Homogeneous Reaction Kinetics of Carbohydrates with Viologen Catalysts for Biofuel Cell Applications

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Homogeneous Reaction Kinetics of Carbohydrates

with Viologen Catalysts for Biofuel

Cell Applications

Hilary Bingham

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Homogeneous Reaction Kinetics of Carbohydrates with Viologen Catalysts for Biofuel Cell Applications

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Energy usage is continually on the rise and significant efforts are being extended to provide more renewable energy. One area of exploration is the development of fuel cells, which includes biofuel cells that can extract energy from carbohydrates obtained from biomass. Recently, viologen catalysts have been shown to enhance reaction rates of energy extraction and improve carbohydrate conversion efficiencies. However, characterizing the effects of process parameters such as pH, reactant concentrations, and carbohydrate exposure time to buffer solutions with a rigorous model is lacking.

This thesis characterizes the homogeneous reaction between carbohydrates and a methyl viologen catalyst to provide insights on ways to enhance the reaction rates to produce more energy. Specifically, the rate of formation of reduced methyl viologen (MV\(^{+}\)) in the presence of carbohydrates was measured based on changes in the MV\(^{2+}\) concentration, carbohydrate concentration, pH, and carbohydrate exposure time. A rigorous mechanistic model of the reaction rate was developed and showed a first-order dependence on OH\(^{-}\) concentration, a zero-order dependence when the MV\(^{2+}\) concentration was >> 0.5 mM, and a 3-fold increase in the reaction rate when glucose was pre-incubated in a pH 12 buffer solution for 100 minutes. The pre-incubation effect had a strong dependence on pH. The mechanistic model agreed well with experimental data.

This thesis also addresses the decomposition of viologen catalysts. MV\(^{2+}\) decomposition experiments showed a trend seen previously in literature that the rate of decomposition increases with an increase in MV\(^{2+}\) concentration, OH\(^{-}\) concentration, and temperature. The data and mechanistic model suggest second order dependence of both MV\(^{2+}\) and OH\(^{-}\) concentrations under conditions in this thesis (MV\(^{2+}\) concentrations of 100-300 mM and OH\(^{-}\) concentrations of 0.001 M and 0.01 M). An activation energy was found from MV\(^{2+}\) decomposition to be 145 kJ/mol. MMV\(^{+}\) decomposition was shown to decompose anywhere from 6.2 – 16.1 times slower than MV\(^{2+}\). Therefore, MMV\(^{+}\) decomposes slower and is more stable than MV\(^{2+}\). It was also found that MV\(^{2+}\) is more stable than IPV-Cl and IPV-Br. An analysis was performed to find the recommended operating range for MV\(^{2+}\)/glucose biofuel cells under different conditions while ensuring that at least a viable amount of energy could be produced.

Keywords: fuel cell, viologen, kinetics, model, carbohydrate
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1. INTRODUCTION

1.1 Energy Demand

Energy usage is continually on the rise and significant efforts are being extended to provide more renewable energy. Nearly 90% of energy consumed comes from nonrenewable resources such as petroleum, coal, natural gas, and nuclear with the remainder from renewable resources such as solar, wind, and hydropower (Administration, 2016a; Hepbasli, 2008). Renewable resources, which continue to grow in utilization, still have many sustainable challenges related to economics, efficiency, supplies, and storage. Thus, continued efforts are needed to address these and other challenges to optimize the use of renewable energy (Athanasios Angelis-Dimakis, 2011). One area of exploration is the development of fuel cells, which includes biofuel cells that utilize biomass as the feedstock (M. Q. L. Hao, Xianhua; Feng, Mengnan, et. al., 2014; W. Liu, Mu, & Deng, 2014; Watt, 2014b; Wheeler et al., 2009; Xuan, Leung, Leung, & Ni, 2009; Yang, Liu, Hao, & Zhang, 2015).

1.2 Fuel Cells

1.2.1 How a Fuel Cell Works

Many types of fuel cells have been developed such as hydrogen, methanol, or biofuel cells. Figure 1-1 shows the overall idea of how a hydrogen fuel cell works (Dervisoglu, 2017). Hydrogen
fuel, the source of energy in this case, is fed into the cell. Electrons transfer from the fuel to the electrode (anode) and move from the anode to the cathode, producing an electric current and energy source. Biofuel cells utilize a biological fuel, such as a carbohydrate, and often require a catalyst on the anode side in order to facilitate the transfer of electrons from the fuel to the electrode.

![Figure 1-1. Hydrogen fuel cell schematic taken from (Dervisoglu, 2017).](image)

There are challenges with using biofuel cells as a source of energy. One challenge is the small amount of energy fuel cells produce in comparison to other energy sources. In fact, the use of the electricity generated from biofuel cells produces enough energy for small electronic or medical devices, but still not enough power for high power applications such as transportation (Watt, 2014b). A reliable source of biofuel also needs to be available. Another challenge is the catalyst that oxidizes the biofuel must be stable, inexpensive, and efficient in order to transfer the electrons from the biofuel source to the anode of the biofuel cell (Wheeler et al., 2009).
1.2.2 Introduction to Viologen Catalyst

In general, a $C_n$ carbohydrate has a maximum of $4n$ available electrons that can be extracted to produce energy. For example, the $C_6$ carbohydrate glucose ($C_6H_{12}O_6$) has 24 available electrons. Since electron extraction from carbohydrates is difficult in the absence of a catalyst, both biological and non-biological catalysts have been explored to enhance reaction rates and efficiencies (Evans, 1929; Isbell & Frush, 1973; Isbell, Frush, & Martin, 1973).

Recently, viologen catalysts (methyl viologen shown in Figure 1-2) have been shown to enhance reaction rates and improve carbohydrate conversion efficiencies. In one study, the electron extraction rates of four different carbohydrates at two different temperatures in the presence of viologen catalysts were measured. They were predicted to be fast enough to produce viable amounts of power, (30 mA/cm²), based on approximate calculations with the rate constant of MV and glucose at 55°C (Watt, 2014a). In another study, it was shown that a high electron extraction efficiency was achieved at viologen/carbohydrate ratios greater than 10, and a simple mechanistic model was described (Watt, Hansen, Dodson, Andrus, & Wheeler, 2011). In addition, it has been shown that the oxidation efficiency with an O₂-uptake vial method and the use of viologen catalysts was greater than 40% for some carbohydrates (Wheeler et al., 2009).

![Figure 1-2. Structure of methyl viologen (SigmaAldrich, 2018).](image)

Figure 1-3 schematically shows the interaction of a viologen catalyst, in this case methyl viologen (MV), with a $C_n$ carbohydrate in the presence of an electrode. Basically, in the solution surrounding an electrode, a carbohydrate reduces MV$^{2+}$ (oxidized MV) to MV$^+$ (reduced MV) and
other products as shown by pathway 1. If an electrode is present, the electrode can extract electrons from \( \text{MV}^+ \) and regenerate \( \text{MV}^{2+} \) as shown in pathway 2. Other viologens would undergo a similar process. There are two key rates and efficiencies associated with Figure 1-3. First, there is the homogeneous rate at which the carbohydrate reacts with \( \text{MV}^{2+} \) and the associated efficiency to form \( \text{MV}^+ \). Second, there is the heterogeneous rate at which \( \text{MV}^+ \) reacts with the electrode and the associated efficiency. The limiting step for a biofuel cell utilizing a carbohydrate feedstock in the presence of viologens appears to be the homogeneous reaction rate at which electrons are extracted from the carbohydrate to form \( \text{MV}^+ \) (Watt, 2014a).

![Figure 1-3. Schematic diagram of electron transfer with methyl viologen catalyst.](image)

The most efficient process for the homogeneous reaction in an alkaline solution would be for a \( C_n \) carbohydrate to transfer all \( 4n \) electrons to form \( \text{MV}^+ \) with carbonate (\( \text{CO}_3^{2-} \)) as the final carbon product. The efficiency would be cut in half if only \( 2n \) electrons were transferred and the final carbon product were formate (\( \text{CO}_2^- \)). Each formate still contains \( 2n \) electrons (i.e., the carbons have not been fully oxidized). Additional side reactions or decomposition reactions would further affect the efficiency (pathway 1).

The optimal conditions such as pH, concentration, and temperature to maximize the rate and efficiency of electron extraction from the carbohydrate to the catalyst need to be determined.
The products and byproducts formed are also important to assess. Additionally, the degradation process, or decomposition reactions shown by pathway 1, may interfere with the effectiveness of MV$^{2+}$ as a catalyst to transfer all electrons. For this work, the homogeneous rate of reduced MV (MV$^+$) formation for the reaction of MV$^{2+}$ and a carbohydrate was measured. The effects of pH, MV concentration, glucose concentration, and glucose incubation time on the extraction rate were identified and modeled. The decomposition rates of MV$^{2+}$ were determined to assess the potential reduction in efficiency with the carbohydrate extraction of electrons.
2. BACKGROUND

2.1 Biofuel Cells

2.1.1 Types of Biofuel Cells

As mentioned before, one area of high interest is the development of fuel cells as a method for utilizing renewable resources. There are multiple types of biofuel cells that will convert a feedstock, such as carbohydrates, into energy. The simplest type is the direct biofuel cell that does not utilize a catalyst. However, direct biofuel cells are not as viable since they are extremely limited in the feedstock reaction rates and extraction efficiency. Generally, a catalyst is needed to enhance the reaction rates and efficiency. Microbial and enzymatic fuel cells, in which a biological microbe or enzyme acts as a catalyst, provide opportunities for producing energy in the presence of various feedstocks. Advantages of microbial and enzymatic fuel cells include feedstock specificity and a reduction in byproducts; in contrast, challenges include low feedstock conversion efficiencies, catalyst attachment, transport limitations, and environmental limitations (e.g. pH) (Rismani-Yazdi, Carver, Christy, & Tuovinen, 2008; Santoro, Arbizzani, Erable, & Ieropoulos, 2017). Biofuel cells with non-biological catalysts can also be utilized while mitigating some of the challenges associated with biological catalysts. However, catalyst degradation and non-specific (e.g. complex) reactions can potentially lead to side products (Farrington, Ledwith, &
Stam, 1969; Rieger & Edwards, 1988). In all cases, there are opportunities to mitigate many of the challenges in order to help optimize biofuel cells.

### 2.1.2 Carbohydrate Feedstock and Viologen Catalyst

Optimizing biofuel cell performance using an efficient catalyst and an abundant and inexpensive fuel source are important in developing a viable and valuable source of energy. Exactly how much energy that replacement fuel cells could provide has not been determined. Hao et al. have reported a power density of a nickel foam anode and viologen catalyst cellulose fuel cell as 450 mW/m², which is better than the 59-143 mW/m² reported by researchers at Penn State University converting cellulose to electricity in a microbial fuel cell. (M. Q. L. Hao, Xianhua; Feng, Mengnan, et. al., 2014; Ren, Steinberg, & Regan, 2008). Liu et al. reported a power density of about 500 W/m² in a biomass fuel cell, a value almost 3000 times higher than in a microbial fuel cell (W. Liu et al., 2014). If optimized, biofuel cells could potentially replace part of the large consumption of energy by the power they produce.

This research specifically focuses on biofuel cells that utilize biomass as the feedstock. Typical feedstocks for biofuel cells have included various constituents of wood, alcohols, agricultural crops, biogas, and solid waste (Administration, 2016b). For example, carbohydrates obtained from agricultural crops or industrial waste streams have been utilized as a feedstock. Carbohydrates provide a good source of energy because they are abundant, renewable, have high energy density, and are convenient as fuels. There are approximately 180 billion tons of biomass available each year. About 75% of that biomass is carbohydrates, of which only about 5% is used by man. The rest of those carbohydrates are returned to soil. Lichtenthaler believes carbohydrates are the most industrially and economically viable option to replace energy produced from
petrochemical sources (Lichtenthaler, 2006). Due to the large amounts of available carbohydrates and their high-energy density, carbohydrates are a great option for use in a biofuel cell.

One challenge with biofuel cells is the catalyst. As mentioned above, for the biofuel cell to be efficient and produce sufficient power, the catalyst must be stable, inexpensive, and efficient (Wheeler et al., 2009). The discovery of viologens as a catalyst provides an opportunity to overcome this challenge. An important role of a catalyst is to transfer as many electrons as possible from the biofuel to the electrode. Viologen catalysts have the potential to transfer 24 of the available electrons from glucose to the viologen (Watt, 2014b). Hansen’s work shows these catalysts transferred anywhere from 12 to 24 of the available electrons (Hansen, 2012). However, uncertainties including the number of electrons transferred by the viologen catalyst, the side reactions that occur, and the optimal conditions need to be addressed.

As shown in Figure 1-3 and described above, the optimal process would be for an n-carbon carbohydrate to form carbonate and transfer 4n electrons to MV$^{2+}$ (the maximum available). However, if glucose is only able to react until formate is produced, only 12 electrons will be transferred to MV$^{2+}$ with the remaining 12 electrons stored in formate. A formate fuel cell could be used as well to then utilize the remaining 12 electrons. This would greatly increase the electron transfer efficiency. Zeng et al. showed the feasibility of a formate fuel cell, such that this optimization is possible (Zeng, Tang, & Zhao, 2014).

2.2 Degradation of Viologen Catalysts

In addition to viologens extracting electrons, viologen decomposition can occur. This can greatly reduce the electron transfer efficiency. For example, Farrington et al. proposed that the oxidized form of MV (MV$^{2+}$) decomposes into the reduced form (MV$^+$) at pH higher than 10 by the following reaction (Farrington et al., 1969).
\[
3MV^{2+} + 2OH^- \rightarrow MMV^+ + 2MV^+ + H_2O + CH_2O + H^+ \tag{2-1}
\]

This reaction has a first order dependence of MV\(^{2+}\). Another proposed mechanism for this reaction with data taken at lower temperatures (24 °C) is described by Rieger and Edwards. In their mechanism, MV\(^{2+}\) has a second order dependence and hydroxide remains first order, although data showed the hydroxide dependence as high as 1.4. (Anne L. Rieger, 1988). Additionally, MMV\(^+\) is known to be more stable than MV\(^{2+}\) (Smith 2007). If the decomposition occurs rapidly enough, decomposition would compete with the ability of the viologen to extract electrons from the carbohydrate. Replacing MV\(^{2+}\) with MMV\(^+\) could potentially decrease the rate of decomposition. However, it may decrease the rate of electron transfer from the glucose as well. This will need to be addressed further.

### 2.3 Challenges with Carbohydrates

The literature describes some complexities, apart from decomposition of MV\(^{2+}\), which may occur with the carbohydrates. The chemistry of carbohydrates is very complex. For example, various researchers previously proposed reaction mechanisms that included the formation of an enediolate from glucose. In these mechanisms, an equilibrium forms between different forms (cyclic and linear) of glucose and an enediolate (Anderson et al., 2012; Sowden & Schaffer, 1952; Vuorinen & Sjostrom, 1982). Glucose (G) and fructose (F) upon reaction with a hydroxide ion form rapid equilibrium species through which both glucose and fructose can convert to each other. A known interconversion exists between glucose, fructose, and mannose. The conversion to mannose was shown to be much slower (Maclaurin & Green, 1969). Maclaurin and Green also determined that each of those carbohydrates form different enediolates because the rates of enolization were not proportional to the kinetic rates between glucose, fructose, and mannose.
(Maclaurin & Green, 1969). These enediolates are not very stable and can therefore form and react quickly (Anderson et al., 2012; Maclaurin & Green, 1969; Vuorinen & Sjostrom, 1982). There is also some discrepancy as to where exactly in the reaction scheme these enediolates form. Anderson, et al. described a reaction mechanism with ionization and two forms of enolization of glucose before the redox reaction with glucose occurs (Anderson et al., 2012). Vuorinen and Sjostrom reported that two enediolates formed between the glucose and fructose path, as well as another enediolate that fructose forms (Vuorinen & Sjostrom, 1982).

The rate of formation of the enediolate and subsequent products was found to be first order and consequently the enediolate and subsequent products builds up at a very slow constant rate over time at pH 11. This reported constant rate of enolization implies that the enediolate of glucose only degrades by 1.4% over the 28 hours that the rate was reported. The enediolate is believed to be the form of glucose that reacts with MV$^{2+}$ (Isbell, Frush, Wade, & Hunter, 1969). Longer glucose incubation times ($t_i$, the time glucose sits in the buffer before MV$^{2+}$ is added) may relate to faster rates for the electron transfer from glucose to MV$^{2+}$. This is addressed in Chapter 3 and an explanation of why is also provided. Glucose incubation times may also effect the efficiency of the reaction. Enediolate decomposition to other unknown species is shown in the literature (Vuorinen & Sjostrom, 1982). Other side products, such as acids, have been shown to form as a result of reactions of glucose with potassium phosphate, NaOH, or MV$^{2+}$. (Lindberg & Theander, 1968; Novotny, Cejpek, & Velisek, 2008). Longer glucose incubation times may increase the rate of MV$^{2+}$ reaction with glucose, but other side products could form ‘dead-end’ products and reduce the efficiency at which glucose can transfer its electrons to MV$^{2+}$.  

11
2.4 Previous Applicable Work

Others have investigated the effects of pH and temperature on the reaction of viologens with a carbohydrate. As for pH effects, viologens oxidize glucose and other carbohydrates under alkaline conditions (Hansen, 2012; D. C. Hansen, Pan, Stockton, Pitt, & Wheeler, 2012). However, the ideal pH for biofuel cell applications is still unknown. Hao et al. reported that increasing both the OH⁻ and MV²⁺ concentration resulted in faster kinetics for cellulose oxidation (M. Q. Hao, Liu, Feng, Zhang, & Wang, 2014). Additionally, initial work of this research group here at BYU has led to a simplified rate law (Equation 2-2) for the overall process of the reaction of viologens with a carbohydrate (Equation 2-3) in which just formate is produced.

\[
rate = k_1 [Glu][OH^-] \quad (2-2)
\]

\[
C_6H_{12}O_6 + 18OH^- \rightarrow 6HCO_2^- + 12e^- + 12H_2O \quad (2-3)
\]

This rate law implies that increasing glucose or OH⁻ concentrations would lead to a higher rate of MV⁺ formation, which has been seen. However, a more detailed rate law is still needed since Equation 2-2 is a simplified rate law. A dependence on MV²⁺ has been seen, but the reaction conditions that provide an MV²⁺ dependence as well as the extent of the effect of carbohydrate and viologen concentrations need to be investigated further.

Temperature dependence has been explored in some studies. In a glucose/air fuel cell with a MnO₂ cathode and MV²⁺ catalyst, a 350% increase in power output was reported at 32°C compared to room temperature (Orton & Scott, 2015). Therefore, a higher temperature than room temperature is desired with glucose. Watt published that higher temperatures (40-55°C) are more useful for carbohydrates with five carbons or more, but carbohydrates with fewer than five carbons react with viologens rapidly at room temperature (Watt, 2014a). We must understand this relationship better to determine the optimal temperature. An optimal temperature where the
carbohydrate reacts the quickest, it is a feasible temperature for applications, and there are no negative side effects to the chosen temperature is desired.

2.5 Objectives

In summary, studies have shown that viologen catalysts are capable of extracting electrons from carbohydrates, viologen catalysts do degrade, carbohydrates form enediolates which can react with viologen, increased OH⁻ and MV²⁺ concentrations increase the rate of MV⁺ formation, and increasing temperature can greatly increase the rate of MV⁺ formation. This work expands on this knowledge by measuring and modeling the rate of MV⁺ formation for the reaction of MV²⁺ and glucose in detail, as well as measuring and modeling decomposition rates of oxidized MV²⁺.

Objective #1: The first experiments of this study were performed to measure and model the rate of MV⁺ formation for the reaction of MV²⁺ and glucose. Initial work of this the biofuel cell group at Brigham Young University has led to a simplified rate law (Equation 2-2, shown above) for the overall process (Equation 2-3, shown above) in which just formate is produced. This objective was to measure rate data as a function of pH, reactant concentrations, temperature, and glucose incubation time of MV²⁺ reacting with glucose. A mechanism and model was then developed based on pseudo steady-state analysis. This mechanism and model are more rigorous than the initial simplified rate law proposed previously. A nonlinear regression of the data was used to verify that the data fit the model well.

Objective #2. The second objective of this research is to characterize the decomposition rates of oxidized MV²⁺ and compare those to the rate of MV⁺ formation. An important part of a biofuel cell is the stability and efficiency of the catalyst. The decomposition rate of the oxidized viologen will determine the stability of the viologen. The amount of available viologen decreases with decomposition and minimizes the amount of oxidized viologen available to react with the
carbohydrate and deliver the electrons to the electrode. The rate of reaction of the carbohydrate with the viologen must be significantly quicker than the rate of decomposition of the viologen for viologens to sustainably generate electricity from a fuel cell. A study was conducted with varying viologen concentrations, hydroxide concentrations, and temperatures to model the kinetics of decomposition. Experiments were run at pH values of 11 and 12, temperatures of 40°C and 50°C, and a concentration of MV$^{2+}$ from 0.1-0.3 M. The rates of MV$^{2+}$ decomposition were measured and the MV$^{2+}$ decomposition rate was analyzed to assess the potential reduction in efficiency with the carbohydrate extraction of electrons. Preliminary studies on the rate of decomposition of MMV$^+$ were also performed to compare to those of MV$^{2+}$.

Gathering data and developing a rate law and mechanism for the interaction of the viologen with the carbohydrate as a function of pH, initial concentrations of carbohydrate and viologen, temperature, and incubation time and coupling that information with the rate of MV$^{2+}$ decomposition will provide guidance towards developing an optimized biofuel cell. The reaction rate expressions developed in this study are also used to estimate a current density that can be achieved in a fuel cell, assuming that the homogeneous reaction is limiting (i.e., the electrochemical reaction is fast relative to the homogeneous reaction).
3. **HOMOGENEOUS REACTION KINETICS WITH EFFECT OF INCUBATION TIME**

3.1 **Background**

The work described in this chapter has been submitted to the Journal of Applied for publication. One key aspect of sustainable biofuel cells utilizing carbohydrates as the feedstock is the need for sufficiently fast oxidation rates to enable extraction of available electrons from the fuel at current densities that will meet the power requirements for the application(s) of interest. As mentioned in Chapter 1, in general, a $C_n$ carbohydrate has a maximum of $4n$ available electrons that can be extracted to produce energy. For example, the $C_6$ carbohydrate glucose ($C_6H_{12}O_6$) has 24 available electrons. Since the rate and extent of electron extraction from carbohydrates is very low in the absence of a catalyst, viologen catalysts have been explored and shown to enhance reaction rates and improve carbohydrate conversion efficiencies.

Both the homogeneous reaction of a viologen catalyst with a $C_n$ carbohydrate as well as the heterogeneous reaction of the viologen and carbohydrate in the presence of an electrode are schematically shown in **Figure 1-3**. Since the homogenous step seems to be the rate determining step, understanding the homogeneous reaction rate is critical towards identifying process improvements that can enhance the reaction rate and associated efficiency. This chapter focuses on assessing and characterizing the homogeneous reaction rates of $\text{MV}^{2+}$ with various carbohydrates, with a specific emphasis on how pH, $\text{MV}^{2+}$ concentration, carbohydrate
concentration, and exposure time to buffer solutions affect the rate of reaction when using glucose as a model carbohydrate. An understanding of these various effects can provide design parameters for operating a viable biofuel cell.

3.2 Materials and Methods

Two studies were performed to characterize the initial homogenous reaction rate between a carbohydrate and MV$^{2+}$. Focusing on the initial reaction rate provides mechanistic insights on how the various process parameters noted above initially play a role in the reaction between a carbohydrate and MV$^{2+}$. The first study, performed by Dr. Gerald Watt from the BYU chemistry department and some of his students, measured the initial reaction rates of various carbohydrates and MV$^{2+}$. This data was included to provide a comparison of reaction rates between different carbohydrates. The second study measured the initial reaction rates of MV$^{2+}$ with glucose to develop a rigorous model for characterizing the homogeneous reaction as a function of the various conditions noted above. For all studies, methyl viologen hydrate (MV$^{2+}$, 98% purity) was utilized. Six-carbon carbohydrates (glucose, fructose, mannose, galactose, and rhamnose), five-carbon carbohydrates (ribose, arabinose, and xylose), and the three-carbon carbohydrate dihydroxyacetone (DHA) were evaluated in this study.

For the first study, the initial rate of MV$^+$ formation was measured at pH 11 (0.50 M potassium buffer solution) in the presence of each of the carbohydrates noted above. The carbohydrates were equilibrated in the buffer solution for 5 min in a 3.5-ml cuvette under anaerobic conditions. An anaerobic MV$^{2+}$ solution was then added to start the reaction. The reactions were performed at 50°C with initial concentrations of 23.6 mM carbohydrate and 11.8 mM MV$^{2+}$. Absorbance (or amount of light absorbed at a certain wavelength) was measured as a function of time at a wavelength of 603 nm using a spectrophotometer with a heating block to maintain the
desired temperature. The initial slope of the absorbance versus time curve was converted to a rate of MV$^+$ formation with use of an extinction coefficient of 13000 M$^{-1}$ cm$^{-1}$.

For the second study, the influence of pH (11 and 12), carbohydrate concentration (1 and 2 mM), and exposure time (0 to 100 minutes) to buffer solutions were examined at several values of the MV$^{2+}$ concentration (2, 4, 8, and 16 mM). Glucose was used as the model compound for these experiments, and all experiments were conducted at 50°C. Separate stock solutions of glucose and MV$^{2+}$ were prepared with deionized water, purged with nitrogen to remove oxygen, then placed in an incubator to preheat the solutions to 50°C. Sodium hydroxide was added to a potassium phosphate solution to obtain the desired pH and was then purged with nitrogen to remove oxygen. The buffer was placed in 3.5-mL cuvettes in an oxygen-free glovebox and preheated in an incubator oven to 50°C. The glucose stock solution was then added to the buffer solution by a gas tight syringe to obtain the desired starting glucose concentration. Glucose remained in the buffer solution for incubation times ranging from 0 to 100 min. The MV$^{2+}$ stock solution was then added with a gas tight syringe to start the reaction. Absorbance data at a wavelength of 730 nm were measured using a spectrophotometer while a heating block maintained the desired temperature. After each run, the pH was measured to confirm that the pH was constant during the experiment.

The initial rate of MV$^+$ formation for each study was determined by converting the linear absorbance data observed during the first 2-3 minutes to an MV$^+$ concentration with use of an extinction coefficient. The extinction coefficient for MV$^+$ has been extensively studied at an absorbance of 600 nm and a wide range of values have been reported- 13,000 M$^{-1}$ cm$^{-1}$ was used in our initial experiments. However, for the subsequent set of experiments, the absorbance was measured at 730 nm to enable measurement of higher MV$^+$ concentrations. This was necessary
since the signal at 600 nm becomes saturated at relatively low concentrations. The extinction coefficient using known MV\(^+\) concentrations at 730 nm was 2737 ± 42 M\(^{-1}\) cm\(^{-1}\).

### 3.3 Results

#### 3.3.1 Rate Constants for Various Carbohydrates

The initial rate of MV\(^+\) formation \((r_{MV^+,i})\) at pH 11 for the various carbohydrates is shown in Table 3-1 with \(r_{MV^+,i}\) initially modeled as:

\[
r_{MV^+,i} = k(C)_i(OH^-)
\]  

Here, \((C)_i\) is the initial concentration of the carbohydrate and \(k\) is the second-order rate constant. The \(r_{MV^+,i}\) values in this table were recorded by Dr. Gerald Watt and his students. Table 3-1 also shows the calculated values of \(k\) for the various carbohydrates.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th># of Carbons</th>
<th>(r_{MV^+,i}) (µM s(^{-1}))</th>
<th>(k) (M(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td>3</td>
<td>35.4</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Ribose</td>
<td>5</td>
<td>14.2</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>Xylose</td>
<td>5</td>
<td>3.78</td>
<td>0.16</td>
</tr>
<tr>
<td>Arabinose</td>
<td>5</td>
<td>3.54</td>
<td>0.15</td>
</tr>
<tr>
<td>Fructose</td>
<td>6</td>
<td>4.72</td>
<td>0.20</td>
</tr>
<tr>
<td>Galactose</td>
<td>6</td>
<td>3.30</td>
<td>0.14</td>
</tr>
<tr>
<td>Glucose</td>
<td>6</td>
<td>1.39</td>
<td>0.059</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>6</td>
<td>1.23</td>
<td>0.052</td>
</tr>
<tr>
<td>Mannose</td>
<td>6</td>
<td>1.01</td>
<td>0.043</td>
</tr>
</tbody>
</table>

As noted, \(r_{MV^+,i}\) (and the associated \(k\) values) for the monosaccharides generally increased as the number of carbons in the carbohydrates decreased. The values of \(r_{MV^+,i}\) and \(k\) for ribose and DHA were difficult to assess since they occurred much more rapidly relative to the other
carbohydrates- only lower bound estimates are provided. For the C6 carbohydrates, fructose and galactose had $r_{MV^+,i}$ and $k$ values of approximately 3-4 times higher than those of glucose, rhamnose, and mannose. A possible explanation for the faster fructose rate is provided later in the discussion.

### 3.3.2 Rate of Reaction Between Glucose and Methyl Viologen

The second set of experiments focused on glucose as the model compound to provide a more rigorous characterization of $r_{MV^+,i}$ based on pH, MV$^{2+}$ concentration, carbohydrate concentration, and exposure time to buffer solutions. Figure 3-1 shows $r_{MV^+,i}$ at pH 12 in the presence of initial MV$^{2+}$ concentrations varying from 2 to 16 mM. Here, glucose was incubated in buffer solution from 0-100 minutes prior to the reaction with MV$^{2+}$. The experiments showed good repeatability. As is evident, the glucose incubation time had a significant impact on $r_{MV^+,i}$, with an increasing rate with incubation time. The solid lines in Figure 3-1 represent the rigorous model described in the discussion section. As seen, $r_{MV^+,i}$ appears to be slightly dependent on the MV$^{2+}$ concentration at zero incubation time, particularly at 2 mM.

Figure 3-2 shows $r_{MV^+,i}$ as a function of incubation time at (a) pH 11 and 12 and (b) glucose concentrations of 1 and 2 mM. The solid lines represent the rigorous model described in the discussion section. Based on Equation 3-1, the ratio of $r_{MV^+,i}$ at pH 11 relative to pH 12 at each time point should be $(0.001M)/(0.01M) = 0.1$ for the dependence of pH to be appropriately accounted for in Equation 3-1. For the three incubation time points evaluated at both pH values shown in Figure 3-2a, the average ratio is 0.23 which is a little higher than this expectation.
Figure 3-1. Initial rate ($r_{MV^+\text{,i}}$) of reduced methyl viologen ($MV^+$) formation in the presence of 2 mM glucose versus glucose incubation time at pH 12 and 50°C. Initial $MV^{2+}$ concentrations were 2, 4, 8, and 16 mM. Previous to starting the reaction, the glucose was incubated in the buffer solution for various times ranging from 0 to 100 minutes. The symbols represent the data and the solid lines represent Equation 3-12.

As shown, the strong pH effect is generally captured by both the model and the data even though the model slightly under predicts the effect. As for the effect of glucose on $r_{MV^+\text{,i}}$, the ratio of $r_{MV^+\text{,i}}$ at 1mM glucose relative to 2 mM glucose at each time point should be (1 mM)/(2 mM) = 0.5 for the dependence of glucose to be appropriately accounted for in Equation 3-1. Figure 3-2b shows an average ratio of 0.46 which is consistent with expectations. Thus, $r_{MV^+\text{,i}}$ has a first-order dependence on pH and carbohydrate concentration.
Figure 3-2. Initial rate ($r_{MV^+}$) of reduced methyl viologen ($MV^+$) formation in the presence of 16 mM MV$^{2+}$ versus glucose incubation time at 50°C. Previous to the reaction, the glucose was incubated in the buffer solution for various times ranging from 0 to 100 minutes. (a) 2 mM glucose at pH 11 (squares) or pH 12 (circles). (b) pH 12 at 1 mM glucose (squares) or 2 mM glucose (circles). Solid lines represent Equation 3-12.

Interestingly, the glucose exposure time to buffer prior to the reaction with MV$^{2+}$ had a significant impact. To further explore whether other carbohydrates are affected by the incubation time, fructose incubation was studied under the same conditions as glucose incubation (pH 12 with an initial fructose concentration of 2 mM and an initial MV$^{2+}$ concentration of 8 mM). As shown in Figure 3-3, the fructose reaction rate decreased with incubation time, which is opposite to the increase observed for glucose. At an incubation time of zero, the fructose reaction rate was much higher than that of glucose, similar to the findings in Table 3-1 at pH 11. Figure 3-3 also shows the fit of the rigorous model for the glucose data. As discussed below, it appears that there is a conversion between glucose and fructose when the carbohydrate is exposed to a buffer solution and this conversion can account for the observed results. Further studies would need to be performed to assess incubation time effects on other carbohydrates.
Figure 3-3. The initial rate ($r_{MV^+,i}$) of reduced methyl viologen ($MV^+$) formation in the presence of 2 mM fructose and 8 mM MV$^{2+}$ (triangles) or 2 mM glucose and 8 mM MV$^{2+}$ (circles) versus incubation time at pH 12 and 50$^\circ$C. Previous to starting the reaction, the carbohydrate was incubated in the buffer solution for various times. The solid line represents Equation 3-12.

3.4 Discussion

The initial reaction rates shown in Table 3-1 provided insights into general trends of the reaction of carbohydrates with MV$^{2+}$. The reaction rates generally conform to the following trend for mono-carbohydrate oxidation by MV$^{2+}$: hexoses $<$ pentoses $<$ trioses. The carbohydrate experiments shown in Table 3-1 were first analyzed by a second-order rate law as shown by Equation 3-1. This rate model properly accounted for the effects of pH (hydroxide concentration) and carbohydrate concentration. However, the slight dependency on MV$^{2+}$ concentration observed in the second study is not accounted for in Equation 3-1. In addition, the effect of the carbohydrate incubation time is not accounted for. When seeking to design a biofuel cell, it is important to characterize kinetic regimes in order to optimize the system. Thus, there is a need to develop a more rigorous rate model to account for the incubation time as well as the potential effects of MV$^{2+}$ concentration.
3.4.1 Reaction Mechanism

Figure 3-4 shows a proposed reaction mechanism developed by this study of MV$^{2+}$ reacting with glucose (G) and fructose (F). The mechanism shows that glucose (G) and fructose (F), upon reaction with the hydroxide ion, form rapid equilibrium species denoted as G’ and F’. Thus, glucose and fructose can interconvert. Rapid equilibrium has been described in the literature (Vuorinen & Sjostrom, 1982). MacLaurin and Green studied the kinetics between a glucose, fructose, and mannose system and determined that the reaction path between the glucose and fructose is the fastest between those carbohydrates, confirming interconversion between F and G. It was also determined that distinct enediolates form between F’ and G’ (not shown) and other pathways (shown as $E_G$ and $E_F$). As the enediolates are not very stable, they can form and react quickly (Anderson et al., 2012; Maclaurin & Green, 1969; Vuorinen & Sjostrom, 1982). Only through enediolate formation of $E_G$ and $E_F$ from G’ and F’, respectively, and not via enediolate formation between G’ and F’, was the mechanism and model consistent with the experimental results. The enediolates $E_G$ and $E_F$ are the suggested species that react with MV$^{2+}$. In addition, enediolate decomposition to other unknown species denoted as $D_G$ and $D_F$ was included, which is consistent with enediolate decomposition previously described (Vuorinen & Sjostrom, 1982).

![Figure 3-4. Proposed reaction mechanism for glucose, fructose, and their enediolates with methyl viologen (MV$^{2+}$). $G'$ and $F'$ are equilibrium species, E represents an enediolate, and D represents a decomposition product.](image-url)
3.4.2 Model for Initial Rate of MV\(^+\) Formation (\(r_{MV^+,i}\))

The following method and equations were used to obtain a more rigorous rate expression for \(r_{MV^+,i}\). First, based on Figure 3-4, \(r_{MV^+,i}\) was defined according to

\[
r_{MV^+,i} = k_{MF}(E_F)(MV^2+) + k_{MG}(E_G)(MV^2+) \tag{3-2}
\]

A pseudo-steady state hypothesis analysis was used to approximate the concentrations of \(E_G\) and \(E_F\) since enediolates are at very low concentrations relative to other species due to rapid formation and depletion (Anderson et al., 2012; Maclaurin & Green, 1969). The rapid equilibrium assumptions of \(G' = K_G(G)(OH^-)\) and \(F' = K_F(F)(OH^-)\) and a pseudo-steady state assumption for \(E_G\) and \(E_F\) were applied to predict the approximate concentrations of \(E_G\) and \(E_F\) according to Equations 3-3 and 3-4, respectively.

\[
E_G = \frac{k_{G,F}K_G(G)(OH^-)}{k'_G + k_{MG}(MV^2+)} \tag{3-3}
\]
\[
E_F = \frac{k_{F,F}K_F(F)(OH^-)}{k'_F + k_{MF}(MV^2+)} \tag{3-4}
\]

Here, \(k'_G = k_{G,r} + k_{G,d}\) and \(k'_F = k_{F,r} + k_{F,d}\). For this study, it was assumed that \(k'_G \approx k_{G,r}\) since decomposition products of glucose are relatively small (Vuorinen & Sjostrom, 1982). It was also assumed that \(k'_F \approx k_{F,r}\) is small over the incubation time studied since \(r_{MV^+,i}\) increased with incubation time and significant degradation of fructose would lead to decreasing \(r_{MV^+,i}\). These predictions were then substituted into Equation 3-2 to obtain \(r_{MV^+,i}\) when glucose is initially present as shown in Equation 3-5.
Here, \( k_{G,ratio} = k'_G / k_{MG} \) and \( k_{F,ratio} = k'_F / k_{MF} \) and \((G_0)\) is the initial glucose concentration. Interestingly, the ratio \((F)/(G_0)\) is present in Equation 3-5 and this ratio can change, especially during the incubation period where interconversion between fructose and glucose can occur. Thus, a method to estimate \((F)/(G_0)\) prior to the time \(MV^{2+}\) is added is required for parameterizing \(r_{MV^+,i}\) based on the data. Clearly, if glucose did not convert to fructose during incubation, then \((F)/(G_0)\) would be zero.

During glucose incubation in buffer in the absence of \(MV^{2+}\) (\(k_{MG}\) and \(k_{MF}\) are not applicable), Equations 3-6 and 3-7 give the change in the total glucose concentration, \((G^T) = (G) + (G')\), and the total fructose concentration, \((F^T) = (F) + (F')\), with incubation time \((t)\).

\[
\frac{\partial G^T}{\partial t_i} = -(k_{G,f} + k_1)(G') + k_2(F') + k_{G,r}(E_G) \tag{3-6}
\]

\[
\frac{\partial F^T}{\partial t_i} = -(k_{F,f} + k_2)(F') + k_1(G') + k_{F,r}(E_F) \tag{3-7}
\]

The rapid equilibrium assumptions of \((G^T) = (G)[1 + K_G(OH^-)]\) and \(G' = K_G(G)(OH^-)\) for glucose and \((F^T) = (F)[1 + K_F(OH^-)]\) and \(F' = K_F(F)(OH^-)\) for fructose are then substituted into Equations 3-6 and 3-7. In addition, Equations 3-3 and 3-4 (with \(MV^{2+} = 0\)) are also substituted to give the resulting Equations 3-8 and 3-9.
Equations 3-8 and 3-9 thus characterize the interconversion between glucose and fructose during incubation in a buffer solution in the absence of MV$_2^+$.

Equations 3-8 and 3-9 were solved to estimate $(F)/(G_0)$ with $t_i$. At the initial time, only glucose was considered present since this represented the experimental procedure. Based on Equations 3-8 and 3-9, $d(G) = -d(F)$. Thus, $(G) = (G_0) - (F)$. Substituting this relationship into Equation 3-9 and dividing both sides by $(G_0)$ gives

$$\frac{d[(F)/(G_0)]}{dt} = a - b [(F)/(G_0)]$$ \hspace{1cm} (3-10)

Where

$$a = \frac{k_1K_G(OH^-)}{[1 + K_F(OH^-)]}$$ \hspace{1cm} (3-11a)

$$b = \frac{(k_1K_G + k_2K_F)(OH^-)}{[1 + K_F(OH^-)]}$$ \hspace{1cm} (3-11b)

Integration of Equation 3-10 gives $(F)/(G_0) = [(F)/(G_0)]_{ss}(1 - e^{-bt_i})$ where $[(F)/(G_0)]_{ss}$ is the steady state value of $[(F)/(G_0)]$ and is equivalent to $a/b$. To estimate $[(F)/(G_0)]_{ss}$ and $b$, values for $k_1$ and $k_2$ were obtained from literature at 50°C and were 0.055 min$^{-1}$ and 0.026 min$^{-1}$, respectively (Vuorinen & Sjostrom, 1982). In addition, $K_F$ and $K_G$ were evaluated at 50°C as 20.4 M$^{-1}$ and 16.2 M$^{-1}$, respectively (Vuorinen & Sjostrom, 1982). Therefore, $[(F)/(G_0)]_{ss} = 0.63$, $b$
is 0.012 min\(^{-1}\) at pH 12, and 0.0014 min\(^{-1}\) at pH 11. This model for \((F)/(G_0)\) along with the assumption that \([k_{G,\text{ratio}} + (MV^{2+})] = [k_{F,\text{ratio}} + (MV^{2+})]\) was substituted into Equation 3-5 to give:

\[
 r'_{MV^+,i} = \frac{k_{G,f}K_G(G_0)(OH^-)(MV^{2+})}{k_{G,\text{ratio}} + (MV^{2+})} \left[ 1 + \left( \frac{k_{F,f}K_F}{k_{G,f}K_G} - 1 \right) \left( \frac{F}{G_0} \right) \right] \left( 1 - e^{-bt_i} \right) \quad (3-12)
\]

When fructose is initially present, a similar equation to Equation 3-12 is obtained except all terms with F are changed to G and all terms with G are changed to F.

The value for \(k_{F,f}\) relative to \(k_{G,f}\) was determined from Equation 3-12 and Figure 3-3. Basically, the right term in brackets in Equation 3-12 is unity at \(t_i = 0\). When \(r'_{MV^+,i}\) is independent of \(MV^{2+}\) then:

\[
 \frac{r'_{MV^+,i \ (\text{starting with } F)}}{r'_{MV^+,i \ (\text{starting with } G)}} = R = \frac{k_{F,f}K_F(F_0)}{k_{G,f}K_G(G_0)} \quad (3-13)
\]

From Figure 3-3, \(F_0\) and \(G_0\) were equivalent such that \(R\) is 7.7. Thus, \(k_{F,f} = 6.1 \ k_{G,f}\) according to Equation 3-13. Therefore, the only unknowns in Equation 3-12 are \(k_{G,f}\) and \(k_{G,\text{ratio}}\). All data in Figure 3-1 were regressed to Equation 3-12 to obtain \(k_{G,f} = 5.2 \times 10^{-3} \text{ s}^{-1}\) and \(k_{G,\text{ratio}} = 0.38 \text{ mM}\).

With the known ratio of \(k_{F,f}\) to \(k_{G,f}\), the value of \(k_{F,f}\) was determined to be \(3.1 \times 10^{-2} \text{ s}^{-1}\).

### 3.4.3 Data and Model Consistency

The data for the rate of \(MV^+\) formation as a function of \(t_i\), presented in Figure 3-1, have a nearly linear trend. This can be explained by Equation 3-12 where the part of the model that is effected by incubation time is \((1 - e^{-bt_i})\). When \(b\) is small, \(e^{-bt_i} \approx 1 - bt_i\). This is consistent for the values of \(b\) noted above. Therefore, \((1 - e^{-bt_i}) \approx bt_i\), which shows the model is consistent.
with the near linear trend of the experimental data. Another consistency of Equation 3-12 with the data is shown in Figure 3-3 where $r_{MV^+,i}$ decreased with incubation time starting with fructose and $r_{MV^+,i}$ increased with incubation time starting with glucose. When starting with glucose, the $[k_{G,f}K_F/k_{G,f}K_G - 1]$ term is positive, leading to an increase in $r_{MV^+,i}$ with incubation time. However, when starting with fructose, the term becomes $[k_{G,f}K_F/k_{F,f}K_F - 1]$, which is negative, leading to a decrease in $r_{MV^+,i}$ with incubation time.

Figure 3-5 shows the predicted initial rate versus the experimental rate for all of the data. The line shows where each data point would lie if the experimental results and the model were in complete agreement. The solid lines in Figures 3-2 and 3-3 also show the model fits for the experimental data. In general, the model did very well in capturing the dependence of $MV^{2+}$ concentration, pH, carbohydrate concentration, and incubation time on $r_{MV^+,i}$. Generally, when the $MV^{2+}$ concentration is $>> k_{G,ratio}$ then the $MV^{2+}$ dependence disappears. Thus, there is a first-order dependence on $MV^{2+}$ concentration at very low concentrations and no dependence at higher concentrations when $MV^{2+} > 10k_{G,ratio}$ (around 3.8 mM for the carbohydrates in this study).
Figure 3-5. Actual vs predicted initial rate ($r_{MV^+,i}$) of reduced methyl viologen ($MV^+$) formation of data in Figure 3-1. The MV$^{2+}$ concentration ranged from 2-16 mM and the incubation time ranged from 0-100 min. Each point represents an experimental data point and the line shows where each data point would lie if the experimental results and model were the same values.

To compare with the carbohydrate studies shown in Table 3-1 at pH 11, which utilized an MV$^{2+}$ concentration of 11.8 mM and an incubation time of 5 minutes, Equation 3-12 becomes $r_{MV^+,i} = (1.03)k_GK_G(G)(OH^-)$. By comparing this with Equation 3-1, the glucose $k$ value in Table 3-1 is equivalent to $1.03k_{G,f}K_G$. Thus, with $K_G = 16.2$, the equivalent glucose $k_G$ for Table 3-1 would be $3.5 \times 10^{-3}$ s$^{-1}$, which is generally consistent with the fitted parameter. However, it should be noted that the fitted $k_G$ came from a much larger set of experimental data over a wider range of experimental conditions.

Characterization of the homogenous reaction rate of MV$^{2+}$ and glucose provides guidance on how to optimize the design of a biofuel cell. For example, from the results of the change in $r_{MV^+,i}$ for fructose versus glucose, one may want to allow glucose to incubate before feeding the solution into the biofuel cell, but the same process with fructose would decrease the rate of electron extraction from the carbohydrate. The model for $r_{MV^+,i}$ also provides insights into the effects of pH, MV$^{2+}$ concentration, and incubation time that can be useful for design consideration.
3.5 Conclusions

Values for $r_{MV^+,\ell}$ (and the associated $k$ values) for various monosaccharides were reported (Dr. Watt’s data). The value of $r_{MV^+,\ell}$ generally increased as the number of carbons in the carbohydrates decreased. Using glucose as the model carbohydrate, a rigorous model was developed to show the dependence of MV$^{2+}$ concentration, pH, carbohydrate concentration, and incubation time on $r_{MV^+,\ell}$. Model predictions agreed very well with experimental data. The effects of incubation time for a glucose system were consistent with interconversion between glucose and fructose. The OH$^-$ concentration dependence was first order; thus, a pH increase from 11 to 12 would result in a 10-fold increase in the reaction rate. Similarly, the glucose dependence was first order. The effect that the concentration of MV$^{2+}$ has on $r_{MV^+,\ell}$ is a bit more complicated. There is MV$^{2+}$ dependence at lower concentrations; however, as the concentration of MV$^{2+}$ increases (to values about 10-fold larger than the $k_{G,\text{ratio}}$, or 3.8 mM), $r_{MV^+,\ell}$ is no longer dependent on the MV$^{2+}$ concentration. The model for $r_{MV^+,\ell}$ also provides insights into the effects of pH, MV$^{2+}$ concentration, and incubation time that can be useful for biofuel cell design consideration.
4. DECOMPOSITION OF VIOLOGEN CATALYST FOR BIOFUEL CELL APPLICATIONS

4.1 Background

As mentioned before, both fuel technology and alternative sources of energy are being researched and developed to help meet rising energy demands. One specific fuel technology that has been developed is the biofuel cell. One challenge with biofuel cells is that in order to produce viable amounts of electricity, a catalyst is needed. A proper catalyst increases the rate at which electrons are extracted from the fuel source and deposited to the electrode of the biofuel cell. Watt identified viologens as an effective catalyst for carbohydrate oxidation in a biofuel cell (Corbin & Watt, 1990; Wheeler et al., 2009). One downside to viologen catalysts is the potential degradation issues at high pH or MV$^{2+}$ concentration (Farrington et al., 1969; Rieger & Edwards, 1988). This chapter addresses the significance of MV$^{2+}$ degradation and the impact that it may have for biofuel cell applications.

Farrington et al. proposed that the oxidized form of MV$^{2+}$ decomposes into the reduced form of MV$^{+}$ at pH higher than 10 by the following reaction (Farrington et al., 1969).

$$3MV^{2+} + 2OH^- \rightarrow MMV^+ + 2MV^+ + H_2O + C_2H_2O + H^+$$  \hspace{1cm} (4-1)

The proposed reaction has several steps in which the reaction gives a first order dependence of both MV$^{2+}$ and OH$^-$, shown by Equation 4-2. The conditions for the experiments performed by
Farrington were an MV\(^{2+}\) concentration of 1.0 to 100 mM and OH\(^-\) concentrations of 0.01-0.5 M (pH 12 to 13.7).

\[ r_{MV^{+},i} = 2k[OH^-][MV^{2+}] \]  

(4-2)

Another proposed mechanism for the reaction of MV\(^{2+}\) degradation with data taken at lower temperatures (24\(^\circ\)C) is described by Rieger and Edwards (Rieger & Edwards, 1988). This mechanism proposes an initial rate of formation of MV\(^+\) \( (r_{MV^{+},i}) \) from the decomposition of MV\(^{2+}\) at high pH as

\[ r_{MV^{+},i} = 2k[OH^-][MV^{2+}]^2 \]  

(4-3)

The mechanism proposed by Rieger states that the rate of decomposition of MV\(^{2+}\) has a second order dependence on the MV\(^{2+}\) concentration and a first order dependence on the hydroxide concentration at high pH. However, Rieger reported that the order of the hydroxide concentration ranged from 1.0 to 1.4 although 1.0 was recommended (Rieger & Edwards, 1988). For these studies, the MV\(^{2+}\) concentration ranged from 8.2 to 66 mM and the OH\(^-\) concentration ranged from 0.03 to 1.78 M (pH 12.5 and higher); only initial rate data were collected.

This paper presents MV\(^{2+}\) degradation data at 40\(^\circ\)C and 50\(^\circ\)C instead of at the 24\(^\circ\)C that Rieger reported, as well as at lower pH values than Rieger reported. An activation energy was found from both experimental data reported in this chapter at 40\(^\circ\)C and 50\(^\circ\)C as well as Rieger’s data at 24\(^\circ\)C. This study also proposes a mechanism and model for the decomposition of MV\(^{2+}\), which addresses the variability in the dependence of the decomposition of MV\(^{2+}\) on the
concentrations of OH\textsuperscript{-} and MV\textsuperscript{2+}. Some insight into decomposition with time, not just initial rate, is also provided.

4.2 Materials and Methods

For all experiments, a pH 11 or pH 12 potassium phosphate (monobasic) buffer was used. The methyl viologen (MV\textsuperscript{2+}) is 98% pure (Acros Organics, Fisher Scientific, Waltham, MA). Because oxygen can oxidize MV\textsuperscript{+} back into MV\textsuperscript{2+}, all experiments were conducted under anaerobic conditions; therefore, careful lab procedures were followed to ensure that the solutions used for the experiments were oxygen free. A 1.0 M potassium phosphate buffer was made using deionized water. NaOH was added until the desired pH of 11 or 12 was reached. The buffer solutions were then purged with nitrogen before being placed in an anaerobic chamber. Inside the chamber, 3.5-mL cuvettes were filled with the desired volume of buffer. The cuvettes were capped, removed from the chamber, and placed inside an incubator set at the desired temperature of 40°C or 50°C. A stock solution (2 M) of MV\textsuperscript{2+} in deionized was also prepared. The MV\textsuperscript{2+} solution was then purged with nitrogen and heated to the desired temperature of 40°C or 50°C. The buffer cuvette was placed in the spectrophotometer, which contained a heating block to maintain the desired reaction temperature. Through a gas-tight syringe, MV\textsuperscript{2+} stock solution was added to the cuvette to obtain a desired overall concentration of 0.1, 0.2, or 0.3 M MV\textsuperscript{2+}. Absorbance data with time were collected.

Absorbance data were collected at 730 nm as a function of time and an extinction coefficient of 2737 M\textsuperscript{-1} cm\textsuperscript{-1} was used to convert absorbance into concentrations using Beer’s law. This extinction coefficient value had been determined with previous work in the lab using known MV\textsuperscript{2+} concentrations as mentioned in Section 3.2. Depending on the conditions of the experiment,
different absorbance with time results were obtained, with slightly different shapes to the curve. However, for this work, initial slopes were evaluated in all cases by using the data points in the initial slope range, which were always linear.

Additional studies were performed to measure the decomposition of additional viologens to compare their stability to the stability of MV$_{2}^{+}$. The oxidized monomethyl viologen (MMV$^{+}$) was synthesized and provided by Dr. Gary Watt in the Chemistry department. The protocol for measuring MMV$^{+}$ decomposition was the same as that for MV$_{2}^{+}$. However, an extinction coefficient for the reduced form of MMV$^{+}$ (denoted as MMV) was needed at 730 nm in order to measure absorbance with time and convert that to a concentration using Beer’s law. The following method was used to calculate the MMV extinction coefficient. First, a pH 11, 1 M KH$_{2}$PO$_{4}$ buffer solution was made by dissolving the necessary amount of KH$_{2}$PO$_{4}$ into deionized water and adding NaOH until the desired pH was obtained. A 10 mM MMV$^{+}$ solution was made by weighing the desired amount of MMV$^{+}$ and adding it to deionized water. Both solutions were purged with nitrogen. Additionally, a 10 mM sodium dithionite (DT) solution was made in the anaerobic glovebox with buffer solution as the solvent. After all solutions were prepared, 50 µL DT was added to 1.5 mL buffer and the absorbance of the solution was measured (Figure 4-1). The absorbance at 315 nm was used to verify the concentration of DT found using an extinction coefficient of 8043 M$^{-1}$cm$^{-1}$ for DT at 315 nm, reported by Yousafzai and Eady (Yousafzai & Eady, 2002).

Next, 100 µL MMV$^{+}$ and 5 µL DT were added to a new 3.5-mL cuvette with 2 mL buffer under anaerobic conditions. A spectrum of MMV was obtained and the absorbance at 600 and 730 nm was recorded. It should be noted from Figure 4-1 that DT does not absorb at either of these wavelengths. After the spectrum from the initial DT addition was obtained, incremental
amounts (typically 3 µL) of DT were added and a spectrum was obtained after each addition of DT to measure the absorbance at both 600 and 730 nm following the addition of each incremental amount.

Figure 4-1. Spectrum of 10 µL DT in buffer solution.

One example of a wavelength vs. absorbance plot is shown in Figure 4-2, taken after 7 µL of DT was added to the MMV+ solution.

Figure 4-2. Spectrum of 100 µL MMV+ and 7 µL DT.
A plot of DT concentration versus absorbance (divided by 2) at the desired wavelength was obtained for each run. The slope of this plot is equal to the extinction coefficient of MMV at the given wavelength. The y-axis is the absorbance divided by 2 because one mole of DT reacts with 2 moles of MMV$^+$.  

Figure 4-3. DT Concentration versus absorbance/2 at 730 nm. The slope is the extinction coefficient of MMV at 730 nm.  

A total of 4-5 runs were completed and used to find the average extinction coefficient of MMV at both 600 and 730 nm. Table 4-1 shows the measured extinction coefficient for each run as well as the averages. The extinction coefficient of MMV at 600 nm was $9930 \pm 413 \text{ M}^{-1} \text{ cm}^{-1}$ and the extinction coefficient of MMV at 730 nm was $1955 \pm 85 \text{ M}^{-1} \text{ cm}^{-1}$. MMV$^+$ decomposition studies were only performed at 730 nm, although the value at 600 nm was also obtained since many researchers use 600 nm for viologen studies.
Table 4-1 MMV Extinction Coefficient (M⁻¹ cm⁻¹) at 600 and 730 nm

<table>
<thead>
<tr>
<th></th>
<th>Extinction Coefficient at 600 nm</th>
<th>Extinction Coefficient at 730 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10196</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>9298.9</td>
<td>1859.7</td>
<td></td>
</tr>
<tr>
<td>10402</td>
<td>2080.5</td>
<td></td>
</tr>
<tr>
<td>9596.6</td>
<td>1896.3</td>
<td></td>
</tr>
<tr>
<td>10158</td>
<td>1982.2</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>9930 ± 413</td>
<td>1955 ± 85</td>
</tr>
</tbody>
</table>

Once an extinction coefficient for MMV was found, studies of the decomposition of MMV⁺ were performed with the same method described above for MV²⁺. MMV⁺ decomposition studies were performed at initial concentrations of 0.1, 0.2, and 0.3 M, pH 12, and 50°C.

The decomposition rates of isopropyl viologens stabilized with bromine or chlorine (IPV-Br and IPV-Cl) as well as MV²⁺ were also measured, except these rates were measured electrochemically. These experiments were conducted by another BYU graduate student, Meisam Bahari, and are included in this thesis for comparison purposes later in the chapter. For these electrochemical experiments, solutions of each viologen solution were prepared and deoxygenated. Inside an anaerobic glove box, IPV-Br, IPV-Cl, or MV was injected into a pH 12 buffer solution so the final viologen concentration of the solution was 50 mM. The solution was put in a heating block set at 50°C and left to allow the decomposition reaction to occur. A small amount of the solution was taken at incremental times and injected into an electrochemical cell, which was also in the glove box. Current with time was measured. The measured current from the electrochemical cell was converted to the concentration of the solution. This was done at multiple times to obtain concentration versus time data.
4.3 Results

Figure 4-4 shows MV$^+$ absorbance as a function of time for a 0.2 M MV$^{2+}$, pH 11, 40°C run measuring the decomposition of MV$^{2+}$. Note that Figure 4-4 has 3 different slopes. Many runs, especially those at pH 12 and 50°C, were linear during the entire time the reaction proceeded (until absorbance reached a value of 2, which was the limit of the spectrophotometer). The experimental conditions at lower concentrations, pH, and temperature allowed data to be collected over longer periods of time before reaching an absorbance of 2. This gave some greater insight into how the decomposition reaction proceeds. More explanation is given below in the discussion section. As previously stated, the initial rate of MV$^+$ formation, based on the initial slope and the extinction coefficient, was obtained for each experiment. As shown in Figure 4-4, the initial slope represents the maximum decomposition rate since this is the region in which the rate of MV$^+$ formation was the largest.

![Figure 4-4. Absorbance versus time (s) of MV$^+$ following decomposition 0.2 M MV$^{2+}$ at pH 11 and 40°C.](image)
Figure 4-5 shows the initial rate of $\text{MV}^+$ formation (nM/s) versus the initial $\text{MV}^{2+}$ concentration (M). For this figure, each filled circle represents the initial slope obtained from each decomposition experiment. Experiments were conducted at 40°C and 50°C, pH 11 and 12, and an initial $\text{MV}^{2+}$ concentration of 0.1, 0.2, or 0.3 M. The line represents a model (described more in the discussion section) that predicts the initial rate for at each temperature, pH, and $\text{MV}^{2+}$ concentration. Good agreement is observed. As previously mentioned and shown in literature, an increase in $\text{MV}^{2+}$ concentration, pH, and temperature all increase the initial rate of $\text{MV}^+$ formation. More specifically, an increase in pH from pH 11 to pH 12 increases the initial rate of $\text{MV}^+$ formation by about 100-fold. If the rate had a first order dependence on $\text{OH}^-$ concentration, the rate would only increase by 10-fold. Therefore, it is clear that a second order dependence exists for the $\text{OH}^-$ concentration for the initial rate of $\text{MV}^+$ formation.

Figure 4-5. Initial rate of $\text{MV}^+$ formation (nM/s) versus the initial $\text{MV}^{2+}$ concentration (M). Each filled circle represents an experiment. The line represents Equation 4-8 (described more in the discussion section) that predicts the initial rate.
As mentioned before, a few additional studies of other viologens were performed to compare the stability of MV$^{2+}$ to MMV$^+$, IPV-Br, and IPV-Cl. **Table 4-2** shows the rates of MMV formation at initial MMV$^+$ concentrations of 0.1, 0.2, and 0.3 M, pH 12, and 50°C.

<table>
<thead>
<tr>
<th>[MMV$^+$]$_0$ (M)</th>
<th>Rate (nM/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>45</td>
</tr>
<tr>
<td>0.1</td>
<td>41</td>
</tr>
<tr>
<td>0.2</td>
<td>277</td>
</tr>
<tr>
<td>0.2</td>
<td>231</td>
</tr>
<tr>
<td>0.3</td>
<td>891</td>
</tr>
<tr>
<td>0.3</td>
<td>896</td>
</tr>
</tbody>
</table>

The data for the rate of MMV formation was taken under the same conditions as **Figure 4-5d**. By comparing the formation rates of MV$^+$ and MMV at the three viologen concentrations, the rate of MV$^+$ formation is much higher than the rate of MMV formation. At 0.1 M viologen, the rate of MV$^+$ formation is about 700 nM/s, while the rate of MMV formation at 0.1 M is only about 43 nM/s. Additionally, at 0.2 M and 0.3 M, the rates of MV$^+$ formation and the rates of MMV formation are about 2600 nM/s versus 254 nM/s and 5500 nM/s versus 894 nM/s. MMV$^+$ is shown to be a more stable viologen than MV$^{2+}$.

**Figure 4-6** shows the percentage of the concentration of the reduced viologen divided by the initial concentration of the viologen (either MV$^{2+}$ or MMV$^+$). This data from this figure was collected at 0.3 M viologen, pH 12, and 50°C. Note that the MMV$^+$ decomposes much slower than the MV$^{2+}$. 

40
Figure 4-6. Percentage of the concentration of the reduced viologen divided by the initial concentration of the viologen. The top solid line is for MV$_2^+$ decomposition and the bottomed dotted line is for MMV$^+$ decomposition.

Table 1-3 and Figure 4-6 show rates of the decomposition of MMV$^+$ as well as the percent of the viologen that decomposed. These results showed that MMV$^+$ is more stable than MV$_2^+$. For the data used in Figure 4-6 (pH 12, 50°C, and 0.3 M viologen), MMV$^+$ decomposed 6.4 times slower than MV$_2^+$. For all the runs shown in Table 1-3, MMV$^+$ decomposed anywhere from 6.2 – 16.1 times slower than MV$_2^+$. This results again show that MMV$^+$ decomposes slower and is more stable than MV$_2^+$.

The decomposition of IPV-Br, IPV-Cl, and MV$_2^+$ (denoted as MV-Cl) were measured using the electrochemical method explained in section 4.2 and the results are displayed in Figure 4-7. The figure shows the percent of the reduced species relative to the initial oxidized species that decomposed as a function of time. The percentage for IPV-Br is shown by the diamonds, the percentage for IPV-Cl is displayed as triangles, and the percentage for MV$_2^+$ is shown by squares. The MV$_2^+$ decomposed more slowly than the IPV-Br or IPV-Cl.
Figure 4-7. Decomposition of IPV-Br, IPV-Cl, and MV-Cl measured electrochemically and displayed as a percentage of the reduced viologen. Initial concentrations of viologens was 50 mM.

*Figure 4-7* shows the decomposition results of IPV-Cl and IPV-Br compared to MV$^{2+}$, measured electrochemically. The figure shows the percent of the species that decomposed as a function of time. These results (collected by Meisam Bahari, BYU graduate student) show that MV$^{2+}$ is more stable than IPV-Cl and IPV-Br since the percentage of MV$^{2+}$ that decomposed with time was lower than the percent degradation of IPV-Cl and IPV-Br.

These studies on the decomposition rates of MMV$^+$, IPV-Cl, and IPV-Br were performed to compare potential options of other viologens to MV$^{2+}$ for a biofuel cell application. MMV$^+$ was found to be the most stable viologen of the four viologens studied in this thesis and future work will further address the use of MMV$^+$ instead of MV$^{2+}$ for biofuel cell applications. The reaction of MMV$^+$ with a carbohydrate would need to be studied to determine the feasibility of using it in a biofuel cell.
4.4 Discussion

4.4.1 MV$^{2+}$ Decomposition Model

As mentioned above, the absorbance versus time graph displayed in Figure 4-4 has 3 distinct slopes in the range of times considered. The reaction mechanism proposed below does not fully explain why 3 slopes are seen. It is possible that an equilibrium is reached prior to initiation of a subsequent reaction, but this has not been confirmed. Although the mechanistic model described below does not account for all 3 slopes, the initial slope was used for modeling and represents the worse-case scenario since the initial slope shows the greatest rate of decomposition.

A mechanistic model was developed to describe the initial decomposition of MV$^{2+}$ and link the experimental work reported above with the work of Rieger and the work of Farrington (Farrington et al., 1969; Rieger & Edwards, 1988). Equation 4-4 through Equation 4-7 represent the proposed mechanism for the decomposition of MV$^{2+}$.

\[
\begin{align*}
MV^{2+} + OH^- & \xrightarrow{k_1} R^+ + H_2O \\
\xleftarrow{k_2} \\
R^+ + OH^- & \xrightarrow{k_3} ROH \\
\xleftarrow{k_4} \\
ROH + MV^{2+} & \xrightarrow{k_5} MV^+ + R^{2+} + OH^- \\
\xleftarrow{k_6} \\
R^{2+} + MV^{2+} + 2OH^- & \xrightarrow{k_7} MMV^+ + MV^+ + CH_2(OH)_2 \\
\xleftarrow{k_7}
\end{align*}
\]
The mechanism is a modification of the mechanism proposed by Rieger (Rieger & Edwards, 1988), which also only modeled the initial decomposition rate, to allow for a variation in the order of the rate law for both MV$^{2+}$ and OH$^-$ concentrations. The modification expands the second reaction proposed by Rieger into two reactions (Equations 4-5 and 4-6) to show that the rate law can be second order with respect to both MV$^{2+}$ and OH$^-$ concentrations, first order with respect to both MV$^{2+}$ and OH$^-$ concentrations, or have some transition between the two, depending on the MV$^{2+}$ concentration and pH.

From a pseudo-steady state hypothesis on ROH, R$^+$, and R$^{2+}$, Equation 4-8 is obtained.

$$r_{MV^+,i} = \frac{2k_1k_3k_5(OH^-)^2(MV^{2+})^2}{k_2k_4(H_2O) + k_2k_5(MV^{2+})(H_2O) + k_3k_5(MV^{2+})(OH^-)}$$  (4-8)

Here, $r_{MV^+,i}$ represents the initial rate of MV$^+$ formation due to the decomposition of MV$^{2+}$. The conditions of the experiments in this work were low MV$^{2+}$ concentrations and low pH values, essentially meaning that $k_2k_5(MV^{2+})(H_2O)$ and $k_3k_5(MV^{2+})(OH^-)$ could be negligible compared to $k_2k_4(H_2O)$. In such cases, Equation 4-8 simplifies to

$$r_{MV^+,i} = \frac{2k_1k_3k_5(OH^-)^2(MV^{2+})^2}{k_4k_2(H_2O)} = 2k_0(OH^-)^2(MV^{2+})^2$$  (4-9)

Here, $k_0 = k_1k_3k_5/[k_4k_2(H_2O)]$. Thus, according to the proposed mechanism, the initial rate is expected to be second order in OH$^-$ and second order in MV$^{2+}$ for low MV$^{2+}$ concentrations and low pH values. Equation 4-9 fits the data from this thesis well; therefore, MV$^{2+}$ concentrations up to 0.3 M and pH up to 12 are categorized as low and still allow for the $k_2k_5(MV^{2+})(H_2O)$ and
$k_3k_5(MV^{2+})(OH^-)$ terms to be considered negligible compared to $k_2k_4(H_2O)$. Equation 4-9 is the applicable rate law any time $k_2k_5(MV^{2+})(H_2O)$ and $k_3k_5(MV^{2+})(OH^-)$ terms are negligible compared to $k_2k_4(H_2O)$.

All data points for $r_{MV^+,i}$ in Figure 4-5 were regressed in Python using a model of $r_{MV^+,i} = 2k_0(MV^{2+})^\alpha(OH^-)^\beta$ to assess the order dependence of the experimental results and to compare that dependence with the predicted order in Equation 4-9. The Python fit was $\alpha = 2.55$ and $\alpha = 2.02$ for 40°C and 50°C, respectively, as well as $\beta = 1.99$ and $\beta = 2.01$ at 40°C and 50°C, respectively. This led to the assumption that $\alpha$ and $\beta$ were each 2 since an $\alpha$ value of 2.55 is not seen or common for rate laws and all other parameters were consistent with Equation 4-9. The value for $k_0$ was 0.31 s^{-1} M^{-3} at 50°C and 0.092 s^{-1} M^{-3} at 40°C. As shown in Figure 4-5, the data fit very well with a second order hydroxide rate law. This can easily be seen by comparing the pH 11 versus pH 12 data in Figure 4-5 at both 40°C and 50°C. At both temperatures, the initial rate of MV\(^+\) formation increased 100-fold as the pH increased from 11 to 12. A 10-fold increase would be seen with a first order hydroxide rate law and the 100-fold increase would be seen with a second order hydroxide rate law. As mentioned in the introduction section, the effect of the MV\(^{2+}\) concentration on the rate of MV\(^{2+}\) decomposition was observed as second order by Rieger and first order by Farrington. Both reported a first order OH\(^-\) dependency (although Rieger mentioned the OH\(^-\) dependency increased to an order of 1.4 for some experiments without a mechanistic explanation) and the initial rate data in this thesis showed second order OH\(^-\) dependency.

Equation 4-8 can describe how a varying dependence on OH\(^-\) concentration is possible, although Rieger’s rate law cannot be fully derived from this equation. The rate law presented in Equation 4-8 shows the possibility of second order dependence by Equation 4-9. To get a first order dependency of each species, the last term on the bottom of Equation 4-8
\((k_3 k_5 (MV^{2+})(OH^-))\) must dominate. One instance in which this would happen is at high MV\(^{2+}\) and high OH\(^-\) concentrations, or when \(k_3 k_5 (MV^{2+})(OH^-) \gg k_2 k_4 (H_2O)\) and \(k_2 k_5 (MV^{2+})(H_2O)\). These conditions simplify **Equation 4-8** to

\[
 r_{MV^{+},i} = 2k_1 (OH^-)(MV^{2+})
\]  

(4-10)

**Equation 4-10** is a rate law that is first order in both OH\(^-\) and MV\(^{2+}\). Under any conditions in which \(k_3 k_5 (MV^{2+})(OH^-)\) is much larger than the other two terms in the denominator of **Equation 4-8**, **Equation 4-10** will be the resulting rate law.

Although neither **Equation 4-9** nor **Equation 4-10** are the rate laws that Rieger proposed, there is a transition region between first and second order MV\(^{2+}\) and OH\(^-\) and **Equation 4-8** is still consistent conceptually with the work of Rieger. It is possible that with the range of Rieger’s data, what appeared to fit best was first order OH\(^-\) and second order MV\(^{2+}\) because she was in the transition region between the two since the pH was very high for the reported studies of Rieger. The \(k\) values at various points from Figures 1 and 2 in Rieger’s paper were found using **Equation 4-3** and then plotted. This plot shows that in the higher pH range the \(k\) values are basically constant (as they should be), but at the lower pH range, a linear trend is seen and the \(k\) values are not constant. Figure 1 in Rieger’s paper was obtained by collecting data at a single lower pH value of 12.5 with varying MV\(^{2+}\) to verify the MV\(^{2+}\) dependence of 2. Figure 2 was performed at a low MV\(^{2+}\) value with varying high pH to verify the OH\(^-\) dependence of 1 (Rieger & Edwards, 1988). It would have been valuable to vary both pH and MV\(^{2+}\) at the same time to rigorously validate the model. It should be noted that Rieger’s model does not show the possibility of a transition between both first and second order for both MV\(^{2+}\) and OH\(^-\) although there is clear second-order OH\(^-\)
dependence from this work and a proposed first-order dependence in MV$^{2+}$ from the Farrington work.

Farrington proposed a rate law that was first order for both MV$^{2+}$ and OH$^-$, although conditions for the experiments were not always at high MV$^{2+}$ concentrations and high pH values. However, experimental data were not utilized to validate the proposed rate law. In addition, it was also not clear if data were evaluated at initial rates or at later times. As previously noted, Figure 4-4 shows that the rates change with time. For this work and the work of Rieger, initial rates were modeled, which represents the maximum rate that would occur.

Again, Equation 4-8 allows for first or second order OH$^-$ and MV$^{2+}$ dependence as well as a transition region (such as 1.4 order in OH$^-$ mentioned by Rieger), which is in agreement conceptually with the work reported by Rieger and Farrington. However, further work is needed to refine the decomposition mechanism. For instance, Equation 4-7 (also proposed by Rieger) is not an elementary reaction and needs to be decomposed into elementary reaction steps for proper kinetic analysis. Also, Figure 4-4 shows several possible reactions could be occurring at different times and this work only captured the initial reaction kinetics. It is possible that other reactions orders could occur during different regions. However, understanding the initial reaction kinetics is valuable since this appears to be the time at which the highest decomposition rate occurs.

4.4.2 MV$^{2+}$ Decomposition Model Analysis

Figure 4-8 shows the rate data for pH 11 at both 40°C and 50°C that was previously shown in Figure 4-5. The filled circles represent data points, the lines are the model (Equation 4-9, second order hydroxide), and the open circles represent where the model would be if it were first order in hydroxide. It is clear to see that the assumption of second-order hydroxide fits the data at pH 11 much better than a first-order hydroxide model.
Figure 4-8. Initial Rate of MV⁺ formation (nM/s) versus the initial MV²⁺ concentration (M) with conditions of (a) pH 11, 40°C and (b) pH 11, 50°C. The filled circles represent data points. The line represents the model values with second-order hydroxide concentration (Equation 4-9). The open circles correspond to what the initial rate values would be if the model were first order in hydroxide concentration.

Figure 4-9 is a plot of the actual initial rate of MV⁺ formation versus the predicted initial rate of MV⁺ formation with the solid line representative of where each point would lie if the model and experimental data were exactly the same values. This figure along with Figure 4-5 both show that the decomposition rate model is a good fit.

Figure 4-9. Actual versus predicted initial rate of MV⁺ formation (nM/s). Each point represents an experiment. The solid line is where all points would lie if the model and experimental data were exactly the same.
As mentioned previously in section 4.1, Rieger and Edwards performed decomposition experiments for \( \text{MV}^{2+} \) at 24°C. Work presented in this chapter reported decomposition rates of \( \text{MV}^{2+} \) at 40°C and 50°C. In order to solve for an activation energy for the rate of formation of \( \text{MV}^{+} \) from the decomposition of \( \text{MV}^{2+} \), a \( k_0 \) value from Rieger’s data at 24°C was needed. Using data from Figure 1 from Rieger (pH 12.5, varying low \( \text{MV}^{2+} \) concentration data- similar to this study), an average \( k_0 \) value of \( 5.9 \pm 1.0 \times 10^{-3} \text{ s}^{-1} \text{ M}^{-3} \) was obtained based on Equation 4-9. Using the three \( k_0 \) values from the 24°C, 40°C, and 50°C data, an activation energy for the rate of formation of \( \text{MV}^{+} \) from the decomposition of \( \text{MV}^{2+} \) was found by plotting \( \ln(k_0) \) versus \( 1/\text{Temperature} \) shown in Figure 4-10. The slope is -17498, which corresponds to an activation energy of 145 kJ/mol.

![Figure 4-10](image)

**Figure 4-10.** Plot of \( \ln(k_0) \) versus \( 1/\text{Temperature} \) from \( k_0 \) values at 24°C (Rieger’s data), 40°C, and 50°C to find the activation energy for the decomposition of \( \text{MV}^{2+} \).

### 4.4.3 \( \text{MV}^{2+} \) Decomposition Model Application

The purpose of the work done for this chapter was to measure and model the decomposition rate of viologens and compare the decomposition rates to the rate of electron extraction for carbohydrates in order to determine if a biofuel cell is capable of producing viable amounts of electricity without appreciable decomposition of the catalyst. For comparison purposes, the reaction between \( \text{MV}^{2+} \) and glucose is used. Previously, in Chapter 3, an initial rate model for the
rate of MV\(^+\) formation for the rate of electron extraction for glucose was derived. By combining that rate law with the initial rate of MV\(^+\) formation from decomposition (which is the maximum rate), Equation 4-11 is derived.

\[
\frac{dr_{MV^+}}{di} = \frac{k_G k_C(G)(OH^-)(MV^{2+})}{k_{MG} + (MV^{2+})} + 2k_0(MV^{2+})^2(\text{OH}^-)^2 \tag{4-11}
\]

With rearrangement of Equation 4-11, Equation 4-12 and Equation 4-13 result.

\[
r_{MV^+} = \frac{k_G k_C(G)(OH^-)(MV^{2+})}{k_{MG} + (MV^{2+})} [1 + f] \tag{4-12}
\]

\[
f = \frac{2k_0}{k_{MG} k_C(G)} \frac{(MV^{2+})(\text{OH}^-)}{[MV^{2+}] + [OH^-]^2} \tag{4-13}
\]

Equation 4-12 assumes the maximum rate for decomposition, which represents the worse-case scenario. In order for the rate of electron extraction to be significantly greater than the decomposition rate, \(f\) must be \(< < 1\). If \(f = 0.1\), this signifies that the amount of MV\(^+\) that is formed from decomposition is about one tenth of the amount of MV\(^+\) formed from electron extraction.

Figure 4-11 is a plot of the ratio of the initial MV\(^{2+}\) and glucose concentrations versus \(f\). This figure shows the safe operating region (below the 0.1 line) for different pH values and \([MV^{2+}]_0/[G]_0\) ratios. From part (c) of this figure, it is determined that at pH 11 and initial concentrations of glucose of 100 mM or less, a safe operating range occurs at \([MV^{2+}]_0/[G]_0\) ratios of at least 10 or less. In other words, it is fairly easy to stay within feasible operating ranges with biofuel cell conditions of pH 11 and initial concentrations of glucose of 100 mM or less with a \([MV^{2+}]_0/[G]_0\) ratio of 10. Increasing the concentration of glucose at pH 11 requires \([MV^{2+}]_0/[G]_0 \ll 1.6\) at 5 M glucose (the high end of possible glucose concentrations) or higher ratios at lower glucose concentrations. For pH 12, operation at \([MV^{2+}]_0/[G]_0 \leq 4\) is recommended for a glucose concentration of 100 mM, and at \([MV^{2+}]_0/[G]_0 \leq 0.5\) for a glucose
concentration of 5 M. The objective of Figure 4-11 is to show the recommended operating range for MV$^{2+}$/glucose biofuel cells under different conditions while ensuring that at least a viable amount of energy could be produced, or that the decomposition is not significant compared to the $r_{MV^+}$ from the MV$^{2+}$ and glucose reaction.

Figure 4-11. Operating graphs for an MV$^{2+}$/glucose biofuel cell all for 50°C. The solid line in each graph represents an f value of 0.1, the value at which the operating biofuel cell should stay under for efficient operation. (a) pH 12 with initial glucose concentrations of 1mM (circles), 10 mM (dashes), 40 mM (squares), 80 mM (triangles), and 100 mM (diamonds). (b) pH 12 with initial glucose concentrations of 0.1 M (circles), 1 M (dashes), and 5 M (squares). (c) pH 11 with initial glucose concentrations of 1 mM (circles), 10 mM (dashes), 40 mM (squares), 80 mM (triangles), and 100 mM (diamonds). (d) pH 11 with initial glucose concentrations of 0.1 M (circles), 1 M (dashes), and 5 M (squares).

To emphasize, the rate of MV$^+$ formation data was analyzed at the highest decomposition rate (which occurs at the initial time), meaning the suggested operating ranges presented in Figure
4-11 are worse-case scenarios and it is possible that these operating ranges could broaden in actual operation. Figure 4-11 is under conditions of 50°C and pH 11 or 12.

Although an analysis for biofuel cell applications was not performed at pH 13 or higher (which is the range of the Rieger work), it is very unlikely that the operation conditions in a MV²⁺ biofuel cell would be pH 13 or higher. The reason for this is that the decomposition of MV²⁺ at pH 13 would be at least 10-fold or larger compared to pH 12 depending upon the OH⁻ dependence at the higher pH. For example, at pH 13, the rate of decomposition of MV²⁺ would be 10-fold greater than the rate at pH 12 if the OH⁻ dependence was first order, and 100-fold greater than the rate at pH 12 if the OH⁻ dependence was second order. As mentioned, Rieger observed a first-order dependence at the higher pH. Rieger’s model fit the high pH data very well. However, the model proposed in this chapter fits lower pH data (pH 11 and pH 12), which is within the range that would be desirable to run a biofuel cell since the MV²⁺ decomposition is much slower at lower pH.

4.5 Conclusion

MV²⁺ decomposition experiments showed a trend seen previously in literature that the rate of decomposition increases with an increase in MV²⁺ concentration, OH⁻ concentration, and temperature. The data and mechanistic model suggest second order dependence of both MV²⁺ and OH⁻ concentrations under conditions examined in this thesis (MV²⁺ concentrations of 100-300 mM and OH⁻ concentrations of 0.001 M and 0.01 M). A mechanistic model was proposed, with the equation of \( r_{MV^{+},d} = 2k_0(MV^{2+})^2(OH^-)^2 \) for these conditions and a value of \( k_0 = 0.31 \text{ s}^{-1} \text{M}^{-3} \) at 50°C and \( k_0 = 0.092 \text{ s}^{-1} \text{M}^{-3} \) at 40°C. Similar to that reported by Rieger, the MV²⁺ concentration was found to be second order in the rate law for the decomposition of MV²⁺ developed in this thesis. Rieger’s reported 1-1.4 OH⁻ concentration dependence is also
conceptually consistent with the proposed rate law due to the operating conditions (low MV$^{2+}$ concentration, high pH). Decomposition of MV$^{2+}$ over longer periods of time may become more complex than the mechanism reported here because the slope was seen to change when some experiments ran as long as 15 hours. Future work will need to address the complexity that seems to exist for the decomposition of viologens over long time periods. This analysis used the initial slopes, which are the steepest and represent a worse-case scenario.

An activation energy was found for MV$^{2+}$ decomposition using data from this thesis at both 40$^\circ$C and 50$^\circ$C, as well as data from Rieger’s paper at 24$^\circ$C. The activation energy was found to be 145 kJ/mol. MMV$^+$ was found to be more stable than MV$^{2+}$, with the rate of decomposition anywhere from 6.2 – 16.1 times slower than MV$^{2+}$. It was also found that MV$^{2+}$ is more stable than IPV-Cl and IPV-Br since the percentage of MV$^{2+}$ that decomposed with time was lower than the percent degradation of IPV-Cl and IPV-Br.

An analysis was performed to find the recommended operating range for MV$^{2+}$/glucose biofuel cells under different conditions while ensuring that at least a viable amount of energy could be produced. It was found that it would be fairly easy to stay within feasible operating ranges (conditions in which the decomposition is not significant compared to the $r_{MV^+,i}$ from the MV$^{2+}$ and glucose reaction) with biofuel cell conditions of pH 11 and initial concentrations of glucose of 100 mM or less. Increasing the concentration of glucose at pH 11, the bounds of the safe operating area occurs at $[MV^{2+}]_0/[G]_0 \approx 1.6$ at 5 M glucose concentration. For pH 12, it would be recommended to operate at $[MV^{2+}]_0/[G]_0 \leq 4$ for a glucose concentration of 100 mM and $[MV^{2+}]_0/[G]_0 \leq 0.5$ for a glucose concentration of 5 M.
5. SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Biofuel Cell Applications

To this point in the thesis, the focus has been on the homogeneous reaction between the carbohydrates and MV$^{2+}$ in which the carbohydrate is oxidized and MV$^{2+}$ is reduced to form MV$^+$. A detailed analysis of glucose as a model carbohydrate was performed, and appropriate rate expressions were developed. The intent, of course, is to use oxidation of the glucose to generate electric power in a biofuel cell. The electrochemical reactions that take place in the fuel cell are:

\[
MV^+ \rightarrow MV^{2+} + e^-
\]

\[
O_2 + 2H_2O + 4e^- \rightarrow 4OH^-
\]

The cathode is a standard oxygen electrode and will not be discussed further in this chapter. The reaction at the anode is the oxidation of MV$^+$, which regenerates MV$^{2+}$ as shown in Pathway 2 of Figure 1-3 in Chapter 1, producing energetic electrons in the process. Assuming that the homogeneous reaction is limiting (i.e., the electrochemical reaction is fast relative to the homogeneous reaction), the reaction rate expressions developed in Chapter 3 can be used to estimate the current density that can be achieved in a fuel cell. The rate expressions presented in Chapter 3 indicate that the rate increases with increasing pH and glucose concentration. In addition, it is necessary to have sufficient MV$^{2+}$ to catalyze the desired reaction. Consequently, preliminary calculations assumed the following conditions: pH 13, 50 °C, 0.5 M glucose and an MV$^{2+}$ to glucose ratio of five. A 2-mm thick porous fuel-cell anode with a superficial area of 10

55
cm², a current perpendicular to the direction of fluid flow, and a biofuel cell operating with a low single-pass conversion and recycle were also assumed. With these assumptions, it is estimated that a fuel cell is capable of a current density of approximately 300 mA/cm², based on the superficial area. This estimated current density is significantly larger than values reported in the literature for glucose fuel cells (Li, Liu, Liu, & Zhang, 2016; Liu et al., 2013; Liu, Li, Yang, Liu, & Zhang, 2016; Scott, Tsang, Chetty, Aloi, & Liaw, 2011).

5.2 Summary of Work

The work in this thesis covered many key points dealing both with the initial rate of formation of MV⁺ ($r_{MV⁺,t}$) from the reaction of a carbohydrate and viologen as well as the rate of decomposition of viologens. The work done for this thesis included outcomes such as:

- Provide values for $r_{MV⁺,t}$ (and the associated $k$ values) for various monosaccharides
  - $r_{MV⁺,t}$ (and the associated $k$ values) for the monosaccharides generally increased as the number of carbons in the carbohydrates decreased
- Develop a rigorous model using glucose as the model carbohydrate and MV²⁺ as the viologen to show the dependence of MV²⁺ concentration, pH, carbohydrate concentration, and incubation time on $r_{MV⁺,t}$
  - $r_{MV⁺,t}$ is independent of MV²⁺ when the concentration of MV²⁺ is greater than 4 mM
  - $r_{MV⁺,t}$ has first-order dependence on the OH⁻ concentration
  - $r_{MV⁺,t}$ has first-order dependence on the glucose concentration
- \( r_{MV^+,i} \) increases with increasing incubation time due to the formation of fructose

- Find a value for the extinction coefficient of MMV⁺
  - Value of the MMV⁺ extinction coefficient at 600 nm is 9930 M⁻¹ cm⁻¹
  - Value of the MMV⁺ extinction coefficient at 730 nm is 1955 M⁻¹ cm⁻¹

- Report rates of decomposition for MV²⁺, MMV⁺, IPV-Br, and IPV-Cl
  - MMV⁺ was more stable than MV²⁺, which was more stable than IPV-Br or IPV-Cl

- Develop a mechanism and model for the rate of MV²⁺ decomposition
  - \( r_{MV^+,i} \) due to decomposition has a second-order dependence on both MV²⁺ and OH⁻ concentrations at pH 11 and pH 12

- Find a value for the activation energy for the rate of MV²⁺ decomposition
  - Activation energy for \( r_{MV^+,i} \) due to decomposition is 145 kJ/mol

- Provide recommended operating range for MV²⁺/glucose biofuel cells under different conditions while ensuring that at least a viable amount of energy could be produced
  - At pH 11 and glucose concentrations of 100 mM or lower, operating conditions are very broad (decomposition does not play a significant role)
  - At pH 11 and glucose concentrations of 10 M and below, \([MV^2+]_0/[G]_0\) must be less than 1.25
  - At pH 12 and glucose concentrations of 100 mM or lower, \([MV^2+]_0/[G]_0\) must be less than 4
5.3 Lessons Learned During Experiments

The ability to execute the experiments consistently took some time for the team. Over the course of many experiments, multiple things were learned that will be helpful for those continuing this project. First off, it is highly recommended to keep an up-to-date lab journal and record extra information that you may not even think will be helpful that day. By doing so, the factor(s) that contributed to error or inconsistency in the data was determined multiple times. One example of this is pH inconsistencies. Although the specific pH value after each experiment was not necessarily used, recording the pH after each experiment helped to catch that the buffer was too weak for some experiments. After fixing the problem and continuing to record the pH, it was confirmed that the buffer was strong enough for a given experiment and ruled that option out when some inconsistencies arose later on.

Keeping the experiments oxygen free is crucial to obtain accurate results. To help with this goal, make sure to purge the solutions for 15 minutes and then vacuum the air space. Also, follow procedures for the glovebox precisely and always allow time for the oxygen level to fall again after opening the inside door (about 20 minutes). It was noticed that if the cuvettes and/or solutions were left in the incubator for more than a week, there would sometimes be what appeared to be oxygen contamination. It is recommended to not let the cuvettes with the prepared amount of buffer solution sit in the incubator for over a week. They should be used within the week after they are prepared in the glovebox. Also, the septa

- At pH 12 and glucose concentrations of 10 M and below, \([MV^{2+}]_0/[G]_0\) must be less than 0.5
on the vials of solution (whether it is glucose, MV, or MMV) should be replaced weekly. This means the solutions get re-purged every week.

Another issue dealt with was the consistency of the amount of stock solutions that were added to the cuvettes before running an experiment. The gas tight syringes work well, but be cautious to use them very precisely and remove all air bubbles before using them in a solution.

The experiments were conducted at multiple temperatures. Although the spectrophotometer has a heating block in it, it is important to heat the solutions up to the desired temperature beforehand. It took approximately 2 hours in the incubator or 10 minutes in the heating block to heat the cuvettes up to 50°C. It is recommended to specifically measure the time needed to heat up to the desired temperature with a thermometer and always wait at least that amount of time.

5.4 Recommendations for Future Work

One part of the work focused mainly on measuring initial rate data and developing a mechanism and model that captured the essence of what was occurring at the onset of the reaction between glucose and methyl viologen. As mentioned before, this reaction is complicated, and there are still unknowns about the reaction over long periods of time. One recommendation for future work is that reaction rates over longer periods of time should be measured. Additional understanding of the effects of viologen concentration, carbohydrate concentration, pH, and temperature needs to be developed for the longer time periods.

Another key point of the thesis was to measure decomposition rates of MV$^{2+}$ and MMV$^+$. Preliminary decomposition results from this thesis show that MMV$^+$ is more stable than MV$^{2+}$. 
This higher stability could allow for the biofuel cell to operate under higher pH conditions, which is beneficial for producing a greater amount of electricity. Therefore, it is recommended that MMV\(^+\) is further explored as a replacement for the MV\(^{2+}\) catalyst. However, the use of MMV\(^+\) may have undesirable effects on the rate or efficiency and therefore needs further exploration.


