change and renewable energy technologies across the disciplines. (**a**) The average percentage of pages and standard deviation. (**b**) Page counts for location in the textbook.

In addition to the number of pages, their location may influence the likelihood of encountering the page in classroom education, depending on the depth and breadth included in the introductory level course. Figure 8.3b indicates a general trend, especially for the Biology and Chemistry textbooks, showing a slight increase at the end. Often accounting for the increased content in the final third, applications chapters (including environmental implications) are added frequently to the end of the book. I speculate that this may be intended to allow students to utilize previously developed content knowledge through the preceding sections of the course (and textbook). Especially with introductory-level materials, the volumes tend to emphasize broad content knowledge, and thus, have large numbers of pages and correspondingly many chapters (see Figure 8.2) on varying topics within the discipline. With most relevant content discussion in the final third of the book, I hypothesize that these materials may not be included in assigned reading or course content. Additionally, Biology textbooks are shown to have on average the highest amount of content presented for the effects of climate change (on average less than two percent of pages), followed by a highly variable level in Chemistry, and minimal (less than one-half of one percent of pages) in Physics (see Figure 8.4).

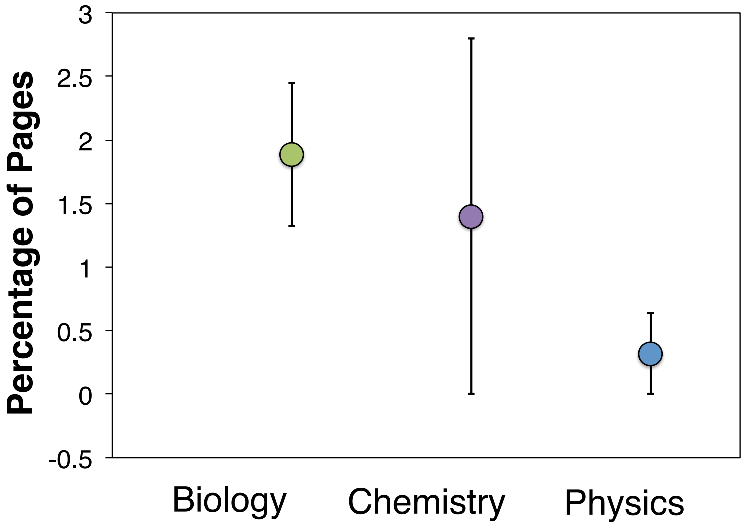


Figure 8.4. The presentation of the effects of climate change and renewable energy technologies by discipline. Normalized by textbook length to percentage of pages, I find the largest variability in the chemistry books, while biology and physics show significant differences. I note here the presentation in introductory Physics courses to be on average less than one-half of one percent of pages.

To investigate the presentation of renewable energy technologies, I include the normalized page counts (as percentages) comparing the discussion of fossil fuels and renewable technologies in Figure 8.5a. The results indicate less than one percent of pages including coverage allotted to either fossil fuels or renewable energy technologies on average across the disciplines. The high variations between textbooks create large standard deviations for these numbers. For the Biology and Chemistry textbooks, the average presentation of fossil fuels is greater than that of renewable technologies. With large error bars, the opposite is suggested in Physics.

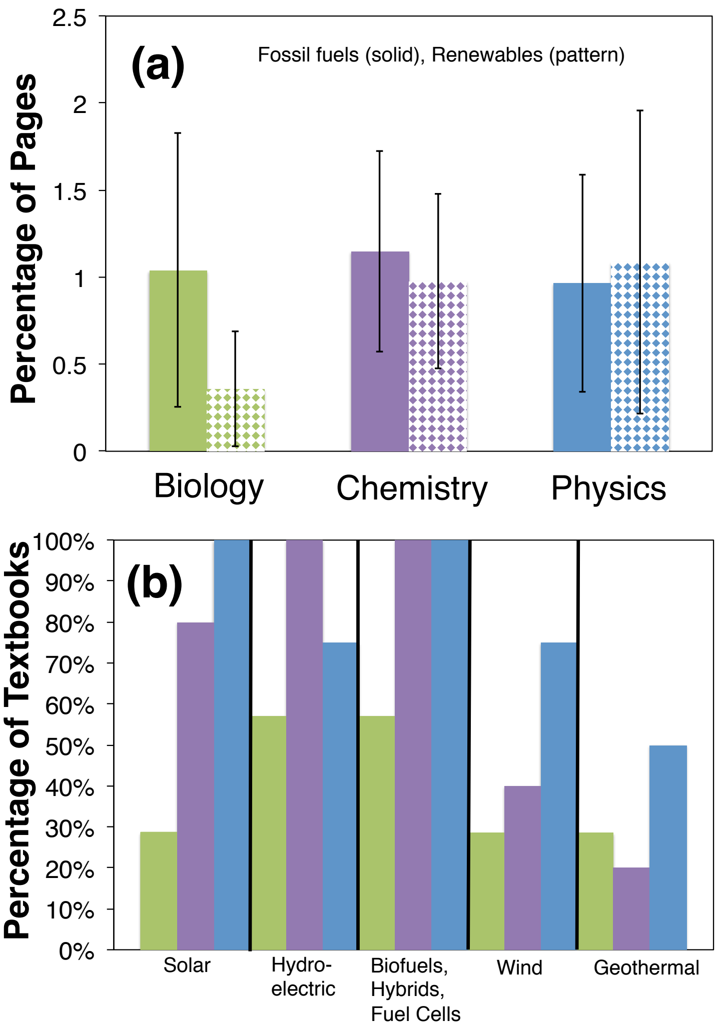


Figure 8.5. A comparison of fossil fuel and renewable energy technology presentation. (**a**) Percentage of pages. (**b**) Individual technologies included by discipline based on the presented categories. This figure does not include a section for "other" technologies.

Further defining the renewable energy technologies presented, Figure 8.5b presents the normalized quantity as a percentage of textbooks including discussion categorized into solar, hydroelectric, biofuels/hybrids/fuel cells, wind, and geothermal. I created these categories based on the most common and popularly discussed technologies and note that the figure does not include a category for "other," that while lacking specificity, could be included as well. Among the three disciplines, the data suggest the least emphasis placed on renewable energy technologies in Biology. Notably, the transportation category (biofuels/hybrids/fuel cells) shows 100% presentation in Chemistry and Physics. As may be expected by the discipline, Biology textbooks focus on the impacts of climate change as compared to the presentation of renewable technologies. However, for the renewable energy technologies presented, the Biology textbooks present the greatest emphasis on those related to biomass, including alternative transportation fuels (see Figure 8.6).

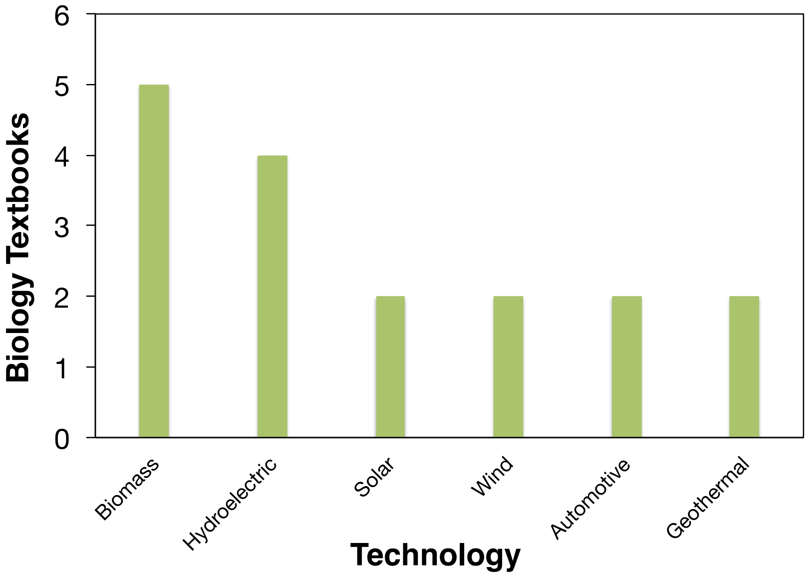


Figure 8.6. Biology textbooks demonstrate a tendency to focus on biomass and related biological resources as technologies. For example, these biomass resources were defined to include algae, reducing deforestation (and use of forests for carbon capture), ocean fertilization, photosynthesizing organisms at the output of coal-fired power plants, marshes for carbon capture, and plant biofuels. As compared to Figure 8.5b, I present a distinction between the biomass resource of plant biofuels and a category of "automotive." The automotive category includes for example, improving efficiency in transportation through hybrid vehicles. Not shown on the chart are other technologies including geoengineering approaches presented including atmospheric shading, CO_2 scrubbers (abiotic), wave energy, carbon sequestration, and cap-and-trade systems. These technologies are each *n*=1 through the analysis of biology textbooks.

Alternative fuels and other technologies related to energy use in the transportation sector are emphasized heavily in Chemistry and Physics. I present additional information supplementing the presentation of specific renewable energy technologies for the Biology, Chemistry, and Physics textbooks in Figures 8.6, 8.7, and 8.8, respectively.

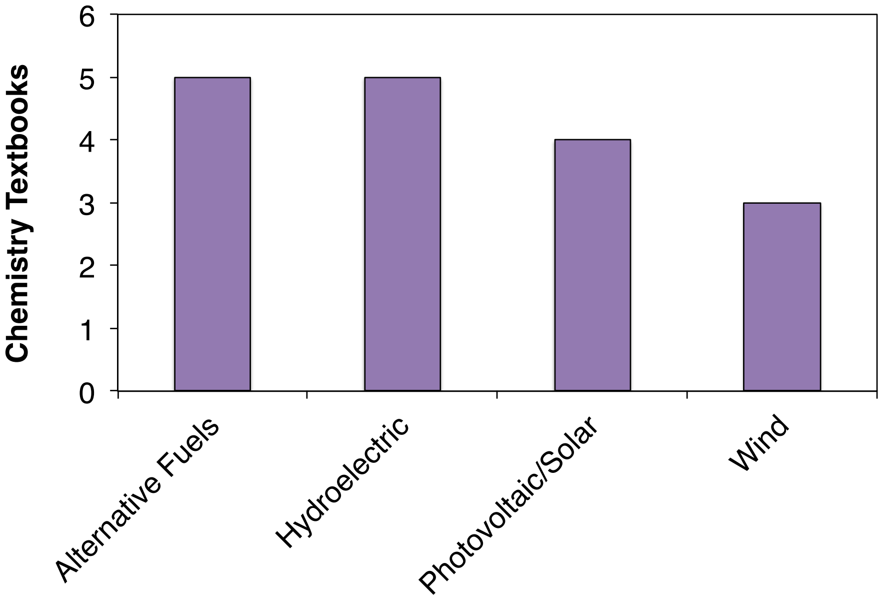


Figure 8.7. Chemistry textbooks favor several technologies including alternative fuels. As categorized here for the chemistry books, the alternative transportation fuels include biofuels, E85, methane, hybrid vehicles, and hydrogen fuel cells. This represents a distinct categorization scheme from Figure 8.6, where the focus of the technologies in the biology textbooks centered on biomass-based sources. Here I categorize the alternative fuels differently than the "automotive" in Figure 8.6 for clarity and to reflect the apparent emphases placed by the textbook authors. The other technologies listed here include geothermal, bacteria for energy, and methane hydrates (each *n*=1). Within these or related discussions, there was also a focus in the chemistry textbooks on an overall energy efficiency (*n*=3, not included on chart).

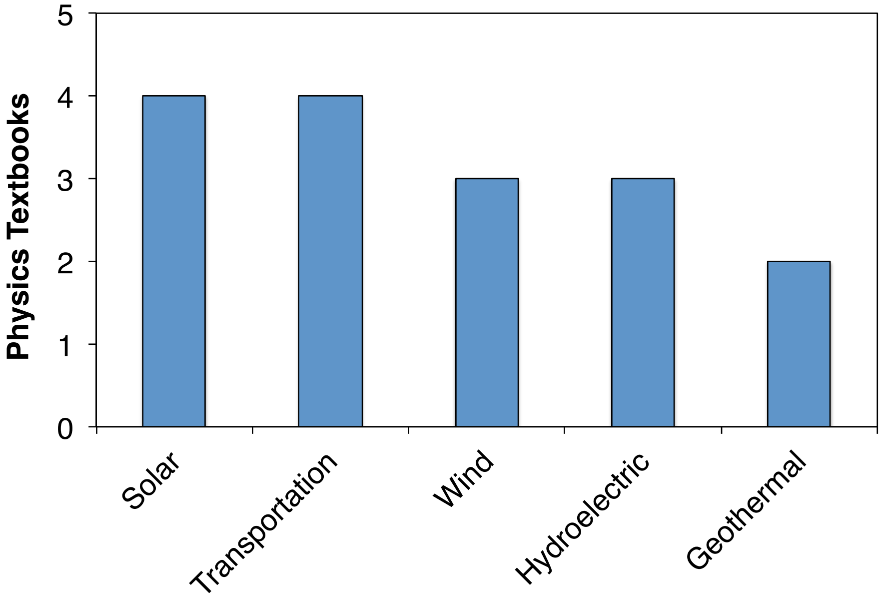


Figure 8.8. Physics textbooks focus on solar and general transportation technologies. In this case, the transportation technologies category includes alternative fuels, fuel cells, hybrid vehicles (including gasoline/electric and flywheel hybrid), lithium-ion batteries (commonly used in hybrid vehicles), supercapacitors (for transportation), and magnetic levitation technologies (for transportation). Following the pattern of the other disciplines, I present an "other" category; in this case, I find a presentation of compressed air energy storage for the n=1 listed.

In the context of current scientific research, the boundaries dividing instruction in these disciplines are called into question. With respect to the crosscutting issues of climate change and energy facing society today, I find a discrepancy in the terminology used for "global warming" or "climate change" that crosses these disciplinary lines. The minimal presentation (<5%) of related topics questions whether the educational materials used in introductory-level undergraduate science courses are providing adequate exposure to the scientific aspects of a pressing societal issue. For science majors, the introductory courses are often taken concurrently and may present a lack of agreement in the discussion (including basic terminology). For non-majors students, the textbook may accompany the course fulfilling a basic science requirement. In either case, it may be essential to build a solid foundation upon which students integrate new knowledge and make judgments on climate change and the utilization of energy technologies.

A general pattern for the location of these topics in the textbooks is observed with the least presentation in the middle section of the book. This content distribution has implications on the likelihood of assigned content including climate change and energy topics. The tendency towards presentation in the final third of the book may decrease the possibility of students encountering this material during normal activities. The foundational study from Mauna Loa for atmospheric CO_2 shows near uniform inclusion, however other supporting evidence is presented at varying levels. As anticipated, renewable energy technologies are most heavily favored for discussion in the introductory-level Chemistry and Physics textbooks, while the effects of climate change have the largest presentation in Biology.

8.4 Conclusions

Students can encounter discussion and debate on climate and energy in everyday life, particularly through the mainstream media. This study provides a general quantification of the information presented in a representative set of widely used introductory-level Biology, Chemistry, and Physics university textbooks from major U.S. publishers. The traditional disciplinary lines influence specific discussions, but the overall trends reveal a small percentage of pages allotted to the topics related to energy and climate across the disciplines. The study in this context is limited primarily to quantified presentations of topics. Greater in-depth analysis on specific content may in certain instances reveal discrepancies between disciplines or misconceptions perpetuated. Overall, this research should elicit concern as to the adequacy of the educational presentation of climate change and renewable energy technologies. Bridging the gap between the information encountered by students in daily life and in the classroom should be a priority for education as students assimilate new knowledge within their preexisting constructs.

CHAPTER 9

FOCUSING ON NUCLEAR ENERGY AS AN OUTLIER IN THE ENERGY AND CLIMATE DISCUSSION

This chapter is being prepared for publication in collaboration with Bina Vanmali.

9.1 Introduction

9.1.1 Overview

The politicization of science, and especially climate change, may have far reaching implications for STEM education in the United States. Here I analyze the presentation, including type of information and bias, of nuclear technologies, a prominent and currently utilized alternative to fossil fuels, in an effort to better understand how these resources may alter opinions and policies favored by the general public in the future. This study reveals crosscutting and discipline-specific themes from undergraduate introductory-level Biology, Chemistry, and Physics textbooks. I find a negative presentation of nuclear energy, except in Physics, where the presentation is relatively more favorable. When presented, other nuclear applications, (*i.e.* medicine) are in a favorable manner. The educational study of nuclear energy is relevant for its own social and scientific merits, but also for the probable impact of educational materials on the perception of the general public with respect to energy policy and safety.

9.1.2 Background

Nuclear energy often galvanizes the conflicting nature of science and technological issues in modern society, with discussion and controversy prevalent throughout most of the world (Jho et al., 2014). As climate change and current energy technologies demand the increasing implementation of alternatives to fossil fuels, scientists, educators, and policy makers must understand the intersection of technology and education for the general public. Education in nuclear technologies influences perceptions of important societal topics including energy and medicine, as well as citizenship participation in energy policy and nuclear terrorism issues (DOE/NSF Nuclear Science Advisory Committee, 2004). The pressures of climate change, increasing energy demands from the global population, and international trade make both national energy independence and low-carbon sources desirable. The sources for energy technology information forming the opinions of the general public create a complex social and educational issue.

While nuclear science remains "vigorous and stimulating," warning signs are cited, including decreased Ph.D. graduates, lack of diversity in the field, and widespread public misconceptions of 'nuclear' (DOE/NSF Nuclear Science Advisory Committee, 2004). Despite these findings and the importance of the subject, little evaluation of nuclear energy presentation in introductory-level and non-technical education appears in the literature. Established curricula development and evaluation methods, for example that developed by the European Nuclear Education Network (ENEN), focus on developing and maintaining expertise in nuclear technologies (Safieh et al., 2011), particularly through higher education. Similar evaluation is done in the United States by the Committee on Nuclear Engineering Education as well as other literature studies of higher education (Lartigue and Martinez, 2008; Tamalis et al., 2009; Zeisler et al., 2005). A recent recommendation argued "the United States' leadership in science and technology demands enduring attention to adequate science education – not only the education of undergraduates, graduate students, and postdoctoral fellows, but also the education of precollege students and the broader public" (DOE/NSF Nuclear Science Advisory Committee, 2004).

As the decades-long debate between coal and nuclear has lessened, environmental groups are focusing on divesting from fossil fuels (due to climate change issues) in lieu of fighting against nuclear, now a “fading issue” (Weart, 2012, p244) and transitioning to carbon-neutral renewables. Although nuclear opens up possibilities of self-reliant energy and decreased carbon emissions, undercurrents of aversion based on perceived risks have been documented in young adults (Kilinc et al., 2013). In the U.S., the popular public opinion surveying organization, the Gallup Poll, found in March 2015 that 51% of Americans favor and 43% oppose the use of nuclear energy for electricity (Gallup News Service, 2015). Similar studies in other countries have found approximately 40% accepting of nuclear power in order to tackle climate change (Bird et al., 2014), but similar or lower after the recent disaster in Japan for studies of individual countries and worldwide (Bird et al., 2014; Kim et al., 2013; Gallup Poll, 2015). Educational interventions, although showing increases in content knowledge, find difficulty in changing attitude or decisions related to nuclear energy technology (Jho et al., 2014). Researchers attribute the lack of enthusiasm for nuclear energy in teachers to be based on past tragedies and accidents, particularly in Japan (Hirose et al., 1999). This aversion often mirrors the general population and creates a barrier for communication (Hirose et al., 1999). Even entertainment media shows a shift over four decades from nuclear to biological sources as catalysts for television shows, as nuclear is “no longer scary enough” (Weart, 2012, p252).

Although, for example, studies have investigated the presentation and understanding of nuclear energy in educational materials and activities for schoolchildren (Hirose et al., 1999; Stylianidou et al., 2002; Bodzin, 2012; Jho et al., 2014), teacher education (Benzer et al., 2014), and concept associations among the general population (Greenberg and Barnes Truelove, 2011; Truelove et al., 2014), an understanding of nuclear energy in non-specialized and non-technical education at the university level is largely unexplored. I present a detailed analysis of nuclear energy technologies in introductory undergraduate biology, chemistry, and physics textbooks. I chose this set based on the idea that these courses may be the foundation in a science pathway for students or the final science course taken, for example as a general education requirement. I note that these topics are not often a focus of the textbooks and may not be a primary factor in their selection. In this case, I comment on the traditional disciplinary lines being drawn with biology assumed to present the effects of nuclear topics on life, chemistry with nuclear reactions and possible commentary bridging between the disciplines, and physics with the applications of chemistry and the engineering approaches to nuclear technologies. Any book would be assumed to have the opportunity to provide further commentary on implications and social aspects. In all, the crosscutting nuclear topics of socio-scientific debate represent important societal and environmental contexts for developing informed and productive citizens.

9.2 Materials and Methods

Introductory level science textbooks in Biology, Chemistry, and Physics were examined though a page-by-page combing approach to evaluate the content and depth of discussion allotted to nuclear technologies. I explored the content and depth of these discussions based on the context within which it was presented and the included commentary or analysis. I define sufficient information in this case to be presentation of factual information or discussion that in quantities that may suggest or influence the opinion of a reader and minimal discussion to be content on less than two pages of the total. For the purposes of this study, I evaluated seven Biology, five Chemistry, and four Physics textbooks. These textbooks were obtained through the generosity of four major publishing companies. The results are presented in aggregate form with data and quotes as specific illustrative examples. Publishing information about the textbooks is not presented so as to not endorse or discourage the use some books over others. All textbooks have publication dates of 2013 – 2015. The following editions were used: Biology (3rd, 4th, 4th, 4th, 5th, 8th, 10th), Chemistry (3rd, 6th, 7th, 12th, 12th), Physics (2nd, 3rd, 10th, 12th). The average edition number for the textbooks included in this study was greater than 6. Textbooks with many editions typically are more frequently used, updated, and demonstrate a larger distribution in classrooms. From these criteria, I found the least selection in the university Physics textbooks and limited the study to four volumes. In contrast, the biological sciences displayed a great diversity in the scope and focus of the books, thus more were chosen to be representative of this collection.

The textbooks were combed in a page-by-page method by the first author and a research volunteer. The first author evaluated all 16 textbooks used and the second reader evaluated 6. A focused rubric was used for overall comparison between the two readers and detailed notes were taken during the page-by-page analysis. The main text, including footnotes, in-chapter questions, and examples, are considered to be part of the analysis. Excluded sections are end-of-chapter reviews, end-of-chapter questions, figures, and figure captions. Following the page-by-page analysis of the texts, main concepts and themes were determined and aligning statements were compartmentalized into themes for analysis. These topics were used then to compare the distributions and discussion between individual books and as representatives of the three science disciplines. The simplified overall ratings of positive or negative presentation were determined from a detailed analysis of the statements made throughout the textbook and supported by individual evidence for the categories investigated. These ratings serve as a basic reference by which to introduce the topic. Many additional categorizations provide contextual information and quotes are provided as supporting evidence throughout. I note that several topics discussed in the textbooks applied to thematic discussions in more than one category. For example, nuclear waste storage or disasters would count also in the controversial nature section, based on the presentation. For example, a description of long-term storage may include debate surrounding stable geological storage locations.

9.3 Results and Discussion

9.3.1 Overall Attitude Presented

Of the textbooks evaluated (*n*=16), I found a negative attitude presented for existing nuclear energy technologies in approximately half, often citing nuclear power plant disasters and long-term waste storage. In this work, I define factual evidence of disasters and technological challenges as indicative of a negative bias in the rhetoric. Additional description of the assessment techniques is presented in the Methods section and further information outlining the topics classifications of statements can be found in the Appendix (Section 9.5). An additional 30% did not discuss or provide sufficient information to evaluate an overall perspective. In contrast, three Chemistry and one Physics textbooks, having an overall negative approach to nuclear energy, present the possibility of fusion energy technologies in an optimistic manner, citing benefits including an abundant supply of fuel and little waste generation. Addressing the possibility of nuclear dangers, if the fusion reactors "were turned off, it would shut down completely and instantly, without any danger of a meltdown" (Chemistry).

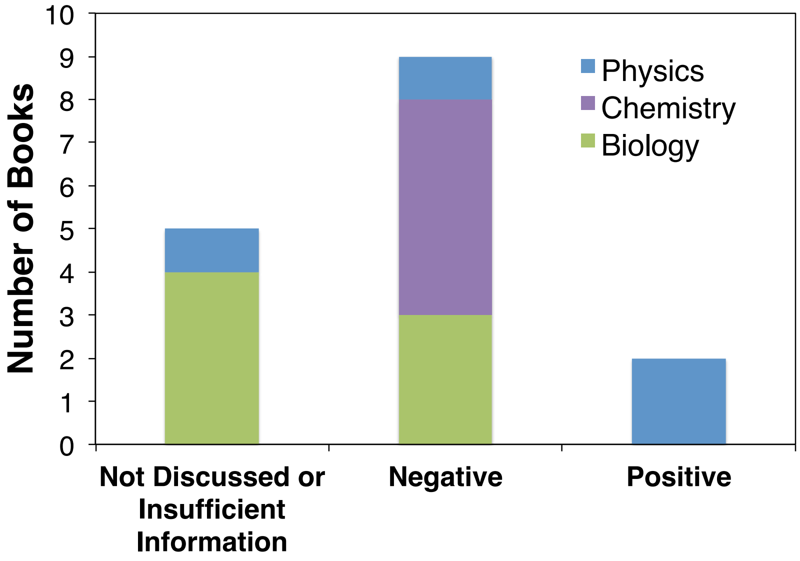


Figure 9.1. The overall presentation of current nuclear energy technologies across the introductory university Biology (*n*=7), Chemistry (*n*=5), and Physics (*n*=4) textbooks investigated.

In addition to a fusion future, two Physics books present the current nuclear energy infrastructure (fission-based technologies) with a positive attitude. Supporting evidence includes the idea that "when safety concerns are met and problems of storing radioactive wastes are solved, watch for renewed interest in this form of power that doesn't pollute the atmosphere" as comparisons are drawn with the emissions from coal-fired power plants. I categorize this quote as positive for fission technologies based on the author's diction downplaying the current safety concerns and storage issues.

Major benefits of nuclear technologies are less carbon emissions and greater energy efficiency as compared to fossil fuels. In these books, the lack of pollution is emphasized by the lack of fossil fuel-based air pollutants (in particular, sulfur oxides). Other benefits cited include greater per-unit-mass energy production and waste containment. However, a comparison is drawn with the use of other technologies as "there is a lot of coal in Earth's crust, and water is easily backed up behind a dam"

(Physics). This statement, for example, indicates a negative view of nuclear based on the comparison with the mass of coal or ease of use for hydroelectric power.

9.3.2 Relative Presentation

The relative emphasis of nuclear technologies is presented quantitatively based on the frequency of appearance in Figure 9.1. These books, Biology (n=7), Chemistry (n=5), and Physics (n=4) represent introductory-level university textbooks from major publishers with wide distribution.

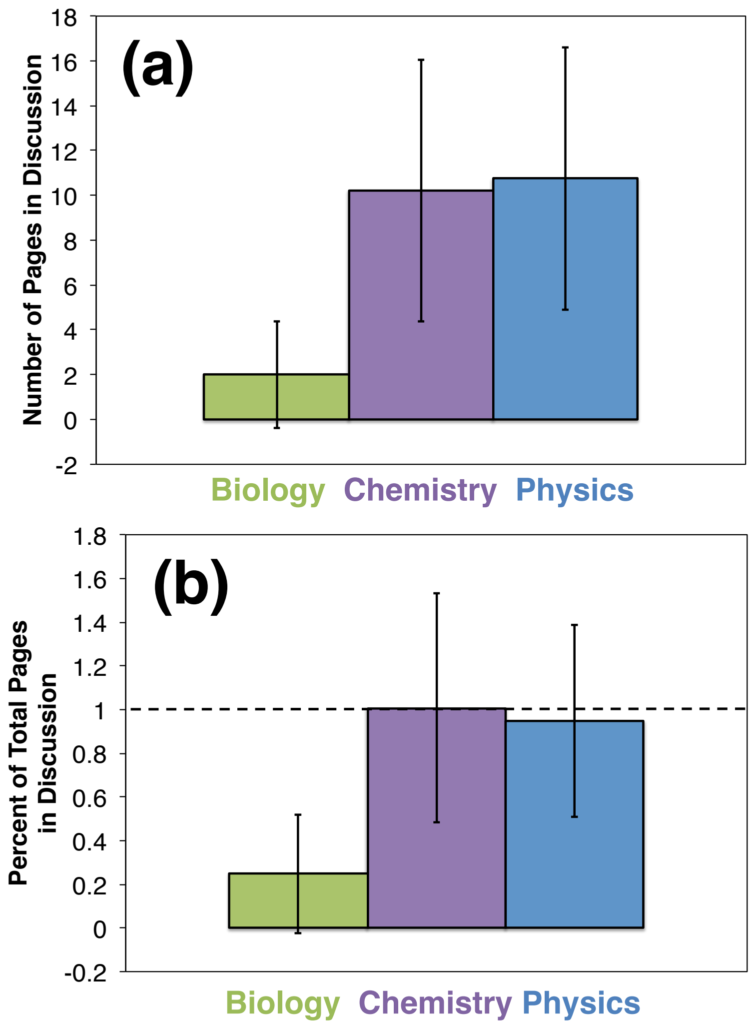


Figure 9.2. **(a)** Average number of pages including discussion of nuclear energy or technologies in textbook and **(b)** nuclear content normalized to the total main text pages in the textbook, including a dashed line to indicate 1%. Note: These include topics related to nuclear areas such as energy generation, disasters, weapons, and medical applications.

Nuclear technologies appear on average on less than 1% of pages. The results of Figure 9.2a are normalized to the total number of pages in the main text of the book (Figure 9.2b). Biology texts included in this study appeared on average approximately 100 pages and 300 pages less than Chemistry and Physics, respectively, and Figure 9.2b suggests a significantly more limited presentation.

Of the textbooks included in this study, half either lack or include a minimal discussion of nuclear technologies (content on less than two pages of the total). These include three Biology (lacking) and minimal discussions in three additional Biology, one Chemistry, and one Physics textbook. For those with minimal content, the discussion varies widely between the three disciplines. The Biology books (n=3) focus on the interactions of radiation and living organisms, often including health harm. Similarly, the Physics book presents the average radiation to which a person would be exposed to per year and a comparison of efficiencies of types of energy generating plants. In contrast, the Chemistry book with minimal content introduces the fission process, half-lives of by-products, long-term storage, and fusion as a future power source. I observe here content fitting along the traditional disciplinary lines, but the amount of content is defined as minimal in my classification system. Although this list of topics appears to be extensive, these may be as little as a single sentence in the book.

9.3.3. Topic Distribution

The location of appearance in the textbook plays a large role in the possibility of the information appearing in the content of a particular course (Tsaparlis et al., 2013). This importance may increase for minimally presented topics. Commonly, materials covered in textbooks, especially at the introductory level, focus heavily on the beginning and middle chapters, while rarely reaching the end. Figure 9.3 illustrates the average distribution as textbook pages are grouped into thirds.

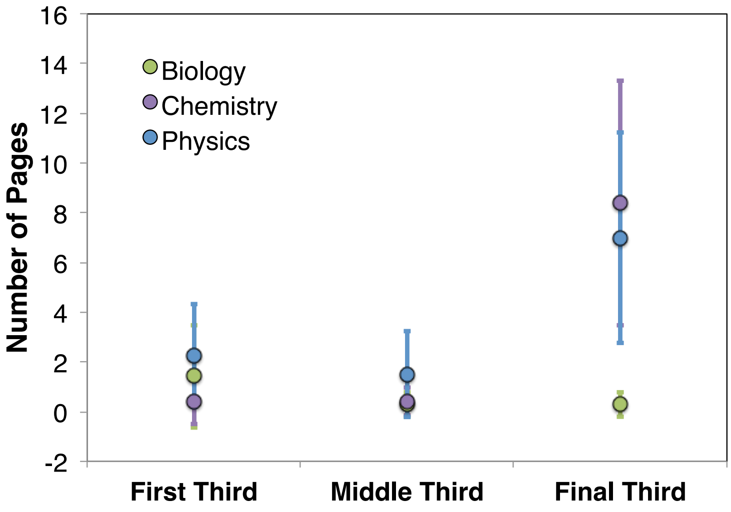


Figure 9.3. Average distribution and one standard deviation for presentation of nuclear-related discussion per third of the textbooks.

Thematic content presentation provides more detailed representation of the apparent aims or motivations of authors, as seen per book in Table 9.1. The main themes reflect key topics emphasized across the disciplines.

Table 9.1. Distribution of content and themes related to nuclear technologies across representative textbooks of introductory-level university Biology, Chemistry, and Physics textbooks. Those with content are indicated by gray shaded boxes. The main themes include (1) nuclear disasters, (2) requirements for waste disposal and long half-lives of nuclear by-products, (3) applications and effects on living organisms, especially humans, (4) the controversial nature and debate surrounding nuclear energy, especially from a societal perspective, (5) comparisons between nuclear energy and fossil fuels (often energy per mass), and (6) fusion research and as a future possibility in energy.

Textbook	Disasters	Waste Disposal and Half-Lives	Biological Implications	Controversial Nature	Comparisons to Fossil Fuels	Fusion
Biology 1						
Biology 2	▨		▨			
Biology 3	▨	▨	▨	▨	▨	
Biology 4						
Biology 5	▨	▨	▨			
Biology 6						
Biology 7	▨		▨	▨		
Chemistry 1		▨				▨
Chemistry 2	▨	▨	▨	▨	▨	▨
Chemistry 3	▨	▨	▨			▨
Chemistry 4	▨	▨	▨		▨	
Chemistry 5	▨	▨	▨	▨	▨	▨
Physics 1	▨	▨		▨	▨	▨
Physics 2		▨			▨	▨
Physics 3			▨		▨	
Physics 4	▨	▨		▨	▨	▨

The main themes identified are most prevalent in the Chemistry and Physics, and one Biology book. Although half of the books have minimal discussion, significant content is covered, indicating efficiency and brevity. These 16 textbooks provide insights into the presentation of nuclear technologies in undergraduate introductory science education. Here, I discuss prevalent themes, in order of frequency of appearance.

9.3.3.1 Disasters

The most common argument against the utilization of nuclear energy technologies is a major disaster. A total of 10 (of 16) books present the possibility of nuclear disaster with 8 naming specific nuclear incidents, as seen in Table 9.2.

Table 9.2. A comparison of the distribution of each of three major disasters in the textbooks in which nuclear plants are discussed. The categories include the nuclear disasters at (1) Fukushima Daiichi, Japan, March 11, 2011, (2) Chernobyl, Ukraine, April 26, 1986, and (3) Three Mile Island, Pennsylvania, United States, March 28, 1979. Content presented is indicated by gray shading.

Textbook	Fukushima Daiichi	Chernobyl	Three Mile Island
Biology 3	▨		
Biology 5	▨	▨	
Chemistry 2	▨	▨	
Chemistry 3		▨	
Chemistry 4	▨	▨	
Chemistry 5	▨	▨	▨
Physics 1	▨	▨	
Physics 4	▨	▨	

Beyond that presented in Table 9.2, one additional Biology textbook briefly discusses the use of bioremediation techniques decontaminating after a nuclear disaster in Japan, although the nuclear plant is not named. These disasters most often are represented in a cause and effect scenario with safety failures triggering environmental and health impacts. This has implications on the importance of disaster severity and historical content in contemporary science education. For example, parallels are drawn with the recent Fukushima Daiichi disaster: "the long-term harmful effects of the radiation leakage to the environment are not yet fully assessed, but it is believed to be comparable to that at Chernobyl" (Chemistry). In the United States, the nuclear incident at Three Mile Island occurred over 30 years prior to the publication of these textbooks, and is cited as the incident that "first brought the potential hazards of nuclear plants to public attention" (Chemistry). I speculate that the de-emphasis of the nuclear incident at Three

Mile Island may be based on its comparative severity as a disaster, less recognition by student readers based on the number of years that have passed, or both. I note that for "traditional" undergraduate students today (*i.e.*, ages 18-22), both the Three Mile Island and Chernobyl disasters would have occurred before they were born.

9.3.3.2 Waste Disposal and Half-Lives

The overwhelming consensus among these American textbooks for nuclear fission includes the problems posed by the half-lives of by-products and/or long-term waste storage (*n*=10 of 16). Multiple solutions, including perspectives for long-term storage locations and methods, are presented. The most commonly considered storage location is stable geologic formation (*i.e.,* Yucca Mountain, NV (US Secretary of Energy, 2002)), vitrification (encasement in glass), or similar methods. However, one textbook presents "the ideal disposal site" as the sun, but that requires "100 percent reliability in space technology" (Chemistry). One textbook names Yucca Mountain, NV, a long-debated geological storage location. As news stories often influence engagement in the sciences, especially for adults and young adults (Polman and Hope, 2014). Here, I note a possible disconnect between educational materials and the media because of the lack of precise examples that may be encountered in the media being cited in the textbooks.

9.3.3.3 Biological Implications

Biological implications include effects on individuals and life. For example, "the release of radioactive particles following a nuclear power plant accident can have far-reaching and long-lasting effects on human health" (Biology). However, perspectives besides those linking nuclear plant disasters and cancer are less common, although "nuclear fuel, whether used to generate power or to produce bombs, has been linked to cancer" (Biology). Each of the Biology books focuses on the detrimental impacts of nuclear radiation on living organisms. In contrast, the beneficial uses of nuclear technologies on living organisms (*i.e.,* imaging) are included in one Biology, one Physics and two Chemistry textbooks. This implies a negative bias in the Biology books and an avoidance of the life science in the Physics books. In contrast, the Chemistry books more heavily favor interdisciplinary discussions.

9.3.3.4 Controversial Nature

Here, I define controversial nature as the explicit presentation of the complex interactions between science and society. Several textbooks focus on presenting the "complex political, environmental, and economic debate about the deployment of nuclear chemistry" (Chemistry), especially so the students can "participate in some of the most important policy discussions of our time in an informed manner" (Physics). This common theme allows students to compare the relative risks and emissions of nuclear and fossil fuel generating stations (Biology). Other topics include safety and storage concerns of radioactive wastes (addressed previously).

The theme of nuclear controversy is heterogeneous. For example, "it seems likely that the controversy will continue for some time" (Chemistry) or "when safety concerns and problems of storing radioactive wastes are solved, watch for renewed interest" (Physics). The approach towards the controversy suggests an alignment with the overall attitude.

9.3.3.5 Comparisons to Fossil Fuels

Half of the textbooks (n=8) present a comparison with fossil fuels. The "safety of nuclear power" is presented, for example, as weighing against the "certainty of continued carbon dioxide emission and the unknown health hazards of global climate change resulting from fossil fuel use" (Biology). The most common comparisons include the differences in carbon emissions or climate change implications (*n*=1 Biology, *n*=2 Chemistry, *n*=2 Physics) or mass of fuel per energy output (*n*=2 Chemistry, *n*=2 Physics). The favorable approach for the mass per unit energy includes "a large quantity of energy that can be obtained from a small mass of fuel and the absence of chemical pollution of the kind associated with fossil fuels" (Chemistry) and "kilogram for kilogram, you get several million times more energy from uranium than you do from coal or from falling water" (Physics). Although infrequently discussed, similarities include negative environmental impacts (thermal pollution) and comparative efficiencies of energy generation (each *n*=1).

9.3.3.6 Fusion

Present in only the Chemistry and Physics textbooks (*n*=7 of 9), the dominant themes surrounding fusion are the benefits and technical challenges. Presented as fueling other energy sources through solar fusion (to photosynthesis, oil) (Physics) and mentioned without support in another (Chemistry), the remainder of the discussions include the benefits and technical challenges of fusion. Among these statements, a fusion future proposes "energy from nuclear fusion is thought by many to be the most likely means of satisfying the long-term energy needs of modern industrialized society" (Physics). Additionally, three textbooks (1 Chemistry, 2 Physics) provide contrasts between fusion-based bombs (thermonuclear) and the "sustained and controlled release of energy" in power plants (Physics). Notably lacking from the books (n=14 of 16) were examples of actual facilities or experimentation in the fusion arena. Specific named facilities include ITER, the proposed international fusion facility, and the National

Ignition Facility at the Lawrence Livermore National Laboratory, found in two Physics textbooks (one mentioning ITER). This presentation suggests that discussions in the future of nuclear energy technologies are limited to concepts and ideas and lack tangible structures or examples across the disciplines.

9.3.3.7 Possible Misconceptions

Although peripheral to the scope of this research study, this comparison introduces the possibility of future studies for content and interpretations that may lead to misconceptions or misinterpretations. Based on the methodological assessment of the authors or example, I provide two preliminary examples. Two books (both Chemistry) present the use of nuclear technologies for generation in the United States, one stating 10% of energy and another 20% of electricity. In contrast, a Biology book discussed 87% of energy for humanity coming from fossil fuels. With this statement, the book implied a comparison with nuclear included in the remaining 13% percent of worldwide energy production. For reference, the U.S. Energy Information Administration reports 19% of electricity production in 2014 from nuclear (U.S. Energy Information Administration, 2015). Of total energy consumption, however, approximately 8.5% from nuclear is reported for 2014 (U.S. Energy Information Administration Beta, 2015). In this case, further assessments and commentary may be necessary for accurate discussion, especially when comparing the disciplines.

A second example, from a Physics book, shows that “when safety concerns are met and problems of storing radioactive wastes are solved, watch for renewed interest in this form of power that doesn’t pollute the atmosphere.” Similarly, a Chemistry book indicates that “although fission reactors do not generate chemical pollution, they do produce highly hazardous radioactive waste.” This commentary presents a possible distinction in how I define pollution and waste in education. According to these examples, agreement must be considered for whether radioactive waste is considered chemical waste and how that may differ from the chemical waste produced by fossil fuel power plants. The method of presentation, whether factual or based on opinion, is of great importance for potentially influencing student readers. These examples may have important implications on the interpretations of students and the ways in which I discuss energy in the educational setting.

9.4 Conclusions

The presentation of controversial and politically important material may require special attention on the part of authors and educators. In the textbooks selected from those prominent in introductory-level Biology, Chemistry, and Physics, I find minimal emphasis placed on nuclear energy and technologies often with strong negative biases. The international public opinion of nuclear energy has been decreasing in recent years, particularly since the Fukushima disaster (Kim et al., 2013), creating a possible reflection of the general opinion in the ideas expressed by the textbook authors. At the time of the disaster, those in he nuclear industry were expecting a rebirth in the technology (Miller et al., 2013). Re-starting nuclear reactors in Japan and nuclear power increases in India have spurred widespread public protest (Miller et al., 2013). Significantly, I observe only minimal discussion allotted to nuclear technologies, with content on average on less than 1% of textbook pages across the disciplines. From this study, I find that the specific statement-based presentation of nuclear energy technologies in introductory-level undergraduate Biology, Chemistry, and Physics education varies by book. I observe thematic trends presented, including: disasters, waste disposal, biological implications, comparisons to fossil fuels, the nuclear controversy, and a fusion future. Presentation by discipline provides unique perspectives on these messages. For example, I observe a disciplinary crossover in the presentation of the biological implications of nuclear technologies, with medical applications discussed in Chemistry, not Biology books.

This study raises several questions. First, as scientists and educators, I must consider the scope of the different disciplines, especially with respect to key socio-scientific issues crossing these lines. In this case, I utilize nuclear as a representative topic crossing disciplinary boundaries. The advantages and disadvantages to compartmentalized presentation versus overlapping content must be considered, especially as science research leans heavily on interdisciplinary collaboration. From an educational perspective, science communication, starting in the educational process, may be key for developing productive and informed citizens on important socio-scientific issues, such as nuclear technologies and energy generation.

9.5 Appendix

Supporting information for generalizing the choices of discussion related to nuclear technologies included in introductory-level Biology, Chemistry, and Physics textbooks. As a source of reference information on nuclear technologies, I provide a generalized list of what I consider to be common topics presented in introductory-level Biology, Chemistry, and Physics textbooks. These results are for general reference only and provided in no particular order.

- Current technologies – fission, bombs, medical imaging, medical cancer treatment, spacecraft power
- Future technology - fusion
- Nuclear disasters – Three Mile Island, Chernobyl, Fukushima Daiichi
- Comparisons with emissions from fossil fuel or renewable energy sources – energy per unit mass comparisons
- Biological effects – cellular and DNA damage, cancer
- Storage of radioactive wastes, Yucca Mountain, Nevada
- Implications on policy discussions
- Half-lives of radioactive materials, radioactive wastes
- Percentage of energy supply in U.S.
- Type of fission reactors and component parts, history of reactor development
- Mining and supply of raw materials – uranium, deuterium
- Thermal pollution of waterways
- Costs of nuclear power
- Public perception, controversial nature of nuclear, placement in global energy demands

Supporting information for defining the scientific and social biases presented in the textbook. I present the data for each textbook (in no particular order) indicating the classifications of the data for the positive, negative, and neutral classifications of information presented. This information supports the data in Figure 9.1 and related discussion. As shown in Figure 9.1, four Biology textbooks and one Physics textbook did not discuss nuclear technologies or present sufficient information to determine a particular bias. Topics presented and specific quotes are utilized to illustrate. Grouped topics into one box represent the continuation of ideas in one discussion (i.e., one paragraph or page).

Table 9.3. The classification of presented information for Biology textbook "#3." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
"…unless energy sources such as nuclear fusion become a reality" (suggesting need for renewable energy)	Extended study of the Fukushima Daiichi nuclear disaster	
	Presents comparison with "the certainty of continued carbon dioxide emission" and climate change from fossil fuels; suggests renewable energy sources as solution	

Table 9.4. The classification of presented information for Biology textbook "#5." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Medical uses of radiation (localized cancer treatments)	Radiation causing cell changes, DNA damage, causing cancer	
	Widespread impacts of nuclear plant disasters on human health; example of Fukushima Daiichi disaster	
	Separate discussion of disadvantages of nuclear energy, example of Chernobyl disaster, radioactive waste storage, nuclear as a nonrenewable energy source	

Table 9.5. The classification of presented information for Biology textbook "#7." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Provides energy, no greenhouse gas emissions	Dangerous waste products, reactor meltdowns, example of nuclear disaster in Japan in 2011	A political, not scientific decision. "To take a position, you should know about global warming, radioactive waste, and the costs and benefits of other technologies for making electricity – all scientific issues."
	Linking nuclear fuel to cancer; workers at risk (mining or processing, nuclear plant accidents, bomb testing, bomb use); most people will never be exposed to a nuclear disaster or bomb drop	

Table 9.6. The classification of presented information for Chemistry textbook "#1." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
*Nuclear fusion: less radioactive wastes, shorter half-lives	Problems with nuclear power – produces radioactive wastes with long half-lives; need to store safely to not contaminate the environment Provides example of Yucca Mountain, Nevada	Fusion as an experimental technology
		Percentage of U.S. energy from nuclear

Table 9.7. The classification of the presented information for Chemistry textbook "#2." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Cancer treatment; medical imaging; archaeological dating; investigate chemical reaction mechanisms	Military applications for many nations	Nuclear energy includes "political, environmental, economic debate"
Nuclear energy useful for remote locations like spacecraft	Radioactive decay; cites Chernobyl and Fukushima Daiichi disasters; Use of iodine supplements post-disaster; "several cases of thyroid cancer… exposure from the Chernobyl accident"; Plutonium-239 long half-life; very long-term storage needed for plutonium waste; "land contaminated with plutonium cannot be inhabited again for thousands of years without expensive remediation efforts"	Description of types of reactors (examples of fission, fusion, weapons, breeder reactors) and component parts (moderators/control rods)
Large amounts of energy released, "absence of chemical pollution of the kind associated with fossil fuels"	"nuclear energy presents us with great technical challenges and hazards"	Chemistry (as a discipline) is useful for many aspects of nuclear power technologies
*Example of fusion reactor, mostly free of long-lived waste products, seawater extraction of fuel	Fission reactions "do not generate chemical pollution, they do produce highly hazardous radioactive waste."	
Comparison of nuclear energy generation with Hoover Dam (hydroelectric power)	Strip mining of uranium, enrichment; looking for alternative enrichment procedures	
*Discussion of workings of fusion reactor, research approaches, plasma reactor		

Table 9.8. The classification of the presented information for Chemistry textbook "#3." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Energy per unit mass example (lots of energy)	Radiation exposure toxic	Nuclear chemistry has applications in "theoretical, medical, analytical, environmental, safety, and energy solutions"
Applications for energy on space probes	Example of radiation exposure by Chernobyl workers	Fission plants, description of plant
Separate energy per unit mass example	Possibilities of "bomb-like catastrophes" at nuclear fission plants; example from March 11, 2011	Description of workings of fission plant
	Production of radioactive wastes (gas, liquids, and solids); rapid decay for gases, some dangerous; examples of products and cancers caused	
	Long-lived radioactive wastes; "dozens of centuries;" storage procedures, conversion to solid materials, burial in location "stable on the geological time scale;" "scientific and political debate" about locations for storage	
	*Difficulties of utilizing fusion for energy applications; difficult science and engineering issues	

Table 9.9. The classification of presented information for Chemistry textbook "#4." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Mass per unit energy (comparison with gasoline)	Chernobyl and Fukushima Daiichi power plant disasters	Nuclear energy, critical mass discussion, Manhattan Project
Mass per unit energy (comparison with coal)	Description of Fukushima Daiichi disaster; long-term waste storage	Fission to generate electricity
	Effects of radiation on life: radiation damage, increased cancer risk, genetic effects	
	Radiation damage: increased cancer risk (mentioned again), genetic defects (mentioned again), measuring radiation exposure	

Table 9.10. The classification of presented information for Chemistry textbook "#5." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Heavy water reactor using deuterium moderator, doesn't require uranium enrichment	Thermal pollution of waterways, wildlife impacts	Description of nuclear bombs
Production of deuterium by hydroelectric power for heavy water reactors (avoids weapons technology nuclear reactors)	Nuclear as controversial because of safety concerns and waste disposal	Nuclear 20% of U.S. energy; description of fission process; Reactor types: light water reactors; Uranium enrichment
*Description of fusion possibilities	Comparison of cooling system with fossil fuel plants; thermal pollution (another discussion)	Differences between nuclear bombs and nuclear energy generation
	Decreased efficiency of heavy water reactor with neutron leakage	Heavy water reactors; no enrichment; deuterium moderator
	High costs for breeder reactors	Description of natural fission processes
	"Many people, including environmentalists, regard nuclear fission as a highly undesirable method of energy production." Long half-lives of by-products; accidents, Three Mile Island example	Addresses methods other than nuclear power to address greenhouse gas emissions
	Description of Three Mile Island accident; Description of the Chernobyl accident, cancer, deaths; Fukushima, Japan accident description	
	Disposal of radioactive wastes; disposal sites	
	Description of change in public perception of nuclear energy over time	
	Biological effects of radiation	
	Controversial nature of nuclear; nuclear more likely to have accidents than fossil fuel plants	

Table 9.11. The classification of presented information for Physics textbook "#1." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Positive.

Positive	**Negative**	**Neutral**
Nuclear as the most concentrated energy source; doesn't pollute atmosphere	Fukushima Daiichi disaster	Fusion in the sun
"when safety concerns are met and problems of storing radioactive wastes are solved" "power that doesn't pollute the atmosphere"	Waste storage; nuclear weapons; low-level releases; risk of large releases	
Geothermal as a by-product of nuclear		
Comparison of wastes generated and released by fossil fuel and nuclear plants		
Discussion encouraging readers to understand nuclear power		
Abundant energy; saving fossil fuels; no atmospheric emissions		
Comparison of radiation surrounding nuclear and fossil fuel plants		
*Possibilities of fusion technologies in the future		

Table 9.12. The classification of presented information for Physics textbook "#2." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Negative.

Positive	**Negative**	**Neutral**
Efficiency comparisons with fossil fuel plants	Thermal pollution from nuclear plants	Description of fusion process
Energy per unit mass comparison with comparison with fossil fuels	Ease of obtaining energy from other sources	Comparison of schematics for nuclear plants, operating information
*Arguments from supporters of fusion energy	Accumulation of spent rods and nuclear waste over time in on-site storage	Processes occurring in a nuclear reactor; moderators
*Fusion possibilities		History of reactor development

Table 9.13. The classification of presented information for Physics textbook "#4." An asterisk (*) is used to denote discussion in the positive and negative categories omitted from consideration (especially fusion). Overall review: Positive.

Positive	**Negative**	**Neutral**
*Energy from nuclear fusion possibilities, long-term solution	Runaway reactions; Chernobyl accident; Fukushima Daiichi accident; release of radioactive materials	Uranium enrichment for nuclear power
Avoiding greenhouse gas emissions from fossil fuels	Storage of radioactive wastes is "not completely solved"	Similarities between nuclear and fossil fuel plants
Abundance of thorium as a resources; abundance and availability; current efforts in thorium	Countries moving away from fission and towards renewable energy sources	
*Approaches and research directions in fusion technologies	*Large amounts of money put into fusion with little gains, continued "elusive goal"	
Combined heat and power from power plants including "perhaps" nuclear		
"The key to reactor construction" in controlling the nuclear chain reaction and avoiding runaway reactions with moderators (capture neutrons and prevent further fission)		
Energy per unit mass comparison with fossil fuel		
Increasing global energy demands – time will reveal whether alternatives to fission can meet needs		

CHAPTER 10

CONTROVERSY IN BIOLOGY CLASSROOMS – CITIZEN SCIENCE APPROACHES TO EVOLUTION AND APPLICATIONS TO CLIMATE CHANGE DISCUSSIONS

This chapter, in collaboration with Binaben Vanmali, has been accepted for publication in the *Journal of Microbiology and Biology Education* for a special issue on citizen science to be released March 2016. It is presented here in a modified format. Citation information (known): Yoho RA, Vanmali BH. 2016. Controversy in biology classrooms – citizen science approaches to evolution and applications to climate change discussions. 17:107-111. DOI: http://dx.doi.org/10.1128.jmbe.v17il.1026.

10.1 Introduction

The Biological Sciences include topics that are viewed as controversial and even antithetical to the beliefs of some members of the public. Thus, research into new teaching methods, strategies, and tools for controversial topics could strengthen the educational experience as well as offer an assessment of teaching outcomes with respect to both material mastery and general attitudes towards science. When presenting science to general audiences, the primary goals often are to develop a fundamental appreciation of science and the scientific process in addition to specific content knowledge (Lederman et al, 1998; Lederman, 2006; Lederman and Lederman, 2014). This appreciation of science can be developed through engaging students of all ages – from elementary to elderly – in discussion that is explicit and reflective and through hands-on experiences, i.e. research (Abell et al, 2001; Lederman and Lederman, 2004).

Among the topics perceived as controversial in the Biological Sciences, perhaps the most commonly discussed is evolution (Alters and Nelson, 2002;

Labov, 2012; Working Group on Teaching Evolution, 2008). In recent decades, the educational setbacks and gains from teaching evolution in the United States provide a needed frame of reference and offer insights into instructional methods (Griffith and Brem, 2004) for other “hot” topics such as climate change, GMOs, and cloning, among others. Teaching climate change content in a biology classroom is complicated due to many factors as well, some of which are present also when teaching evolution. Biology instructors and researchers regularly encounter the interconnected nature of the environment and organisms; contemporary discussions of climate change therefore should be enriched by fundamental concepts such as evolution and extinction.

Citizen science, the practice of non-scientists collecting data for investigations in an organized manner that yield data to test hypotheses, represents a key method to link science education and environmental education (Backstrand, 2003; Bonney et al, 2009; Bonney et al, 2014; Cohn, 2008; Dickinson et al, 2012; Silvertown, 2009; Wals et al, 2014). Additionally it may create a foundation for enhancing the engagement of citizens of all ages with the sciences by allowing citizens to participate actively rather than passively in science. The expansion of inquiry-based education for encouraging curiosity also shows the rise of publishable research based on well-crafted testable hypotheses, with students participating in the faculty member’s research project (Jarvis, 2013). Citizen science projects in evolution have shown success in allowing ordinary citizens to help test important hypotheses, a notable example being the large-scale Evolution MegaLab project, which spanned 15 countries and involved over 6,000 participants (Worthington et al, 2012). Other notable citizen science examples include eBird and Zooniverse (Bonney et al, 2009; eBird, 2015; Sullivan et al, 2014; Zooniverse, 2015). Additional projects cover a variety of areas, from ecology to comparative genomics (Kawrykow et al, 2012; Satterfield et al, 2015; Silvertown et al, 2011). Recently, developments in smartphone use platforms (Liu et al, 2013) and social networking patterns (Dickinson et al, 2013) represent the ways popular technologies can be integrated into citizen science research. Using mobile devices and social media may be encouraging factors for mass communication and encouraging participation. However, regardless of these advances and expanding projects, the results of such research are not often accepted as traditionally publishable data in the peer review process, but instead as education or outreach only (Bonney et al, 2014). New interest from scientific and educational research perspectives must be utilized to validate the data generated and appreciation for the scientific process developed.

I propose that the development of climate change education in the biology classroom can be better informed by an understanding of the history of the teaching of evolution. A common goal for science educators should be to engender a greater respect for and appreciation of science among students while teaching specific content knowledge. Citizen science has emerged as a viable yet underdeveloped method for engaging students of all ages in key scientific issues that impact society through authentic data-driven scientific research. Where successful, citizen science may open avenues of communication and engagement with the scientific process that would otherwise be more difficult to achieve. Citizen science projects demonstrate versatility in education and the ability to test hypotheses by collecting large amounts of often publishable data. I find a great possibility for science education research in the incorporation of citizen science projects in curriculum, especially with respect to “hot topics” of socioscientific debate based on our review of the findings of other authors.

10.2 Discussion

American instructors in the Earth and Space Sciences who cover "controversial" topics in science must be aware of and respond respectfully to engage students, parents, the community, and their peers (Johnson, 2013). Although environmental concerns receive significant public and media attention, the public has a poor understanding of the science in general, especially as these topics often require drawing upon education from multiple disciplines (Tsurusaki and Anderson, 2010). Several key parallels exist between evolution and climate change, scientifically and through public perception. Beyond discussing extinction or evolution, the topics share many similarities as to how they are presented to students. Citing the teaching of evolution, Hermann (2008) calls upon several key concepts (noted in italics), which we can extrapolate for these purposes to the teaching and learning of climate change:

1. *The issue represents a socioscientific controversy, not a scientific controversy*. That 97% of climate scientists are convinced about the human-caused warming trends while more than one-third of Americans reject the evidence of average global temperature increases (NASA, 2015; Motel, 2014) is an ideological issue in a country that values ideology strongly. Herein, we acknowledge the nature of the debate being limited to "controversial" in the public eye, which classifies it as a socioscientific debate, not a scientific one (Zeidler et al, 2005). The major distinction between climate change and evolution is the nature of the sides – evolution is disagreeable with certain

religious groups and climate change finds opposition primarily in the political arena.

2. *Conceptual frameworks for both span multiple scientific* [and non-scientific] *disciplines.* The teaching of evolution relies on the conceptual understanding of several sub-disciplines. On a larger scale, climate change and its implications require an even greater diversity of knowledge of many fields to understand the vast effects, even outside of the traditional science disciplines, with far-reaching implications into our everyday lives (Forrest and Feder, 2011).

3. *There is debate, disagreement, and uncertainty between opposing sides.* Primarily, we encounter the "sides" of this debate in the media and political arenas. In both situations, the press plays a key role in influencing public opinion to perpetuate these debates. Although outside of our primary focus, legislature and politics often complicate the interactions of civics and education. For example, several states (Louisiana, South Dakota, and Tennessee) have legislature-mandated laws concerning the teaching of climate change, including aspects such as teaching it as a controversy (Johnson, 2013). Creating a circular argument, governmental policy is driven ideally by constituent opinion and may be a direct reflection of citizen opinions.

4. *A clear path or decision does not exist for a "reasonable member of society,"* with "reasonable" being defined in an ethical sense by Kupperman (1985). Controversial topics in the Biological Sciences generate many perspectives and receive commentary from a variety of perceived authoritative sources. It is difficult for a lay audience to create informed,

educated viewpoints; especially when an overabundance of information is available or the perceptions challenge personal beliefs or value systems (Posner et al, 1982). Beyond the quantity of information, creating an organizational structure for analyzing information can be difficult for the lay audience.

In the literature, a number of instructional methods exist for engaging students in general and controversial science topics. With evolution, the instructor's approach can fit into one of four general categories: advocacy, affirmative neutrality, procedural neutrality, and avoidance (Herman, 2008). These include arguing for a side, presenting several positions, allowing students to present sides from resources, and omitting the conversation (Herman, 2008). Regardless of the instructor's choices relative to the topic, the educational approaches for evolution span a representative distribution of methods, including a selection from the nature of science (Cherif et al, 2001; Hildebrand et al, 2008; Lederman, 2006; Lederman and Lederman, 2014; McLelland, 2006; Forrest and Feder, 2011; Working Group on Teaching Evolution, 2008), geological time (Cherif et al, 2001; Kurpius et al, 2015), as well as questioning, case studies, and law (Ferber, 2003; Morishita, 1991). Relatively understated in the literature are studies evaluating the efficacy of newer active learning methods such as citizen science with respect to controversial topics. Among those present, selected examples generally include teaching tools, such as teaching tools for inquiry or active learning for students (Brewer and Gardner, 2013; Bromham and Oprandi, 2006; Dremock, 2002) or teacher training (Crawford et al, 2005).

In the classroom for example, often science educators have found successes in teaching controversial topics (*i.e.*, evolution) to traditionally non-receptive audiences by developing an understanding of the nature of science (Scharmann et al, 2005) and the value of the scientific process. As science teaching continues to move towards many established and/or validated active learning methods in the classroom (Freeman et al, 2014), we must engage students in new ways on key issues, especially those of socioscientific controversy (Aikenhead, 1994). Issues-based teaching favors a science curriculum that politicizes students and promotes "empowerment through civic participation, decision-making and action" (Pedretti, 1999; Pedretti et al, 2008; Zeidler et al, 2005). One method of engaging people in controversial topics is various forms of communication, such as through conversations, argumentation (Erduran and Jiménez-Aleixandre, 2007; Kuhn, 2010), cognitive conflict (Posner et al, 1982), or citizen/expert panel simulations (Albe and Gombert, 2012; Byrne et al, 2014).

To expand on interactive student experiences, citizen science projects have the ability to engage students and the larger community alike. Social and ethical issues offer students opportunities "to attach personal meaning to science concepts, theories and processes, and enable investigations that are closer to student's daily existence" (Zeidler and Sadler, 2008). This may enable educators to bring focus to important ideas by engaging students through emotional and intellectual stimulation, transforming otherwise distant topics into those central to their everyday lives (Zeidler and Sadler, 2008). Although both evolution and climate change include some current citizen science projects, a continued expansion may be an educational opportunity for students of all ages, from the classroom to the community. These projects can expand on the use of inquiry in the classroom to create large-scale research projects. Additionally, students may gain a greater appreciation of science through being involved in areas of non-simulated research, as opposed to the traditional "cookbook" labs.

When considering the use of a citizen science approach for climate awareness and education, a primary advantage is the past success demonstrated in citizen science. Several ongoing citizen science projects mentioned, including those for example of The Cornell Lab (Cornell Lab of Ornithology, 2015) have been shown to generate large amounts of scientifically valuable data while educating participants in the process of science and the specific areas of investigation (Bonney et al, 2009). In North America alone, there may be more than 200 research projects (Cohn, 2008) and several projects (notably eBird and Zooniverse) have led to scores of publications in many fields (Bonney et al, 2014). Additionally, climate change project examples span a range from active participation in parks (National Park Service, 2015) to volunteering computer processing time (Union of Concerned Scientists, 2015). Although some of these projects have turned into published scholarly works, the field of citizen science as a whole is underdeveloped and seen as an educational opportunity (Bonney et al, 2009). Additional opportunities exist for the evaluation of the educational aspects of these projects, as these remain understudied as compared to other teaching techniques (Trumbull et al, 2000), such as those used in active learning or inquiry-based approaches.

Interestingly, studies focusing on the research and educational aspects of citizen science projects for studying local and global impacts of climate change appear to be relatively underdeveloped in the literature. This represents an opportunity, especially for science education researchers. Beyond the possibilities of data generation and publications for the coordinating researchers and organizations, a key aspect of the citizen science project is the education of nonscientists in how science works as well as specific, project-related content (Bonney et al, 2009; Trumbull et al, 2000). Especially in those more controversial of topics, public perception relies strongly on the overall perception of science. With citizen science, we can effect change through engagement to develop an appreciation for science in everyday life.

Using the principles of citizen science, students of all ages can be involved in research. Although ecology, evolution, and climate change are vastly interconnected, such studies have yet to show a strong presence in science education literature. Searches of popular academic literature sites, for example Web of Science and ERIC Social Sciences database, with Boolean key words “citizen science AND education AND climate change” reveal approximately 50 or less total results, most of which relate to developing a productive or knowledgeable citizenry, instead of a citizen science research project. The limited results show a large niche in the educational research for the integration of citizen science in the curriculum and subsequent studies for efficacy or validation. In the case of climate change, this integration would be possible across many disciplines, especially with established topics of socioscientific controversy.

10.3 Conclusions

Many of the controversial topics in the Biological Sciences represent some of the most challenging concepts for communication and education in the sciences. The utilization of a variety of teaching tools to cover these subjects effectively and the continued investigation of new teaching methodologies for the education of students of all ages will be necessary to improve the appreciation for and understanding of subjects for which there is popular opposition. With citizen science in particular, there also exists an additional beneficial outcome: the ability to generate large-scale, publishable data for studies that may exceed traditional limitations. As both scientists and educational researchers, we can expand the opportunities for engaging non-scientists in research in the hope of strengthening the appreciation for and understanding of science in the general population, the "students" of any age. In turn, this positive experience may create excitement for scientific topics and empathy for the preservation of our only habitat.

CHAPTER 11

SCIENCE EDUCATION SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

11.1 Summary of the Problem

Climate change and the implementation of energy technologies form one of the, if not the single, most pressing issues facing human society and the Earth today. The Anthropocene is being defined by the damage humans are causing on the environment in all areas of the planet. Although climate change appears to be an insurmountable issue, certain measures and tangible goals, such as those set at COP21, provide global targets. In order to reach these targets, however, we need to improve overall education and science literacy. The perception of controversy in society needs to be addressed across the science disciplines and throughout the educational process. Issues such as climate change and energy technologies span these disciplines and present educational lessons that have implications on informed citizenship and environmental sustainability. Other areas of academic study, such as business, marketing, literature, art, and law, have applications and influences on these globally important topics.

From the many calls for educational reform in both K-12 and university environments outlined in Chapter 7, the need can be seen for an overwhelming improvement in science education in the United States. The newer techniques like active learning in science education apply directly to addressing issues such as energy technologies and climate change. These are topics that cannot be taught using historical benchmarks; we, and our students, are living these issues and changes right now. Climate change and energy technologies are commonly discussed in day-to-day life, news media, and social media outlets. Press releases from cutting-edge research on the effects of climate change seem to be almost daily occurrences. The required background leading to informed participation in these conversations is influenced by the classroom education and resources available to students. Textbooks, for example, may face more difficulty in presenting such contemporary issues, as the publishing times require a substantial delay for discussing up-to-date information. Many educational approaches are possible for developing informed, active citizens for a new generation. Whether following a pre- or non-science educational path, certain topics of great importance also face challenges in the classroom as socio-scientific issues. As a society, we must prepare our students for the challenges we are creating and facing. Science education is the key to a more sustainable future.

11.2 Summary of the Research

Within the context of science education as an approach to facing large-scale challenges, the research presented here details the presentation of topics related to climate change and energy technologies in undergraduate science textbooks. The investigation of this socio-scientific issue in introductory-level undergraduate Biology, Chemistry, and Physics textbooks revealed overall and discipline-based themes through an analysis of over 15,000 pages. Within this issue, potential causes for confusion among students may begin with the terminology. This research revealed a nearly even split between the use of “global warming” and “climate change,” although these books are current editions published between 2013 and 2015. Based on this research, content related to the research study is presented on less than 4% of textbook pages on average. Additionally, the material is strongly prevalent in the final portion of the book. The beginning of the books demonstrated moderate inclusion, while the middle thirds showed minimal presentation. This research has strong implications on the messages presented to students in the classroom and the likelihood of encountering information on energy technologies and climate change in the material likely to be covered in the course.

The energy technologies discussed follow general trends along the disciplinary lines. For example, the Biology textbooks tended towards presenting those based on biomass and related biological sources. Chemistry and Physics textbooks had a much larger emphasis on alternative fuels and other transportation technologies. Additional technologies, such as hydroelectric, wind, and solar were presented often in the Chemistry and Physics textbooks as well. Based on the traditional disciplinary lines, these do not appear to be surprising observations. However, in the face of minimal science literacy in the general society, interdisciplinary education for these topics may be of increasing necessity. The example technologies, however, are directly applicable to the disciplines. Addressing or including technologies outside the traditional disciplinary lines may become an authorship and editing decision based on the desire to discuss current issues facing society. Conversely, the “other messages,” including the terminology used, may be essential in the future for developing common themes and harmonizing the presentation across Biology, Chemistry, and Physics educational resources.

In addition to the energy technologies and climate change results presented in Chapter 8, a parallel study was presented in Chapter 9 on the presentation of nuclear energy and technologies in introductory-level science textbooks. This topic was separated from the overall energy and climate study because of its marginalized nature as a technology. Although not an underrepresented energy source in terms of percentage generation for many developed nations, the opposition takes many stances. With this, I consider nuclear energy here to be a non-renewable alternative to fossil fuels. As such, it does not function in the same way as fossil fuels in the impacts of energy generation, but also does not present similarities with renewable or more carbon-neutral technologies.

Even as compared to other technologies including fossil fuels, nuclear faces arguably the most vehement opposition in many facets of society. Those in opposition have many stances, a major one stemming from relatively recent large-scale disasters such as Chernobyl and Fukushima Daiichi. These incidents, and Three Mile Island, appeared in over 60% of the textbooks investigated. Such events appear to have made a strong impression on the long-term memory of society. Interestingly, Fukushima Daiichi and Chernobyl appeared in equal numbers of the textbooks (although not always the same textbooks). To me, this prominence in the educational resources indicates that these runaway nuclear disasters, the lives lost, and the long-term environmental impacts had a very strong impression on the textbook authors and editors as well as throughout society. These effects appear to be standing the test of time, as they are appearing in current editions three decades later. To illustrate, the Chernobyl disaster would have occurred years before the majority of college students today would have been born.

Nuclear energy faces other technical challenges in long-term immobilization and storage. The presentation of these issues has the possibility to influence students' opinions as citizens as well. For example, the discussion of long-term waste storage at Yucca Mountain, Nevada is a comparatively recent news topic that is discussed in only one of 16 textbooks studied. Conversely, a general discussion of the need for long-term storage is more widely distributed. In my opinion, this indicates a possible disconnect between the information students may be encountering in daily life (i.e., news and social media) and the educational environments. Specific examples may facilitate the connections made by students between their day-to-day lives and educational materials. A limitation of this research, however, is that the textbooks do not provide an indication of what connections the teacher may draw from the material, including whether or not the topics are covered during class. Understanding and bridging this gap may help to facilitate the development of informed citizens.

The method through which students interact with educational material plays a fundamental role in the educational process. Beyond the textbook, another approach to developing informed citizens in the sciences is to facilitate a greater overall appreciation for science. Towards this goal, citizen science is a scalable method through which non-scientists can participate in the scientific process. Through the citizen science approach, science becomes a way of thinking and approaching the world instead of a set of memorized facts encountered in K-12 or university education.

With the "development of informed citizens" as a theme, new and engaging approaches to science education are of the utmost importance. As seen in Chapter 10, citizen science is a natural extension of the active learning process, allowing students of all ages to engage in different ways with educational materials and the inquiry-based learning process. The gains observed with teaching evolution, perhaps the most historically controversial science topic, by using citizen science can be applied to teaching climate change. This approach provides a natural link between science and environmental education through the participation of non-scientists in collecting data and gathering evidence. Large-scale projects, particularly in research areas related to evolution, can be used as models for expanding the efforts to educate both the general public and introductory-level students on the process of science. Overall, I see great promise in the use of citizen science for developing an appreciation for science and an enhanced understanding of the "hot topics" of socio-scientific controversy.

11.3 Suggestions for Future Research

This research opens many possibilities for future research on the presentation of socio-scientific issues in the classroom. The studies of topics related to energy technologies and climate change in textbooks set a precedent for studying other interdisciplinary issues in educational materials. However, education is not limited to the printed or online volumes. Classroom presentation, instructor dynamics, and peer interaction form essential aspects of many educational experiences. To this end, studies are needed to more completely understand the emphasis placed on energy technologies and climate change in the undergraduate and K-12 science classrooms.

These studies focused on introductory-level Biology, Chemistry, and Physics courses, as they are the “catch-all” science classes including students on both science and non-science plans of study. Environmental science courses would be a useful comparison with the other disciplines. Likely, environmental science would place more emphasis on these topics and may present a more homogenous use of terminology and discussion across the different textbooks available in the discipline. Beyond environmental science, however, other disciplines outside of the sciences play a key role in the development and application of environmental approaches and technologies. For example, business and law are fundamental in the development of start-up companies and the widespread application of energy technologies. Political science and politicians have a great influence over the opinions of citizens and the choices made related to energy and the environment. Additionally, the arts and humanities are the manifestations of the thoughts and ideas in society. As such, these courses and works generated emphasize both cultural norms and alternative viewpoints, and may provide indicators of the feelings towards science and technology.

As discussed in this work, science education is crucial for catalyzing societal change. With respect to socio-scientific issues, special care must to be taken for facilitating discussion without causing belief-based alienation. This type of discrepancy between educational content and educational materials can provide a barrier difficult to overcome in the classroom. Based on the research presented here, a first step may be the adoption of uniform terminology and similarities in approaches for educational materials. An increased appreciation for science in many aspects of life may facilitate the implementation of solutions to improve the health of humans and the environment.

11.3.1 Interviews with University Faculty

Alongside the presentation in educational materials, the classroom plays an equal or possibly greater role in the ideas communicated or developed in a course. Interviews with university instructors teaching introductory and upper-division specialized or interdisciplinary courses may facilitate our collective understanding of the role of these research topics in K-12 and university education. With respect to energy technologies and climate change as a socio-scientific topic, the classroom can be considered the delivery mechanism for other resources including the textbook provided to the students. Additionally, the observations, issues, and teaching techniques of instructors provide a research opportunity for developing a working knowledge of the issues at the forefront of science education.

As a preliminary study in this area, several university faculty members from multiple disciplines at a large public university were interviewed. Following a typological approach with interpretive analyses (based on the work of Hatch, 2002), these qualitative data were coded based on the research and interview questions during the evaluation process to develop key concepts and themes. Several preliminary themes were revealed by these interviews. In general, the goal of many of the courses for the faculty interviewed was to increase the scientific literacy of the students. With respect to energy technologies and climate change, several main trends were observed: (1) avoiding political discussion in the classroom, (2) difficulties for students to utilize a scientific perspective for approaching these issues, (3) students having many perspectives on energy resources, and (4) an emphasis on holistic, order-of-magnitude and qualitative assessments for key concepts. In the future, these data and supporting information will be analyzed further in order to explore the themes and supporting information in greater detail.

11.3.2 High School Science

Although in recent years starting and often completing a university education has become a societal norm, studying the high school classroom is important to developing a fundamental understanding of interdisciplinary socio-scientific issues in the educational system. The underpinnings of worldview development begin early, and therefore, the high school educational system may be equally effective for investigating the presentation of energy technologies and climate change. In contrast to many university courses, high school education often relies on highly structured educational standards and guided curricula determined by organizations external to the school. These guidelines can be set by national or state standards, and create a driving force in the content and methodologies presented in the classroom.

Three fundamental science education research approaches are needed for establishing a baseline knowledge of the presentation of the socio-scientific issues of energy and climate in K-12 education. First, the role of state and national educational standards, frameworks, and “calls to action” must be explored from an educational policy perspective. The unique supervisory role of governmental agencies over K-12 education, especially with respect to energy technologies and climate change, can be revealed through thematic analyses of policy language. Likely, these policies may be able to be traced in a manner similar to that done by Matzke (2015), while creating a phylogenetic analysis of evolution-related legislation. The lineage model may be relevant to the changing dynamics of alternative and renewable energy content as well as climate change/global warming directives.

A second research necessary approach in K-12 education is the analysis of science textbooks. Similar to those discussed in Chapters 8 and 9, a baseline presentation must be established for this invaluable classroom resource. These textbooks may be compared also to the educational standards and policies evaluated. When compared to university education, the K-12 science textbooks are much more stringently adopted, meaning that they are selected by school districts or state level boards of education and not left up to the discretion of the instructor. Even textbook authors and editors rely on the directives of the educational standards. This may facilitate the evaluation of textbooks by providing a more narrow selection offered by the publishing companies or adopted by state or school district.

As necessary with university education, interviews to develop qualitative data on the presentation methods and difficulties encountered by high school science teachers will complete the three-part baseline survey of energy technologies and climate change in high school education. As established, these teachers are defined by much more strict educational standards and guidelines, however, individual teaching methods and approaches utilized in the classroom are essential for understanding. A comparison between the alternative ideas and misconceptions encountered by high school teachers and university instructors may provide information on how to best develop the overarching educational path with respect to these socio-scientific issues.

Following the establishment of baseline information on the presentation and discussion of energy technologies and climate change in the high school classroom, the development of educational materials may be the next logical step for supplementing the materials available. For example, the ideas brought forth in Chapter 10 for citizen science may be broadly applicable for engaging high school students, their families, and the community in the process of scientific exploration and extending the ideas introduced in the textbook and lecture-based activities.

11.3.3 Opinions in the General Population

As opposed to the structured educational environment, an assessment of the concepts and ideas in the general population creates a more difficult task. Educational materials are not typically available or utilized in the general public, so baseline presentations are often found in the news media, social media, and through large-scale surveys. Meta-analyses of socio-scientific issues in news and social media outlets can be found in the literature. Surveys, even those related to energy or climate ideas, are also found in the literature and are the research focus of certain science communication groups (for example, the Yale Project on Climate Change Communication). As the importance of the communication of socio-scientific issues to the general public is of great importance and may be dependent on certain external driving factors (i.e., the visit of the Pope, political debates, and environmental disasters), surveys must be continuously performed. However, the inherent limitation of the survey is a time-delay and potential disconnect between influential events and later data analysis.

With respect to the future work on the continuous need for public opinion assessments, I have developed a partnership with a leading survey center to the trends in opinions and beliefs of certain groups related to alternative and renewable energy technologies and climate change. Beyond this baseline attitude, questions are included for targeting the thought of individuals related to the educational preparedness of society for addressing these issues. For these environmental issues, personal choices in daily life are utilized for correlation of opinions. I anticipate several potential alignments in the data, especially from personal choice indicators and concern about climate change to the implementation of fossil fuel alternatives. With this line of investigation, the positioning of the educational system to develop new technologies and address the environmental issues of climate change may present a greater challenge in assessment. The data generated from this work may provide enhanced insight into the closely held opinions of citizens in the energy-environment-education nexus. I anticipate that this will shape thematic trends in future science education studies and continue those in citizen opinion studies.

Making connections between society and education related to socio-scientific issues, similar ideas can be drawn to those developed for extension lessons in K-12 education. The general population can be engaged in the process of science through observation and project-based learning. For example, the projects such as those from The Cornell Lab of Ornithology, eBird, and Zooniverse, provide scalable models for project-based learning able to engage citizens. The building of metaphorical bridges between science and society may facilitate a feedback loop with positive opinion generating an increased interest in science and funding directed towards research. Just as the role of the educator in the classroom is moving away from the lecturer approach, the science researcher may need to display enhanced communication skills in the future.

Outside of the “ivory tower” communication methods (with journal articles and conference presentations, for example) exists a body of citizens who can be or may become engaged or interested in science. Although citizen science is one perspective, many channels (news, blogs, social media) may provide avenues for communication and research opportunities in education designed for general audiences.

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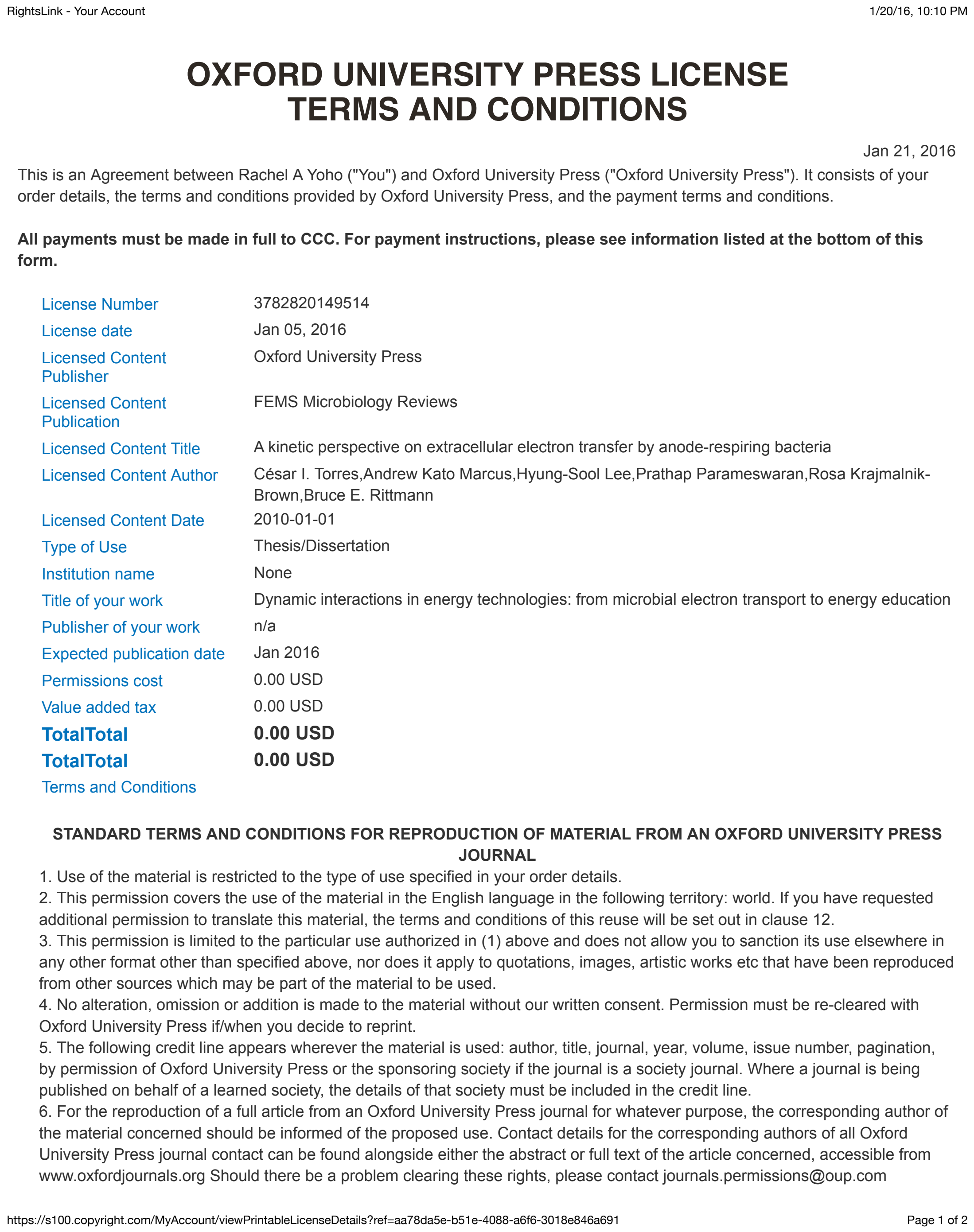

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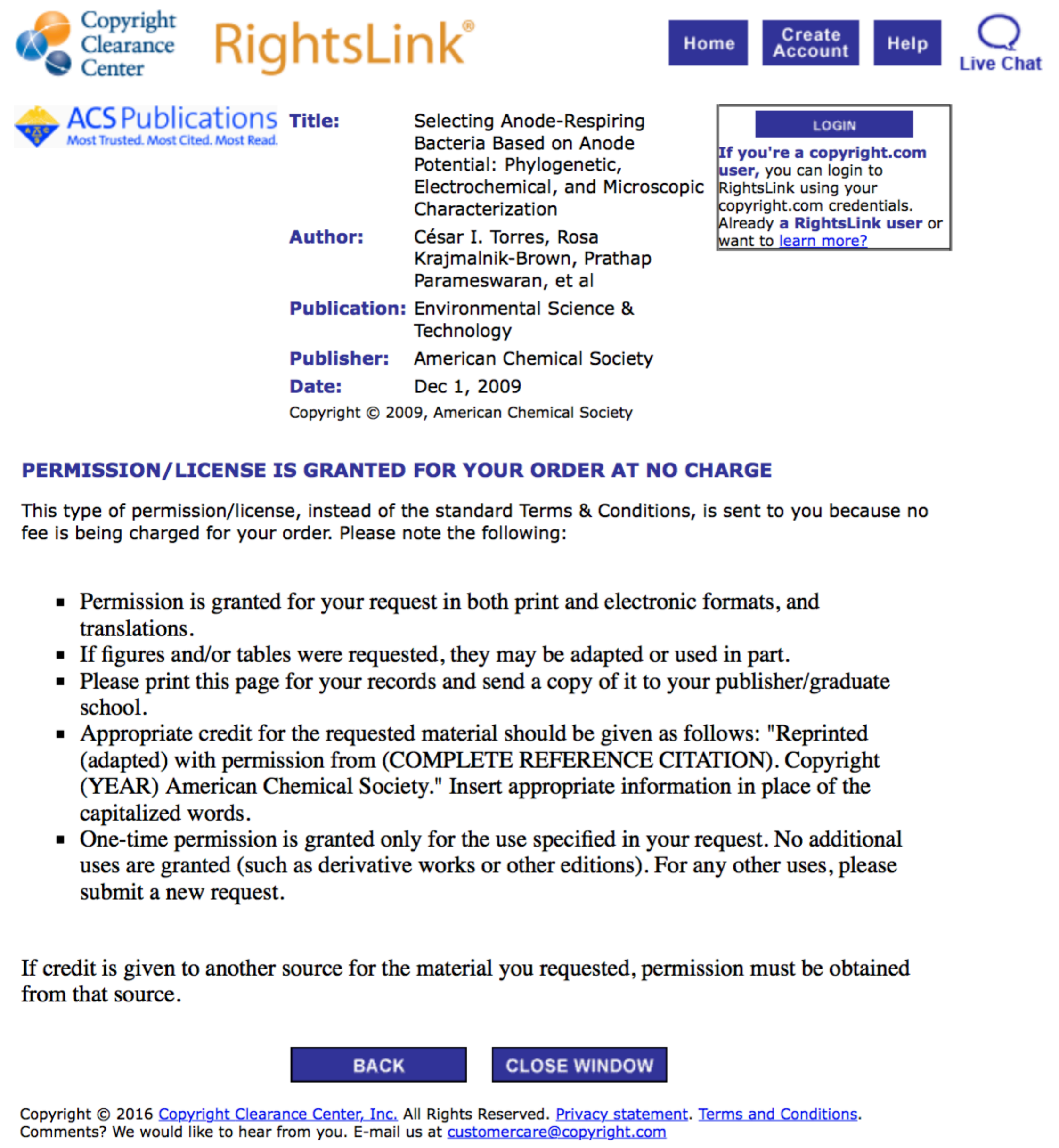

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Publication: Environmental Science & Technology

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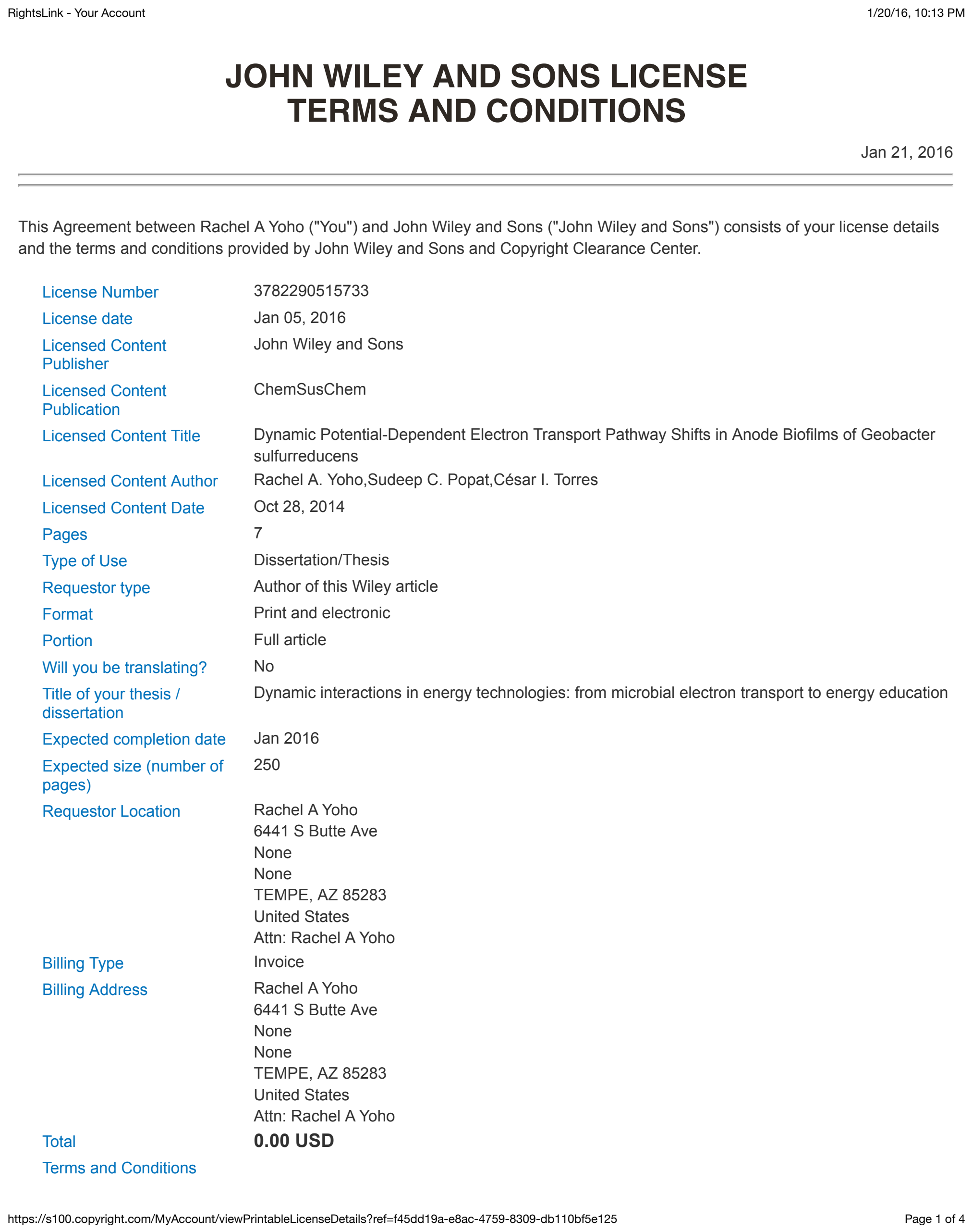

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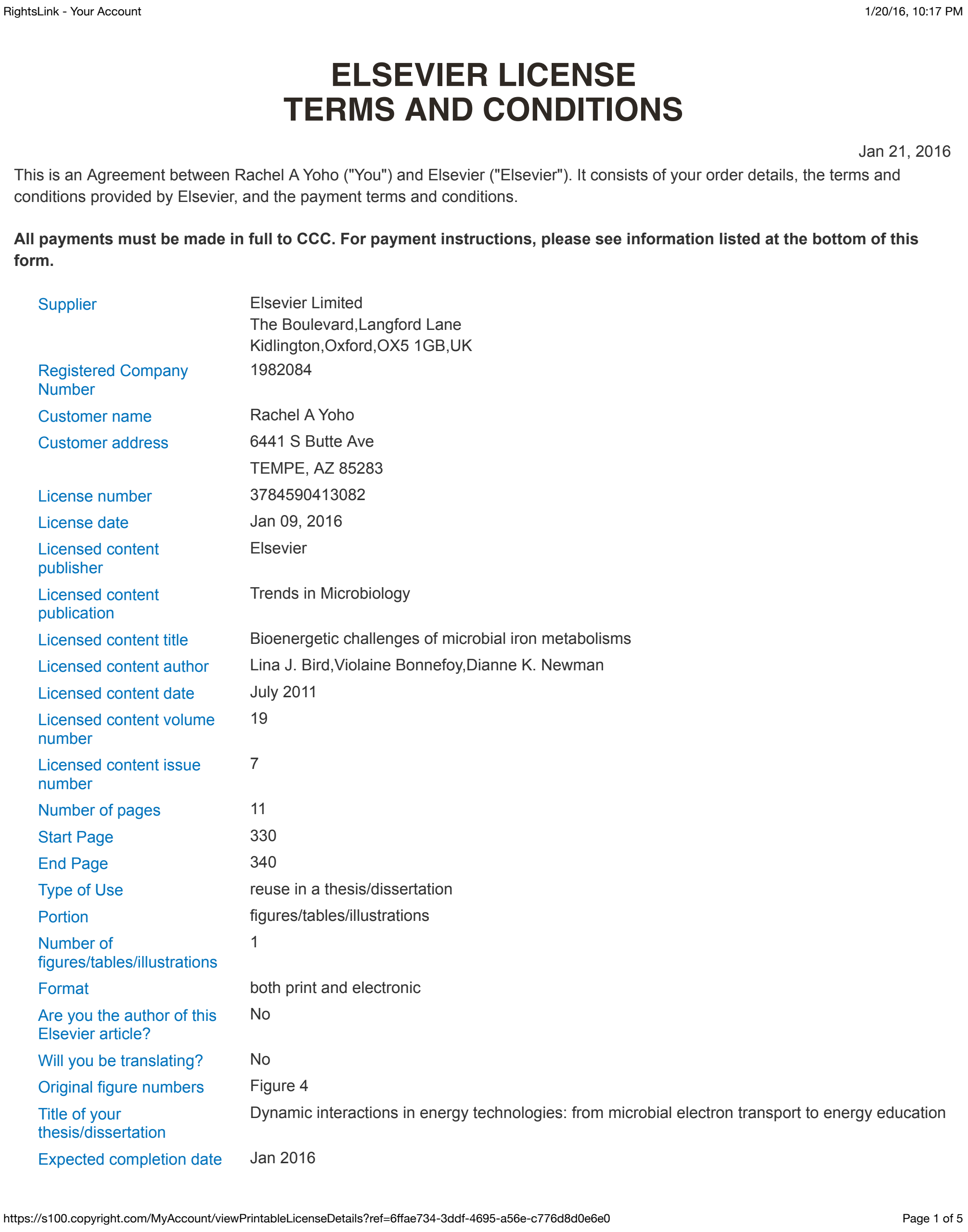

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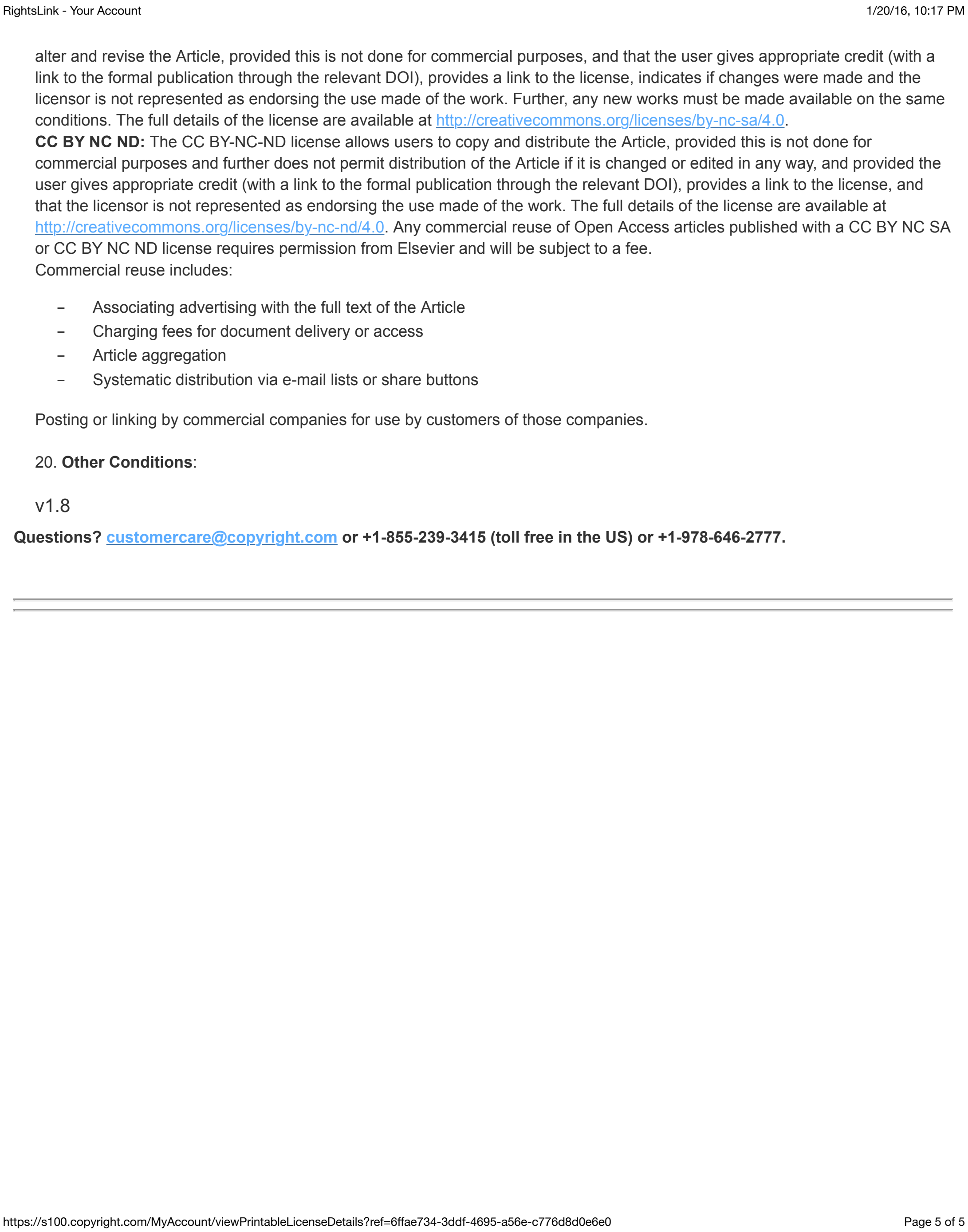

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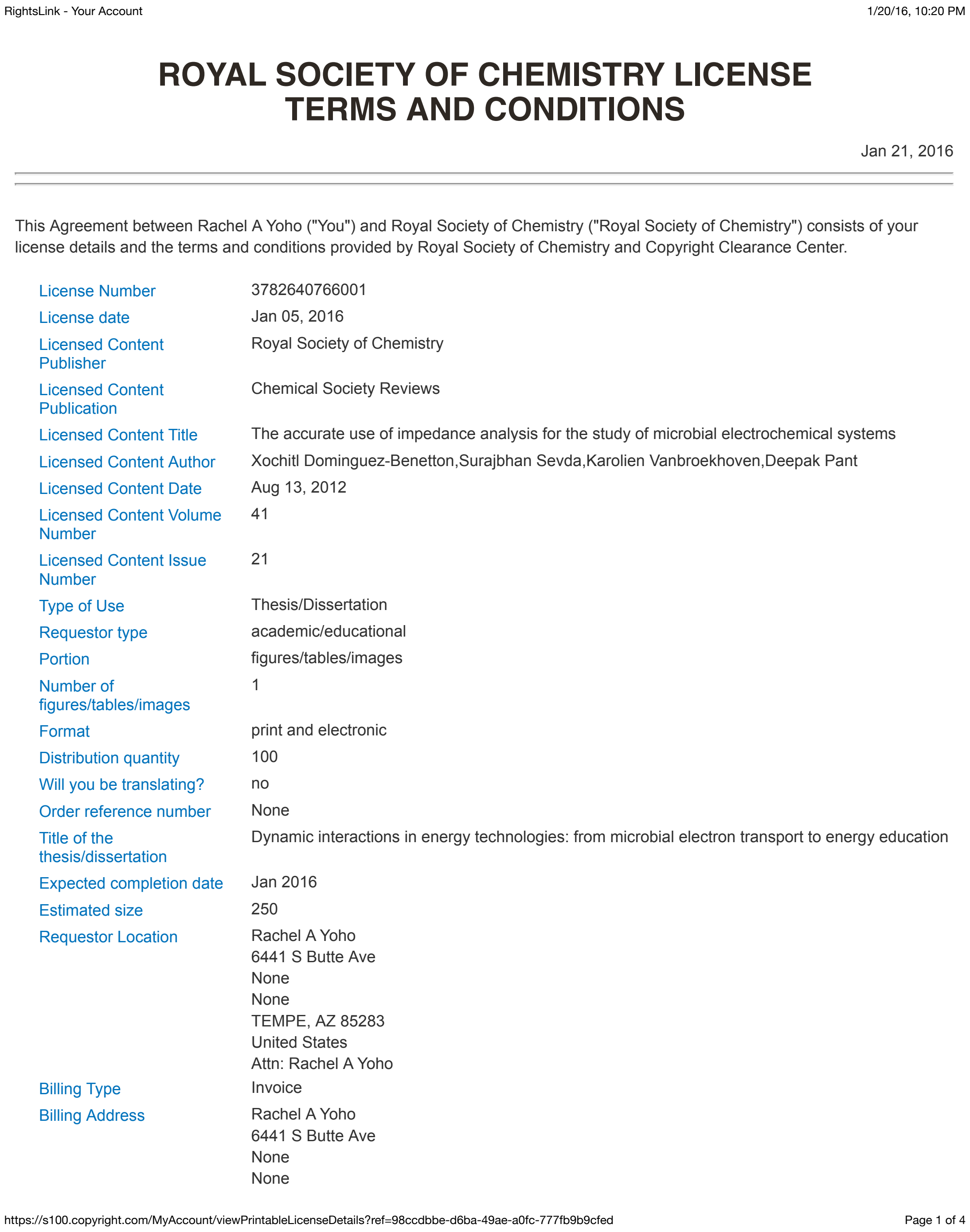

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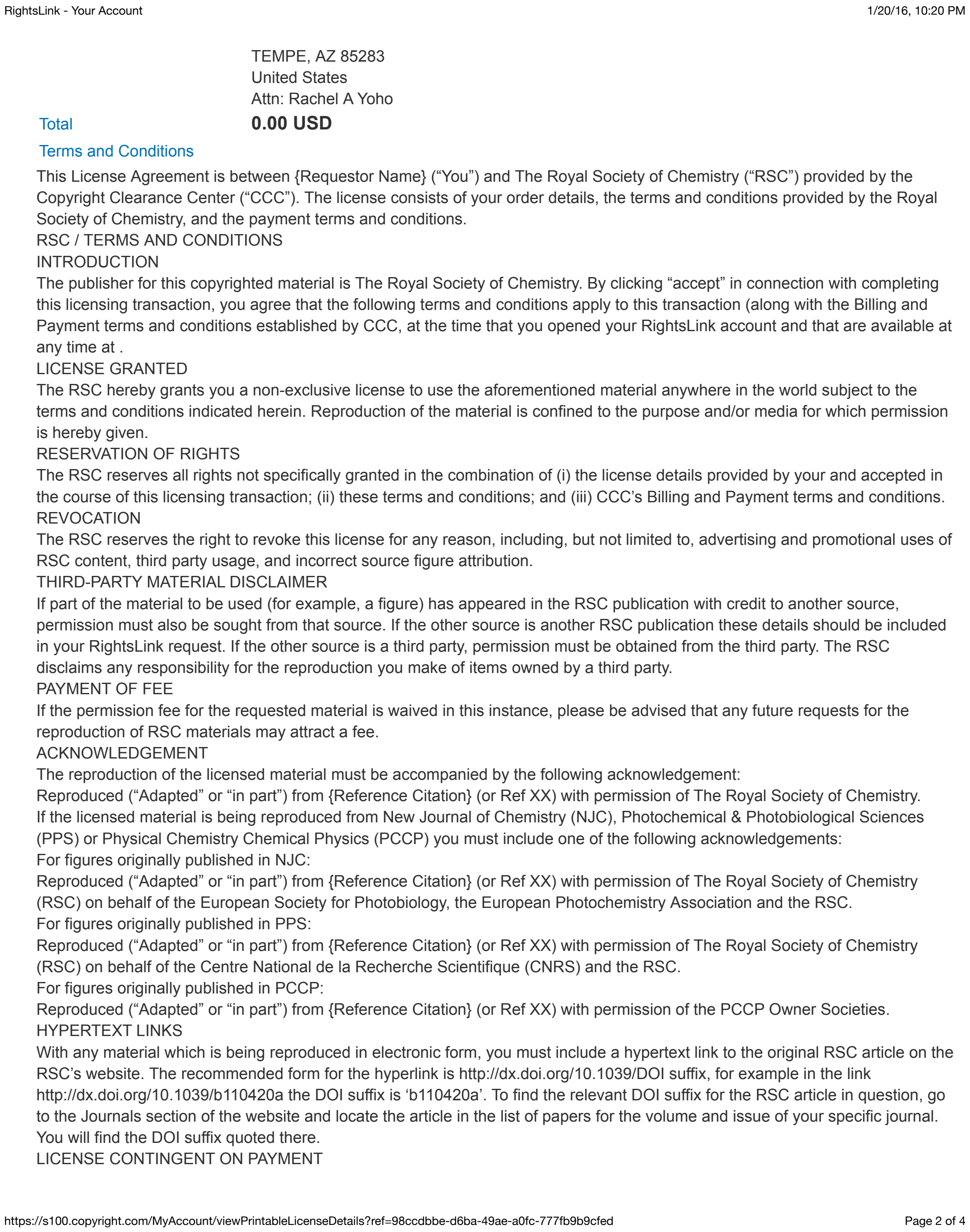

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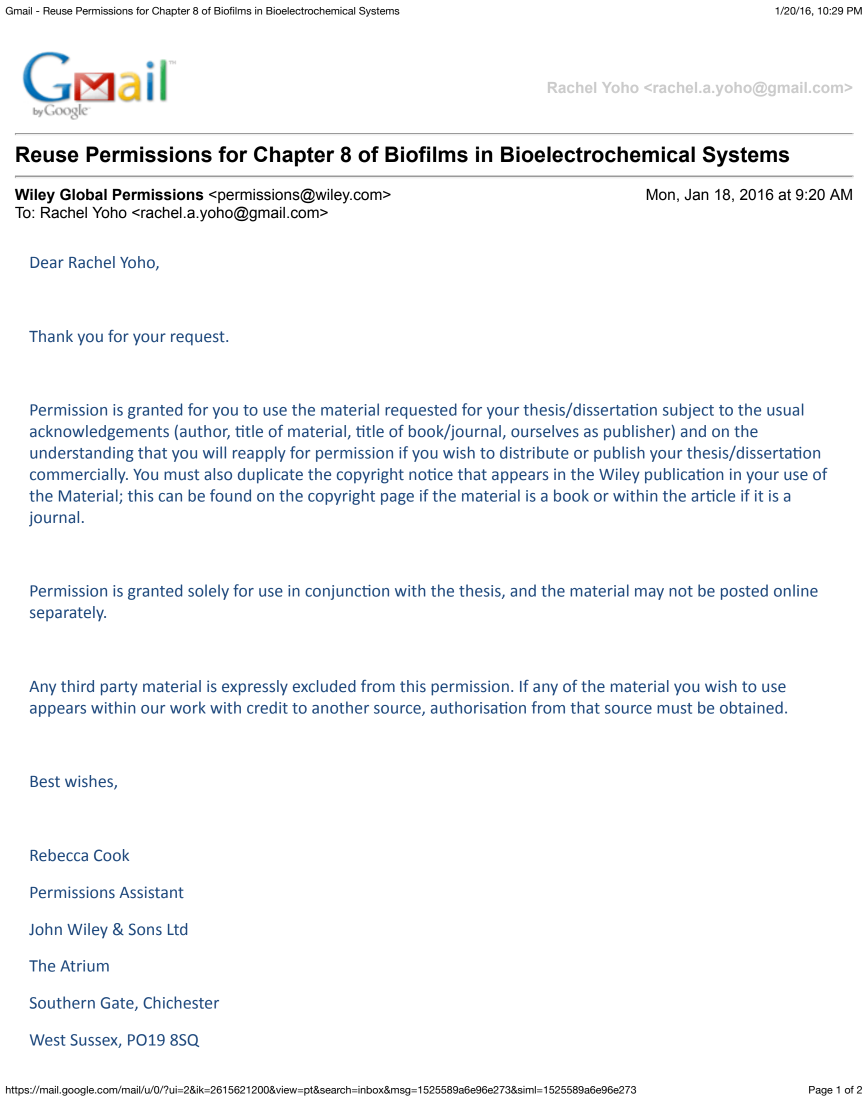

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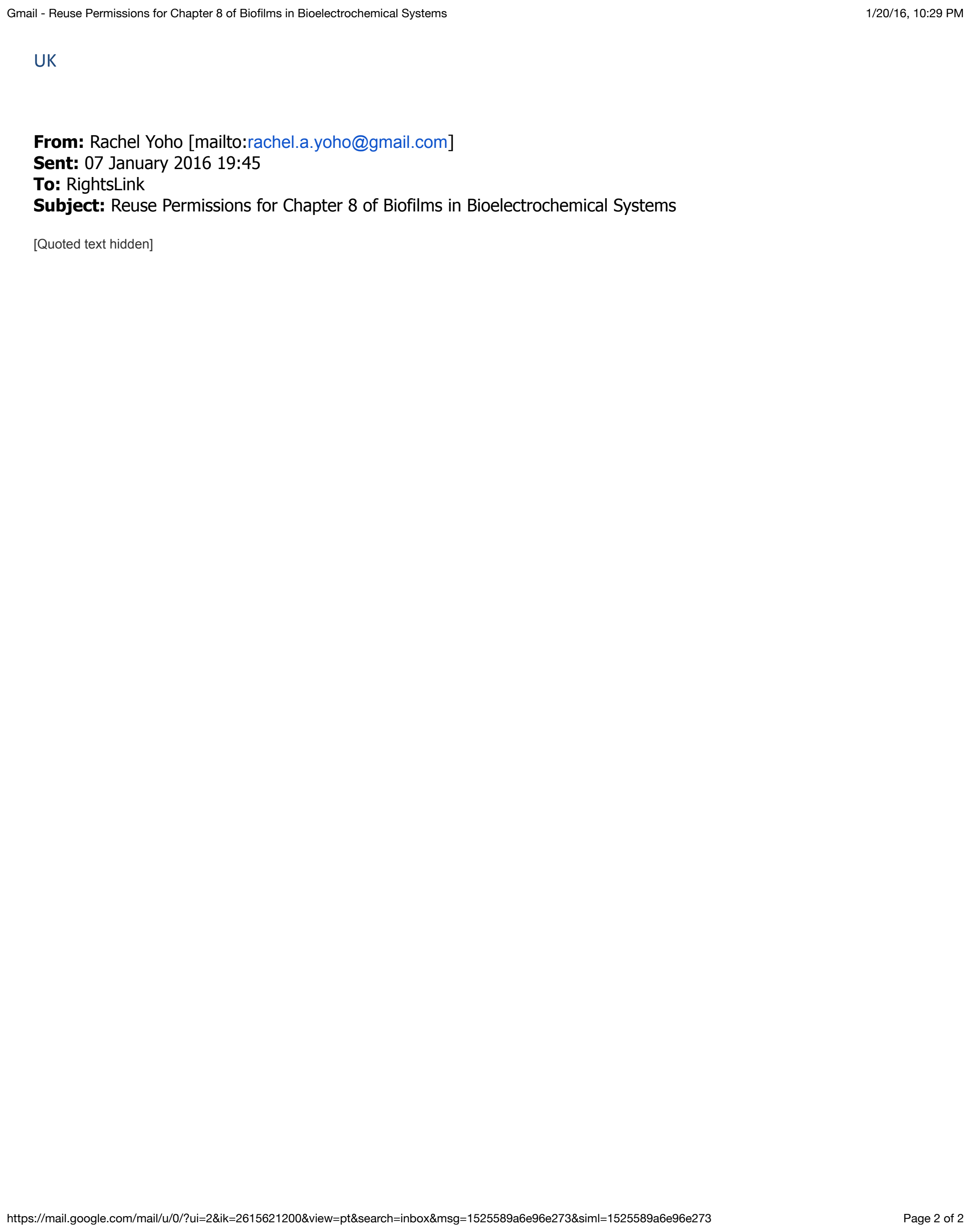

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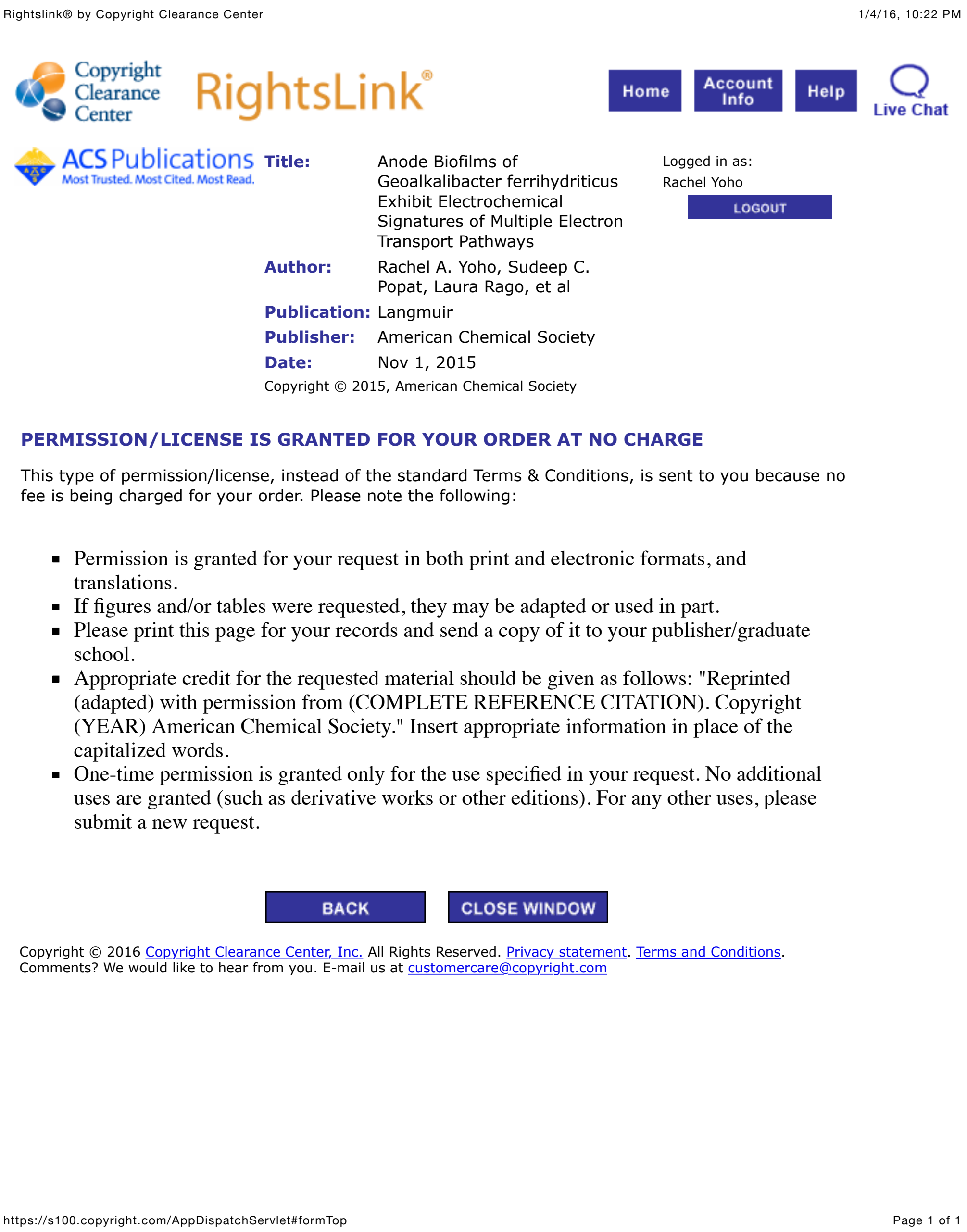

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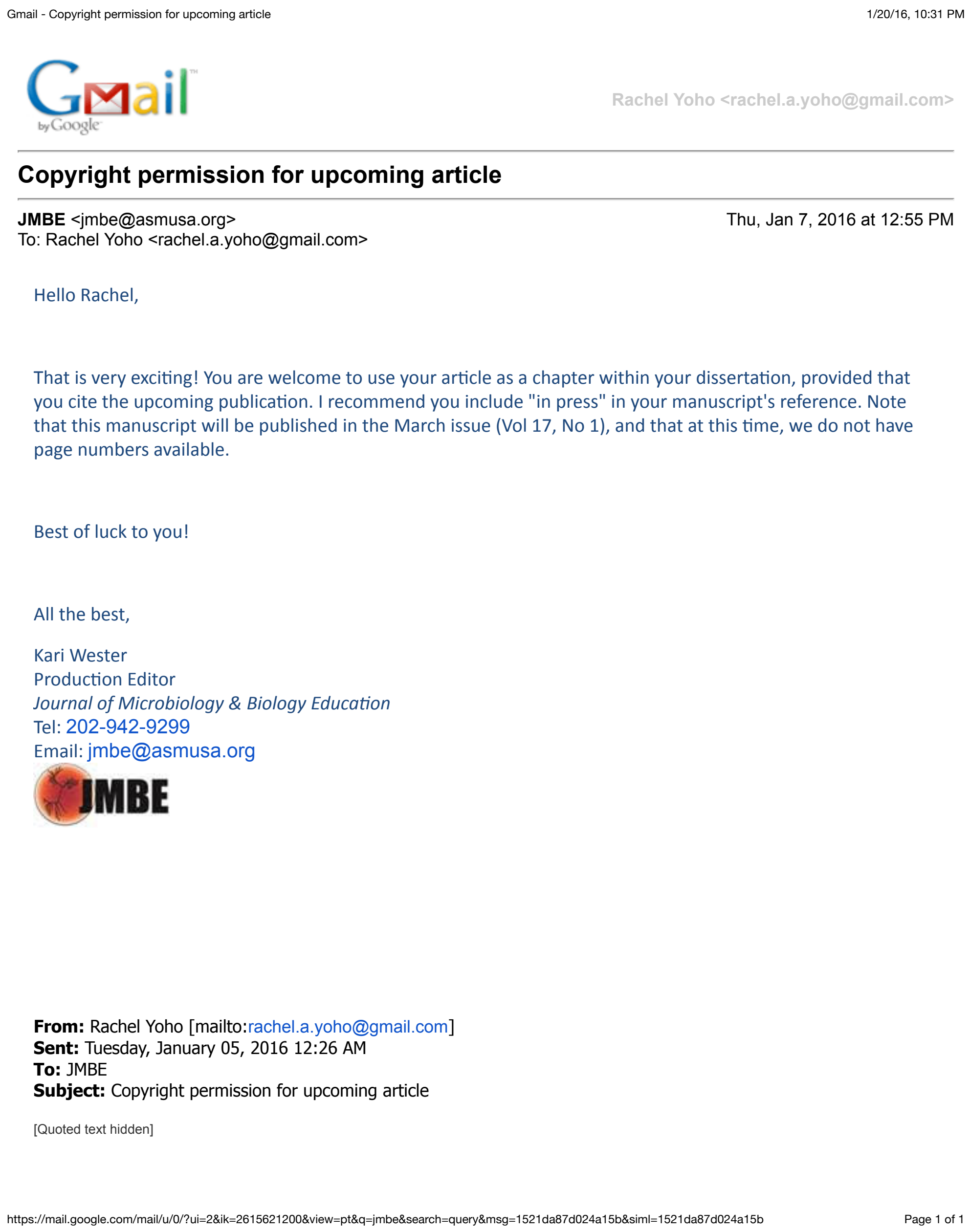

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APPENDIX I

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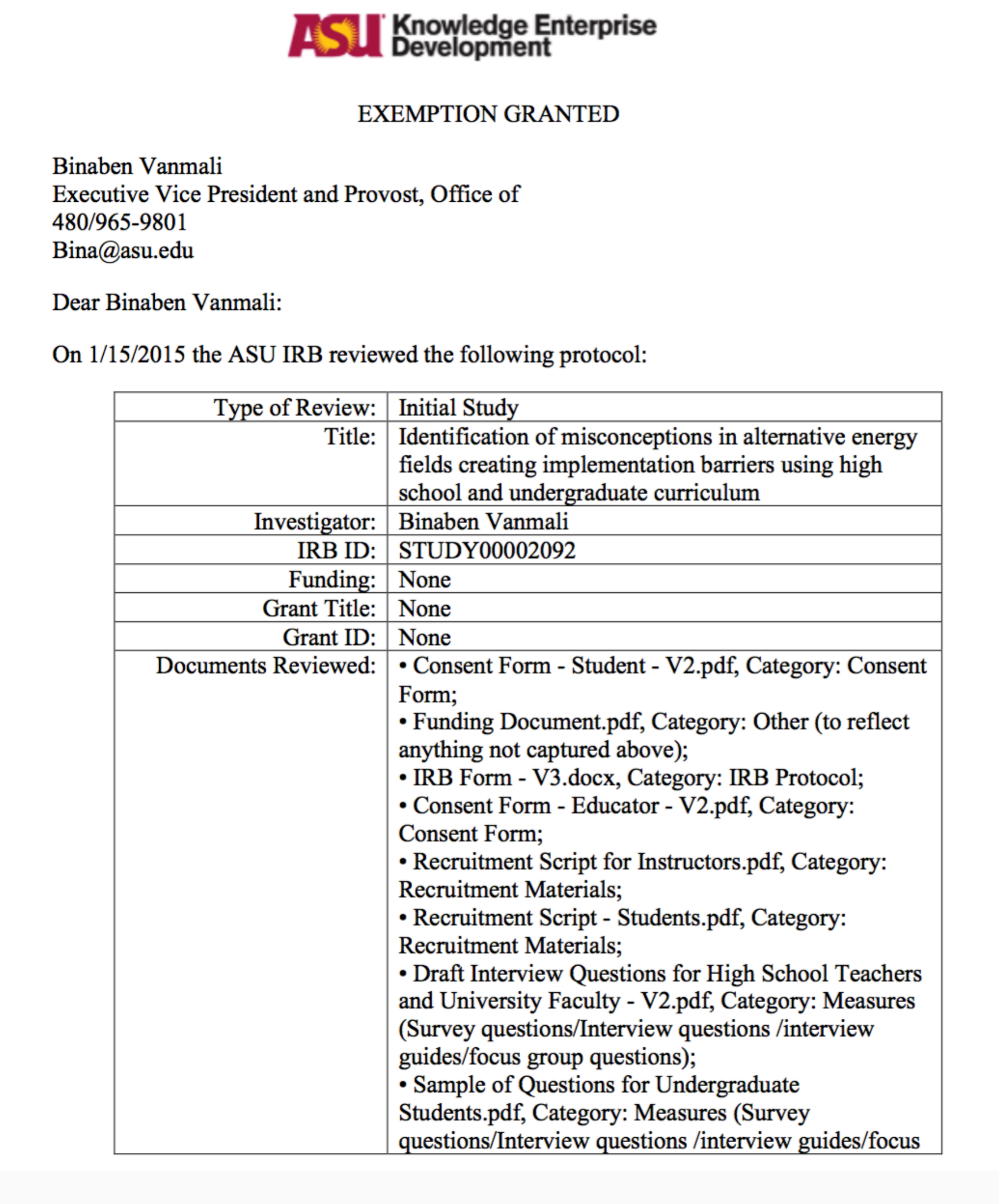

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Type of Review:	Initial Study
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Investigator:	Binaben Vanmali
IRB ID:	STUDY00002092
Funding:	None
Grant Title:	None
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Sincerely,

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cc: Rachel Yoho
Rachel Yoho

BIOGRAPHICAL SKETCH

Rachel Yoho graduated in 2012 from Capital University in Bexley, Ohio with a Biology major and minors in Physics and Spanish. Prior to joining the Biological Design PhD program, she had previous research interests in microbial fuel cells and electrochemical impedance spectroscopy. She was able to combine these interests in the Swette Center for Environmental Biotechnology at Arizona State University. With many years of science fair and science outreach experience, Rachel has been very involved in various science education programs. She was able to include science education research on the communication of climate change and energy technologies as part of her dissertation. Rachel has published research articles in journals such as *ChemSusChem, Langmuir*, and *Journal of Microbiology and Biology Education* and non-technical works in *Science* and the *Naturejobs Blog*. She has presented her work at national and international conferences.