Microbial Fuel Cell Transformation of Recalcitrant Organic Compounds in Support of Biosensor Research

THESIS

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Abstract

Microbial fuel cells (MFCs) have the potential to be used as low-cost, real-time biosensors for drinking water sources. MFCs have been shown to produce current through oxidation of readily degradable organic substrates and the current can be correlated to the substrate concentration. The purpose of this research was to evaluate the transformation of recalcitrant organic compounds, such as aldicarb, in MFCs and to determine if the current generation and current metrics are related to the transformation, through the measured concentrations, of these recalcitrant organic compounds.

Partial transformation of aldicarb was observed over two days in the presence of aerobic bacteria when aldicarb was initially at 1 mg L^{-1} (average concentration difference 13.8%). The aldicarb concentration changed very little when in the presence of anaerobic bacteria, or when added to deionized water or feed media (average concentration difference – anaerobic bacteria 0.7%, feed solution 1.8%, deionized water 2.0%). Aldicarb transformation was greater in MFCs than in the aerobic bacteria solution but only partial transformation was observed (average concentration difference – MFC1 15.9%, MFC3 28.8%). These data confirm that biotransformation of aldicarb does not occur readily.

Aldicarb does not serve as a substrate for the bacteria that generate current in MFCs. This finding was supported by the fact that #1) there were no strong linear regression correlations between the change in aldicarb concentration and the current metrics generated from the MFC, #2) cyclic voltammetry profiles show very little

oxidation potential for aldicarb, #3) the majority of current generation from the aldicarb feed cycles was most likely due to the acetonitrile solvent, and #4) MFCs did not show a tendency to acclimate to aldicarb. It is possible that non-oxidative process, such as interaction with microbial byproducts and enzymes or biological growth, will indirectly affect the bacteria that generate current, which allows for the detection of aldicarb in MFCs.

Acknowledgments

I would like to thank Dr. Willie Harper for all of his guidance and insight. I was extremely fortunate to have such an outstanding thesis advisor. I would also like to thank Lt Col Dirk Yamamoto and Lt Col LeeAnn Racz for their support as committee members, Dr. Dan Felker for his many hours of assistance and instruction in the laboratory, Maj Scott King for his teamwork on this project, Ms. Mekhakhem Kheperu for her work on the monotonous MFC metrics, and Mr. Frank Barosky from the Fairborn Water Reclamation Facility for his assistance. Finally, I want to thank the Air Force Medical Support Agency for funding this project.

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Glossary of Acronyms

10-hr SR	10-hour Subsidence Rate
amu	Atomic Mass Unit
AR	Acceleration Rate
ARB	Anode Respiring Bacteria
C_e	Columbic Efficiency
CEM	Cation Exchange Membrane
COD	Chemical Oxygen Demand
CV	Cyclic Voltammogram
DMMP	Dimethyl Methylphosphonate
DoD	Department of Defense
F	Faraday's Constant
FM	First Moment
GC/MS	Gas Chromatography/Mass Spectrometry
HPLC	High Performance Liquid Chromatography
I	Current
LC/MS	Liquid Chromatography/Mass Spectrometry
mA	Milliampere
mg L^{-1}	Milligrams per liter
mM	Millimole
mV	Millivolts
MFC	Microbial Fuel Cell
PA	Peak Area

PH	Peak Height
ppb	Parts Per Billion
ppm	Parts Per Million
SR	Subsidence Rate
TN	Total Ammonia Nitrogen
V_{An}	Volume of Anode Compartment

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I. Introduction

The vulnerability of drinking water sources from natural and intentional threats within the military and civilian sectors is mitigated partially by water sampling and analysis. The majority of water sampling and analysis is grab sampling and analysis using laboratory equipment. Current monitoring systems using on-line probes have the ability to monitor simple parameters such as temperature, pH and conductivity. The inability to conduct continuous, real-time sampling with cost effective analysis for specific contaminants presents a concern (Hasan et al., 2004). Microbial fuel cells (MFCs) have the potential to bridge these gaps.

MFCs are devices that contain a biofilm capable of producing an electric current through the oxidation of various organic compounds. The biofilm on the anode contains anode respiring bacteria (ARB), which oxidize organic material and transfer electrons to the anode. The electrons produce a current that can be used for electricity generation (Logan, 2008). In addition to current generation, MFCs have been used as biosensors for readily degradable substrates. The current produced by MFCs can be measured using various metrics and potentially used to identify specific chemicals and concentrations in the water (DiLorenzo et al., 2009; Davila et al., 2010; Feng et al., 2013).

The ability of MFCs to oxidize simple substrates has been well documented, along with the correlation between the concentration of these substances and current generation (DiLorenzo et al., 2009; Sharma and Li, 2009; Davila et al., 2010; Nam et al.,

2010; Feng et al., 2013). For organic compounds that are not readily degradable, such as aldicarb and dimethyl methylphosphonate (DMMP), it is unknown if the current production from these potential substrates can be correlated to the change in chemical concentration due to transformation in the MFC. Additional investigation into the transformation of these substances in biotic and abiotic media and into the ability of these compounds to oxidize through cyclic voltammetry experiments is required.

This research will answer the following questions: 1) can MFCs degrade or transform aldicarb and DMMP, 2) can the current generation and current metrics be linearly correlated to the transformation of aldicarb and DMMP, through the measured concentrations, 3) if aldicarb and DMMP is transformed within the MFC, are they substrates for the ARB, and 4) does this research support the proposition that MFCs can be used as biosensors for recalcitrant organic compounds.

II. Literature Review

2.1 Microbial Fuel Cells as Biosensors

MFCs are capable of current generation through the oxidation of organic compounds. MFCs are composed of an anode, a cathode and a wire connecting the anode and cathode with a load, or resistor. Within the MFC, anode respiring bacteria (ARB) grow in a biofilm on the anode, and transfer electrons to a terminal electron acceptor. Organic matter is oxidized within the anode chamber and electrons travel through the anode. Protons are released within the chamber and travel through the cation exchange membrane. In a single chamber MFC, an air cathode is exposed to air and oxygen acts as the terminal electron acceptor. Electrons, protons and oxygen react on the cathode to form water (Logan, 2008). Figure 1 is a basic diagram of a single cell MFC.

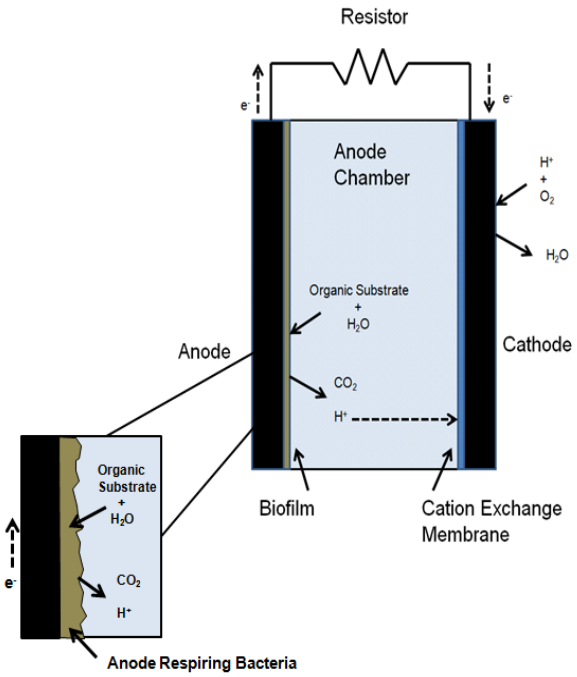


Figure 1. Single Cell Microbial Fuel Cell

The current produced by MFCs can be measured using various metrics such as the maximum current produced or the total current produced over a period of time. These current metrics are related to the type and concentration of substrate provided to the MFCs. This relationship of substrate type, concentration and current production enables the MFCs to be used as a biosensor. Multiple studies have shown that MFCs have the potential to be used to monitor water quality for readily degradable organic compounds.

DiLorenzo et al. (2009) investigated the use single chamber MFCs as a replacement for the traditional 5-day biochemical oxygen demand test. With artificial wastewater as a fuel source, a steady state flow of wastewater at 0.46 cm^3 min^{-1} was supplied to the MFC until a steady current was produced at each concentration. The current output from the MFC was shown to have a linear relationship with the chemical oxygen demand up to 350 ppm with a coefficient of determination (r^2) of 0.96.

Feng et al. (2013) were able to correlate the characteristics of the current production profiles with the concentration of chemical oxygen demand using acetate as a substrate. COD concentrations, ranging from 25 mg L^{-1} to 200 mg L^{-1}, were provided to the MFCs. The current profile metrics of peak height (mA) and peak area (mA-hr) were evaluated at each concentration. Peak height increased linearly from 25 mg L^{-1} to 150 mg L^{-1} and peak area increased linearly from 25 mg L^{-1} to 200 mg L^{-1}.

Davila et al. (2010) used MFCs as toxicity sensors by observing a drop in current production when formaldehyde was introduced to the anode chamber. Concentrations of formaldehyde ranging from 4% to 0.1% were tested and were able to produce a detectable drop in current. Formaldehyde is a biocide and permanently inactivated the biofilm within the MFC.

To date, there is no evidence that MFCs have been used to detect, transform and quantify more recalcitrant organic compounds such as aldicarb.

2.2 Transformation of Aldicarb and DMMP

O-(Methylcarbamoyl)-2-methyl-2-(methylthio)propionaldehyd-oxime, or aldicarb, is a carbamate pesticide and active ingredient in Temik. Aldicarb has been a widely used chemical and the result is the accumulation of aldicarb in groundwater and surface water sources (EPA, 2013). Aldicarb was applied under the soil in granular form and is drawn up from the roots of the target plant where it acts as a systemic pesticide (APVMA, 2001).

Aldicarb has the potential cause acute and chronic toxic effect through oral, dermal and inhalation routes of exposure to humans. Through ingestion, the primary route of exposure, aldicarb is readily absorbed in the gastrointestinal tract. A majority of aldicarb is eliminated within 24 hours through urine, and the rest is slowly eliminated through urine for several days following exposure. Low levels of aldicarb that are not eliminated are evenly distributed through organ tissue. The metabolic pathways for aldicarb include oxidation and hydrolysis to metabolites that are readily eliminated through urine (APVMA, 2001). The metabolic pathway for aldicarb is displayed in Figure 2 (Wilkinson et al.,1983).

The Environmental Protection Agency has established a maximum contamination limit for aldicarb at 0.001 mg L^{-1}. Acute exposure to aldicarb at concentrations exceeding 0.001 mg L^{-1} can lead to nausea, diarrhea and neurological effects due to acetylcholinesterase inhibition. Aldicarb has the potential to cause neurological effects

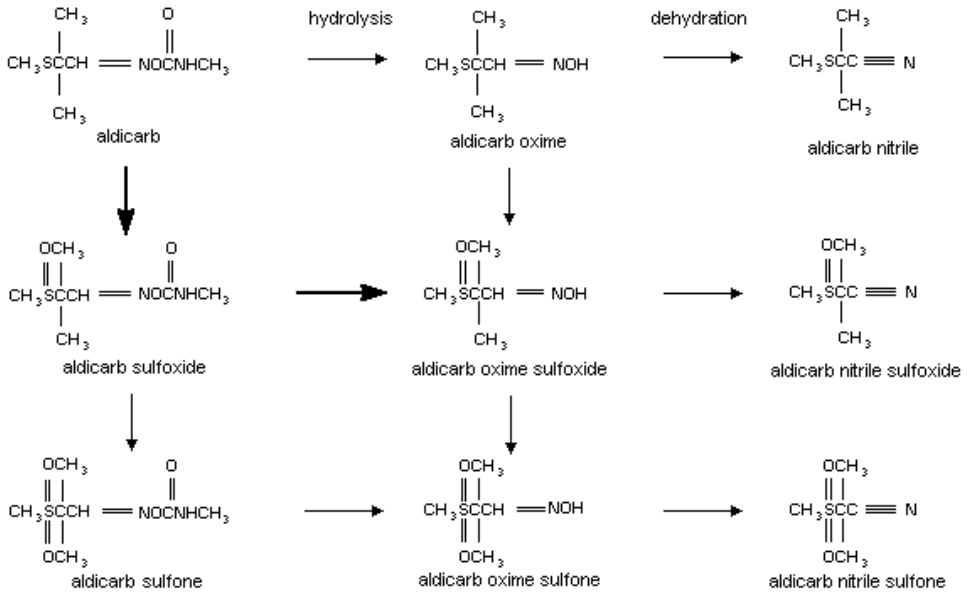


Figure 2. Aldicarb Metabolic Pathway (Adapted from Wilkinson et al., 1983)

such as sweating, leg weakness and papillary effects due to chronic exposure (EPA, 2013).

The biodegradation of aldicarb has been studied thoroughly. Khandaker and Young (2000) studied the transformation of aldicarb in batch reactors under acclimated and unacclimated biological conditions and abiotic conditions. The experiments showed that aldicarb converts to aldicarb nitrile through first-order hydrolysis and dehydration reactions most rapidly in acclimated biological conditions. The acclimated first-order rate constant was 94% greater than the unacclimated rate constant and more than four times greater than the abiotic rate constant. Additionally, Khandaker and Young validated that under anaerobic microbial conditions, aldicarb is converted into aldicarb oxime and monoethylamine through hydrolysis, and then alicarb oxime produces aldicarb nitrile

through dehydration and monoethylamine mineralizes to produce methane and carbon dioxide. Aldicarb nitrile is a persistent metabolite.

Kok et al. (1999) studied the microbial degradation of aldicarb in constant flow reactors. Microbial degradation of aldicarb in aerobic conditions produces aldicarb sulfoxide through rapid oxidation, then aldicarb sulfone through slower oxidation. Complete degradation of aldicarb at 100 ppm was accomplished over four days. The degradation rate was found to increase as the concentration increased to 400 ppm. Beyond 400 ppm, the degradation rate decreased as the concentration increased to 1,200 ppm.

Aldicarb and its degradation byproducts can be detected using high performance liquid chromatography (HPLC). Additionally, the analysis of aldicarb and its byproducts from anaerobic degradation using gas chromatography (GC) was reviewed. With GC analysis using conventional length columns, aldicarb nitrile gives a positive inference for aldicarb (Trehy and Yost, 1984). The use of HPLC for the detection of aldicarb, aldicarb sulfoxide, and aldicarb sulfone in liquid samples has been validated in multiple studies (Damasceno et al., 2008; Wright et al., 1982). The limits of detection for aldicarb, aldicarb sulfoxide and aldicarb sulfone in water and synthetic medium are 0.391/0.440 mg L^{-1}, 0.069/0.192 mg L^{-1} and 0.033/0.068 mg L^{-1}, respectively (Damasceno et al., 2008). Khandaker and Young (2000) used HPLC for the detection of aldicarb and aldicarb nitrile in liquid samples through aldicarb degradation under anaerobic conditions. The minimum concentrations detected for aldicarb nitrile were 5 mg L^{-1} from the graphs presented. Wang et al. (2011) evaluated the degradation of aldicarb and metabolites from water disinfection processes using concentrations of 5 mg L^{-1} for each

aldicarb, aldicarb sulfoxide, and aldicarb sulfone with HPLC/MS analysis. Miles and Delfino (1984) analyzed the degradation of aldicarb using a concentration of 2 mg L^{-1} with HPLC/UV analysis.

Dimethyl methylphosphonate (DMMP) is a nerve agent simulant similar in structure to sarin (Obee and Satyapal, 1998). Literature on the biodegradation of DMMP is limited. In 2005, the DMMP Consortium conducted a review of DMMP research for the Environmental Protection Agency. Information provided on biodegradability was obtained from an unpublished study by Life Science Limited Research in 1990. Using a Modified Sturm-Test, the biodegradability of DMMP in activated sludge was evaluated at 10 mg L^{-1} and 20 mg L^{-1}. After 28 days, DMMP at 10 mg L^{-1} degraded 13% and DMMP at 20 mg L^{-1} degraded 11%.

2.3 Inhibition of Biological Activity

Microbial inhibition can occur due to a variety of factors, such as temperature, pH and nutrient composition. When multiple nutrients make up a substrate, they may act as either competitive inhibitors, in which they are competing for enzymes, or non-competitive substrates, in which there is an additive or multiplicative effect. In addition, high substrate concentrations can affect microbial activity through multiple actions. Inhibitors can affect enzymes, cell functionality and permeability, or the substrate properties (Blanch and Clark, 1997).

Parameswaran et al. (2009) evaluated the synthopic relations between ARB, methanogens and fermentors. When a substrate more complex than acetate, such as ethanol, is supplied to MFCs, it undergoes fermentation to acetate and hydrogen. Other

substrates, such as such sugars, may ferment to ethanol first. Current production occurs from the ARB utilization of the fermentation product acetate, but electrons are lost in the form of hydrogen to methanogenesis. Therefore, the current production is less than expected maximum potential. When the methanogens were suppressed using 2-bromoethane sulfonic acid, hydrogen is either directly utilized by ARB or acetate is produced from homoacetogenesis and there is an increase in current generation. This also illustrates that when the methanogens were not inhibited, they outcompeted the homoacetogens for hydrogen.

Sharma and Li (2009) discussed the inhibition of biological activity in MFCs through the increase of substrate concentrations. MFCs were provided ethanol, acetate and glucose at concentrations ranging from 0.5 mM to 35 mM. Maximum voltage generation at each concentration from 0.5 mM to 8 mM increased from 0.11, 0.12 and 0.14 V to 0.31, 0.34 and 0.39 V for ethanol, acetate and glucose, respectively. As concentrations were increased to 20 mM for each substrate, the maximum voltage decreased 0.26, 0.29 and 0.36 V. Additional increases in concentration led to further decrease in maximum voltage. Sharma and Li hypothesized that the decrease in maximum voltage was caused by two possible factors. First, the increased concentration resulted in an increase of fermentation products, which in turn decreased the pH of the system and inhibited the biological activity. Second, the substrates were used for biological growth, which reduced the current generation.

In addition to the analysis of maximum voltage produced by various substrates and concentrations, Sharma and Li used cyclic voltammetry to measure the substrates ability to oxidize and produce current. The test began by increasing potential from -0.6 V

to 0 V, then decreasing the potential from 0 V to -0.6 V. The oxidation peaks and reduction peaks were evaluated by the maximum current (mA) and the distance between peaks on the potential axis (mV). The magnitude of the peaks correlated well with the substrate and substrate concentration current production in the MFC. Additionally, the separation of the oxidation and reduction peaks correlated well with the substrate's ability to produce current at 0.5 mM. A larger difference in oxidation and reduction peaks on the potential axis indicated a low rate of electron transfer.

Nam et al. (2010) reported the effects of ammonia on MFC current generation. The total ammonia nitrogen (TAN) concentrations ranged from 84 mg L^{-1} to 4000 mg L^{-1}. The highest power density corresponded to 500 mg L^{-1}. At concentrations above 1000 mg L^{-1}, the power density was significantly reduced. Additionally, acetate was provided at a concentration of 32.4 mM in each of the experiments and the concentration of acetate was measured over time. At the 500 mg L^{-1} TAN, the acetate first order decay constant was greatest. The increase in TAN concentration of 500 mg L^{-1} inhibited the anode respiring bacteria utilization of acetate, which decreased current generation. Nam et al. cited that potential causes of inhibition due to ammonia were from ionized NH_3 that may affect the cytosolic enzymes or passive diffusion into the bacteria cells which may alter the internal pH.

III. Materials and Methods

3.1 Experimental Overview

The purpose of this experiment was to measure difference in concentration of aldicarb and DMMP after a two-day feeding cycle in the MFCs, and correlate the difference to the generation of current. The difference in concentration of the two compounds was also measured in separate biotic and abiotic media experiments using four media: deionized water, acetate feed solution, aerobic bacteria solution, and anaerobic bacteria solution. These experiments were designed to investigate the degradability of these chemicals by active bacteria, by abiotic reactions with water (i.e. hydrolysis), or chemicals present in the feed media.

3.2 Microbial Fuel Cells

Twelve single cell MFCs were prepared and maintained prior to the experiments and two MFCs were selected and utilized. MFC 1 was inoculated using activated sludge from the Fairborn (OH) Water Reclamation Facility and MFC 3 was used in a previous experiment and was inoculated using activated sludge from the McKeesport (PA) Water Reclamation Facility (Feng, 2013). Both MFCs were constructed from a previous experiment using three clear acrylic plates (9 in^2, 0.50 in thick, middle plate 2” ID), carbon fiber for the anode (surface area, 20 cm^2; thickness, 1 mm), and four stabilizing bolts (2.25" in length). The anode chambers for MFC 1 and MFC 3 were 25 and 20 mL, respectively. Each anode chamber included a cation exchange membrane (CEM), with a carbon fiber air cathode in contact with the external surface of the CEM. The clips

connected to the anode and cathode were separated by a 47 ohm resistor for MFC 1 and a 100 ohm resistor for MFC 3. Figure 3 shows a labeled MFC used in the experiment.

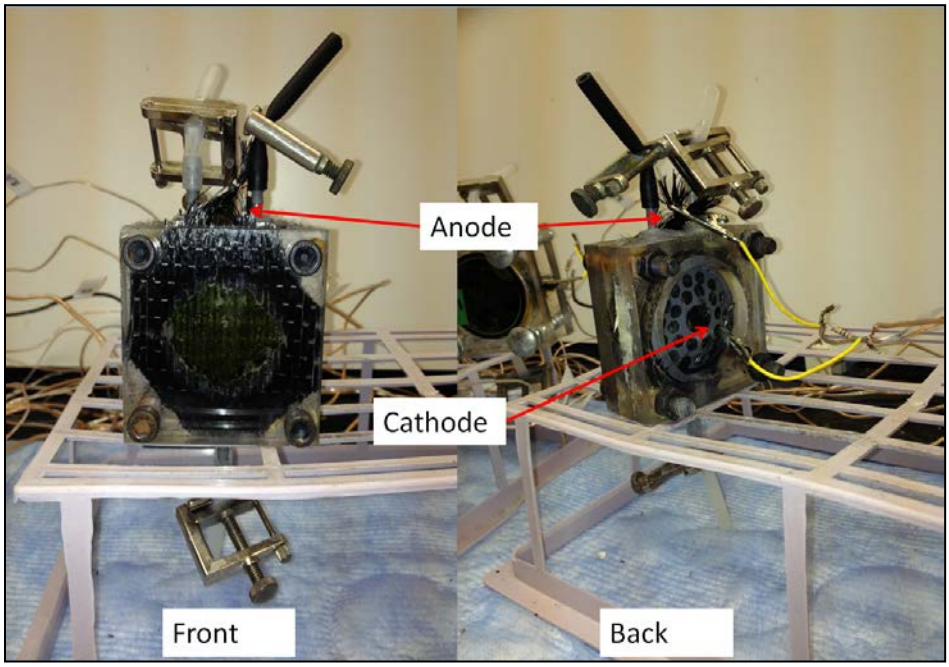


Figure 3. Microbial Fuel Cells

3.3 Fuel Cell Maintenance

The inoculated MFCs were provided acetate feed solution every 48 hours. The feed solution consisted of $C_2H_3NaO_2$ (0.20 g L^{-1}), NH_4Cl (0.38 mM), KH_2PO_4 (10 mM), K_2HPO_4 (1.15 mM), $MgCl_2$ (2.63 mM), $CoCl_2$ (0.15 mM), $ZnCl_2$ (0.07 mM), $CuCl_2$ (0.07 mM), $CaCl_2$ (0.036 mM), $MnCl_2$ (0.079 mM), and deionized water. During each feed cycle, the clamps on each MFC were opened and the solution from the previous feed cycle was drained. The MFCs were rinsed with 10 mL of deionized water. MFC 1 was filled with 25 mL of feed solution and MFC 3 was filled with 20 mL of feed solution. After the clamps were closed, voltage measurements were recorded from the MFCs every 120 seconds using a Keithley Meter Model 2750 and Microsoft Excel ExceLINX add-on.

Figure 4 shows the experimental set-up of the MFCs and Figure 5 shows the screen from the ExceLINX add-on.

Figure 4. MFC Experimental Set-up

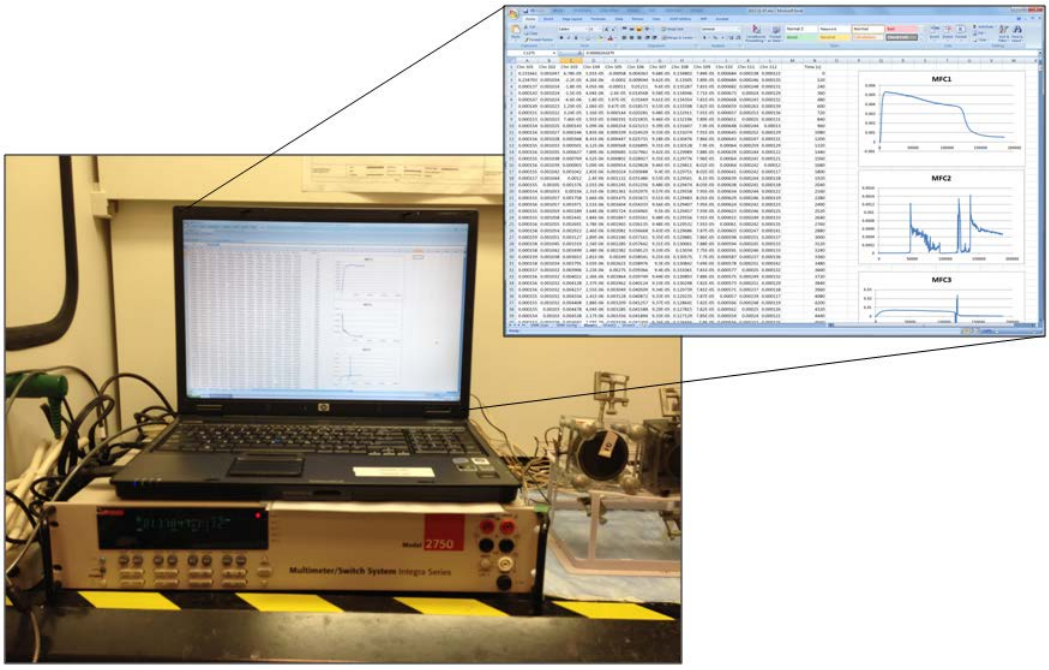


Figure 5. Experimental Set-up with ExceLINX Screen Shot

3.4 MFC Metrics

Current profiles were generated from each feed cycle. Figure 6 shows a typical current profile and the metrics used from each profile: peak height (PH), area, acceleration rate (AR), subsidence rate (SR), 10-hr subsidence rate (10-hr SR), and first moment (FM). Feng et al. (2013) used the metrics PH, area, AR and SR to characterize a current profile. The 10-hr SR is the slope of the line from the peak height to the point in the profile 10 hours later. 10-hr SR was used in order to consistently evaluate the subsidence rate of non-uniform peaks. Current profiles from acetate feed cycles typically provided uniform peaks. The current profiles from feed cycles with aldicarb and DMMP were not similar to the acetate feed cycles as seen in Figures 7 and 8. The metric of FM was used to help account for the shape of the current profile. Second moment was not used for this experiment, but both first moment and second moment are appropriate to use for quantitative shape description (Taylor, 2001). Equation 1 was used to calculate FM for each current profile:

$$\text{FM} = \sum(t_n - t_{n-1})i_{n-1} + \frac{1}{2}(t_n - t_{n-1})(i_n - i_{n-1}) \quad (1)$$

where

$$\text{FM} = first\ moment\ (mA\ hr)$$

$$t = time\ (hr)$$

$$i = current\ (mA)$$

3.5 Aldicarb and DMMP Transformation Experiments in MFCs

The 100 mg L^{-1} aldicarb solution in acetonitrile was purchased from Ultra Scientific Analytical Solutions and DMMP was purchased from Fluka Analytical. When

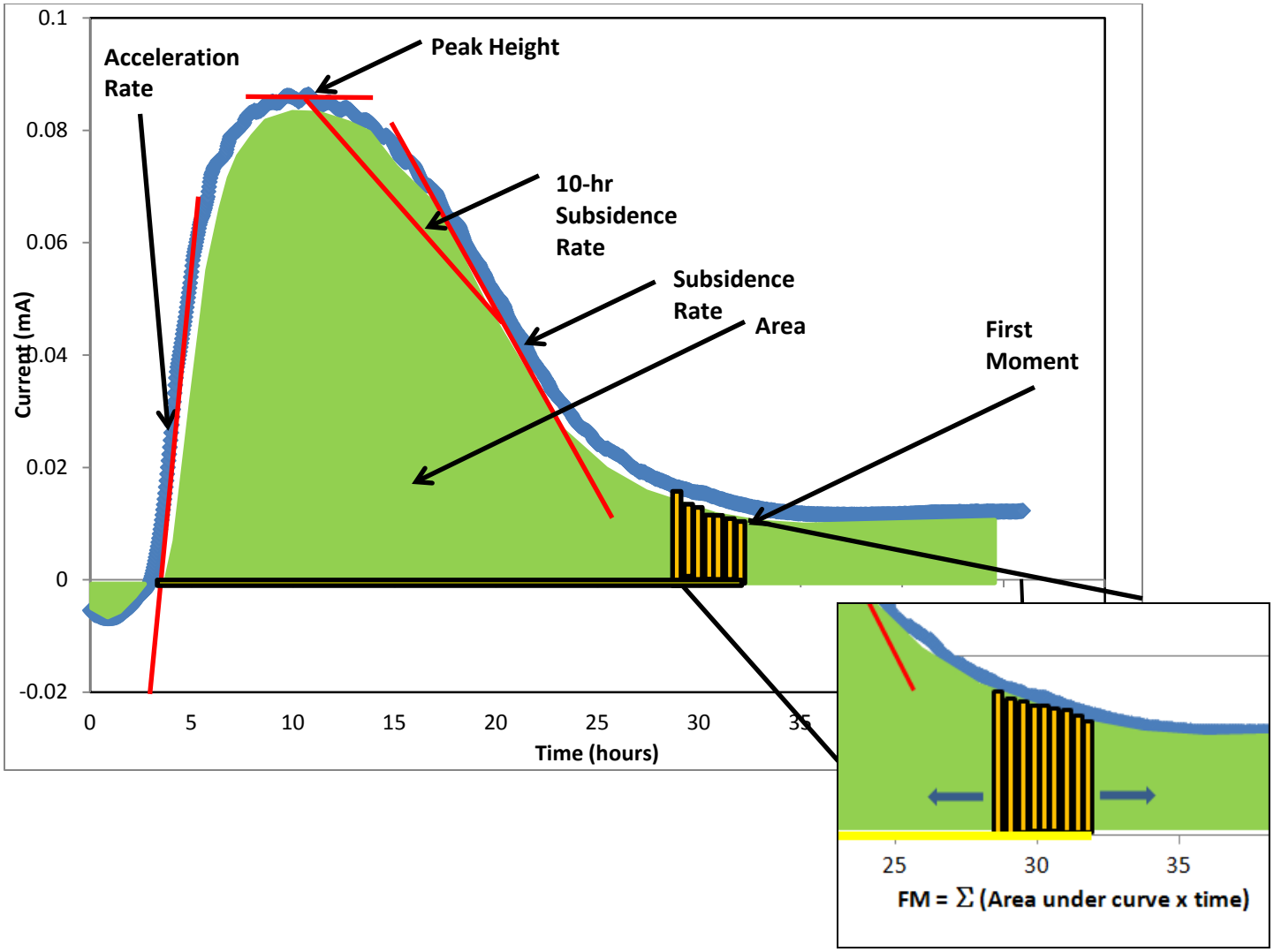


Figure 6. MFC Metrics

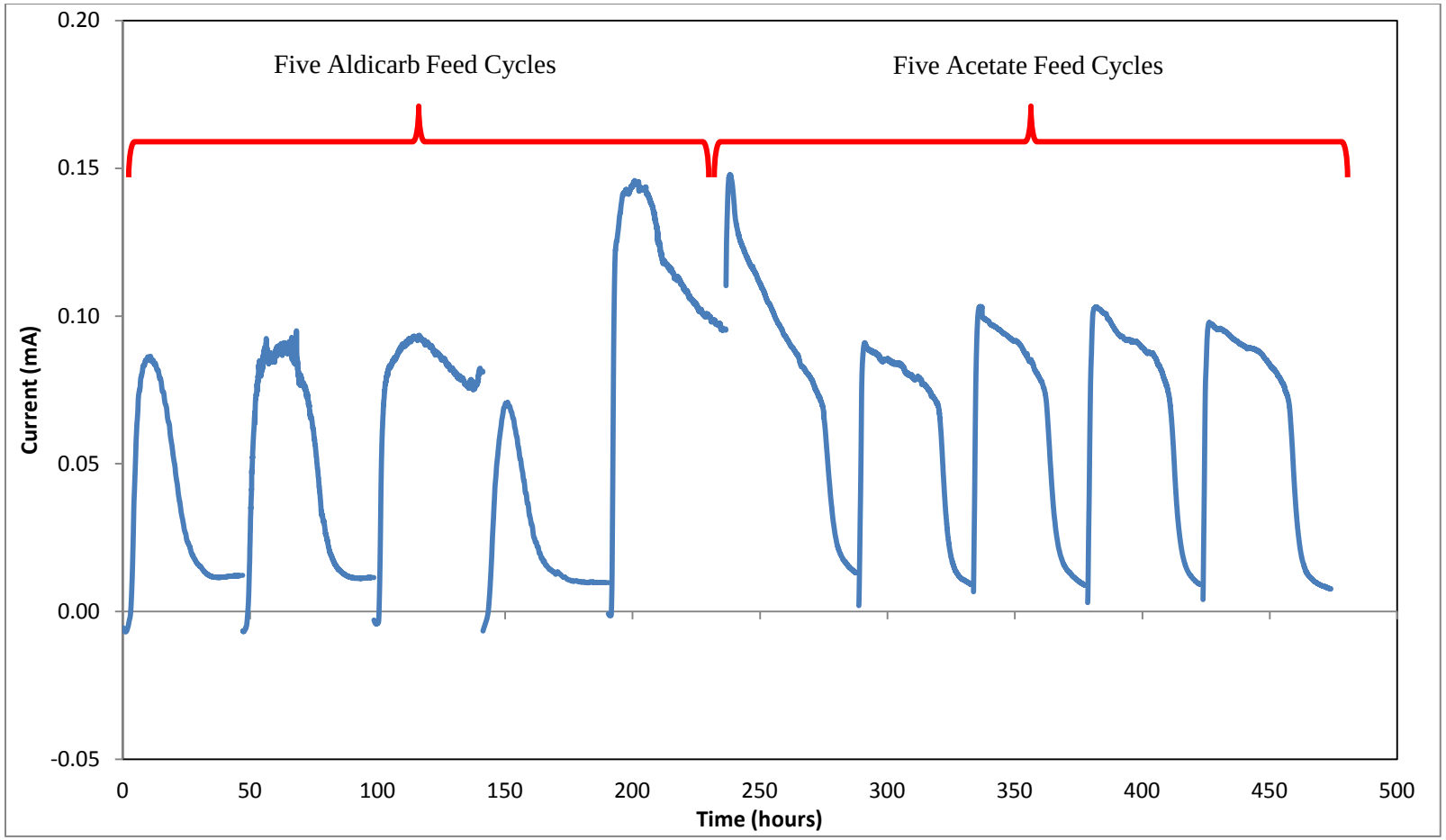


Figure 7. Example of Aldicarb and Acetate Current Profiles

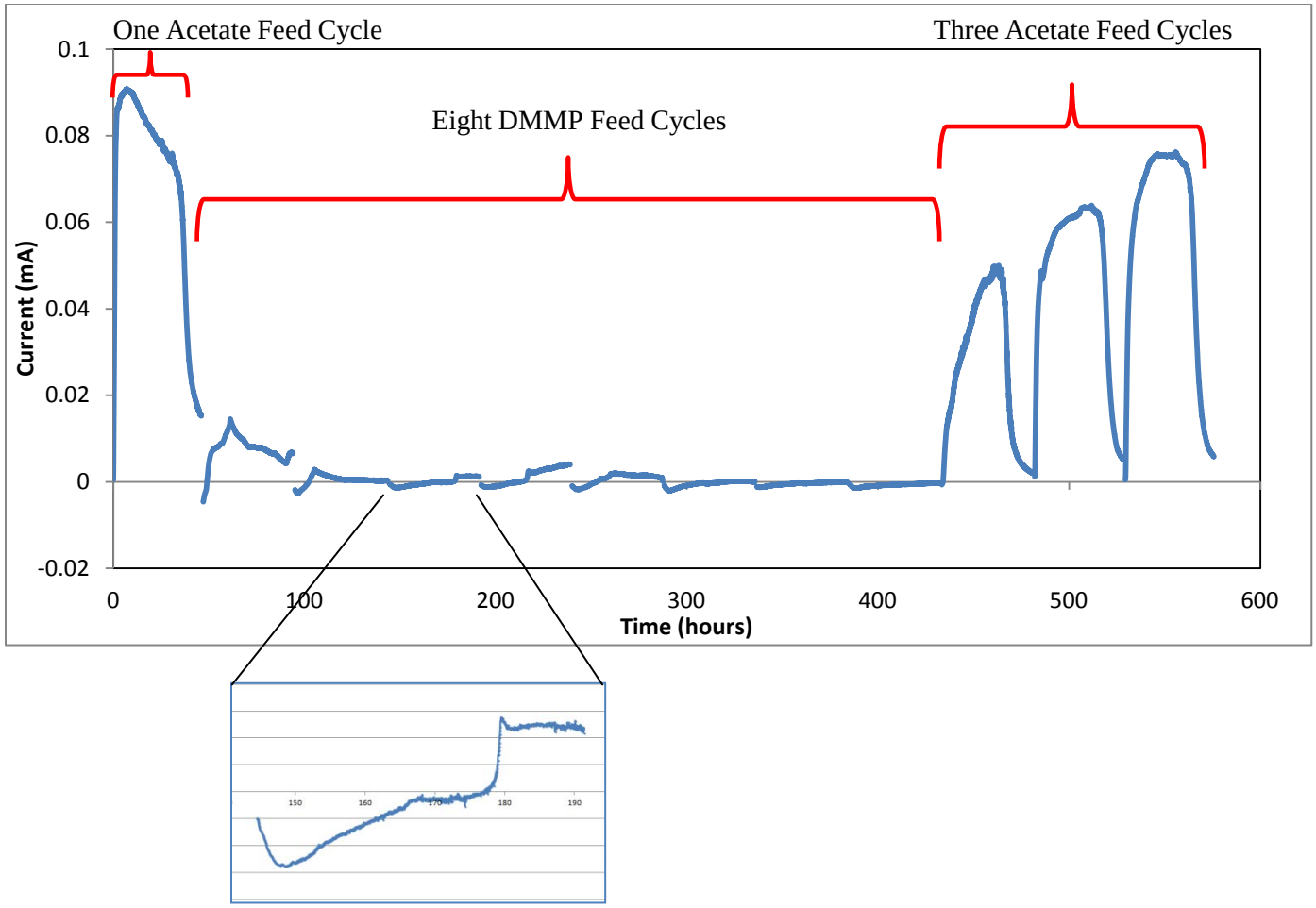


Figure 8. Example of DMMP and Acetate Current Profiles

MFC 1 and MFC 3 produced consistent current profiles (+/- 5% PH for 3 current profiles) from the acetate feed cycles, experiments using aldicarb and DMMP began. Eight feed cycles began concurrently on each MFC, first with 1 mg L^{-1} aldicarb in deionized water. Once the eight feed cycles were complete, the MFCs were given the acetate feed until consistent current profiles were established again. Then, the MFCs began eight feed cycles with 10 mg L^{-1} DMMP in deionized water.

The concentrations of aldicarb and DMMP were analyzed prior to and after each feed cycle. Each sample was collected and filtered using a PVC filter. Aldicarb was measured using an Agilent 1260 Infinity high performance liquid chromatography (HPLC) system with a Zorbax SB C-18 column (2.1 mm ID × 50 mm, 1.8-μm threaded column). The mobile phase began with 95% of 0.1% formic acid in water and 5% of 0.1% formic acid in acetonitrile. Over 5.5 minutes, the mobile transitioned to 85% of 0.1% formic acid in water and 15% of 0.1% formic acid in acetonitrile. From 5.5 minutes to 6.1 minutes, the mobile phase transitioned to 100% of 0.1% formic acid in acetonitrile. The injection volume was 5 μL and the flow rate was 0.25 ml min^{-1}. Aldicarb was detected using an Agilent 6130 Quadrupole liquid chromatography/mass spectrometer (LC/MS). The MS scanned the mass range 120-250 from 0 to 4.8 minutes, 200-216 from 4.8 to 5.2 minutes and 120-250 from 5.2 to 6.1 minutes. An example of the detection of aldicarb at 0.1 mg L^{-1} and 1 mg L^{-1} is shown in Figure 9.

DMMP was measured using an Agilent 6890N Network GC System with an Agilent 5973 inert Mass Selective Detector. Auto-injections were conducted at 4 μl. The separation was performed by an Agilent 19091S-422 HP-5ms GC column that was 30 m x 0.25 mm x 0.25 μm film thickness. Helium was the carrier gas at 0.6378 ml min^{-1}. The

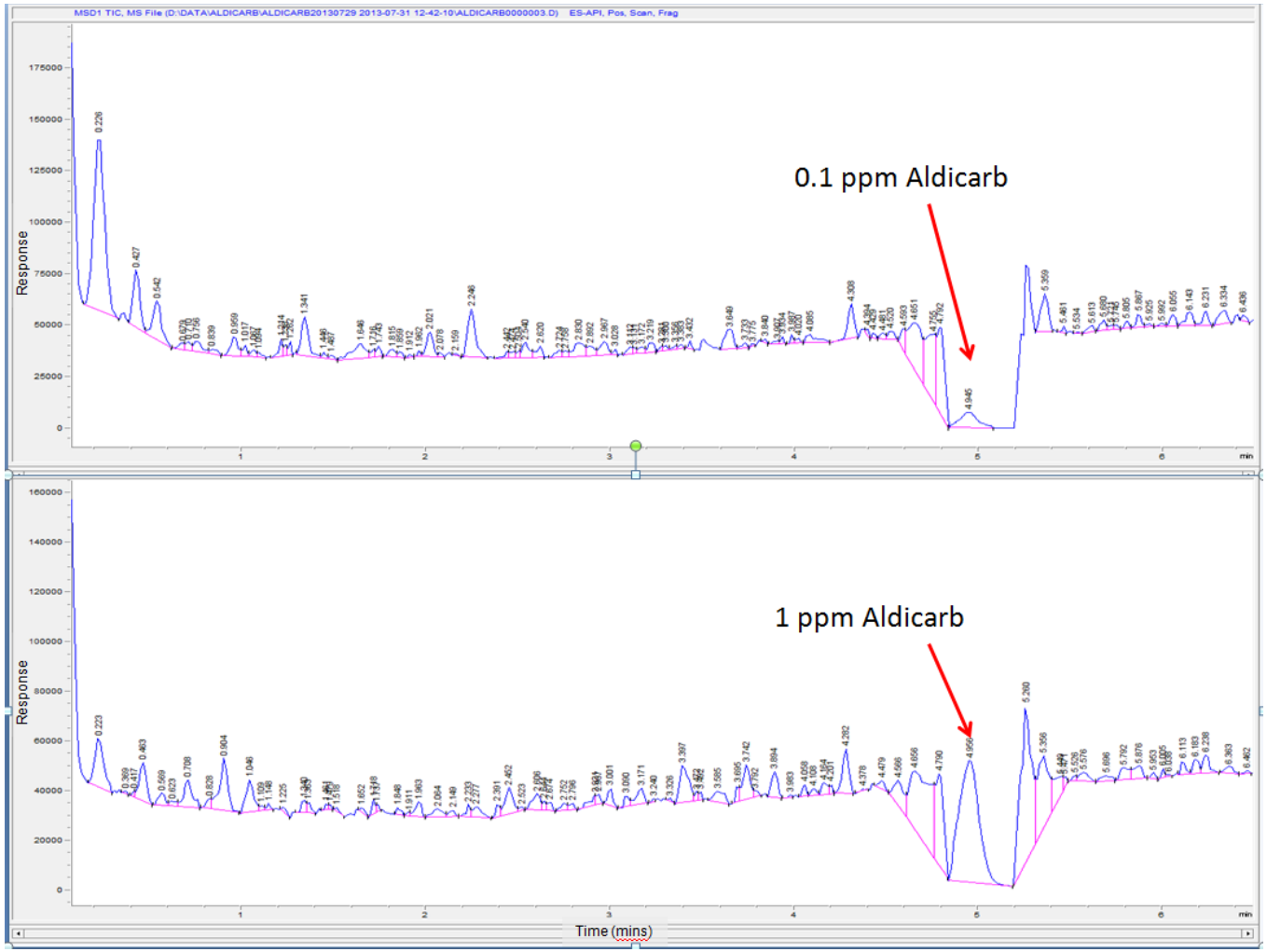


Figure 9. Aldicarb Detection Using LC/MS

initial column temperature was 225^{o}C for 3 minutes. The temperature increased by 15^{o}C min^{-1}, and stopped at 260^{o}C for 2 minutes. The total run time was 7.33 minutes. The mass selective detector was set to a 3 minute solvent delay, and selected ions between the mass range of 30 to 350 amu.

3.6 Aldicarb and DMMP Transformation Experiments in Biotic and Abiotic Media

Aldicarb and DMMP transformation was investigated in deionized water, acetate feed solution, anaerobic bacteria solution, and aerobic bacteria solution. These tests were run over 48 hours and were designed to collect more information about the degradability of these chemicals by active bacteria and abiotic reactions with water (i.e. hydrolysis) or chemicals present in the feed media. Activated sludge was obtained from the Fairborn (OH) Water Reclamation Facility for the aerobic and anaerobic bacteria solutions. 50 mL solutions of 1 mg L^{-1} aldicarb and 10 mg L^{-1} DMMP were prepared in triplicate in each of the four media. The aerobic bacteria solution was comprised of 10 mL of fresh sludge and 40 mL of deionized water and the anaerobic bacteria solution was comprised of 10 mL of refrigerated sludge and 40 mL of deionized water. Each 50 mL solution was left at room temperature for 48 hours. In order to prevent evaporation, the aerobic bacteria solutions were bubbled in a closed loop system with a Master Flex L/S Easy Load II peristaltic pump. Figure 10 shows the experimental set-up for the transformation experiments.

Samples were collected from each solution at the beginning of the experiment, at 24 hours and at 48 hours. Aldicarb samples were analyzed using LC/MS and DMMP samples were analyzed using GC/MS, as described in section 3.5.

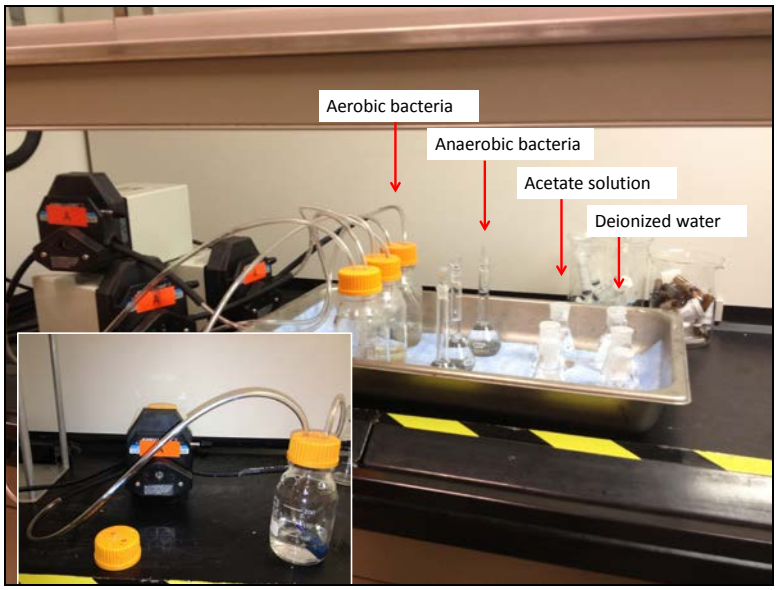


Figure 10. Biotic and Abiotic Media Transformation Experimental Set-up

3.7 Coulombic Efficiency

Coulombic efficiency is the comparison of electrons recovered through the measure of current to the available electrons in the substrate (Logan, 2008). The equation for coulombic efficiency can be expressed as:

$$C_E = \frac{Coulombs\ recovered}{Total\ coulombs\ in\ substrate} \tag{2}$$

or:

$$C_E = \frac{8 \int_0^{t_b} I\ dt}{F v_{an} \Delta \mathrm{COD}} \tag{3}$$

where $\int_0^{t_b} I\ dt = current\ obtained\ over\ time$

$F = Faraday's\ constant$

$v_{an} = volume\ of\ liquid\ in\ anode\ compartment$

$\Delta \mathrm{COD} = chemical\ oxygen\ demand$

Using the principle of coulombic efficiency and Equation 3, the numerator and denominator were calculated separately to evaluate the actual electrons donated through current generation and the potential of aldicarb through the difference in concentration.

3.8 Cyclic Voltammetry Experiments

Cyclic voltammetry (CV) was used to determine the oxidation potential of the substrates. Using a Parstat 2273 Advanced Electrochemical System, voltage was cycled from -0.8 V to 1 V at a scan rate of 50 mV sec^{-1} while measuring current. A three electrode system was used in an electrochemical glass cell, with a glossy carbon as the working electrode, Pt (s) as the counter electrode, and Ag/AgCl (sat) as the reference electrode. Figure 11 shows the CV experimental set-up. Four solutions were measured: deionized water, 1 mg L^{-1} aldicarb, 10 mg L^{-1} DMMP and acetate feed solution.

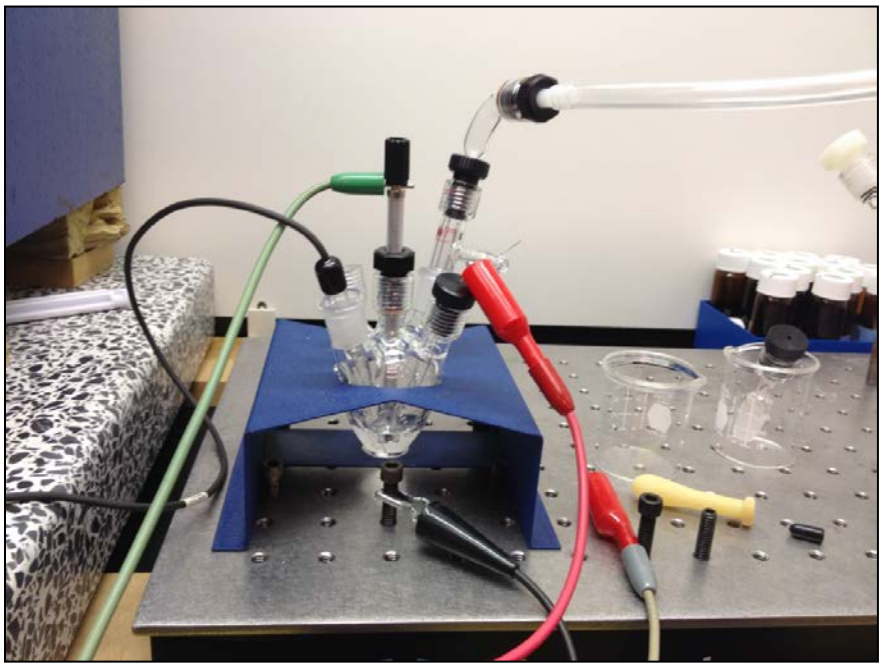


Figure 11. CV Experimental Set-up

IV. Results and Discussion

4.1 Aldicarb Transformation Experiments in Biotic and Abiotic Media

Results from the substrate transformation experiments for aldicarb are summarized in Table 1. The transformation of aldicarb in the anaerobic bacteria solution, the acetate feed solution and deionized water after one and two days was minimal. In the anaerobic bacteria solution, the percent difference in aldicarb concentration after two days ranged from -0.196% to 1.67%, with an average of 0.648%. In acetate feed solution, the percent difference in aldicarb concentration after two days ranged from -3.25% to -0.428%, with an average of -1.48%. In deionized water, the percent difference in aldicarb concentration after two days ranged from -2.26% to -1.81%, with an average of -1.96%.

In the presence of aerobic bacteria, the percent difference in aldicarb concentration after one day ranged from 7.18% to 10.08% and after two days ranged from 10.75% to 16.06%. The average aldicarb concentration decreased by 8.73% after one day and 18.87% after two days. Assuming first order degradation, the calculated degradation rate constant (0.0747 day^{-1}) was comparable to previously determined rate constants (0.060 day^{-1}) (Khandaker and Young, 2000).

Figure 12 compares the transformation of aldicarb within the four media. The data from these experiments support the proposition that the transformation of aldicarb is increased in the presence of aerobic bacteria, and that aldicarb transformation is not related to hydrolysis or chemical reactions with chemicals in the feed media.

Table 1. Aldicarb Transformation in Biotic and Abiotic Media Experiment Results

		Anaerobic Bacteria				Aerobic Bacteria				Acetate Feed				Deionized Water		
			Concentration				Concentration				Concentration				Concentration	
Time (Days)	Sample	Area	mg/L	% Difference	Sample	Area	mg/L	% Difference	Sample	Area	mg/L	% Difference	Sample	Area	mg/L	% Difference
0	Stock	381004	1.040		Stock	409608	1.127		Stock	394611	1.082		Stock	399900	1.098	
1	1	394481	1.081	-3.96	1	383090	1.046	7.18	1	395946	1.086	-0.377	1	398167	1.092	0.482
1	2	383641	1.048	-0.774	2	376642	1.027	8.93	2	380902	1.040	3.87	2	384699	1.051	4.23
1	3	387743	1.061	-1.98	3	372404	1.014	10.07	3	396240	1.087	-0.460	3	386564	1.057	3.71
2	1	379403	1.035	0.470	1	369945	1.006	10.75	1	406108	1.116	-3.25	1	406403	1.118	-1.81
2	2	381672	1.042	-0.196	2	354931	0.960	14.81	2	396128	1.086	-0.428	2	408034	1.123	-2.26
2	3	375316	1.023	1.67	3	350341	0.946	16.06	3	401231	1.102	-1.870	3	406426	1.118	-1.82
1	Average		1.063	-2.23	Average		1.029	8.73	Average		1.071	1.01	Average		1.067	2.81
2	Average		1.033	**0.648**	Average		0.971	**13.87**	Average		1.102	**-1.85**	Average		1.119	**-1.96**
	k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)			
	0.00325				0.0747				-0.00916				-0.00972			

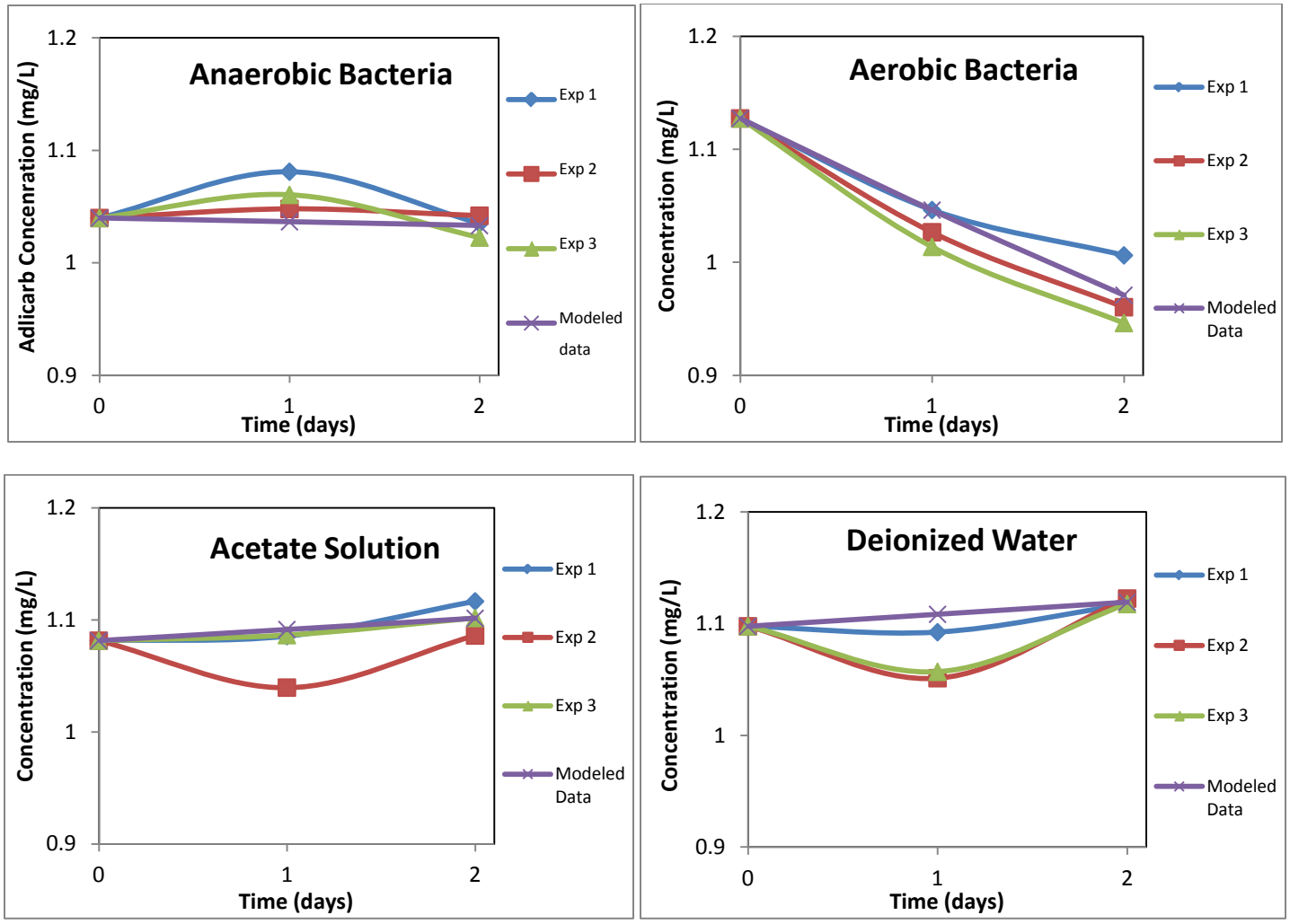


Figure 12. Aldicarb Transformation in Biotic and Abiotic Media

The results presented in Table 1 were from the second experiment conducted. The results from the first experiment were not used. The initial experiment did not include an enclosed system with a peristaltic pump for the aerobic bacteria experiment. The measured concentration of aldicarb significantly increased after two days. It is possible that the water within the aerobic bacteria solution evaporated, causing an increased concentration of aldicarb within the remaining solution. Results from the experiment are presented in Appendix A.

Results from an additional experiment using only anaerobic bacteria are presented in Appendix B. The four solutions for the experiment included: one solution used directly from the refrigerator, one solution left at room temperature for 10 days, one solution left at room temperature for 10 days with 0.1 mg L^{-1} aldicarb, and one solution left at room temperature for days with 10% of the acetate feed solution. The purpose of this experiment was to determine if aldicarb transformation differed with anaerobic bacteria that were acclimated to various conditions. These conditions better replicated the conditions within an MFC. The transformation of aldicarb within these various media did not differ significantly.

4.2 Aldicarb Transformation and MFC Response

The transformation of aldicarb within two MFCs was measured over eight two-day feeding cycles. The change in aldicarb concentrations in MFC 1 and MFC 3 over eight feeding cycles is shown in Figure 13. For MFC 1, only seven feeding cycles were analyzed due to an error in collection on 18 July 2013. The average difference in concentration over the two day feeding cycles for MFC 1 was 15.89%, with a range of

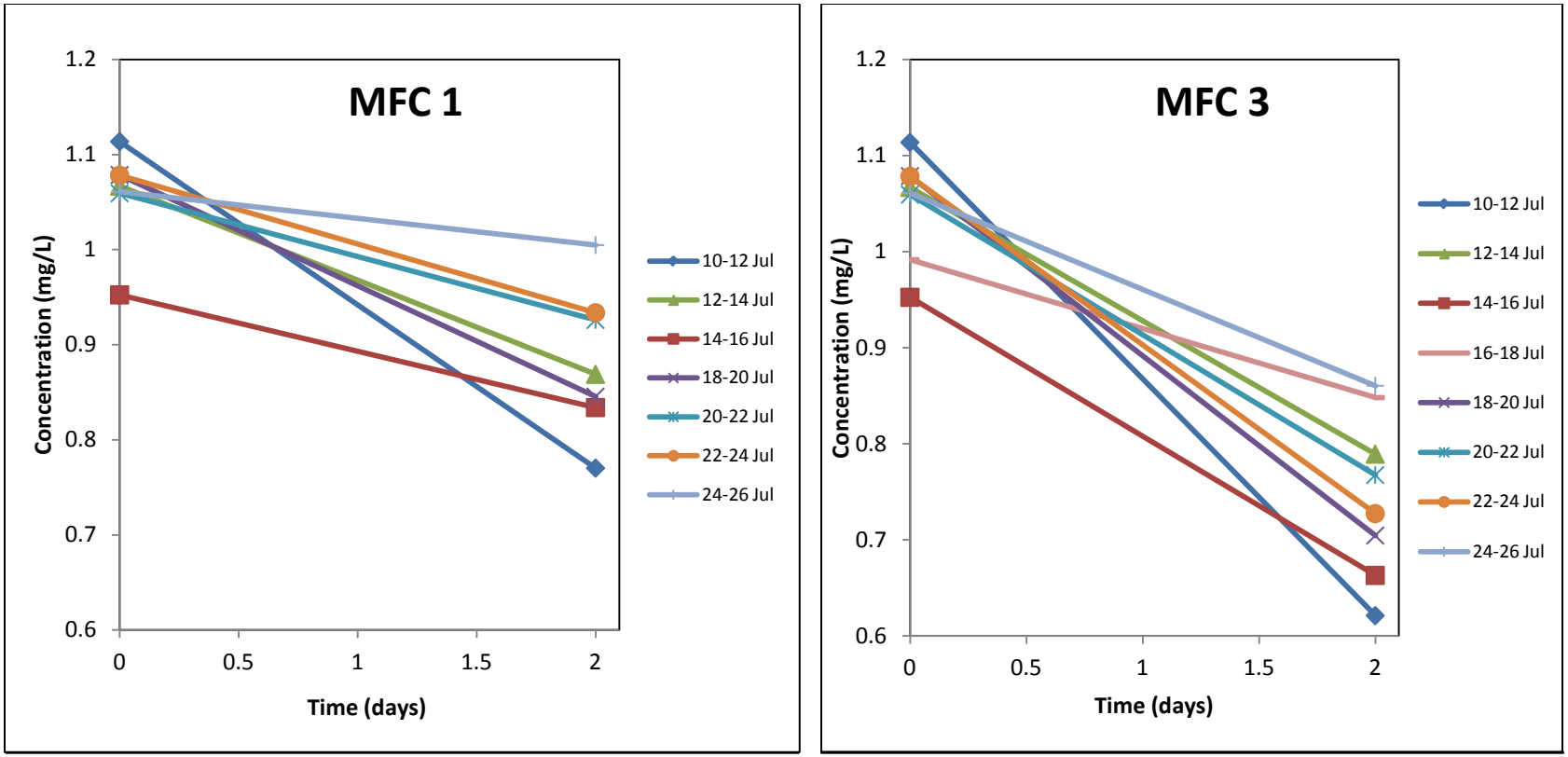


Figure 13. Aldicarb Transformation in MFCs

5.30% to 30.83%. The average difference in concentration for MFC 3 was 28.81%, with a range of 14.47% to 44.24%.

The potential for the loss of aldicarb to sources other than the microbial community was considered. The log octanol-water partition coefficient for aldicarb is 1.359 (WHO, 2013). Aldicarb is relatively hydrophilic and, therefore, the potential loss of aldicarb from adsorption to the interior surfaces of the MFC is minimal. Additionally, aldicarb does not have any readily dissociable functionality and does not have dissociation constant.

The changes in aldicarb concentrations in MFC 1 and MFC 3 were evaluated over a time series to determine if microbial acclimatization effected the transformation. Figure 14 displays the percent reduction of aldicarb over the eight feeding cycles. For MFC 1 and MFC 3, the first feeding cycle had the largest difference in aldicarb concentration. This result has an unknown degree of uncertainty because the stock aldicarb solution may have been diluted from acetate feed solution that did not drain from the MFCs from the prior feeding cycle or from the deionized water rinse. After the first feeding cycle, the MFCs were rinsed with the aldicarb solution instead of deionized water. The aldicarb transformation after the first feeding cycle ranged from 5.30% to 21.61% difference in MFC 1 and 14.47% to 34.67% difference in MFC 3. Figure 14 does not indicate that the microbial community within the MFCs acclimatized to the aldicarb and increased the rate of transformation. The rate of transformation within MFC 3 did not display a consistent increase or decrease, while the rate of transformation within MFC 1 appeared to decrease.

While Figure 14 does not display MFC acclimatization to aldicarb, Figure 15 displays the peak height (PH) of the current profiles of MFC 3 for the acetate feed

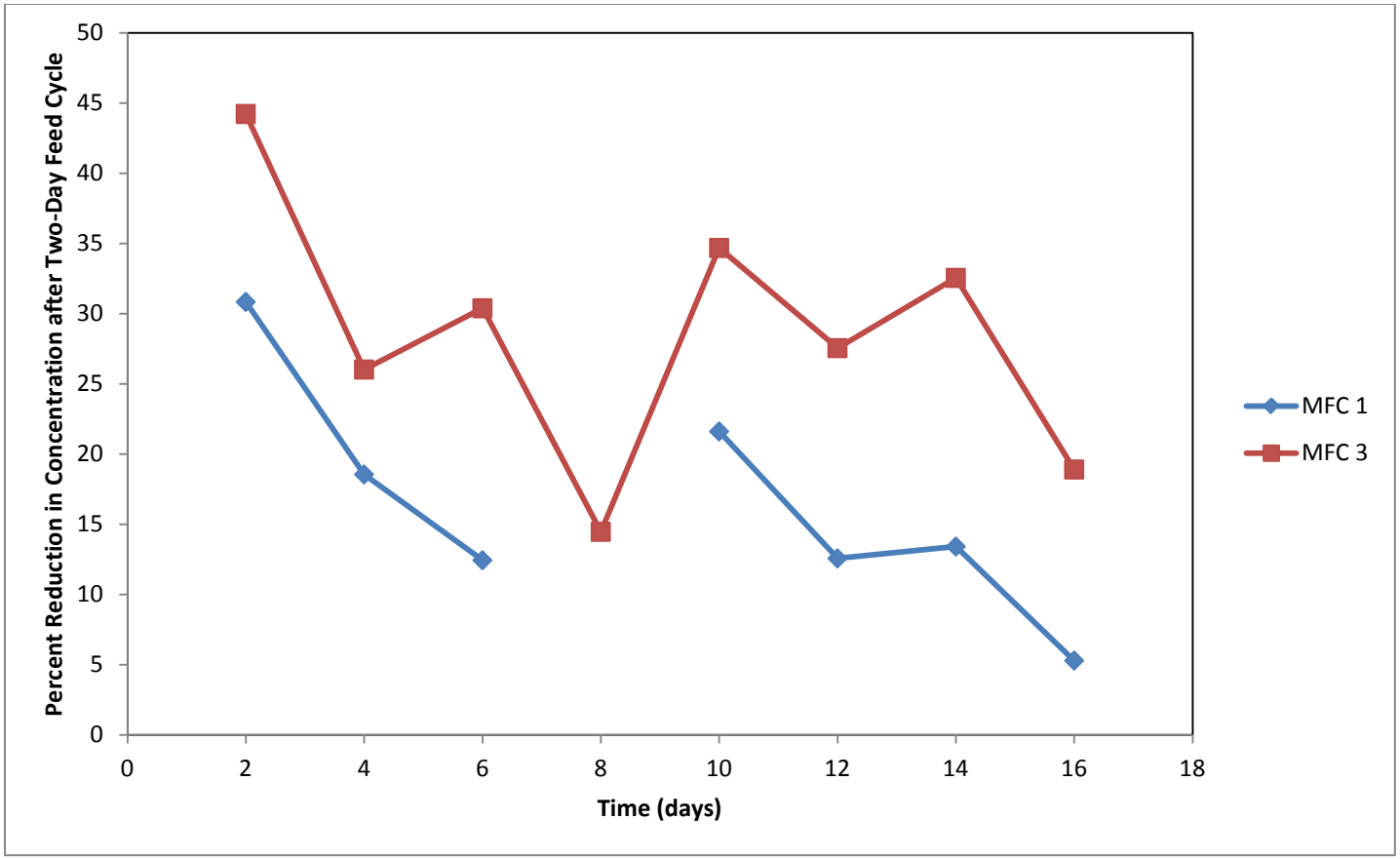


Figure 14. Aldicarb Transformation in MFCs over Eight Feed Cycles

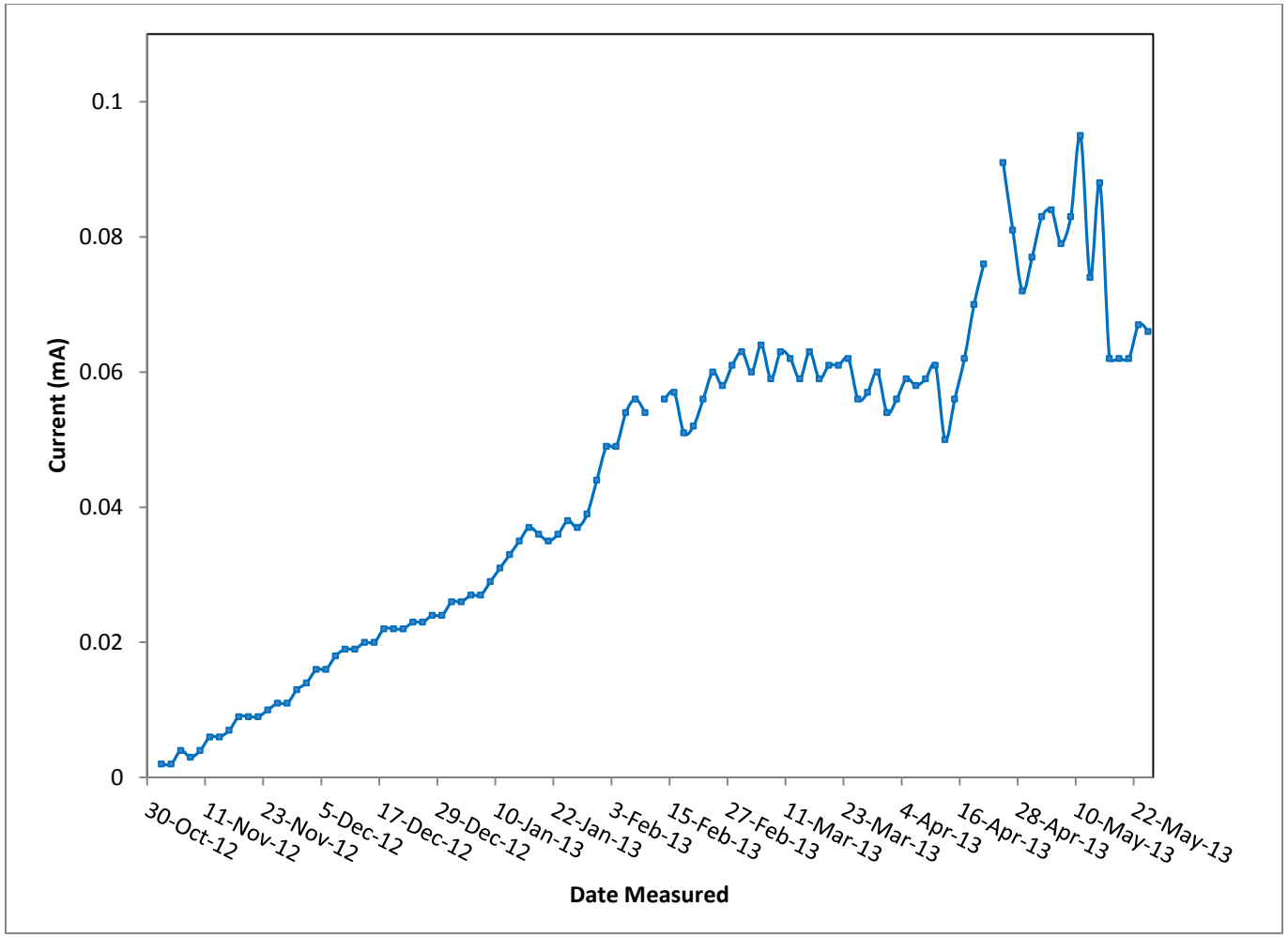


Figure 15. MFC 3 Peak Height Measurements

solution after inoculation. While the measured PH from acetate and the difference in aldicarb concentrations are not a direct comparison, Figure 15 shows the PH from the acetate solution beginning below 0.01 mA in November 2012, and increasing linearly to approximately 0.06 mA in March 2013. This is an example of an MFC acclimating to the given substrate, and producing an increased current over several months with that same substrate.

Figure 16 shows the current profiles of MFC 1 during 10 feeding cycles. For the first 5 feeding cycles displayed, MFC 1 was injected with 1 mg L^{-1} aldicarb in DI and for the last 5 feeding cycles, MFC 1 was injected with the acetate feed solution. Current profiles for feedings prior to 16 July 2013 could not be displayed due to a computer malfunction. The difference in aldicarb concentration in mg L^{-1} is displayed for 4 of the 5 feeding cycles. The feeding cycle ending on 18 July 2013 was not analyzed due to an error in sample collection.

Figure 17 shows the current profiles of MFC 3 during 8 feeding cycles. For the first 3 feeding cycles displayed, MFC 3 was injected with 1 mg L^{-1} aldicarb in DI and for the last 5 feeding cycles displayed, MFC 3 was injected with acetate feed. Current profiles for feedings prior to 16 July 2013 could not be displayed due to a computer malfunction. The current profiles from 18-22 July were not available because the data from the Keithley Meter was not usable. This was most likely due to corrosion build up on the wire connections and clips or loss of contact between the air cathode and the exchange membrane.

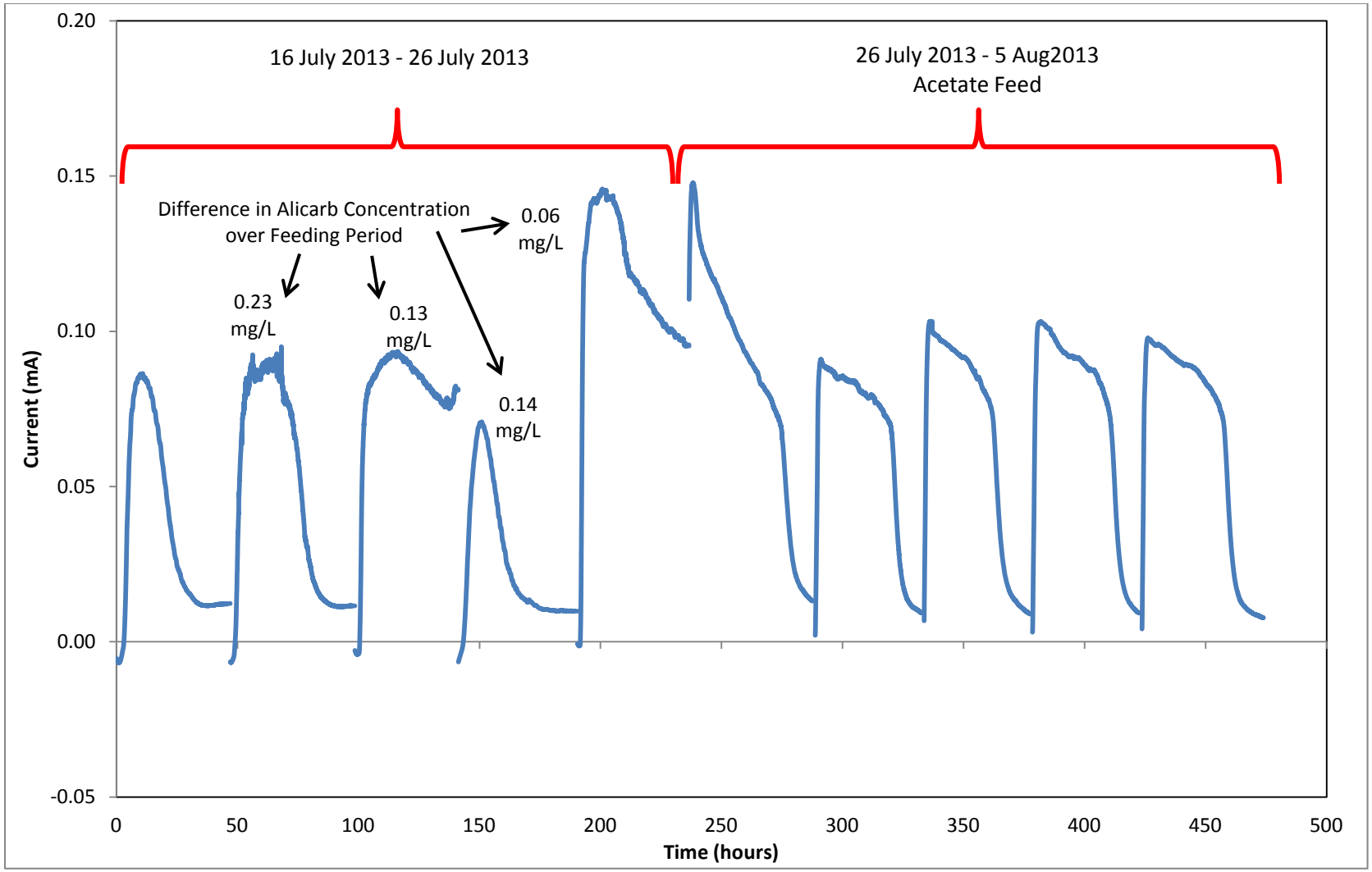


Figure 16. MFC 1 Aldicarb and Acetate Current Profiles

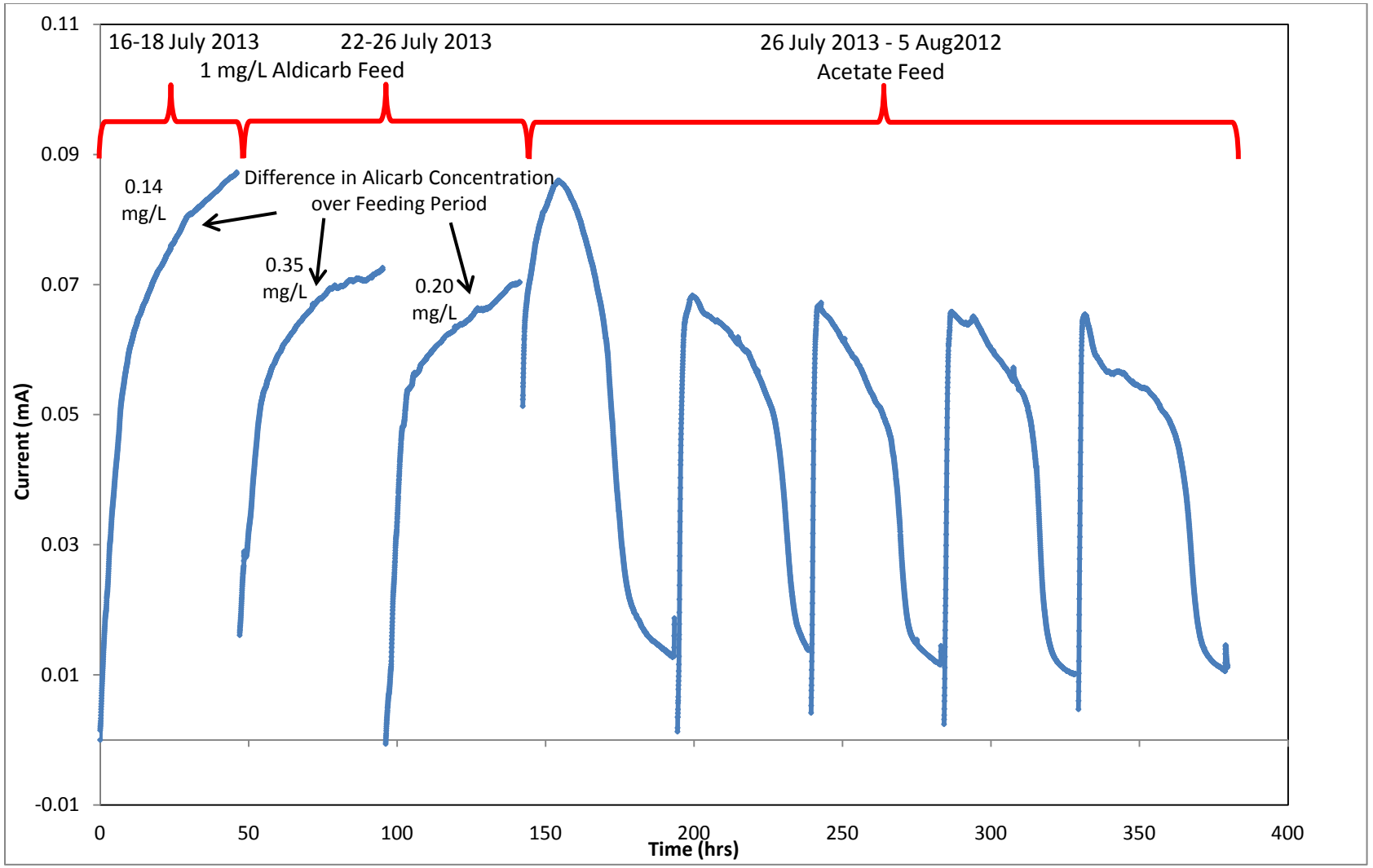


Figure 17. MFC 3 Aldicarb and Acetate Current Profiles

The relationship of the current profiles and the difference in concentration was evaluated using qualitative and quantitative means. A visual evaluation of Figure 16 and Figure 17 does not show a correlation between the difference in concentration and current profiles produced over the feeding cycles. Current metrics (peak height, area, acceleration rate, 10 hour subsidence rate, subsidence rate and first moment) were determined for each of the current profiles and are listed in Table 2 for MFC 1 and MFC 3. The relationship between the difference in aldicarb concentration and the current metrics was evaluated using simple linear correlation. The coefficient of determination (r^2) was calculated for each of the MFC metrics compared to the change in concentration. The results show that there is limited correlation between the change in concentration of aldicarb and the MFC current profile metrics. For both MFCs, the increase of aldicarb transformation correlated more to the decrease in the peak height of the current profile than the increase of peak height. Graphs from JMP statistical software are in Appendix C. These results strongly suggest that aldicarb is not a substrate for anode-respiring bacteria. Aldicarb transformation occurring in the MFCs was likely caused by other mechanisms, possibly including novel abiotic chemical reactions with chemical byproducts generated by ARBs.

4.3 Comparison of Aldicarb MFC and Substrate Transformation Experiments

The magnitude of aldicarb transformation in MFCs was most similar to the transformation in the aerobic bacteria solution, as displayed in Figure 18. While the microbial communities within the MFCs are anaerobic, the anaerobic bacteria solution did not display the same magnitude of transformation as the MFCs. The anaerobic bacteria solution was developed from the activated sludge from a waste water treatment

Table 2. Correlation of Aldicarb Current Metrics and Concentration Difference

MFC 1							
Concentration Difference (mg/L)	**Concentration Percent Difference**	**Peak Height**	**Area**	**Acceleration Rate**	**10-hr Subsidence Rate**	**Subsidence Rate**	**First Moment**
0.233	21.61	0.095	2.44	0.03048	-0.00224	-0.0091	50.3
0.133	12.58	0.093	3.295	0.05548	-0.00074	-0.00089	41.5
0.145	13.42	0.071	1.22	0.01712	-0.00464	-0.00507	48.1
0.056	5.30	0.146	5.189	0.11825	-0.0024	-0.0011	45
Coefficient of Determination with Concentration Difference (R^2)		**0.411**	**0.457**	**0.633**	**< 0.000**	**0.765**	**0.381**

MFC 3							
Concentration Difference (mg/L)	**Concentration Difference (%)**	**Peak Height**	**Area**	**Acceleration Rate**	**10-hr Subsidence Rate**	**Subsidence Rate**	**First Moment**
0.143	14.47	0.087	3.154	0.00719	N/A	N/A	82.7
0.351	32.55	0.073	2.961	0.00464	N/A	N/A	78.3
0.201	18.90	0.07	2.626	0.00945	N/A	N/A	65.8
Coefficient of Determination with Concentration Difference (R^2)		**0.314**	**0.007**	**0.559**	**N/A**	**N/A**	**0.001**

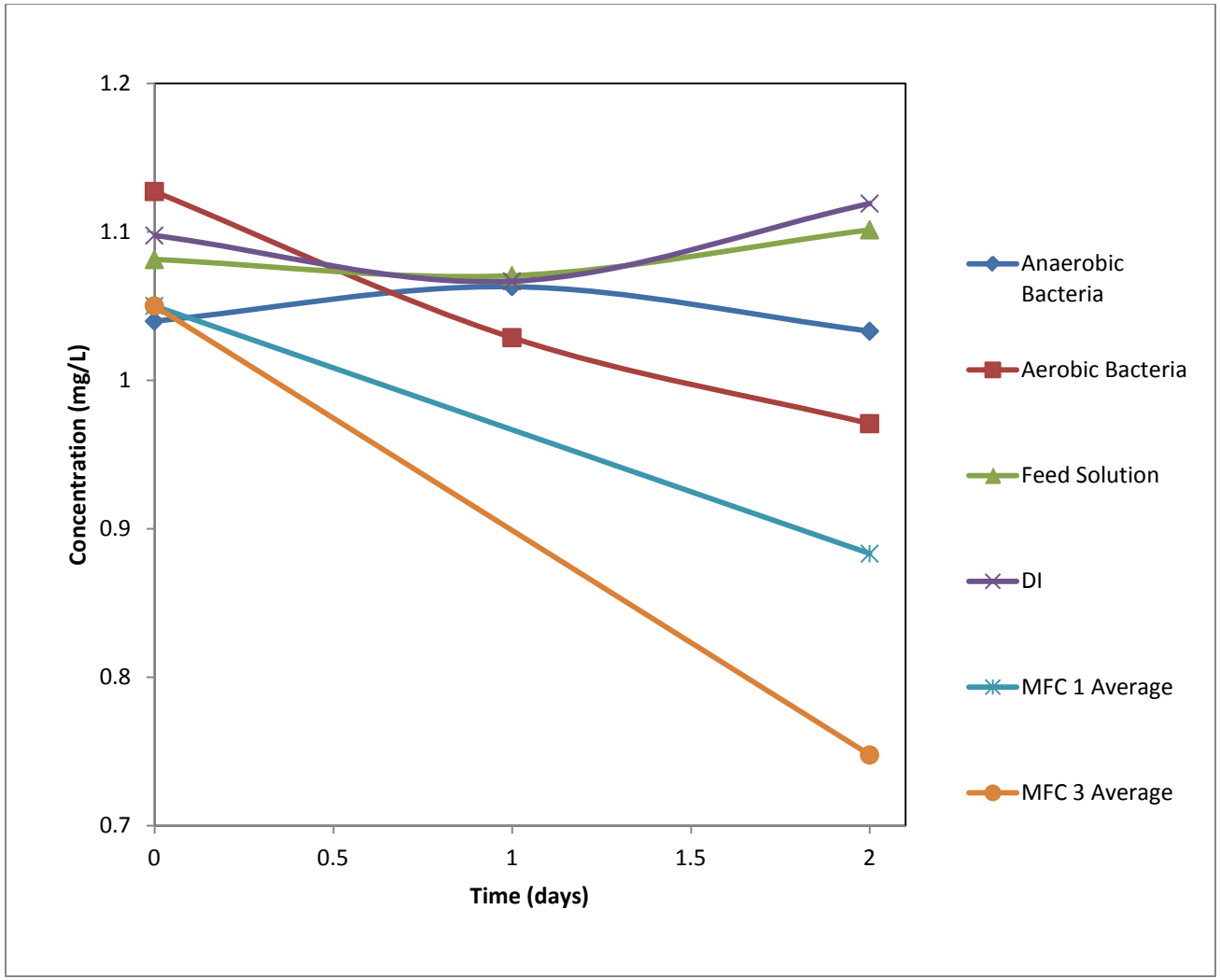


Figure 18. Aldicarb Transformation in MFCs and Media

plant, and was the source of bacteria for inoculating the MFCs. It was speculated that the community may not have been active, due to refrigeration, but the further experiments with anaerobic bacteria discussed in section 4.1 showed that refrigeration did not affect the ability of the bacteria to transform aldicarb.

4.4 Coulombic Comparison of Aldicarb Current Production

Using Equations 2 and 3, the coulombs recovered and the total coulombs in the substrate were calculated in Table 3. The coulombs recovered were calculated using the area under the current profiles for the aldicarb feeding cycles. The total coulombs in the substrate were calculated using the chemical oxygen demand of aldicarb and the difference in aldicarb concentration measured of over the feeding cycle. While Equation 2 is the coulombic efficiency equation, the result was used as a coulombic comparison, because the coulombs recovered were much greater than the total coulombs available. C_e ranged from 7.85×10^5 to 8.59×10^6. This coulombic comparison shows that, in some cases, the electrical charge produced by the MFC was over a million times greater than the available charge from the aldicarb consumed. This would indicate that the current production is a result of a substrate other than aldicarb, which will be discussed further in section 4.6.

4.5 Aldicarb Cyclic Voltammetry

Cyclic voltammetry experiments conducted on an aldicarb solution, an acetate feed solution and deionized water are presented in Figures 19 and 20. Figure 19 displays each of the cyclic voltammograms for aldicarb, acetate feed and deionized water, while Figure 20 displays the voltammograms for aldicarb and deionized water at a decreased

Table 3. Coulombic Comparison of Aldicarb in MFCs

	Aldicarb Concentration Difference	MFC Current Production	Coulombs Recovered	Total coulombs in substrate	C_e
	(ppm)	(mA)	$8 I t_b$	$F v_{An} \Delta COD$	
MFC 1	0.233	2.440	3373	3.46E-03	9.74E+05
	0.133	3.295	4555	1.98E-03	2.30E+06
	0.145	1.220	1686	2.15E-03	7.85E+05
	0.056	5.189	7173	8.35E-04	8.59E+06
MFC 3	0.143	3.154	4360	2.13E-03	2.05E+06
	0.351	2.961	4093	5.21E-03	7.85E+05
	0.201	2.626	3630	2.98E-03	1.22E+06

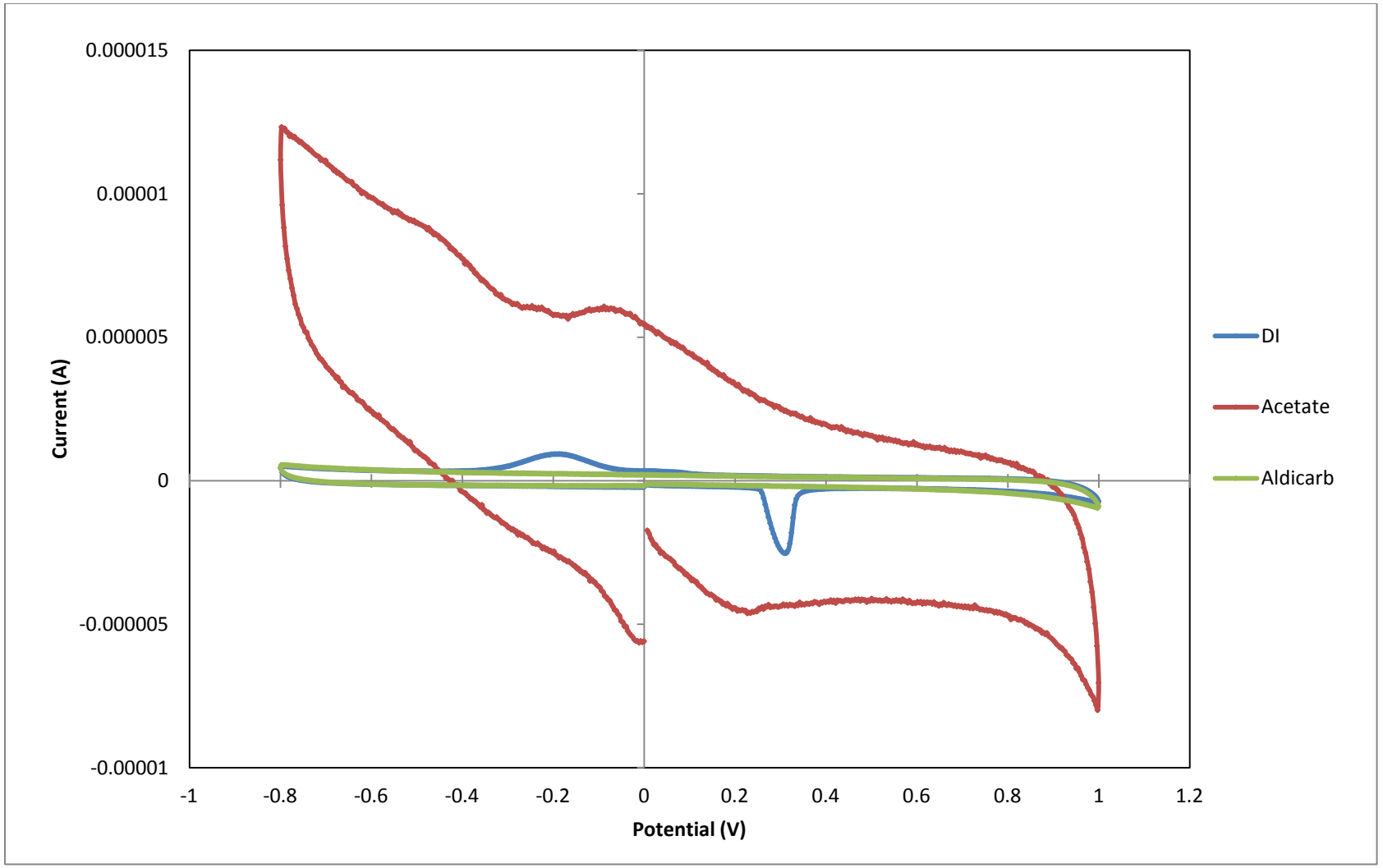


Figure 19. Cyclic Voltammograms for Aldicarb, Acetate and Deionized Water

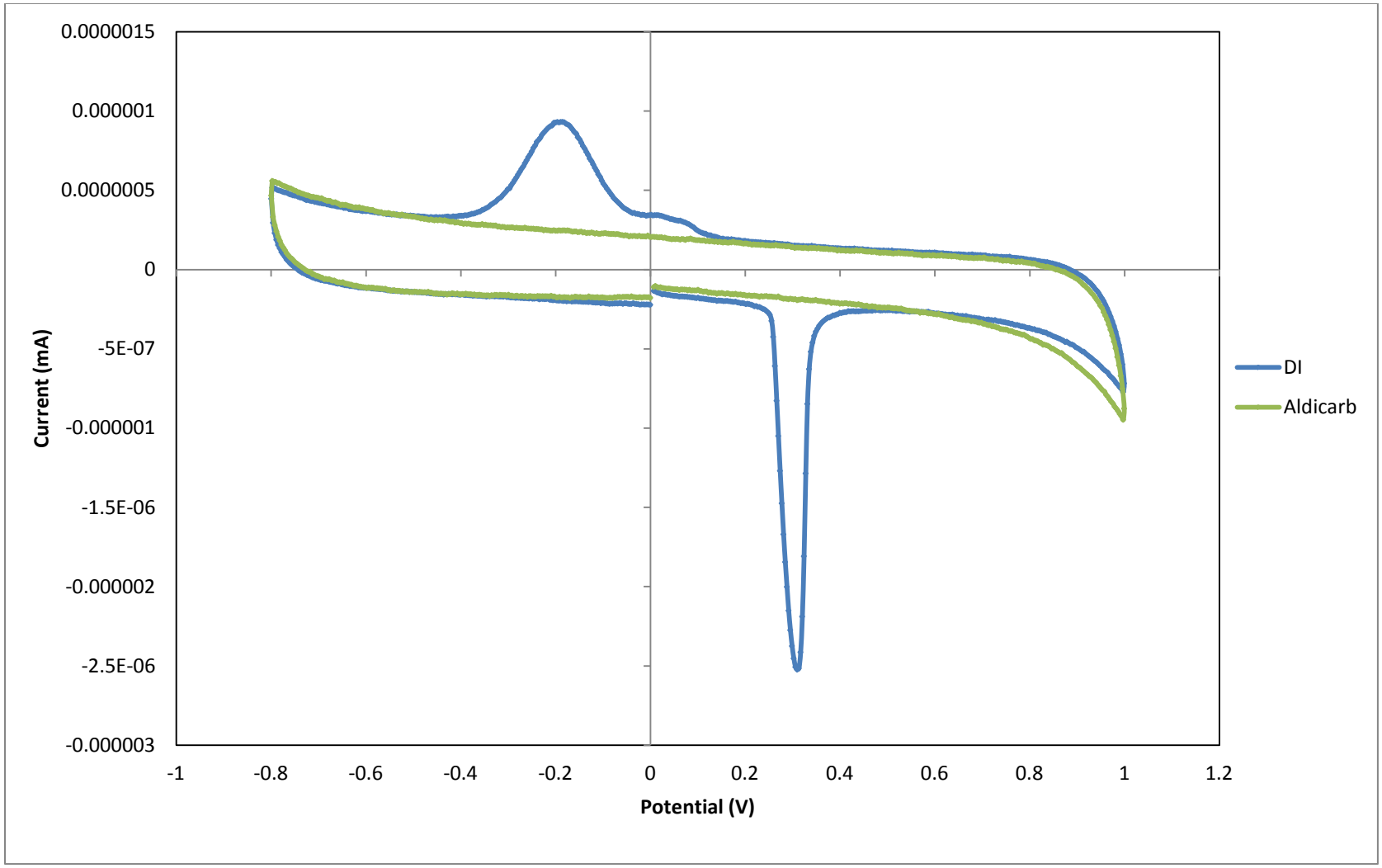


Figure 20. Cyclic Voltammograms for Aldicarb and Deionized Water

scale. The CV for the acetate feed solution displays peaks at 0.2 V and -0.1 V, with current production (-7.5 μA - 12 μA) an order of magnitude greater than aldicarb and deionized water. The CV for deionized water produced oxidation and reduction peaks at 0.3 V and -0.2 V, while the CV for aldicarb did not produce any peaks. The magnitude of current and the lack of redox peaks for the aldicarb CV would suggest that it has a limited oxidation potential.

4.6 Acetonitrile and Water Current Profiles

Aldicarb used in the experiments was dissolved in acetonitrile prior to being diluted in deionized water. The approximate concentration of acetonitrile in a 1 mg L^{-1} aldicarb solution was 1%. Four feeding cycles using 1% acetonitrile solution were conducted in MFC 1 and four feeding cycles using deionized water were conducted in MFC 3. The results from the feeding cycles are displayed in Figures 21 and 22.

The current production from the 1% acetonitrile feed solution was on the same scale as the acetate feed solutions. When compared to Figure 15, the 1% acetonitrile feed solution produced similar peaks to the 1 mg L^{-1} aldicarb feed solution. Figure 22 shows that current production from deionized water significantly differed from the current production of acetate and aldicarb. The current production is approximately an order of magnitude less than acetate and aldicarb feed solutions. This supports the notion that the majority of current production from aldicarb feed solutions results from the acetonitrile.

4.7 DMMP Transformation and Current Production Experiments

The experiments using DMMP were conducted and included in Appendix 2. Transformation experiments in the four media (aerobic bacteria, anaerobic bacteria,

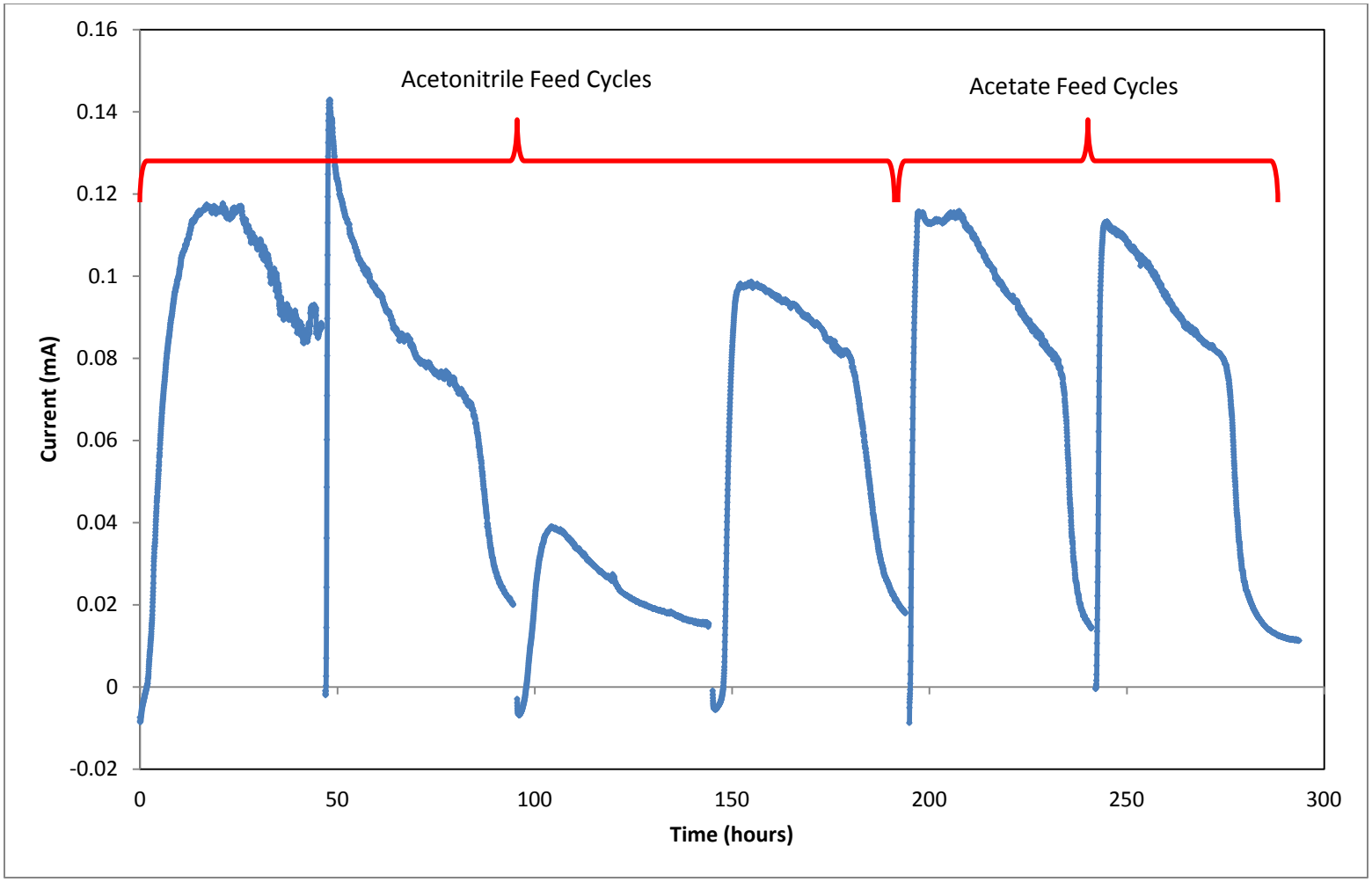


Figure 21. Acetonitrile and Acetate Current Profiles

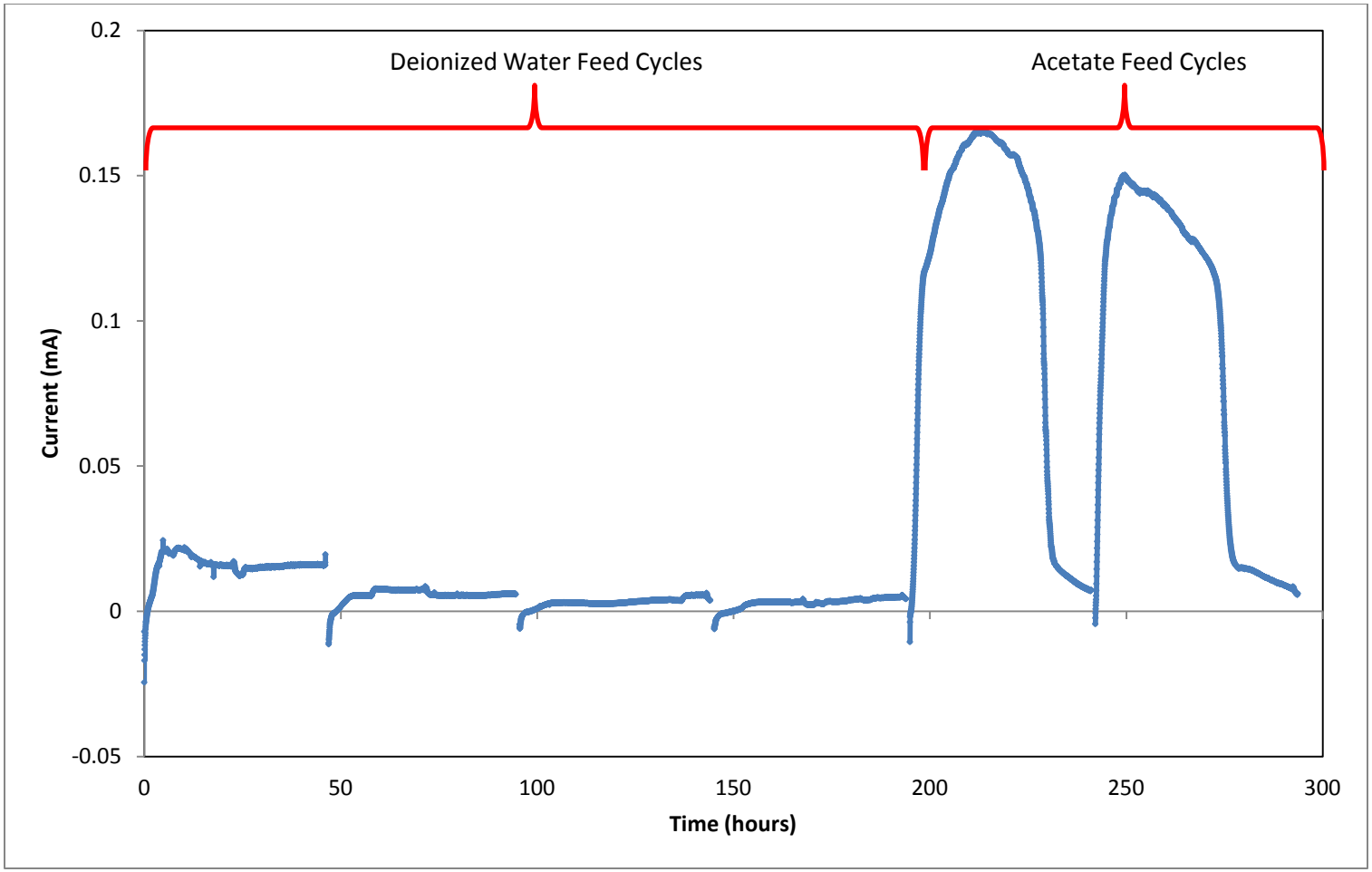


Figure 22. Deionized Water and Acetate Current Profiles

acetate feed, deionized water) using DMMP proved that DMMP transformed most rapidly in deionized water. Due to laboratory limitations, stock solutions of DMMP feed and DMMP feed used in MFC experiments were held for multiple days prior to GC/MS analysis. Sample results from MFC analysis showed DMMP concentrations were higher after a two day feed cycle in the MFC. Most likely, DMMP degraded more rapidly in the stock solution, made up solely of DMMP and deionized water, than in the MFC due to pH conditions. This is supported by the transformation experiments; the experiment in deionized water resulted in a 25.9% loss of DMMP over the two day experiment, while the loss of DMMP in the aerobic and anaerobic bacteria solutions were 17.3% and 9.87%, respectively.

Current profiles from eight DMMP feed cycles are included in Appendix 2. Current production was an order of magnitude less than acetate and resembled the current profiles from deionized water in Figure 22. Based on the limited current production, the lack of microbial degradation in the transformation experiments and supporting data (DMMP Consortium, 2005), it is unlikely that DMMP was degraded or transformed within the MFCs.

V. Conclusion

MFCs are capable of transforming aldicarb. Transformation experiments in biotic and abiotic media proved that, in principle, 1 mg L^{-1} aldicarb can be partially biotransformed in the presence of active aerobic bacteria. The concentration of aldicarb changed by 2% or less in the presence of deionized water, acetate feed solution, or anaerobic bacteria. Partial transformation of aldicarb occurred in two MFCs (average concentration difference – MFC1 15.9%, MFC3 28.8%) which suggests that there may be novel, perhaps abiotic, transformation mechanisms in the MFCs. Aldicarb is unlikely to adsorb to the MFC materials because of its low partitioning coefficient.

Aldicarb is not a substrate for anode-respiring bacteria. This conclusion is supported by the fact that #1) the current metrics did not correlate well with the amount of aldicarb transformation observed in the MFC, and #2) the majority of current generation from the aldicarb feed cycles was most likely due to the acetonitrile solvent, #3) aldicarb has limited redox potential, as shown by the cyclic voltammetry results, #4) the MFCs did not display acclimatization to aldicarb through an increased concentration difference over time, while the current generation from aldicarb increased linearly of several months. Aldicarb may indirectly affect the microbial community in the MFC through interactions with ARB or non-ARB byproducts or enzymes and competition between groups of microorganisms (Parameswaran et al., 2009; Sharma and Li, 2009; Nam, et al., 2011). It is in this way that the effects of aldicarb may become detectable.

Within civilian and military environments, vulnerabilities exist within water storage and distribution systems. Recalcitrant organic compounds such as toxic pesticides may enter and contaminate drinking water sources intentionally or accidently. Monitoring

for a broad range of contaminants at many locations within budget constraints is currently not possible. MFCs show the ability to produce rapid changes in current profiles when exposed to new substrates. This property has the potential to be harnessed into low cost, real-time, continuous detection systems.

Further Research

Prior to the fielding of MFCs within drinking water distribution systems, additional research is required. MFCs have the ability to work as batch or steady state systems. For operational use, a steady state system could provide continuous and real-time data. There are multiple challenges that must be addressed. Drinking water would most likely not have a substrate to produce a stable current. A system could be developed in which a substrate is mixed with the drinking prior to entering the MFCs. Additionally, disinfectant residuals may negatively affect the microbial community within the MFCs. A chemical, such as sodium thiosulfate, may be utilized to neutralize the disinfectant residual. Another key factor that must be determined prior to fielding MFCs is the contact time compounds at various concentrations require in order to detect changes in current. Other research topics include:

- The effect of additional recalcitrant organic chemicals on anode-respiring bacteria and MFCs
- The identity of the metabolites produced in MFCs
- The coulombic potential of other recalcitrant chemicals that may be detected with a MFC

- The temporal effect of corrosion on MFC activity and biosensing effectiveness
- The transformation of chemicals in MFCs deployed in the field under dynamic environmental settings
- The effects of disinfectant residuals on MFCs deployed to monitor treated water sources.

Appendix A. First Aldicarb Transformation Experiment

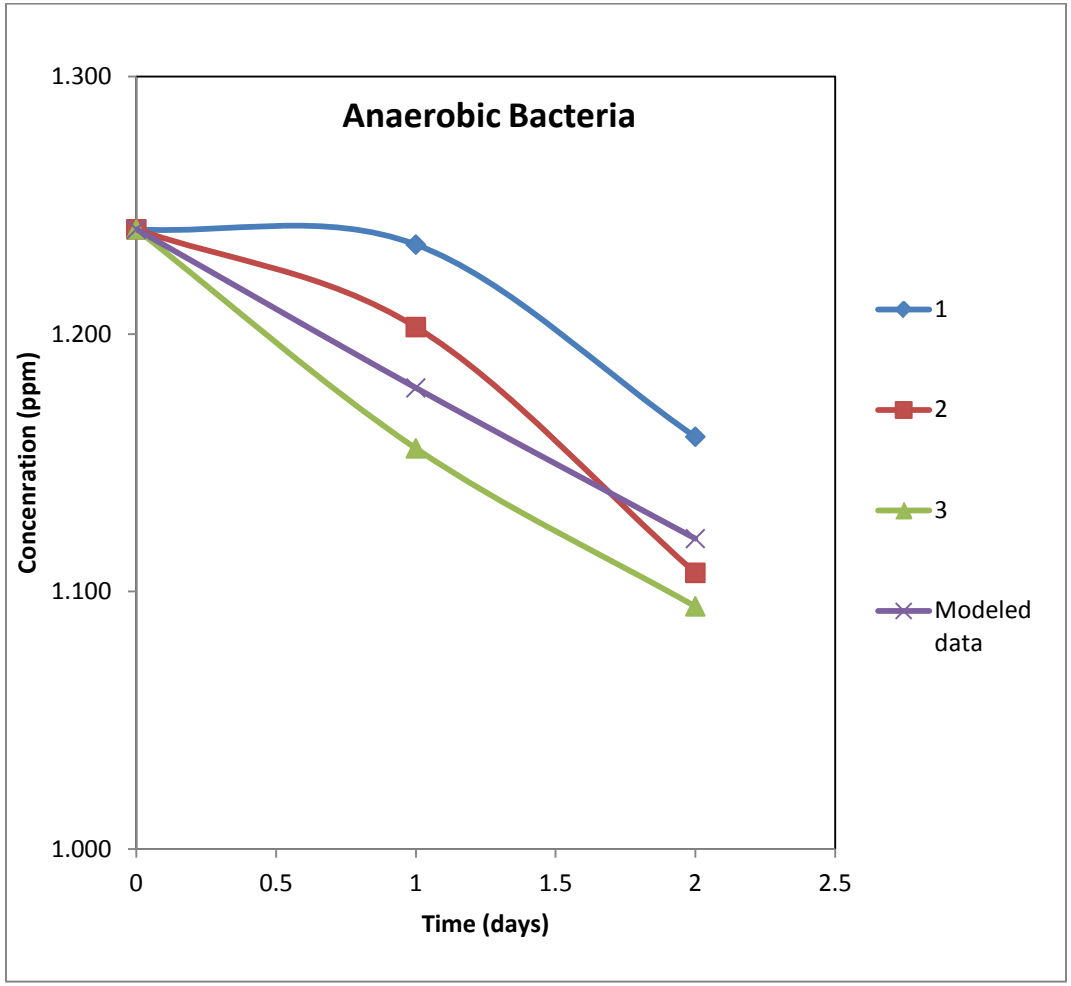


Figure 23. Aldicarb Transformation in Anaerobic Bacteria

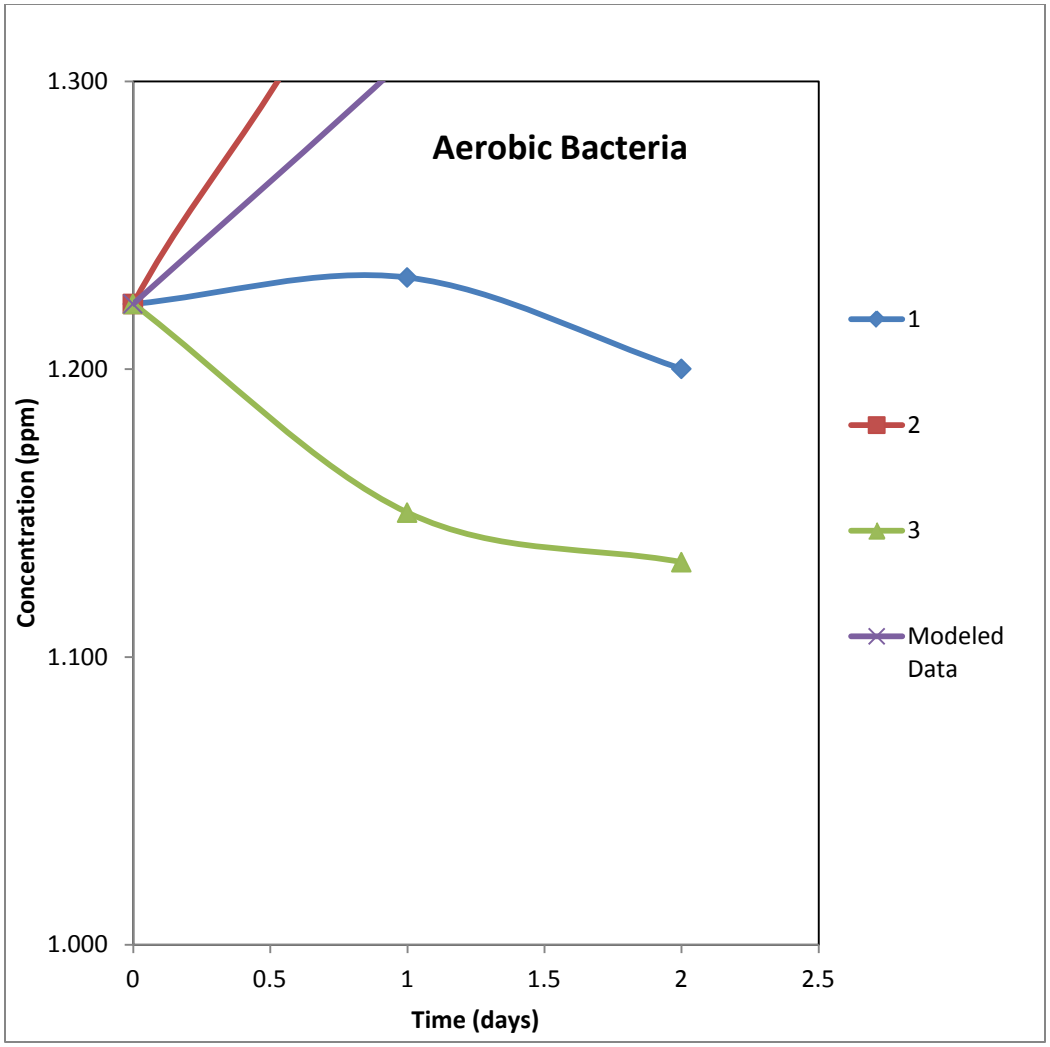


Figure 24. Aldicarb Transformation in Aerobic Bacteria with Possible Evaporation

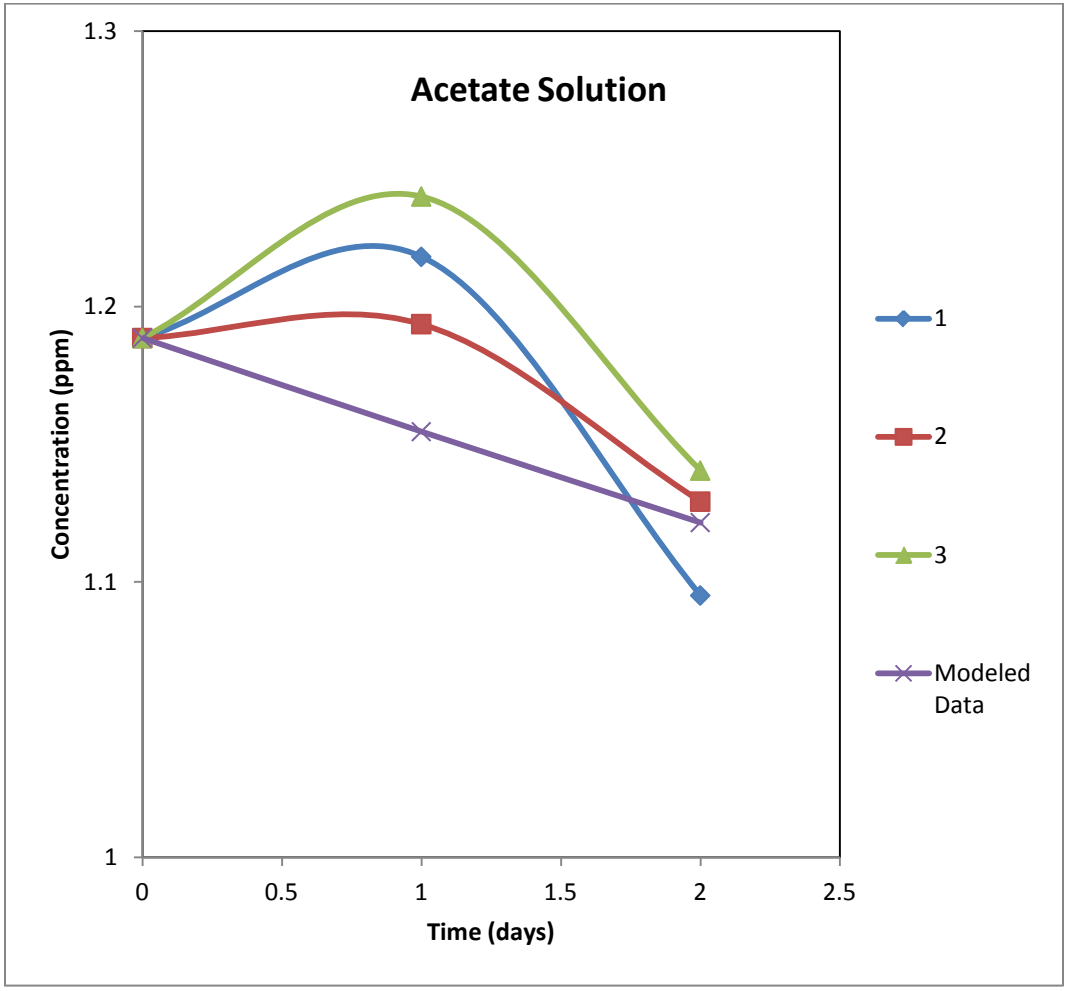


Figure 25. Aldicarb Transformation in Acetate Feed Solution

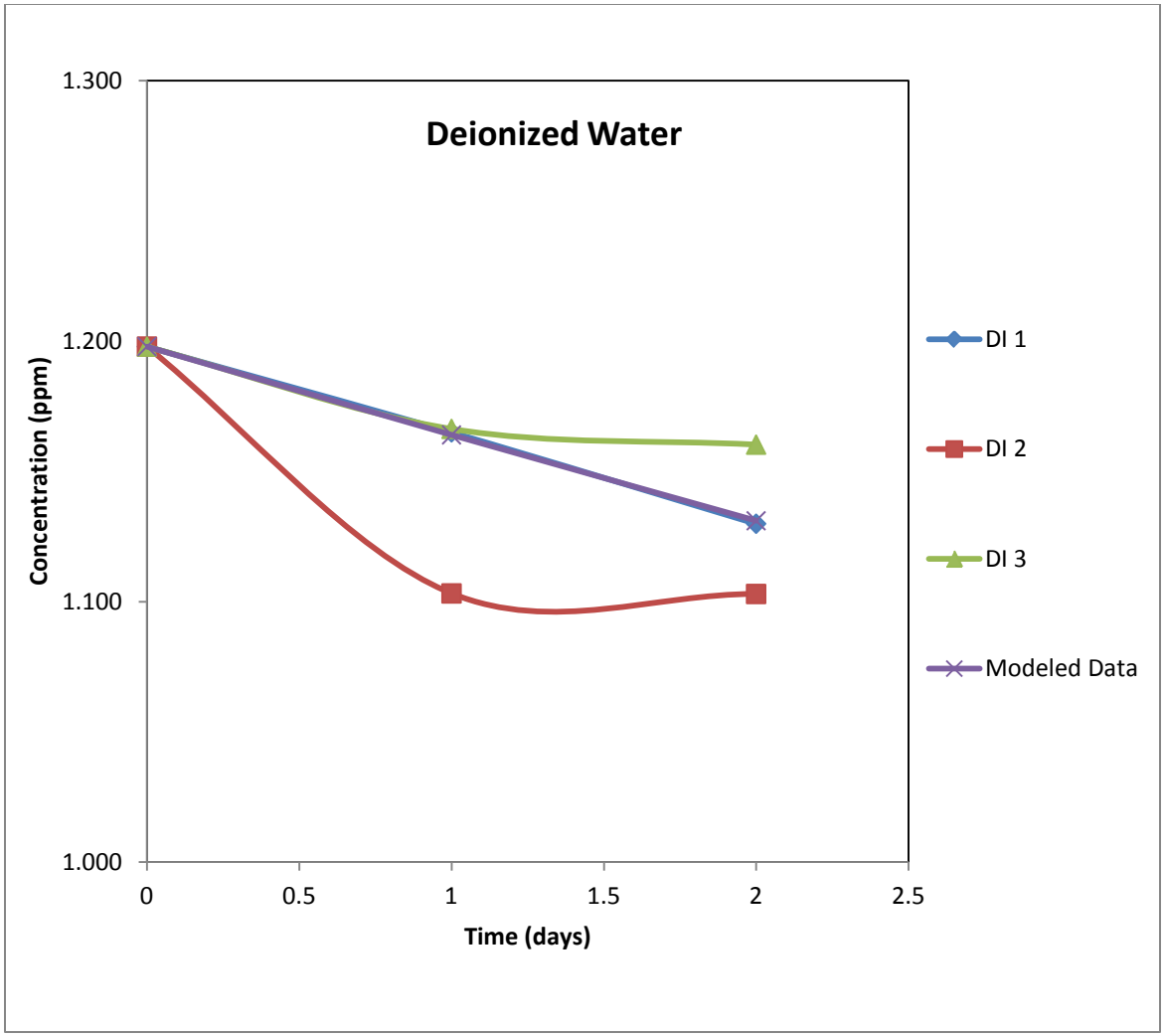


Figure 26. Aldicarb Transformation in Deionized Water

Table 4. First Aldicarb Transformation Experiment

		Anaerobic Bacteria				Aerobic Bacteria				Acetate Feed				Deionized Water			
Time (Days)	Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)	
0	Stock	385146	1.241	% Diff	Stock	379832	1.223	% Diff	Stock	369758	1.188536	% Diff	Stock	372523	1.198	% Diff
1	1	383396	1.235	0.478	1	382550	1.232	-0.753	1	378467	1.218	-2.48	1	362805	1.165	2.74
1	2	373939	1.203	3.06	2	432711	1.402	-14.65	2	371253	1.194	-0.426	2	344550	1.103	7.91
1	3	360040	1.156	6.85	3	358429	1.150	5.93	3	384932	1.240	-4.32	3	363193	1.166	2.63
2	1	361370	1.160	6.49	1	373178	1.200	1.84	1	342169	1.095	7.86	1	352448	1.130	5.67
2	2	345767	1.107	10.75	2	570117	1.867	-52.71	2	352230	1.129	4.99	2	344488	1.103	7.92
2	3	341926	1.094	11.80	3	353347	1.133	7.33	3	355557	1.140	4.04	3	361412	1.160	3.14
1	Average		1.198	3.46	Average		1.261	-3.15	Average		1.217	-2.41	Average		1.145	4.43
2	Average		1.121	9.68	Average		1.400	-14.51	Average		1.122	5.63	Average		1.131	5.58
	k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)			
	0.051				-0.068				0.029				0.029			
0			1.241				1.223				1.189				1.198	
1			1.179				1.308				1.155				1.164	
2			1.121				1.400				1.122				1.131	

Appendix B. Aldicarb Transformation in Four Anaerobic Bacteria Solutions

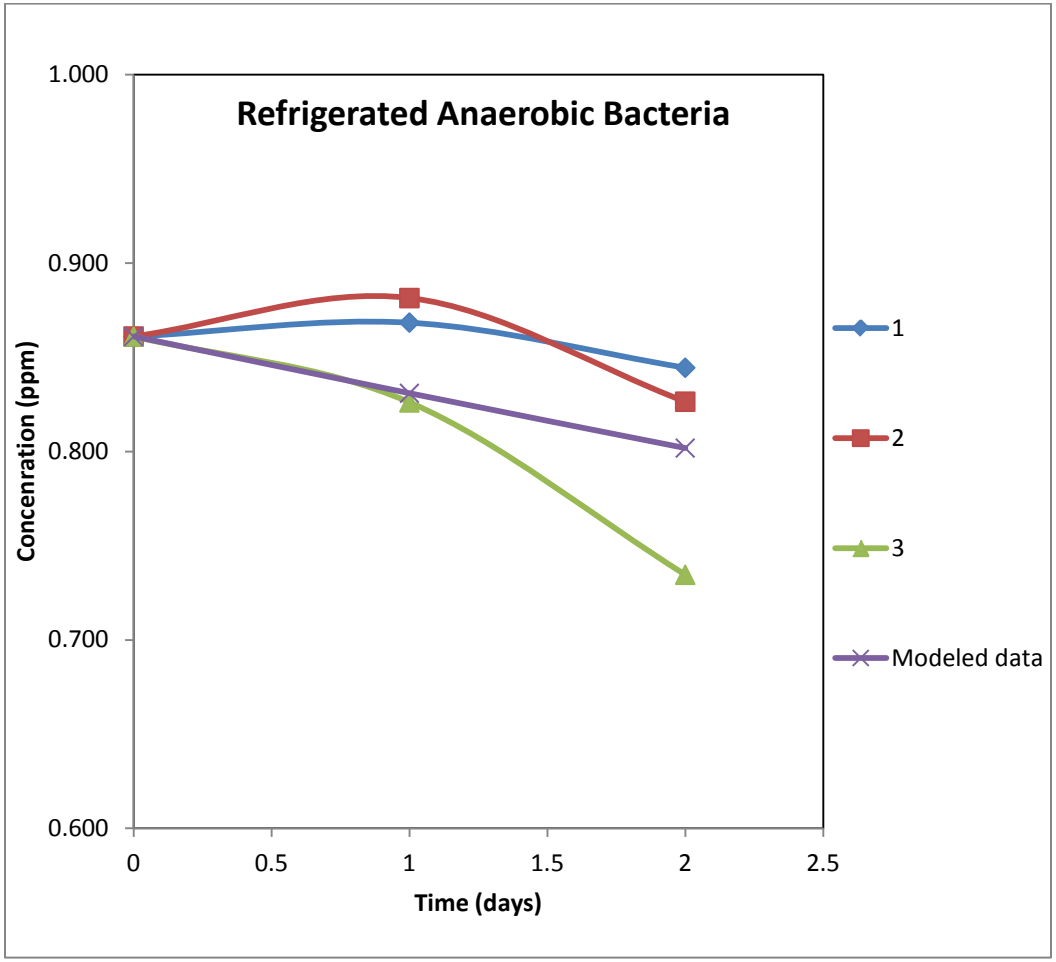


Figure 27. Aldicarb Transformation with Refrigerated Anaerobic Bacteria

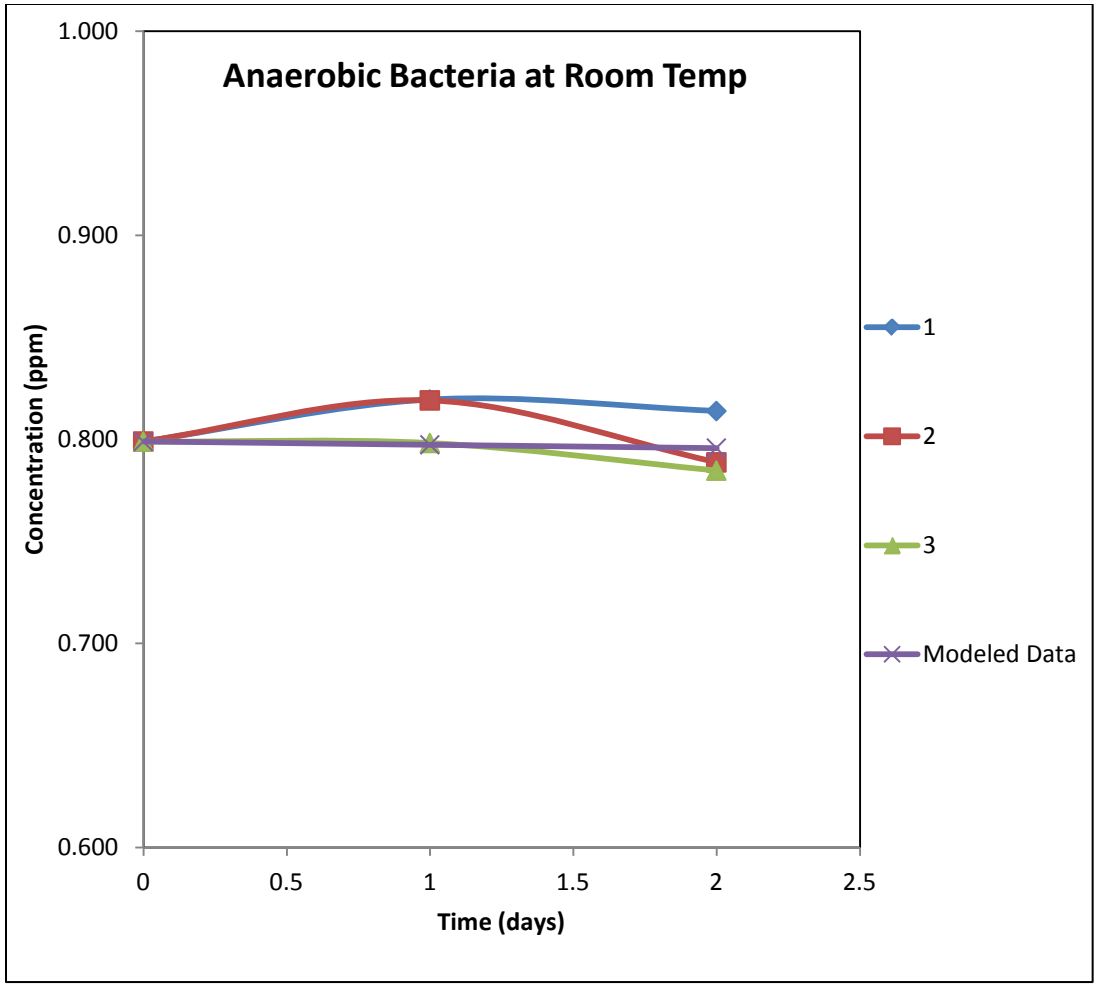


Figure 28. Aldicarb Transformation with Anaerobic Bacteria at Room Temperature

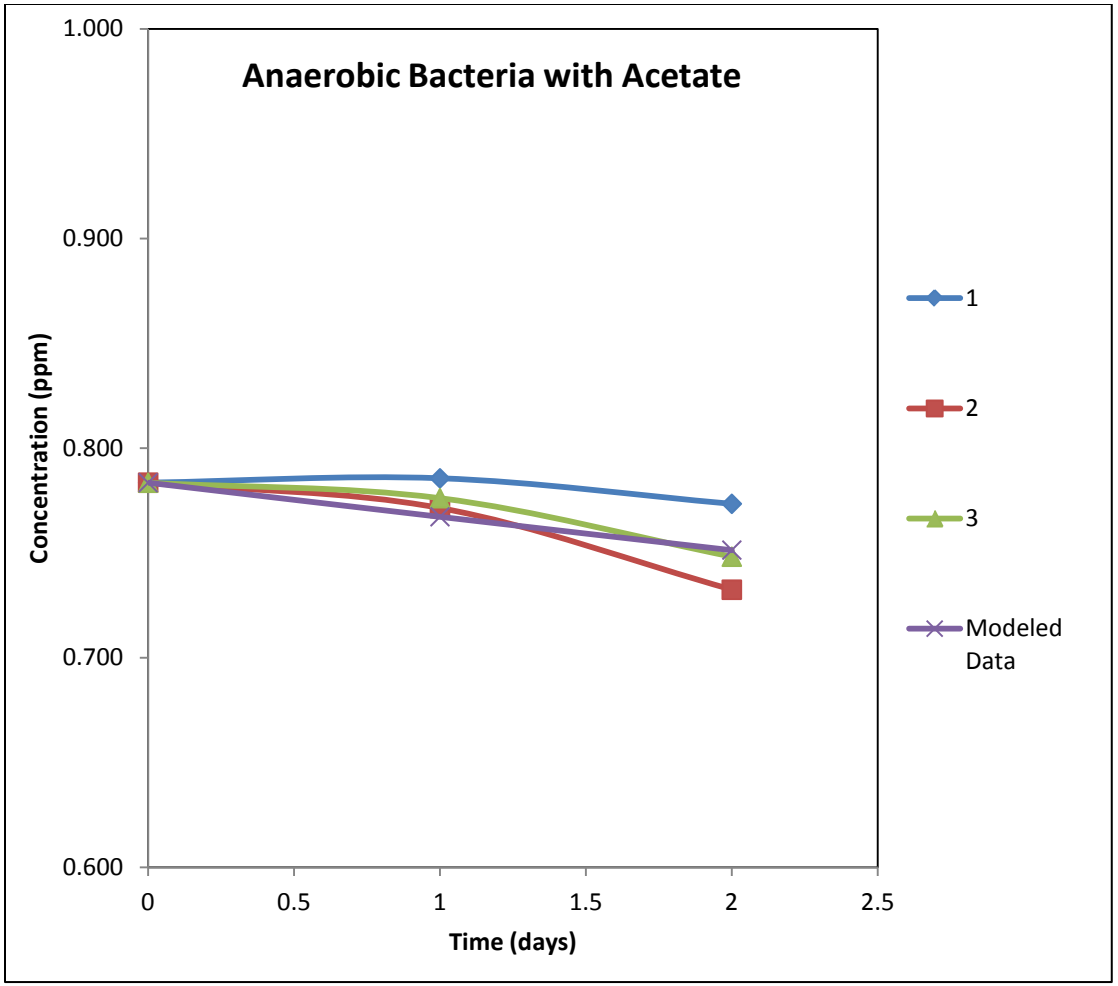


Figure 29. Aldicarb Transformation in Anaerobic Bacteria with Acetate

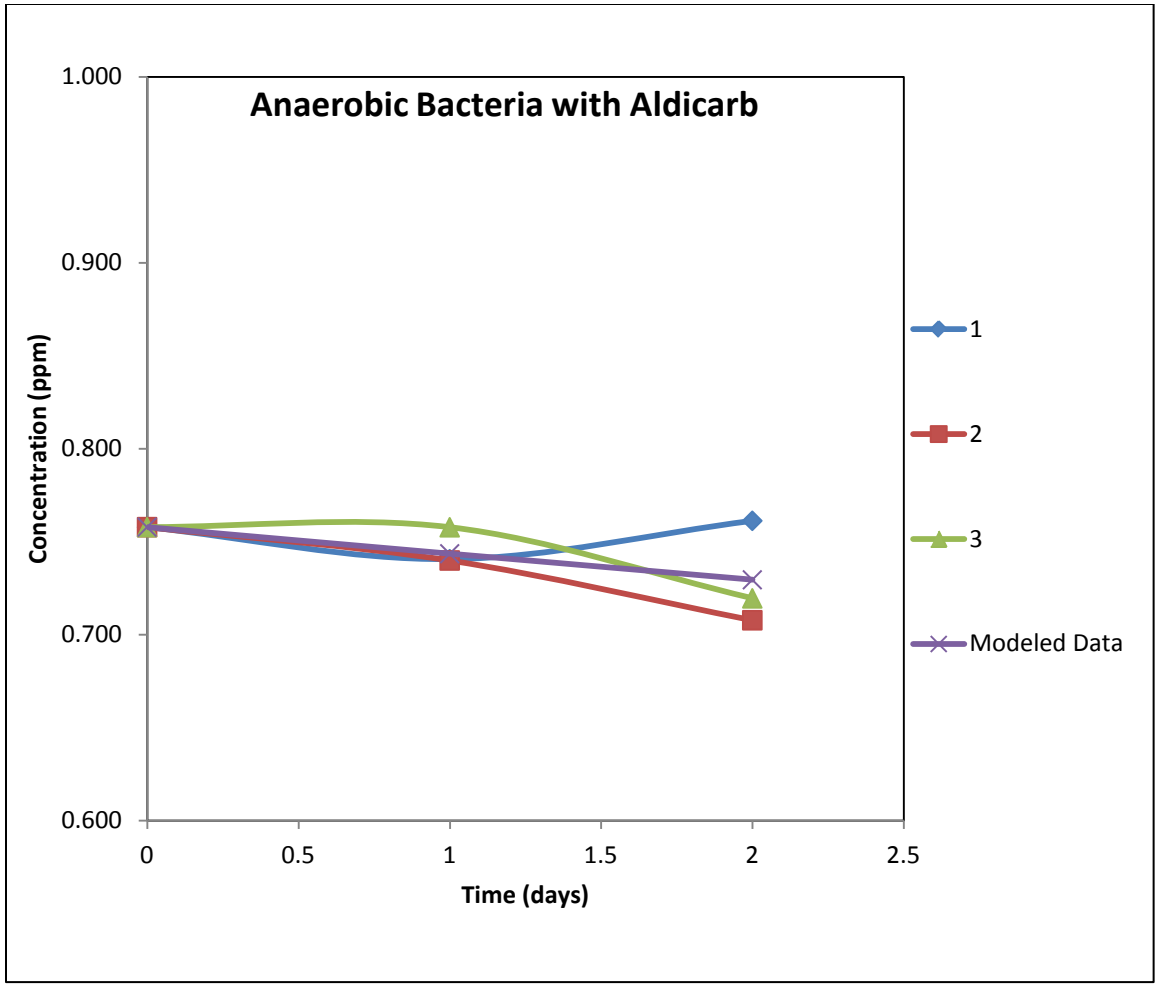


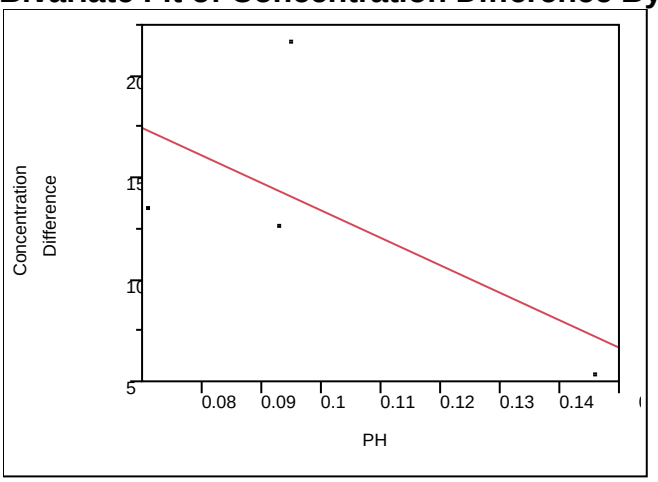
Figure 30. Aldicarb Transformation in Anaerobic Bacteria with Aldicarb

Table 5. Aldicarb Transformation in Four Anaerobic Bacteria Solutions

		Refrigerated				Room Temperature				Room Temp with Acetate				Room Temp with Aldicarb			
Time (Days)	Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)		Sample	Area	Conc (ppm)	
0	Stock	271071	0.86	% Diff	Stock	253479	0.799	% Diff	Stock	249122	0.784	% Diff	Stock	241829	0.758	% Diff
1	1	273166	0.868	-0.859	1	259305	0.819	-2.55	1	249710	0.786	-0.265	1	236944	0.741	2.27
1	2	276849	0.881	-2.36	2	259191	0.819	-2.52	2	245700	0.771	1.54	2	236751	0.740	2.36
1	3	261166	0.826	4.06	3	253282	0.798	0.087	3	247033	0.776	0.941	3	241814	0.758	0.007
2	1	266375	0.844	1.92	1	257715	0.814	-1.87	1	246268	0.773	1.28	1	242798	0.761	-0.45
2	2	261271	0.826	4.01	2	250587	0.789	1.27	2	234659	0.732	6.51	2	227645	0.708	6.60
2	3	235258	0.735	14.68	3	249448	0.785	1.78	3	239124	0.748	4.50	3	230983	0.719	5.05
1	Average		0.859	0.278	Average		0.812	-1.67	Average		0.778	0.739	Average		0.746	1.55
2	Average		0.802	6.87	Average		0.796	0.396	Average		0.751	4.10	Average		0.729	3.73
	k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)			
	0.036				0.002				0.021				0.019			
0			0.861				0.799				0.784				0.758	
1			0.831				0.797				0.767				0.743	
2			0.802				0.796				0.751				0.729	

Appendix C. Statistical Outputs from JMP

Fit Y by X Group
Bivariate Fit of Concentration Difference By PH



Linear Fit
Concentration Difference = 26.870381 - 134.75608*PH

Summary of Fit

RSquare	0.411033
RSquare Adj	0.11655
Root Mean Square Error	6.273141
Mean of Response	13.22633
Observations (or Sum Wgts)	4

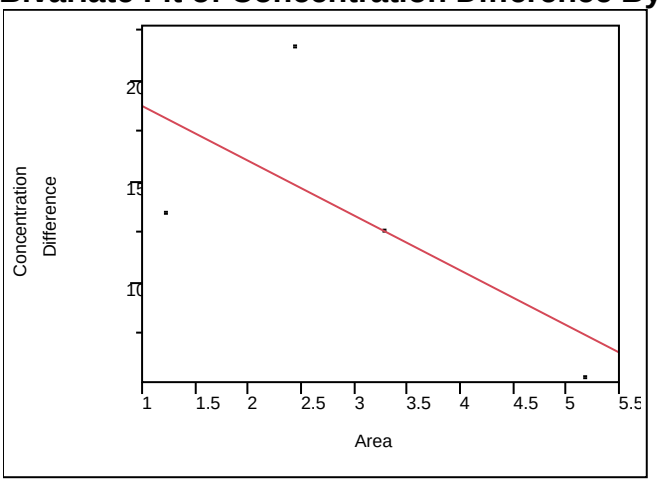
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	54.92704	54.9270	1.3958
Error	2	78.70460	39.3523	**Prob > F**
C. Total	3	133.63165		0.3589

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	26.870381	11.96712	2.25	0.1538
PH	-134.7561	114.0618	-1.18	0.3589

Bivariate Fit of Concentration Difference By Area



Linear Fit

Concentration Difference = 21.431987 - 2.7027862*Area

Summary of Fit

RSquare	0.456762
RSquare Adj	0.185143
Root Mean Square Error	6.024692
Mean of Response	13.22633
Observations (or Sum Wgts)	4

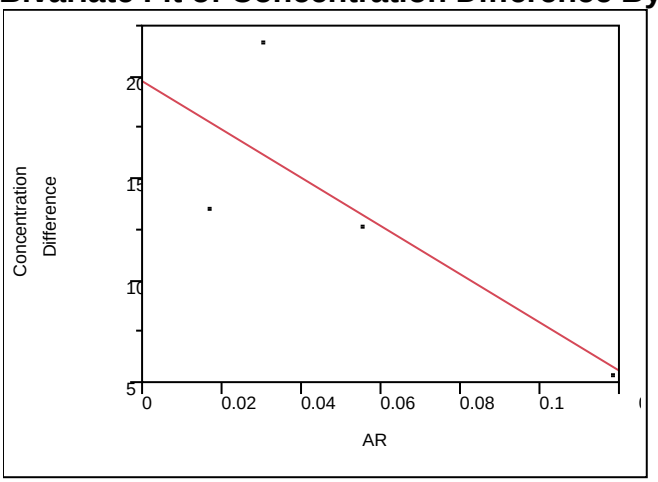
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	61.03783	61.0378	1.6816
Error	2	72.59382	36.2969	**Prob > F**
C. Total	3	133.63165		0.3242

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	21.431987	7.008177	3.06	0.0924
Area	-2.702786	2.084237	-1.30	0.3242

Bivariate Fit of Concentration Difference By AR



Linear Fit

Concentration Difference = 19.776834 - 118.38443*AR

Summary of Fit

RSquare	0.633087
RSquare Adj	0.44963
Root Mean Square Error	4.951324
Mean of Response	13.22633
Observations (or Sum Wgts)	4

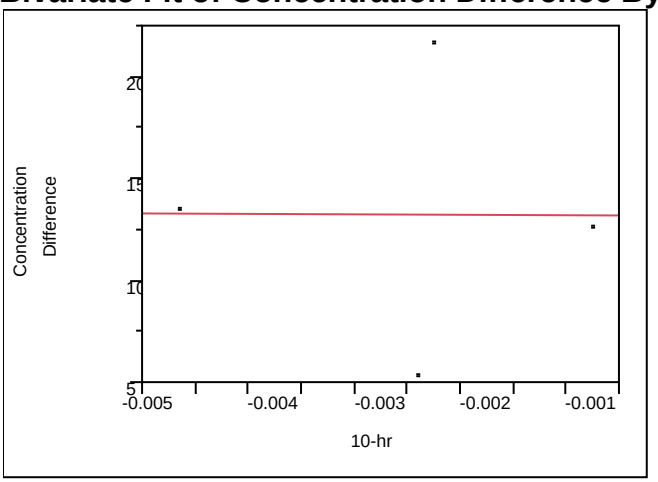
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	84.60043	84.6004	3.4509
Error	2	49.03121	24.5156	**Prob > F**
C. Total	3	133.63165		0.2043

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	19.776834	4.308498	4.59	0.0443*
AR	-118.3844	63.7279	-1.86	0.2043

Bivariate Fit of Concentration Difference By 10-hr



Linear Fit

Linear Fit

Concentration Difference = 13.171612 - 21.842731*10-hr

Summary of Fit

RSquare	2.769e-5
RSquare Adj	-0.49996
Root Mean Square Error	8.173982
Mean of Response	13.22633
Observations (or Sum Wgts)	4

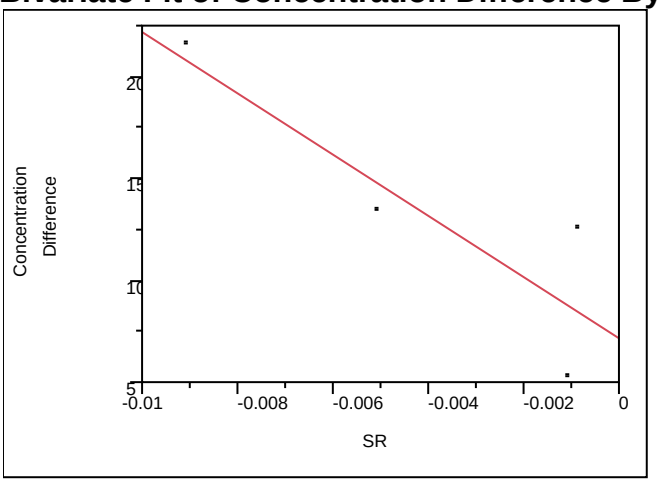
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00370	0.0037	0.0001
Error	2	133.62795	66.8140	**Prob > F**
C. Total	3	133.63165		0.9947

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	13.171612	8.412413	1.57	0.2579
10-hr	-21.84273	2935.291	-0.01	0.9947

Bivariate Fit of Concentration Difference By SR



Linear Fit

Concentration Difference = 7.1535261 - 1503.1688*SR

Summary of Fit

RSquare	0.764784
RSquare Adj	0.647175
Root Mean Square Error	3.964364
Mean of Response	13.22633
Observations (or Sum Wgts)	4

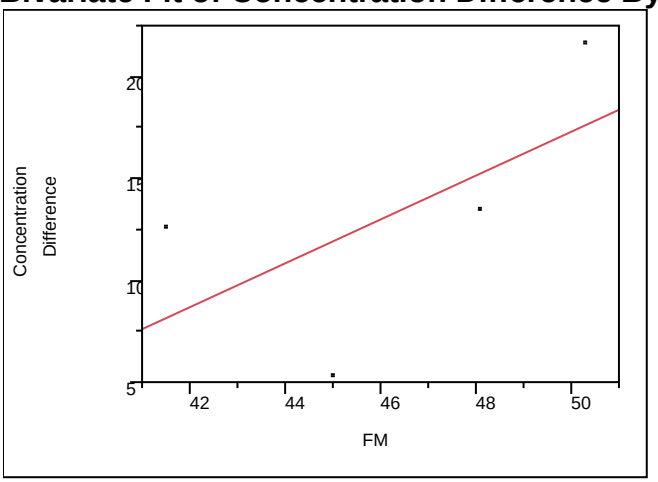
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	102.19928	102.199	6.5028
Error	2	31.43237	15.716	**Prob > F**
C. Total	3	133.63165		0.1255

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	7.1535261	3.098432	2.31	0.1473
SR	-1503.169	589.4641	-2.55	0.1255

Bivariate Fit of Concentration Difference By FM



Linear Fit

Concentration Difference = -36.54723 + 1.0767671*FM

Summary of Fit

RSquare	0.381301
RSquare Adj	0.071952
Root Mean Square Error	6.42953
Mean of Response	13.22633
Observations (or Sum Wgts)	4

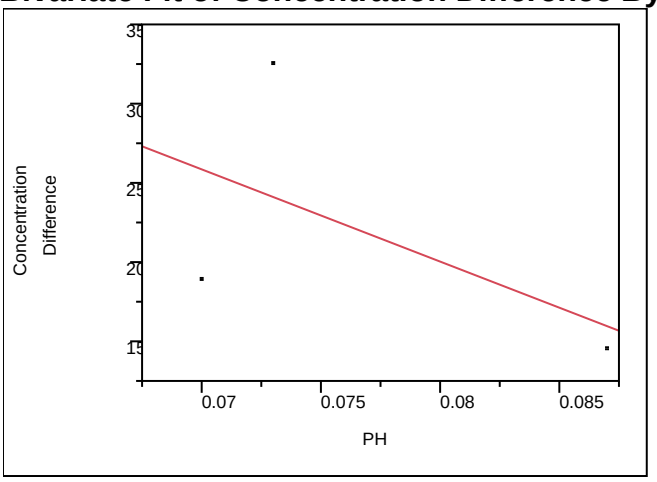
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	50.95393	50.9539	1.2326
Error	2	82.67771	41.3389	**Prob > F**
C. Total	3	133.63165		0.3825

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-36.54723	44.94721	-0.81	0.5016
FM	1.0767671	0.969867	1.11	0.3825

Fit Y by X Group
Bivariate Fit of Concentration Difference By PH



Linear Fit

Concentration Difference = 66.59092 - 581.96955*PH

Summary of Fit

RSquare	0.314194
RSquare Adj	-0.37161
Root Mean Square Error	11.03329
Mean of Response	21.97326
Observations (or Sum Wgts)	3

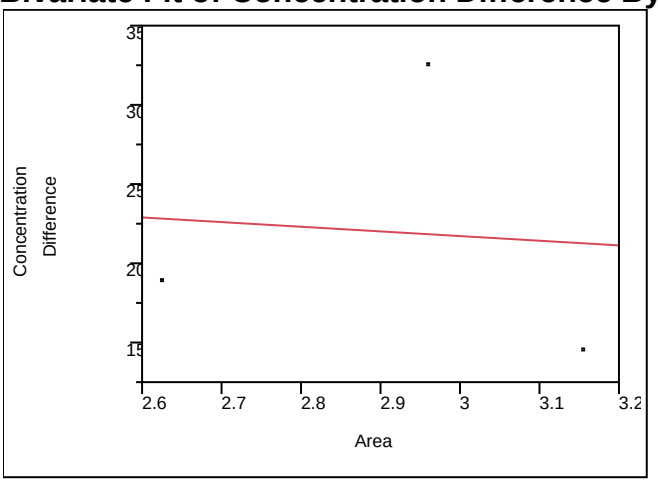
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	55.77071	55.771	0.4581
Error	1	121.73343	121.733	**Prob > F**
C. Total	2	177.50414		0.6212

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	66.59092	66.22578	1.01	0.4982
PH	-581.9695	859.8092	-0.68	0.6212

Bivariate Fit of Concentration Difference By Area



Linear Fit

Concentration Difference = 30.538099 - 2.9395413*Area

Summary of Fit

RSquare	0.006949
RSquare Adj	-0.9861
Root Mean Square Error	13.2767
Mean of Response	21.97326
Observations (or Sum Wgts)	3

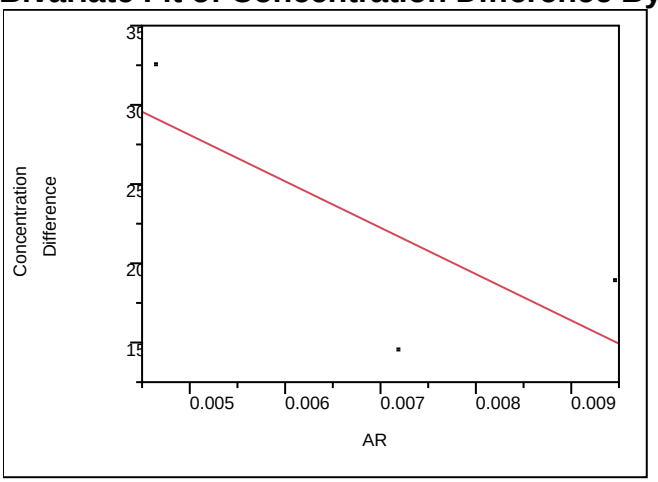
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.23351	1.234	0.0070
Error	1	176.27063	176.271	**Prob > F**
C. Total	2	177.50414		0.9469

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	30.538099	102.6719	0.30	0.8160
Area	-2.939541	35.13969	-0.08	0.9469

Bivariate Fit of Concentration Difference By AR



Linear Fit

Concentration Difference = 42.734358 - 2926.8473*AR

Summary of Fit

RSquare	0.558956
RSquare Adj	0.117912
Root Mean Square Error	8.848001
Mean of Response	21.97326
Observations (or Sum Wgts)	3

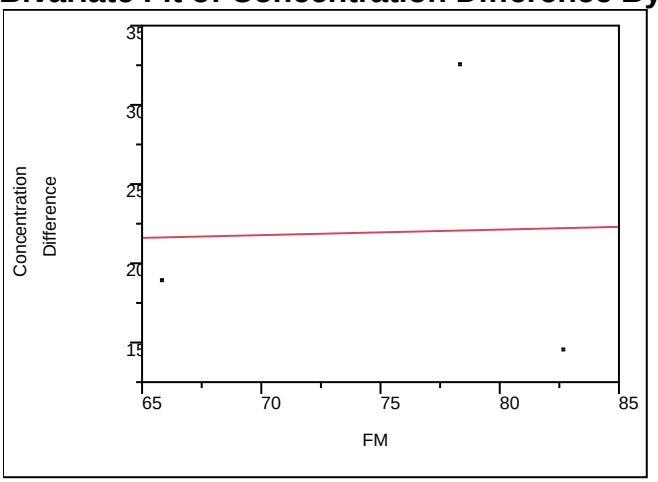
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	99.21702	99.2170	1.2673
Error	1	78.28713	78.2871	**Prob > F**
C. Total	2	177.50414		0.4624

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	42.734358	19.13621	2.23	0.2680
AR	-2926.847	2599.873	-1.13	0.4624

Bivariate Fit of Concentration Difference By FM



Linear Fit

Concentration Difference = 19.33673 + 0.0348747*FM

Summary of Fit

RSquare	0.001053
RSquare Adj	-0.99789
Root Mean Square Error	13.31605
Mean of Response	21.97326
Observations (or Sum Wgts)	3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.18699	0.187	0.0011
Error	1	177.31716	177.317	**Prob > F**
C. Total	2	177.50414		0.9793

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	19.33673	81.55341	0.24	0.8518
FM	0.0348747	1.073945	0.03	0.9793

Appendix D. DMMP Experimental Data

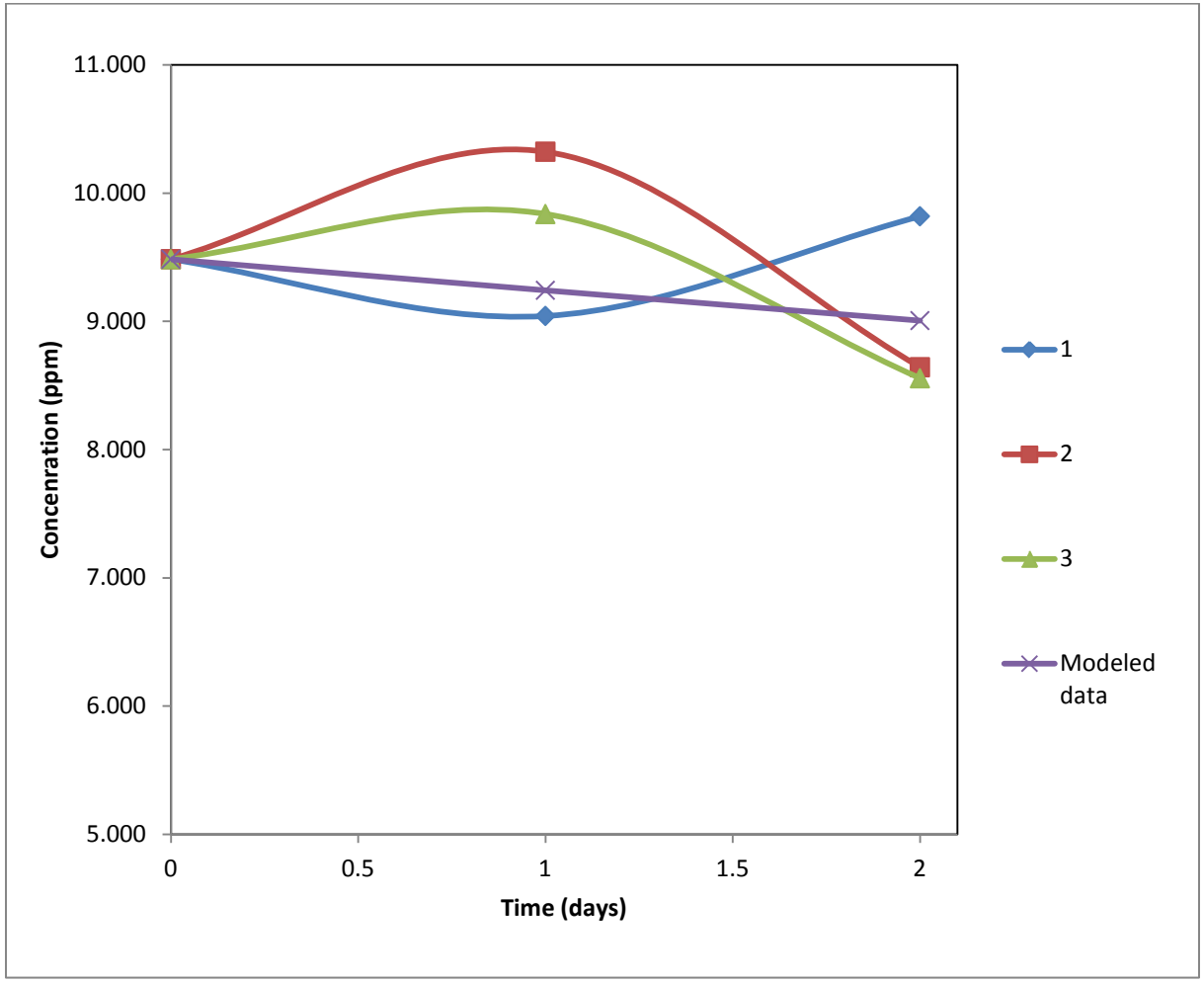


Figure 31. DMMP Transformation in Aerobic Bacteria

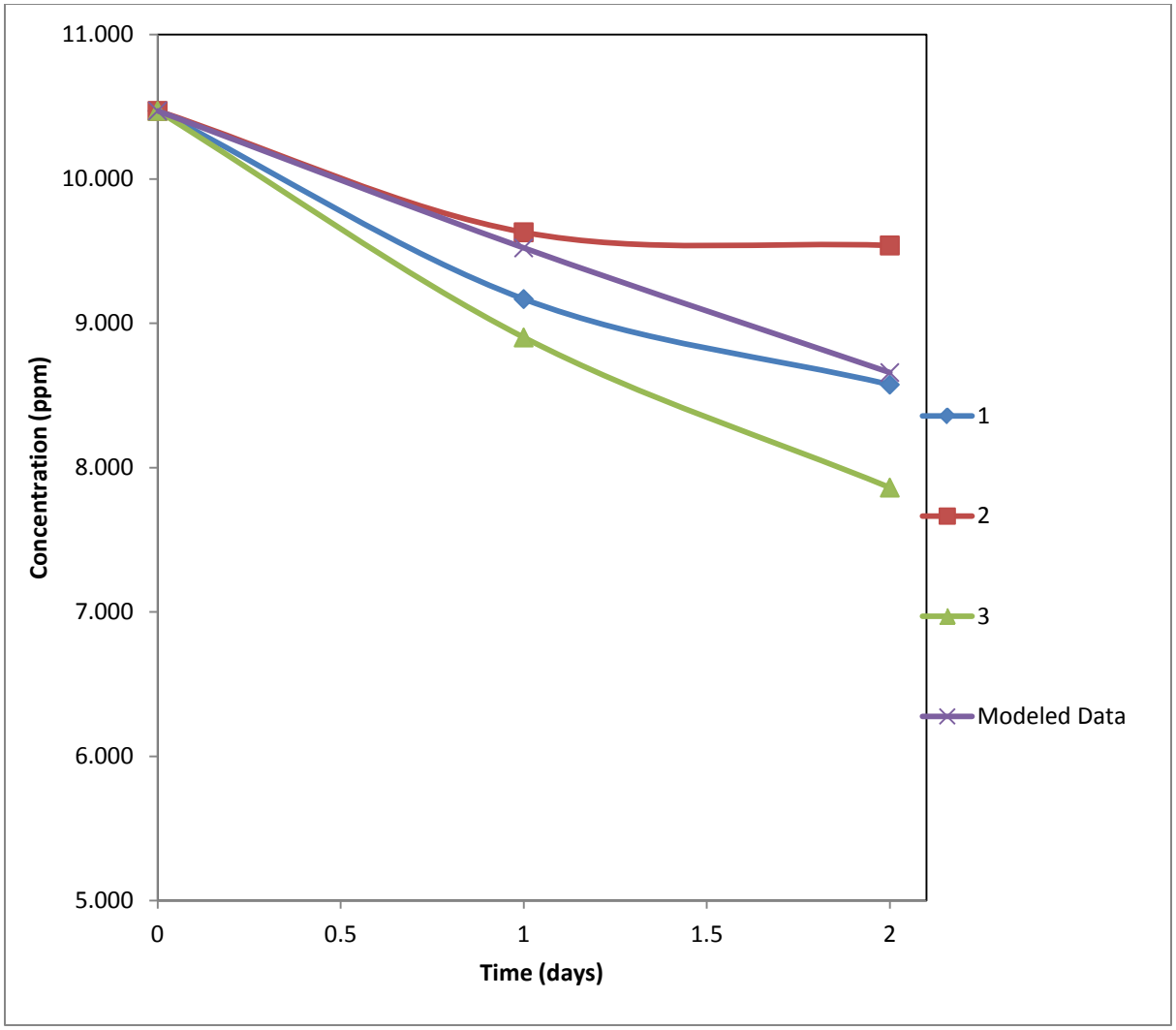


Figure 32. DMMP Transformation in Anaerobic Bacteria

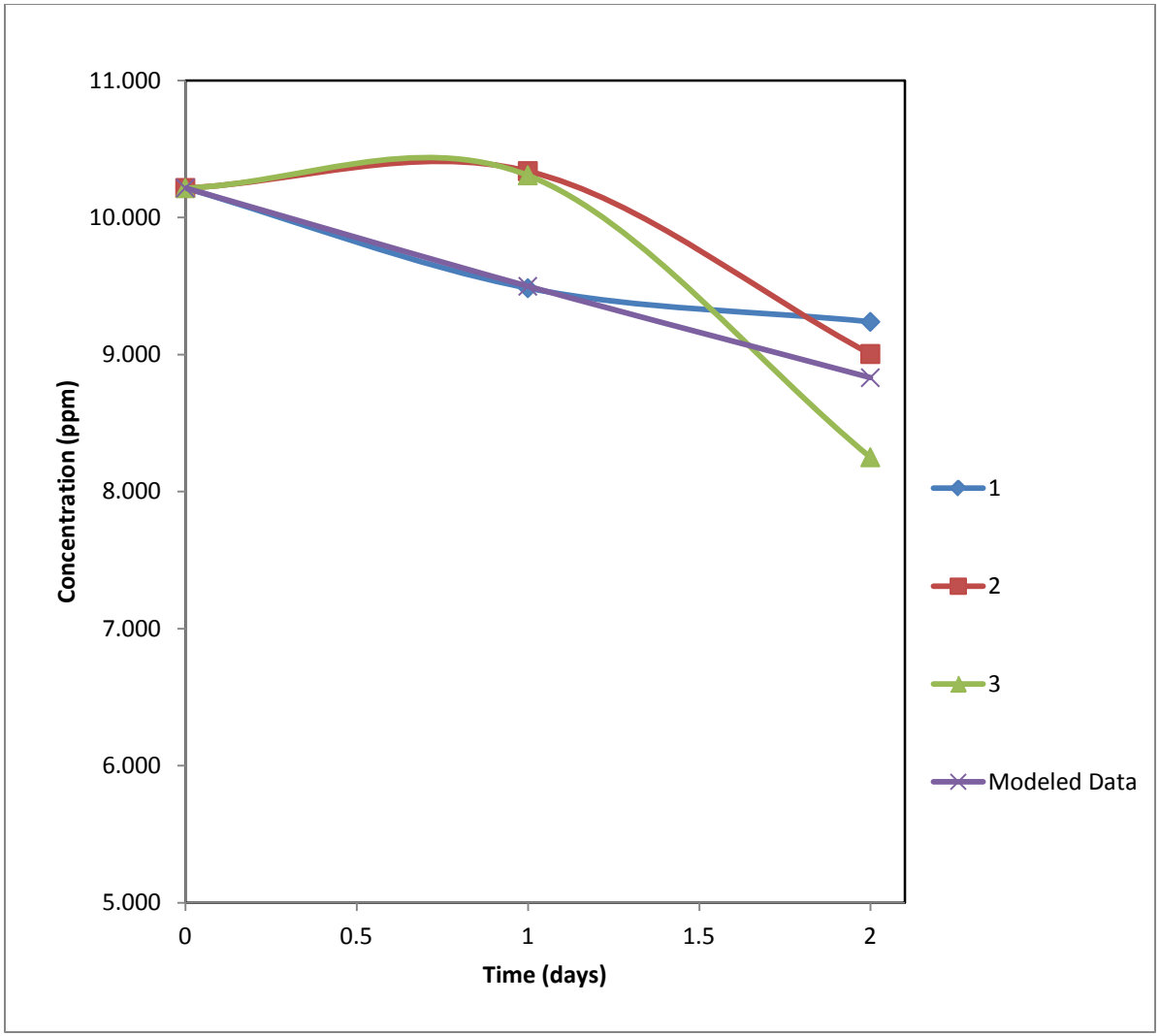


Figure 33. DMMP Transformation in Acetate Feed Solution

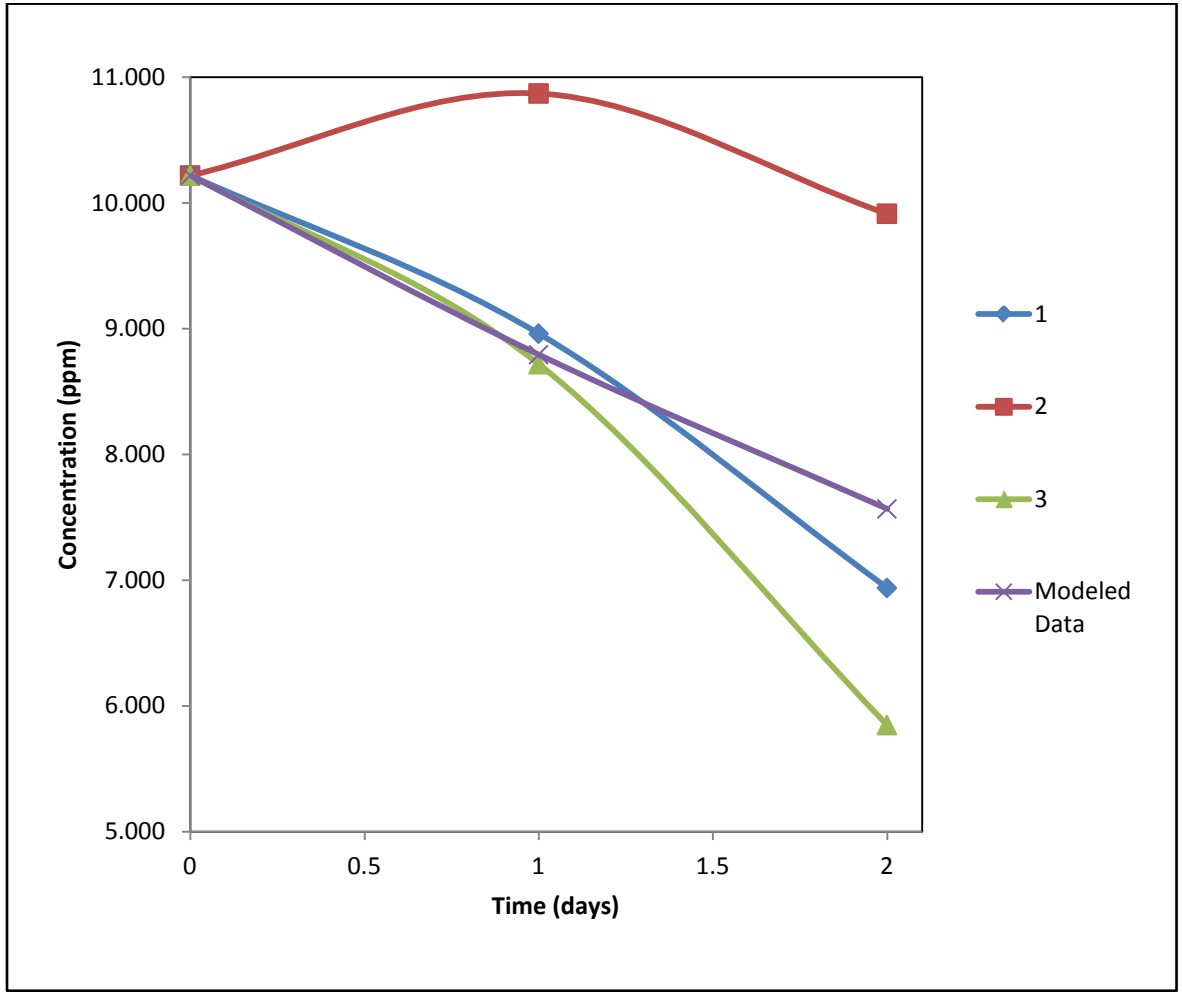


Figure 34. DMMP Transformation in Deionized Water

Table 6. DMMP Transformation Experiments

		Anaerobic Bacteria				Aerobic Bacteria				Acetate Feed				Deionized Water			
Time (Days)	Sample	Area	Conc(ppm)		Sample	Area	Conc(ppm)		Sample	Area	Conc(ppm)		Sample	Area	Conc(ppm)		
0	Stock	2466071	9.49	% Diff	Stock	2722709	10.47	% Diff	Stock	2656087	10.21	% Diff	Stock	2656751	10.21	% Diff	
1	1	2351087	9.04	4.66	1	2383724	9.17	12.45	1	2466053	9.48	7.15	1	2329664	8.96	12.31	
1	2	2683969	10.32	-8.83	2	2504027	9.63	8.03	2	2688108	10.33	-1.20	2	2826343	10.87	-6.38	
1	3	2557665	9.84	-3.71	3	2314569	8.90	14.99	3	2680161	10.30	-0.90	3	2266580	8.71	14.68	
2	1	2553138	9.80	-3.53	1	2229540	8.58	18.11	1	2402178	9.23	9.56	1	1803869	6.93	32.10	
2	2	2247070	8.64	8.88	2	2480271	9.54	8.90	2	2341176	9.00	11.85	2	2577731	9.91	2.97	
2	3	2224732	8.56	9.79	3	2044088	7.86	24.92	3	2145325	8.25	19.23	3	1520416	5.84	42.77	
1	Average		9.73	-2.63	Average		9.23	11.82	Average		10.04	1.68	Average		9.51	6.87	
2	Average		9.01	5.05	Average		8.66	17.31	Average		8.83	13.54	Average		7.56	25.94	
	k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)				k ($days^{-1}$)				
	0.026				0.095				0.073				0.150				

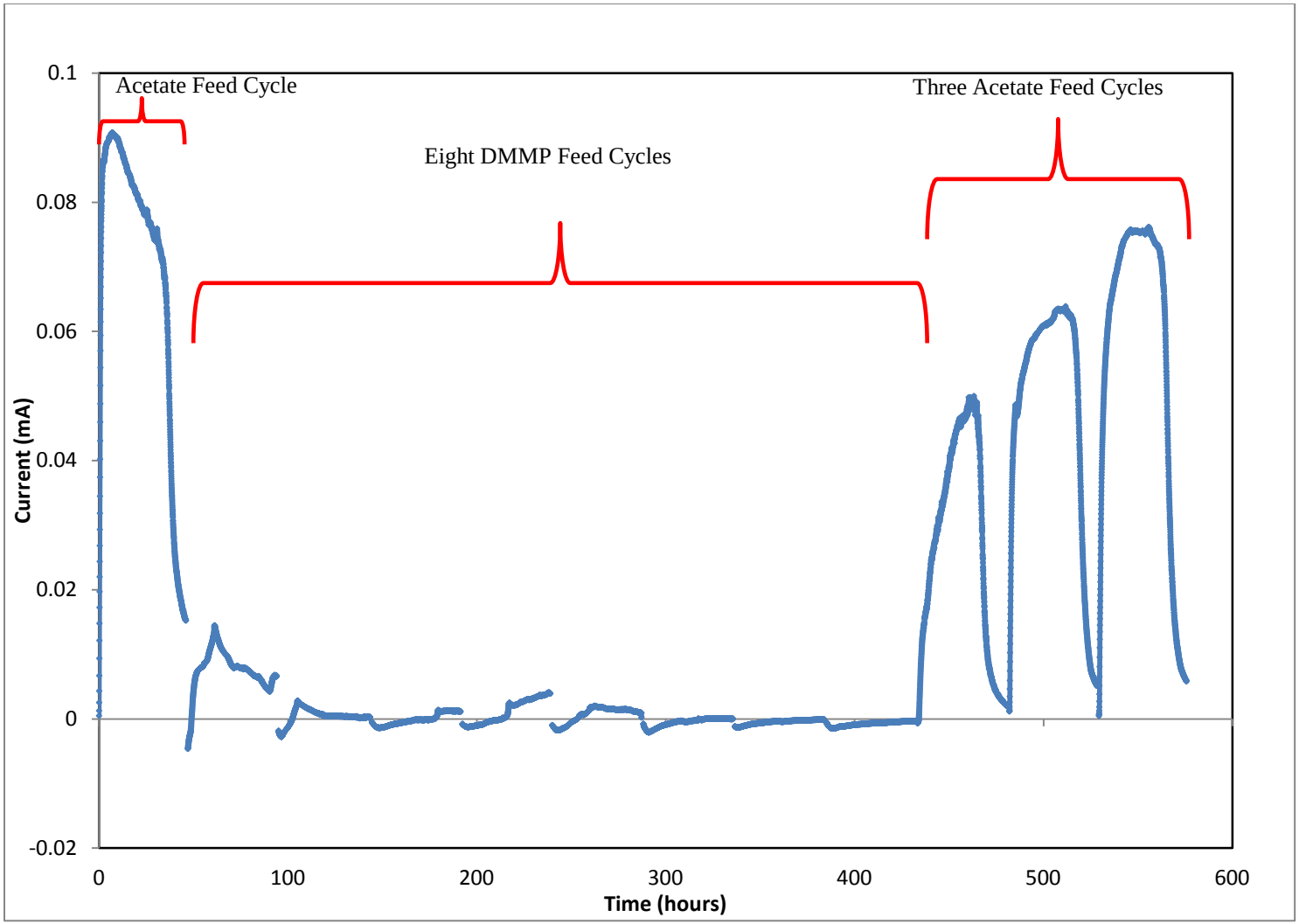


Figure 35. Eight DMMP Feed Cycles on MFC 1

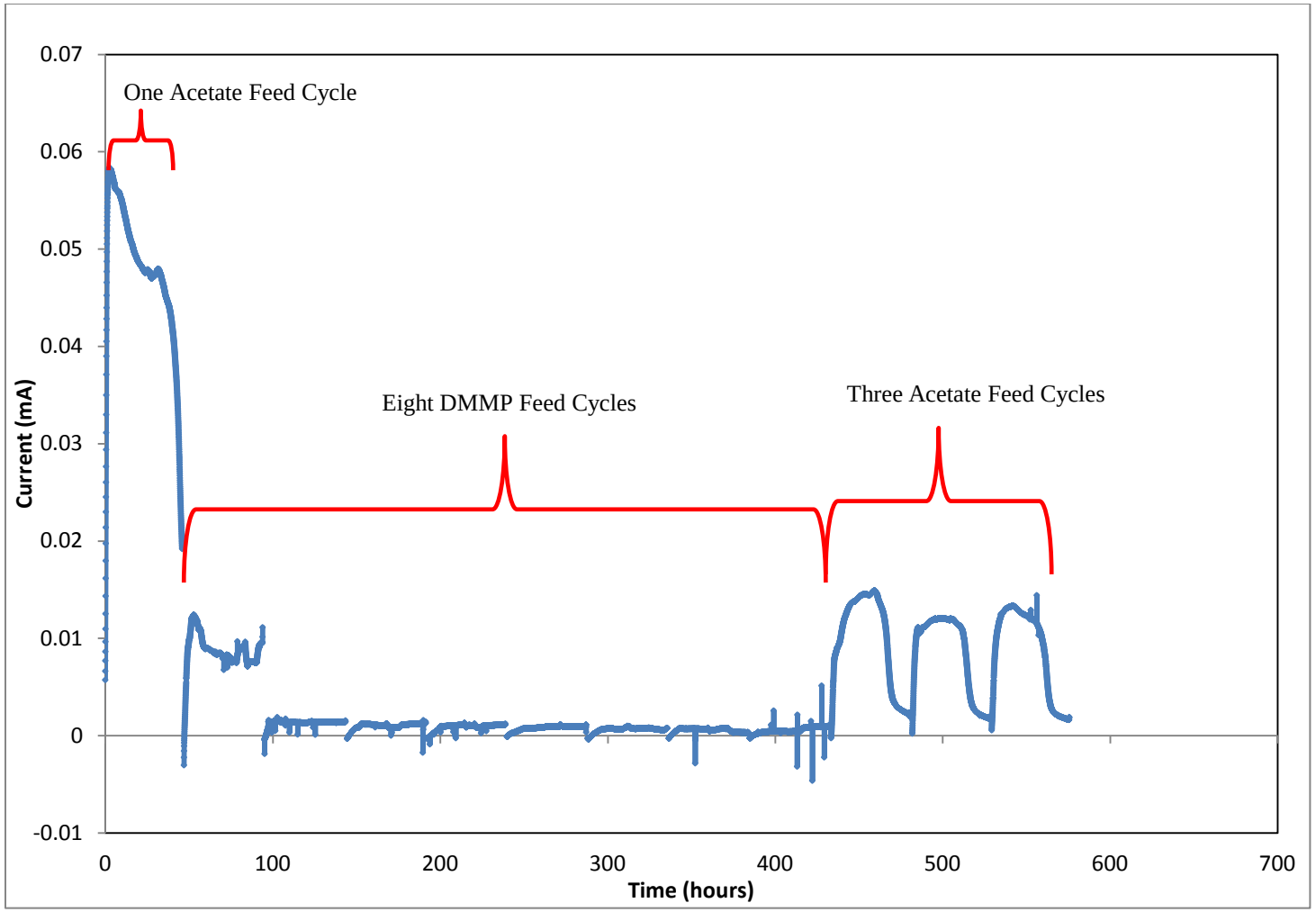


Figure 36. Eight DMMP Feed Cycles on MFC 3

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14. ABSTRACT

Microbial fuel cells (MFCs) have the potential to be used as biosensors for water sources. MFCs have been shown to produce current through oxidation of simple organic substrates and the current can be correlated to the substrate concentration. The purpose of this research was to evaluate the MFCs ability to transform recalcitrant organic compounds and determine if the current generation is related to the transformation.

Aldicarb experiments in four media proved that an active aerobic bacteria solution has the ability to transform aldicarb (average concentration difference 13.8%), while the other solutions tested did not have an effect on the concentration. Additionally, aldicarb transformed within both MFCs (average concentration difference – MFC1 15.9%, MFC3 28.8%).

Current generation metrics from the aldicarb feed cycles could not be linearly correlated to the difference in concentration, and aldicarb was not a substrate for anode respiring bacteria. There is no direct comparison to previous research on MFC inhibition, but the transformation of aldicarb through non-oxidative process, such as interaction with fermentation products or biological growth, inhibits normal microbial activities. This inhibition to the microbial community will affect the current production in an MFC and supports the proposition that MFCs can be used as biosensors.

15. SUBJECT TERMS
Microbial Fuel Cell, Biosensor, Aldicarb

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