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Improvement of Onsite Wastewater Treatment By Use of Electrically Conductive Carbon Cloth

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IMPROVEMENT OF ONSITE WASTEWATER TREATMENT BY USE OF ELECTRICALLY
CONDUCTIVE CARBON CLOTH

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

by
Christopher Daniel Weber
August 2013

Accepted by:
Dr. Kevin Finneran, Committee Chair
Dr. David Freedman
Dr. Tanju Karanfil

ABSTRACT

The purpose of this research is primarily focused on carbon oxidation in domestic septic systems. Small-scale septic waste reactors utilizing electrically conductive carbon cloth were assessed for improving carbon oxidation and therefore the overall efficiency of on-site wastewater treatment systems.

A bioelectrochemical system was developed to enhance carbon oxidation in septic systems using electrically conductive carbon cloth as an anode/cathode bridge to transfer electrons from strictly anoxic septic wastewater to an oxic system outside of the septic reactor. This “septic snorkel” (so named since the system “breathes” via the carbon cloth extends into an aerobic zone) was designed to lower the carbon loading on the leach field, while being simple to deploy at the field level, which would be necessary for regulatory and stakeholder approval. The concept is predicated on previously published work that demonstrated Fe(III) amendment to septic wastewater increased carbon mineralization.

Data with Fe(III) amendment (an analogous system) demonstrated that mineralization of ^{14}C -labeled acetate, lactate, propionate, starch, glucose, and oleic acid increased by as much as 100%, while completely suppressing methane production. Bench scale results using different carbon cloth setups suggested that complete and open circuit configurations improved soluble COD removal by 25% and 23% respectively. Select ^{14}C -labeled compounds were also tested using the carbon cloth system. Interestingly, reactors with carbon cloth alone (no circuitry) showed the highest mineralization for all ^{14}C -labeled compounds. Increases in mineralization ranged from 16% to 82%.

DEDICATION

July 12th, 2013

To my parents Dan and Debbie Weber, my friends David Magjuka, Curtis Vercruysse, Sean Gainey, Jovan Popovic, Nathan Conroy, Catherine Ruprecht, Michael Witmer, Sean Millerick, Kay Millerick, Carina Vargas, and many others, and my advisor Kevin Finneran, I cannot thank you enough for your love, support, and understanding over the past four months and for dragging me to the finish line.

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LIST OF ABBREVIATIONS

| | |
|-------|---|
| CC | Carbon cloth |
| COD | Chemical oxygen demand |
| CWA | Clean Water Act |
| dpm | Disintegrations per minute |
| EPA | Environmental Protection Agency |
| FC | Full circuit |
| HRT | Hydraulic residence time |
| MFC | Microbial fuel cell |
| NPDES | National Pollutant Discharge Elimination System |
| PC | Partial circuit |
| R1 | Bench scale reactor #1: Unamended |
| R2 | Bench scale reactor #2: Open circuit |
| R3 | Bench scale reactor #3: Full circuit |
| sCOD | Soluble chemical oxygen demand |
| SMX | Sulfamethoxazole |
| SRT | Solids residence time |
| SWIS | Subsurface wastewater infiltration system |
| TSS | Total suspended solid |

CHAPTER ONE

1.0 INTRODUCTION

Centralized municipal wastewater treatment facilities are well characterized and designed to handle a variety of flows. While centralized treatment handles the majority of domestic sewage, a recent survey (2007) by the United States Environmental Protection Agency (EPA)^[20] estimated that 20 percent of U.S. housing units were served by septic systems. Discharge limits on municipal treatment facilities on BOD₅, nitrogen, phosphorous and others are federally mandated by the Clean Water Act (CWA) through National Pollutant Discharge Elimination System (NPDES) permitting. However, while the CWA motivates the design criteria for onsite wastewater treatment, regulations and management programs for onsite wastewater systems (i.e. septic systems) are established at the state and local level. Furthermore, these regulations are typically not performance based, and septic tank effluent is highly variable. Growing interest in water resource and public health impact over the past decade has prompted the EPA to evaluate performance based policy for onsite wastewater treatment systems since onsite systems are “known contributors of pathogens and nutrients to surface and ground water.”^[20] Such criteria have been defined numerically by pollutant concentration or mass loading limits and qualitatively by odor or aesthetic characteristics.^[20] However, establishing and implementing new and effective policy has been slowed by public misperceptions, regulatory constraints, and financial barriers. The U.S. Census Bureau estimates that approximately 50% of septic systems were over 30 years old. Furthermore, EPA reviews find that 10 – 20% of systems experience operational failure.^[20]

Failures in septic systems are, in some ways, poorly defined. For example, a septic tank that is subject to infiltration and exfiltration is failing structurally. However, that does not mean

that the system is experiencing hydraulic backup resulting in septage ponding, i.e., a failure of the subsurface wastewater infiltration system (SWIS), which is far more likely to be noticed by the owner. SWIS failure is attributed to a combination of physical, chemical, and biological processes that reduce the infiltration capacity of the system. These processes include colloid swelling, fine migration, ion exchange phenomena, and microbial activity.^[18] It is interesting that while soil clogging is mostly influenced by infiltrative phenomena^[18], it is the absorptive (or percolation) capacity of the soil which is often measured and used as a prescriptive metric in septic system design.^[20] Infiltration into the subsurface is analogous to a slow sand filter, where a clogging layer or biomat forms at the soil interface.^[3] This layer can act as a filter itself, straining out pathogens and providing some degradation of biochemical oxygen demand. Biomat development, which is related to the organic loading rate, causes a decrease in the infiltration rate.^[24]

While different technologies exist, conventional onsite wastewater treatment is typically viewed as a two component operation; septic tank and SWIS. The primary function of the septic tank is to remove settleable solids, oils, greases, and floating debris. Removal rates of these constituents are cited at 60 – 80%.^[20] In a sense, the septic tank acts as a combined clarifier and partial anaerobic digester. The SWIS, colloquially known as the leach field, is responsible for the majority of the carbon, nitrogen, phosphorous, and pathogen removal/transformation. This research is focused exclusively on improving the performance of the septic tank itself, generally in terms of increasing the oxidation of soluble carbonaceous constituents by using an electrically conductive carbon cloth. Ultimately, this would decrease the organic loading rate on the leach field, retarding biomat development.

Anaerobic digestion is classically described in four steps including hydrolysis, fermentation, acetogenesis, and methanogenesis.^[10] While all four stages occur in septic tanks,

hydrolysis and fermentation are the primary forms of metabolism, producing soluble organic acids that largely pass through to the leach field. Complete digestion (i.e. conversion of volatile fatty acids to methane) would reduce the effluent chemical oxygen demand (COD). However, temperatures in septic tanks are often sub-optimal for methanogenesis. Consequently, methane generation is not significant.^[20] At large, centralized municipal treatment facilities, anaerobic digesters are designed primarily to stabilize particulate organic matter. This is regulated by 503B rules in document # EPA/625/R-92/013: Control of Pathogens and Vector Attraction in Sewage. It is a prescriptive legislation requiring either a certain percent reduction in volatile suspended solids or a specific combination of solids residence time (SRT) and temperature.

The crux of this research focuses on shifting the biochemical environment in septic tanks from fermentative to respiratory. This could be accomplished by introducing exogenous soluble electron acceptors. Previous work done by Azam and Finneran^[1] showed that adding different forms of ferric iron to septic tank environments effectively shifted the microbial community towards an iron reducing population and improved carbon oxidation in terms of increased compound specific mineralization. However, septic tank owners would incur an operating cost by adding chemicals (i.e. ferric iron amendments) to their systems. Iron addition could also lead to undesirable system problems in both the septic tank and the SWIS. If the iron precipitated in the tank, it would add to the sludge layer, which would ultimately require more frequent pumping. If the iron precipitated in the SWIS or the distribution laterals, it could clog the system. Left unchecked, this would lead to system failure and could lead to septic ponding.

Some bacteria have adapted cellular machinery and metabolic pathways to use solid phase electron acceptors. One solid phase electron acceptor of particular interest in environmental research is elemental carbon, due to its noncorrosive and electrically conductive properties. These microorganisms have shown the capability to facilitate extracellular electron

transfer via direct attachment^{[14],[23]}, soluble electron shuttles^{[5],[12],[25]}, and bacterial nanowires^{[8],[21]}. Some *Shewanella* sp. exhibited the flexibility to utilize both direct attachment and electron shuttling mechanisms. Interestingly, *Shewanella* sp. are also capable of producing and excreting their own mediators. Flavin mononucleotide and riboflavin were both identified by von Canstein et al.^[25] as the electron shuttles produced. For this reason, the potential for using riboflavin to enhance electron transport was explored during this thesis research.

Microbial fuel cells (MFC) have been studied for their potential to bio-generate electricity using a variety of carbon electrode configurations and geometries. A traditional setup includes two separate chambers; an anode compartment where microbially mediated oxidative processes occur, and a cathode compartment where chemical or microbial reductive processes occur. The chambers are joined by an electrically conductive wire and a salt bridge. Oxidative processes generate electrons that are transferred to the anode. An accompanying reduction at the cathode produces an electric current.^[18] The systems constructed for experiments performed for this thesis rely on similar principles but contain a single chamber with shared carbon electrodes. Results from single chamber MFC configurations using municipal wastewater have demonstrated the potential of high power densities and electricity generation.^[15] While current generation and power densities may be indicator parameters for the overall performance and effectiveness of this system, they were not specific goals. The overall objective of this research to evaluate a method to improve carbon oxidation in septic systems using an electrically conductive carbon electrode. The specific objectives were to 1) design a bench scale reactor configuration capable of evaluating bulk sCOD removal; and 2) design a small scale batch reactor capable of evaluating compound specific oxidation using a ¹⁴C based mineralization assay.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Chemicals and Media

Table 2.1 summarizes the main chemicals and materials used for the batch and mineralization experiments. All other chemicals were reagent grade or higher. Table 2.2.2 summarizes the radiolabeled chemicals used in the mineralization experiments; specific activities were not available.

2.2 Bench Scale Operation and Setup

Three semi batch reactors were operated independently for 85 days. The reactors consisted of 5.7 L plastic Sterilite containers with loose fitting plastic lids. The dimensions of the container were 35.6 cm x 20.3 cm x 11.7 cm. Side view schematics are depicted in Figure 2.1 and Figure 2. Reactors were inoculated with approximately 400 mL of raw septic sludge obtained from Johnson Septic Tank Company.

Reactor #1 (R1) was the unamended, or control, setup. Reactor #2 (R2) contained a 14" x 14" piece of carbon cloth (FibreGlast), which was completely submerged in the liquid and sludge. Reactor #3 (R3) was the experimental setup. It consisted of a 25" x 14" piece carbon cloth submerged in the liquid and sludge layers, which also draped outside the reactor into an aerated water bath (a plastic tub that was slightly larger than the Sterilite container). Aeration was accomplished by using a fish tank aerator (Aqua Culture) fitted with a porous aeration stone. The water bath was maintained at 2 L at room temperature (22-24 °C) and buffered with 10 mM acetate and maintained at a pH around the pK_a of acetic acid (4.76 and less than 7.0) to facilitate electron flow. The total liquid volume in the reactors was kept c.a. four liters. Photos of all three

setups are shown in Figure 2.3. The secondary containment shown in the figure for R3 is the water bath.

Reactors were operated with an average hydraulic retention time (HRT) of 24 hours, which is the minimum operating HRT suggested by the EPA OWTS manual for full scale installations.^[20] This was achieved by manually decanting (via siphoning) 2L of liquid twice daily. In R1, the siphon tube was kept as close to the water surface and sidewalls of the reactors as possible to minimize the suction of solids. In R2, the siphon tube was kept away from the free floating carbon cloth (to avoid clogging the tube) and maintained close to water surface. This location varied since the cloth moved during feeding. In R3, the siphon tube was placed in the middle of the reactor near the water surface. Two liters of fresh media replaced the decanted volume with a 2 L graduated cylinder. The media was composed of three main stocks (A, B, and C) adapted from Bisogni and Lawrence,^[13] whose composition is summarized in Table 2.3. Each stock was prepared in 2 L batches and stored in 2 L glass media bottles (VWR). The feed was prepared by adding 240 mL of stocks A, B, and C to a 10 gallon glass carboy, which was then brought up to 12 L of total volume with tap water. The target sCOD of the feed was 1000 mg/L. The 12 L of feed was sparged with high purity nitrogen through a fish tank aeration stone for approximately 20 minutes before each feed cycle (decant and replace). Enough feed was prepared for two cycles, so the remaining 6 L was kept on ice and covered with aluminum foil to minimize degradation. On days 41 – 44, 2.24g (500 μ M) of riboflavin was added to the 12 L of fresh feed. This achieved a reactor target concentration of 250 μ M.

sCOD and pH were the only operational parameters monitored for the bench scale reactors. sCOD was measured using a slightly modified EPA method (Hach Method 8000, EPA reference 40 CFR 136.3). Approximately 3.5 mL of effluent from each reactor was filtered through a 5 mm, 0.45 μ m syringe filter (Pall) into a 10 mL plastic centrifuge tube. Before

withdrawing volume for sCOD analyses, the pH was measured on an ORION Star A111 pH meter with an ORION Triode probe (Thermo Scientific). Next, two milliliters of filtered effluent was added to a pre-packaged Hach High-Range COD vial (40 – 1500 mg/L). Vials were capped and gently inverted several times to mix. Vials were heated and the contents were digested at 150 °C for two hours and allowed to cool to room temperature before measuring on a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific) at 620 nm. A COD tube filled with 2 mL of ultrapure water served as the spectrophotometer blank. A COD standard curve is in Appendix A.

2.3 Mineralization Experimental Analyses and Setup

Four different series were used to evaluate the extent of carbon mineralization. Each series was setup using 28 mL Balch tubes (Bellco Glass) and sealed with thick butyl stoppers and aluminum crimps (Bellco Glass). The different treatments are here described and subsequently referred to as unamended, carbon cloth (CC), partial circuit (PC), and full circuit (FC). Inoculum was 15 mL liquid from the bench scale reactors. The reactors had not been fed or decanted for two weeks. For the next three days, the reactors were fed and decanted (twice per day) as previously described, and then samples were removed for the batch assay. The reactors were likely to have had effluent sCOD and pH near the averages established during steady state operation. Just before removing inoculum, the reactors were gently stirred to homogenize the solids. Liquid was distributed on the bench top to the pressure tubes in the following sequence: unamended from R1, CC from R2, and PC and FC from R3. All series were run in triplicate. The ¹⁴C carbon compounds listed in Table 2.2 were added individually to the experimental bottles. A 0.2 mL amendment volume of radiolabeled substrate was used for each reactor tube. The total activity added per bottle, which varied by carbon compound, ranged from 40,000 – 345,000 disintegrations per minute (dpm). Unfortunately, the ¹⁴C stocks were prepared by a previous student, and the bottles were not labeled with specific activities; this explains the

considerable variability in the dpm delivered. Reactors were incubated at room temperature and covered to minimize light exposure.

The unamended series contained no circuitry or carbon cloth. The CC series had a piece of carbon cloth (approximately 1.3 cm x 1.3 cm) submerged in the liquid. It should be noted that the cloth frayed and partly lost its structure after cutting such a small piece. The PC and FC series were fitted with 8 gauge stainless steel Lockwire (Malin Co.) achieved by passing the wire through an 18 gauge needle piercing the rubber septum. The partial circuit included a 20.3 cm length of wire that was submerged in the liquid and exposed to the air. The full circuit series included alligator clips at both the (bio)anode and cathode ends. Each clip was attached to a strip of carbon cloth. Figure 2.4 depicts the four series without media or bio-solids in the tubes for easier visualization. Figure 2.5 is a schematic depicting the four series.

One milliliter headspace samples were injected on a Shimadzu GC – 8A equipped with a gas proportional counter (Model GC Ram: IN/US, Tampa, FL). Operational parameters are summarized in Table 2.4. The percent (%) mineralization was tracked by monitoring $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$ as disintegrations per minute (dpm) in the headspace over 5 days following a method previously described by Wei and Finneran.^[28] Percent (%) mineralization was determined using the following equations:

$$K_{app} = 10^{-6.3} \quad (2.1)$$

$$Partition = \frac{H_2CO_3^*}{HCO_3^-} = \frac{10^{-pH}}{K_{app}} \quad (2.2)$$

$$CO_2 \text{ mineralization } (\%) = \frac{A \times B}{C \times D} \times 100 \quad (2.3)$$

where A = dpm/mL headspace; B = headspace volume (mL); C = Partition, according to equation 2.2; and D = total dpm added.

Equation 2.1 is the acidity constant of carbon dioxide in determining the partition value for mineralization calculations.^[11] Equation 2.2 represents the partition, or fraction of CO₂ present in the headspace of a closed system. Direct pH measurements of analogous tubes (without radiolabel) were used to determine the partition at a particular time point. Equation 2.3 is a mathematical expression used for calculating the percent mineralization for a carbon substrate.

2.4 Carbon Cloth Pretreatment

Several studies have evaluated different pretreatment methods of carbon electrodes as a means of improving power production in microbial fuel cells.^{[9][16],[26],[27],[29]} A pretreatment described by Feng et al.^[9] was used in bench and small scale mineralization studies for these experiments. The carbon cloth was soaked in acetone overnight for approximately 24 hours. It was rinsed thoroughly with distilled water before soaking in a 10% (v/v) solution of sulfuric acid for 15 minutes. It was rinsed again with distilled water before heat treating the cloth in a muffle furnace at 450° for 30 minutes.

2.5 Alternate Reactor Design for Mineralization Study

Four different reactor designs were used in an effort to evaluate system performance using mineralization studies. They are not all described in detail here. However, it is worth mentioning that all setups utilizing a screw top media bottle (which were included in all three alternate designs) had problems sealing and maintaining the radiolabeled oxidation products, ¹⁴CH₄ and ¹⁴CO₂, in the reactor. The leaks most likely persisted through holes drilled in the plastic screw stop for circuitry. Preliminary batch tests (Appendix B, Figure B.1) show that there was a higher amount of radioactivity recovered as ¹⁴CO₂ in bottles with butyl stoppers than the screw top bottles, indicating a problem with the seal of the screw top bottles. In contrast,

shows similar radioactivity recoveries in terms of $^{14}\text{CO}_2$ for both circuited and non-circuited Balch tubes.

CHAPTER THREE

3.0 RESULTS

3.1 Bench Scale Reactor Performance

The first ten days of operation had highly variable results in terms of effluent soluble COD (sCOD) and pH. During this time, the reactors were fed more aggressively for the first six days with a target sCOD of 2000 mg/L in an effort to jumpstart microbial activity. Also, the bicarbonate in stock solution C (Table 2.3) was not included, with the intent of avoiding buffering. However, this strategy depressed the pH significantly, which stalled microbial activity. Subsequently, it was decided that a more appropriate strategy would be to operate the reactors in a way such that the control produced effluent with a pH and sCOD concentration similar to full scale septic systems. This was accomplished by including the bicarbonate in stock solution C and reducing the feed concentration to 1000 mg/L sCOD. Effluent quality from various septic tanks has been characterized in several studies. Results presented by Brandes^[7] show average effluent sCOD ranging from 175 – 490 mg/L and a pH ranging from 7.2 – 8.5. The control reactor operated close to within this range for days 41 – 85 with an average effluent sCOD of 429 mg/L and an average pH of 6.98. The entire time series performance of the reactors and feed is shown in Figure 3.1 through

The first forty days of operation showed little difference in performance between the reactor configurations. A spike in sCOD from day 41 – 44 corresponds to a uniform addition of 250 μ M riboflavin in all three reactors. Riboflavin was added in an effort to stimulate the growth of electroactive bacteria, although there were no indicators to confirm or deny their presence. Previous work showed that flavins (as well as quinone derivatives) can act as electron shuttles between certain *Shewanella* sp. and solid carbon electrodes, and that the organism produces the

mediator naturally.^{[12][25]} The addition of riboflavin appeared to have caused a separation in performance between the reactor configurations. As summarized in Table 3.1, both reactors with carbon cloth showed improved sCOD removal after riboflavin addition by 23% and 26% in the open and full circuit setups respectively. *t*-test results at a 0.05 level of significance confirmed this result statistically for pairwise comparisons between the control and open circuit reactor, as well as the control and full circuit reactor (Table 3.2).

3.2 Mineralization Study

The maximum extent of mineralization for acetate, lactate, butyrate, propionate, oleic acid, and starch is summarized in Table 3. for methane and Table for carbon dioxide. Time series plots for each carbon compound for mineralization to carbon dioxide are shown in Figure 3.4 through Figure 3.9. The results presented here showed the highest extent of mineralization for all carbon substrates in the open circuit configuration (CC only). The increase in mineralization compared to the control setup ranged from 16-82%, depending on the carbon donor. There was no discernible trend in terms of performance among the other three reactor configurations. This was in contrast to the expectation that the full circuit tubes would have a higher level of mineralization. The pH increased slightly for all configurations, as shown in Figure 3.10.

Methane peaks were highly variable, so individual series were excluded. This could be an analytical artifact or could be variability in methanogenic activity. However, with the exception of oleic acid, methane generation was negligible. The activity of the oleic acid stock was low (c.a. 2E6 dpm/mL, compared to 1E7 dpm/mL for the other substrates), and responses on the GPC were poorly defined (i.e., peak spreading).

CHAPTER FOUR

4.0 DISCUSSION

Results from the bench scale experiments were encouraging in terms of achieving the goal of this research, which was to improve the performance of septic tanks in terms of carbon oxidation. Soluble COD removal in the reactors with carbon cloth was higher than the control after adding riboflavin midway through the experiment. It was unexpected that the riboflavin had an effect on both the open and closed circuit configurations. Since there were no controls or tests implemented to confirm electric current generation or the presence of electroactive bacteria, the notion that the dominant metabolism shifted away from fermentation and towards respiration via a solid phase carbon electrode is at best speculative. It is possible that the carbon cloth simply provided additional surface area allowing for better attachment and therefore better solids retention. In this case, improved sCOD removal would be attributed to a higher concentration of active bacteria. Although not as biologically interesting, it still has practical relevance. Molecular techniques (e.g., clone libraries) could be used to indicate the presence of electroactive species. Some known genera include *Geobacter*, *Shewanella*, and *Rhodospseudomonas*.^{[5],[17][25]}

Similarly, the mineralization experiments were encouraging in showing improved carbon removal in the open circuit series but not conclusive in terms of establishing a community of electroactive bacteria. Since the inoculum for the various circuitry setups (as described in the methods) came from the three bench scale reactors, there was no way

to ensure a uniform concentration of active biomass in the different series. It might be useful to rerun the experiment with the fresh and homogenous septage from the same source in each series, but this might not be indicative of how an acclimated system would respond. The bench scale reactors were operated for 85 days, which allowed them to develop unique characteristics.

Ultimately, evaluating this technology by running mineralization studies proved challenging from a setup perspective. Aside from problems associated with gas leaking from the bottles, multiple material incompatibilities also added a degree of uncertainty. The alligator clips were made from steel, which were readily oxidized in the cathode chamber. This generated a solid ferric iron layer that coated the carbon cloth, possibly inhibiting electron transfer. The initial setup (some results shown in Appendix C, Figure through Figure C.46) used copper welding wire instead of stainless steel lockwire. The copper corroded on both anode and cathode ends, which influenced the pH. The setup used for the data presented is less than ideal, but it is the most reliable and refined to date.

One other drawback to using mineralization studies is comparing the results of a batch assay to what is actually a semi-continuous system. Septic tanks could best be described as semi-batch systems, which justify the operational feed schedule of the bench scale reactors. However, it was not possible to configure a continuous flow or semi-batch system for mineralization experiments because the substrates were radioactive. Running the different series in batch may illustrate useful and reliable trends, but it is at least worth mentioning that these trends might not be directly transferable to a real septic system. Notably, there was an ascending pH profile for all four reactor configurations in

the mineralization experiment while the pH in bench scale reactors (with some variability) reached steady state.

In tandem, the bench scale bulk sCOD and small scale mineralization experiments indicate that carbon removal efficiency can be improved by adding carbon cloth to model septic tank systems. The mechanism by which this was achieved was not readily apparent, but it was a reproducible trend in terms of sCOD removal and compound specific mineralization studies. A more refined reactor design would easily allow for reliable effluent total suspended solids (TSS) measurements to be made. Qualitatively, effluent from the reactors with carbon cloth was noticeably clearer and free of solids. This might highlight an additional benefit of introducing carbon cloth to a septic system, since leach field clogging is directly related to the organic loading rate and effluent.^{[3][18][24]}

Lastly, this project originally included evaluating the persistence of select pharmaceuticals in septic media. Split peaking in HPLC chromatograms, presumably caused by metabolite formation, eventually stalled further investigation. However, preliminary data for sulfamethoxazole (SMX) was generated and is provided in Appendix D, Figure . Carbon cloth alone in a clean matrix removed SMX the quickest, following what appears to be first order kinetics. Ultimately, the idea of using carbon cloth for its adsorptive properties in septic media was rejected, since high organic loading rates and influent TSS would quickly deplete the adsorptive capacity of the cloth (personal correspondence, Dr. Tanju Karanfil). Electrochemical transformation of trace organics may occur, but further investigation is required.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions have been made as a result of this thesis research.

1. Results from the bench scale reactors demonstrated that adding carbon cloth to a model septic tank system increased carbon oxidation in terms of increased sCOD removal. An average improvement of 23% and 26% in sCOD removal (compared to the control) was observed for the open and full circuit configurations, respectively. It was not clear whether this was caused by a shift in metabolic preference or was a result of increased biomass retention. Riboflavin appears to have had a role in facilitating this this outcome, which suggests some form of mediated electron transfer to the carbon cloth. Electron transfer mechanisms in electrically active biofilms respiring on carbon electrodes provide a basis for an interesting area of study. Continued research on internal and microscale redox gradients^{[4][22]} mediated by metallic like transfer through nanowires or so termed “electron hopping” between cytochromes^[2] might allow for further optimization of the carbon cloth configurations utilized in septic systems.
2. The extent of carbon mineralization was highest for all substrates tested in Balch tubes where carbon cloth was added without any circuitry. Improvement varied by substrate between 16 – 82%. The full circuited series did not perform consistently better than the either partial or non-circuited

series. Better controls (like a single chamber MFC) might provide useful information as to why this was the case.

The following recommendations are offered as a result of this thesis research.

1. Any future bench scale experiments should be refined in terms of setup, operation, and analyses performed. A thick-wall plexiglass reactor fitted with a fixed inlet and outlet appurtenances and connected to feed pump would provide significant operational improvements, allowing for more precise feed delivery and control and more consistent effluent quality. Baffles could be easily implemented, which would more accurately reflect the design of real septic tanks. A rubber lined lid would reduce oxygen intrusion, better preserving anoxic conditions.
2. Several useful analyses and system “checks” were either overlooked or deemed unreliable due to system constraints (i.e., reactor design, manual feed, etc.) Effluent total COD and TSS together would better indicate overall system performance. As described in the Introduction, organic loading and TSS have been directly linked to biomat formation in the infiltration system, which ultimately leads to clogging. Total COD does not directly dictate the organic loading rate, but it would at least be a nice supplemental metric to have in evaluating the total loading on the leach field. Also, in terms of evaluating the systems grossly by solids retention, a test for percent solids at the conclusion of the bench scale experiment would have been insightful.

3. Feed was prepared two cycles at a time and stored on ice with the carboy opening covered with aluminum foil. For quantitative purposes, a check on feed degradation by sCOD analysis between cycles would have been useful.
4. Another control reactor with an inert cloth or surface in the bench scale experiment could have provided important mechanistic information as to why there was improved sCOD removal in the carbon cloth setups, since riboflavin has been shown to facilitate electron transfer in systems utilizing carbon electrodes. If adding riboflavin to a reactor with an inert cloth showed no subsequent improvement in sCOD removal, then the conductive property of carbon cloth had an effect on the system performance.
5. One major oversight in the mineralization study was the lack of a mass balance on ^{14}C . This could be accomplished by measuring total headspace and liquid radioactivity by direct additions to liquid scintillation cocktail. It would have been a simple check and necessary for reliable quantification.
6. A check on the GPC response by using base trapping as an alternative method of quantification would have been, at least, reinforcing. The GPC was erratic at times and samples were often rerun. It is unclear if this is the nature of the instrument, or if poor maintenance is to blame.

TABLES

Table 2.1: Chemicals and materials used for bench scale and mineralization experiments.

| Chemical or Item | Grade/Purity | Vendor |
|-------------------------------|------------------------|-------------------|
| Ammonium chloride | 99.50% | Fisher Scientific |
| AQDS | 99% | Sigma Aldrich |
| Calcium chloride dihydrate | 99% | EM Science |
| Carbon cloth | Braided biaxial sleeve | FibreGlast |
| COD vials | 40 – 1500mg/L | Hach |
| Dextrose | - | Fisher Scientific |
| Potassium phosphate monobasic | Bioreagent | J.T. Baker |
| Riboflavin | 98% | Sigma Aldrich |
| Sodium bicarbonate | 99% | Fisher Scientific |
| Yeast extract | Ultra filtered | Becton Dickinson |

Table 2.2: Radiolabeled substrates used for mineralization experiments.

| ¹⁴C Compound | Vendor |
|--------------------------------|---------------------------------|
| 2- ¹⁴ C Acetate | Moravek Biochemicals |
| 2- ¹⁴ C Butyrate | Moravek Biochemicals |
| U- ¹⁴ C Lactate | Sigma Aldrich |
| 1- ¹⁴ C Oleic Acid | Moravek Biochemicals |
| 2- ¹⁴ C Propionate | Moravek Biochemicals |
| U- ¹⁴ C Starch | American Radiolabeled Chemicals |

Table 2.3: Feed components and their corresponding concentrations. Feed was prepared in 2L batches with a 2L volumetric flask

| Stock Designation | Components | Concentration (g/L) |
|-------------------|---------------------------------|---------------------|
| A | Dextrose | 34.60 |
| B | Yeast extract | 8.63 |
| C | NH ₄ Cl | 4.32 |
| | CaCl ₂ | 4.32 |
| | KH ₂ PO ₄ | 1.36 |
| | NaHCO ₃ | 84.00 |

Table 2.4: Operating conditions and information for the GC and GPC used in mineralization studies.

| | Operating Condition | Description |
|--------------------------|-----------------------|------------------------------|
| Shimadzu GC-8A | Column | Supelco 80/100 carboxen 1004 |
| | Injection temperature | 60°C |
| | Column temperature | 70°C |
| | Column length | 2.0 m |
| Gas Proportional Counter | Exterior oven | 750°C |
| | Carrier gas | Helium UHP |
| | Counting gas | P-10 |
| | Carrier gas flow | 21 - 25 mL/min |
| | Counting gas flow | 15 mL/min |
| | Total flow | 36 - 40 mL/min |
| | High voltage setting | 1375 V |

Table 3.1: Summary of bench scale reactor effluent performance. Note, values represent effluent averages from day 46 – 85. Values in parentheses represent one standard deviation.

| | Feed (SD) | Control (SD) | Open Circuit (SD) | Full Circuit (SD) |
|------|------------------|---------------------|--------------------------|--------------------------|
| sCOD | 923 (28) | 429 (31) | 330 (67) | 316 (66) |
| pH | 8.44 (0.36) | 6.98 (0.12) | 7.11 (0.15) | 7.01 (0.11) |

Table 3.2: Two tailed t-test results for pairwise comparisons between sCOD measurements. A 0.05 level of significance (or p value) was used. The calculated statistic was determined in Excel.

| | Pairwise comparison | | |
|-------------------------|---------------------------|---------------------------|--------------------------------|
| | Control & Open Circuit | Control & Full Circuit | Open Circuit & Full Circuit |
| Calculated statistic | 6.81E-07 | 1.95E-06 | 3.75E-01 |
| Significant (Y/N) | Y | Y | N |

Table 3.3: Summary of maximum methane mineralization observed for radiolabeled studies. Some triplicate responses were highly variable, and most tubes generated injection responses that could be considered negligible or attributed to instrument background noise.

| Circuit Configuration | Maximum % of ¹⁴ C carbon oxidized to ¹⁴ CH ₄ | | | | | | |
|-----------------------|---|----------------------|----------------------|----------------------|----------------------|----------------------|-------------------------|
| | Acetate | Lactate | Butyrate | Propionate | Oleic Acid | Starch | Starch RS4 ^a |
| | CH ₄ (SD ^b) | CH ₄ (SD) | CH ₄ (SD) | CH ₄ (SD) | CH ₄ (SD) | CH ₄ (SD) | CH ₄ (SD) |
| No Circuit | 2.7 (2.0) | 2.2 (1.4) | 5.8 (1.9) | 3.8 (1.1) | 24.6 (7.2) | 5.4 (3.5) | 0.7 (0.2) |
| Open Circuit - CC | 1.2 (0.6) | 2.0 (1.9) | 5.9 (2.8) | 2.3 (0.9) | 14.2 (6.7) | 4.6 (2.4) | 1.0 (0.1) |
| Partial Circuit | 4.9 (6.0) | 4.7 (3.9) | 4.4 (3.7) | 5.3 (0.8) | 25.8 (20.1) | 6.0 (1.1) | 0.8 (0.2) |
| Full Circuit | 2.5 (1.0) | 3.6 (3.1) | 3.7 (3.0) | 3.1 (3.0) | 28.9 (2.7) | 3.0 (3.5) | 0.9 (0.3) |

24

^a “RS4” corresponds to a different experiment, reactor study four. This had a very similar setup to that described in the methods, except copper welding wire was used instead of stainless steel lockwire for the circuitry.

^b SD = Standard deviation

Table 3.4: Summary of maximum carbon dioxide mineralization observed for radiolabeled studies. Some triplicate responses were highly variable, especially at the later time points.

| Circuit Configuration | Maximum % of ¹⁴ C carbon oxidized to ¹⁴ CO ₂ | | | | | | |
|-----------------------|---|----------------------|----------------------|----------------------|----------------------|----------------------|-------------------------|
| | Acetate | Lactate | Butyrate | Propionate | Oleic Acid | Starch | Starch RS4 ^a |
| | CO ₂ (SD ^b) | CO ₂ (SD) | CO ₂ (SD) | CO ₂ (SD) | CO ₂ (SD) | CO ₂ (SD) | CO ₂ (SD) |
| No Circuit | 61.3 (10.9) | 58.1 (6.8) | 49.7 (6.5) | 27.1 (7.4) | 91.5 (5.6) | 40.2 (7.7) | 4.6 (0.6) |
| Open Circuit - CC | 87.8 (1.6) | 67.2 (3.5) | 69.4 (1.0) | 49.2 (13.3) | 127.1 (7.4) | 60.1 (2.8) | 7.3 (1.0) |
| Partial Circuit | 56.8 (7.3) | 49.4 (7.5) | 46.5 (5.9) | 35.3 (1.6) | 67.4 (5.1) | 34.6 (5.4) | 2.7 (0.3) |
| Full Circuit | 67.4 (12.1) | 48.2 (5.6) | 55.2 (2.6) | 36.6 (4.3) | 67.0 (1.8) | 36.5 (17.4) | 13.7 (2.1) |

^a “RS4” corresponds to a different experiment, reactor study four. This had a very similar setup to that described in the methods, except copper welding wire was used instead of stainless steel lockwire for the circuitry.

^b SD = Standard deviation

FIGURES

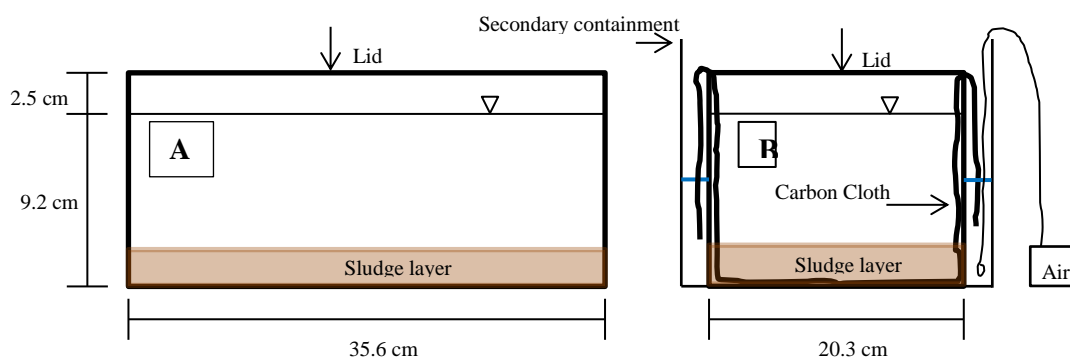


Figure 2.1: Panels A and B show both side views of the batch reactors. Panel B shows the full circuit configuration, where the carbon cloth loosely drapes over the walls of the reactor into the sludge layer. The cloth is exposed externally to an aerated water bath buffered with 10 mM sodium acetate. Full tank dimensions were 35.6 cm x 20.3 cm x 11.7 cm. The reactors were maintained at a 4 L volume, which left about 2.5 cm of free board between the liquid and the top of the tank.

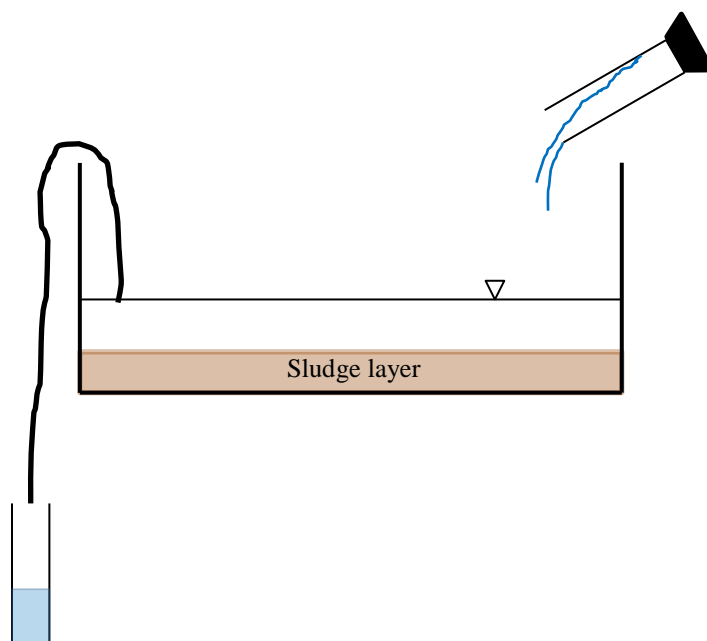


Figure 2.2: Side view schematic of a feed cycle. Liquid was decanted as close to the surface as possible. The volume was replaced with fresh feed using a 2 L graduated cylinder.

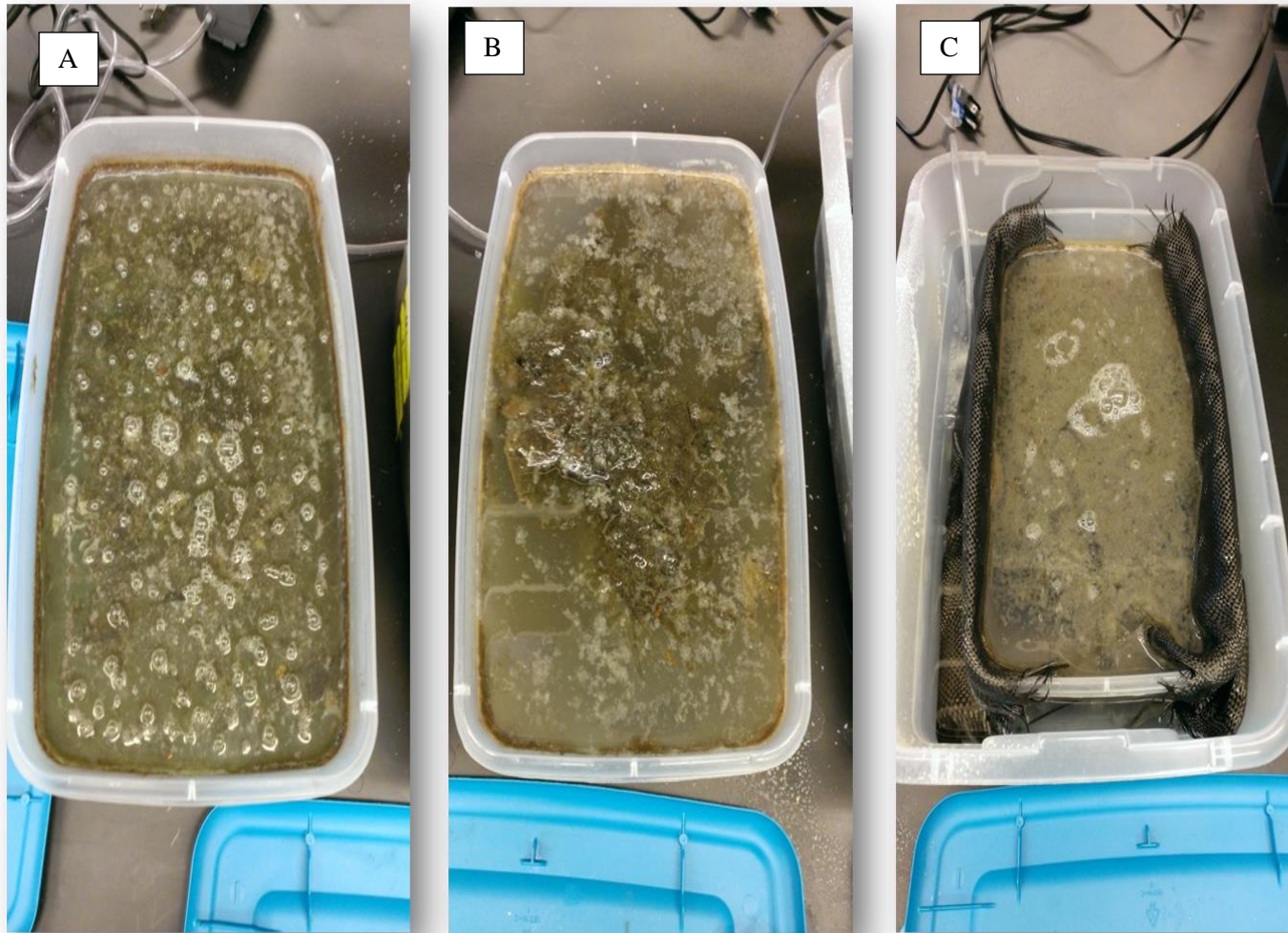


Figure 2.3: From left to right, Reactor #1, 2, and 3 in panels A, B, and C respectively. Plastic lids were removed for better visualization. Note the secondary containment of R3 which was aerated and buffered with acetate at a pH less than 7.0.

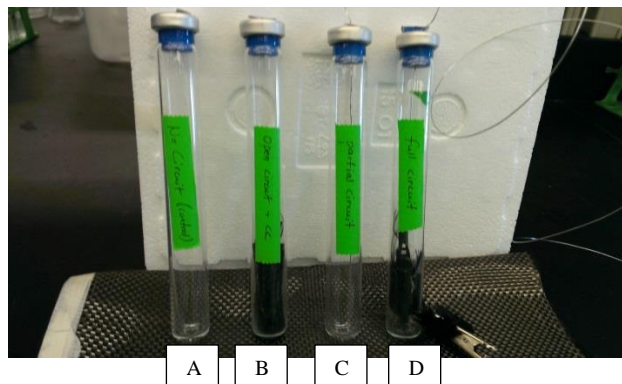


Figure 2.4: Small scale reactor photos taken for each circuit configuration. Panel A shows the unamended, or control, setup. Panel B shows the carbon cloth series, which has a small swatch (~1.3 cm.) of carbon cloth placed at the bottom of the tube. Panel C shows the partial circuit configuration, which is threaded with a 20.3 cm. length of lockwire. Panel D displays the full circuit setup, which is both threaded with lockwire and fitted on either end with alligator clips and carbon cloth.

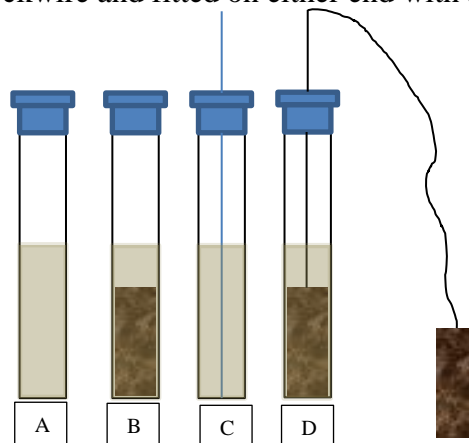


Figure 2.5: Small scale reactor configurations shown as digital sketches. A) Unamended, or control, setup; B) carbon cloth series, which has a small piece (~1.3 cm x 1.3 cm) of carbon cloth placed at the bottom of the tube; C) partial circuit configuration, which is threaded with a 20.3 cm. length of lockwire; and D) full circuit setup, with both threaded with lockwire and fitted on either end with alligator clips and carbon cloth.

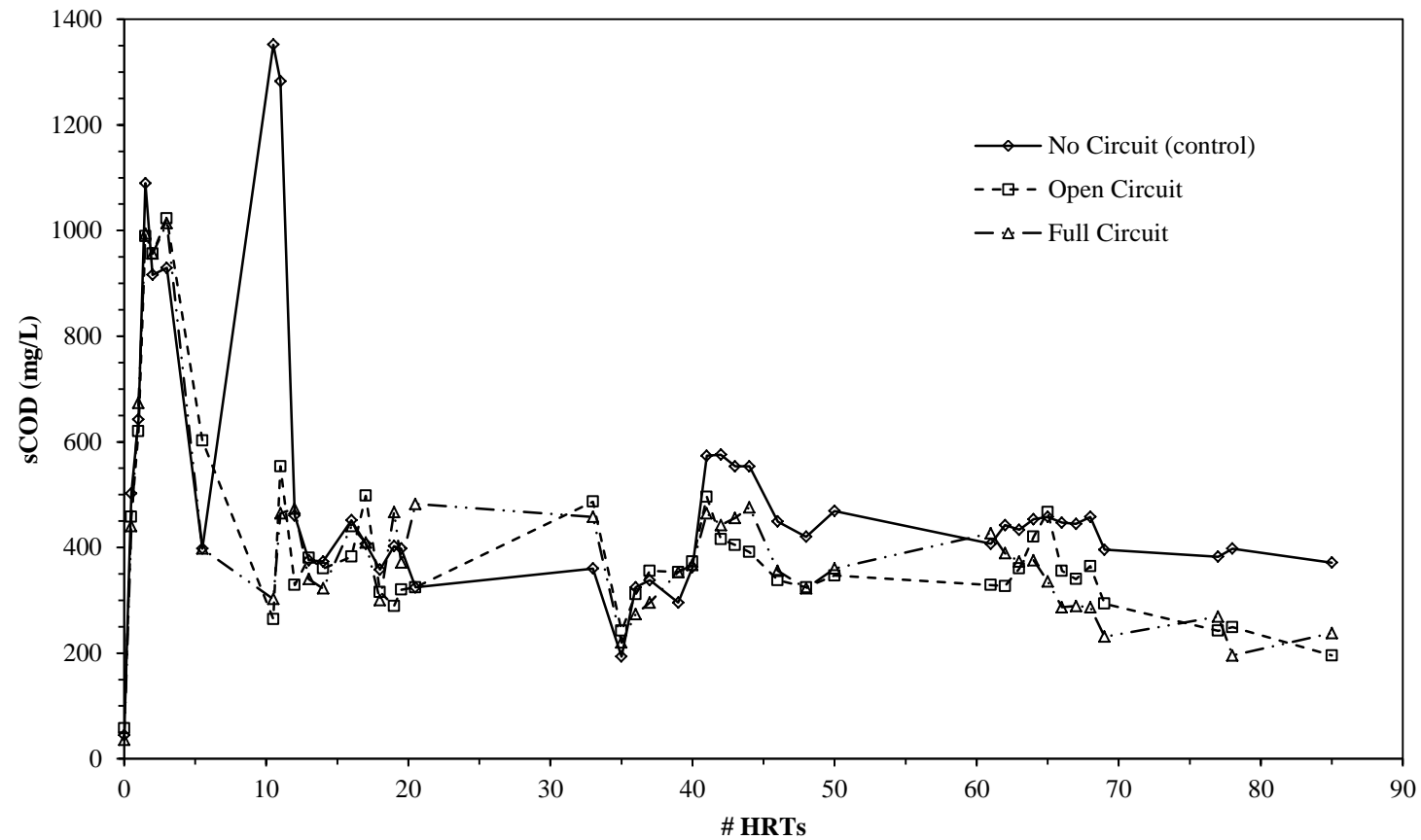


Figure 3.1: Time series performance of the bench scale reactors in terms of soluble COD. The control did not contain any carbon cloth. The open circuit had carbon cloth submerged in the liquid and sludge layers. The full circuit had carbon cloth submerged in the sludge layer and extended outside of the reactor into an aerated water bath.

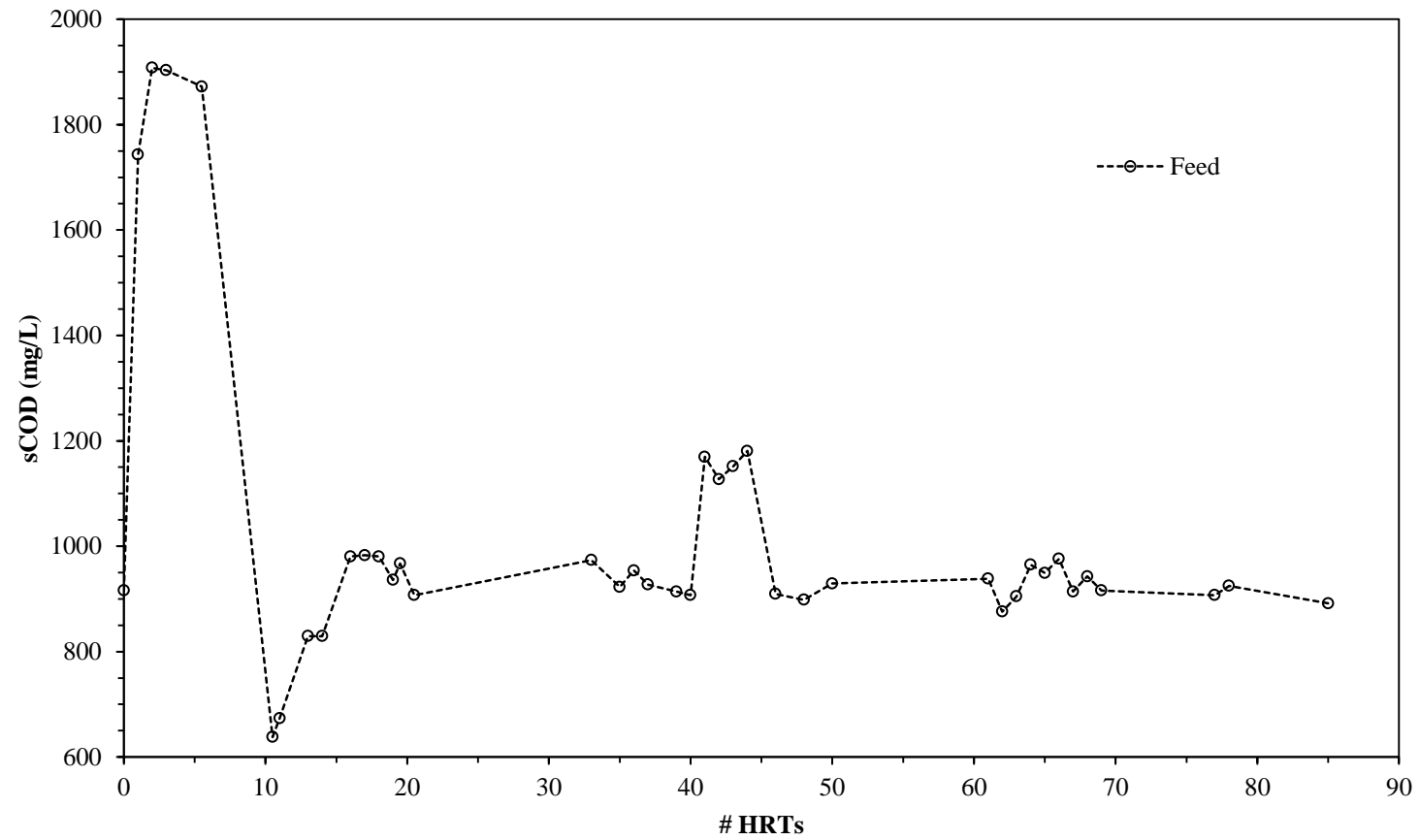


Figure 3.2: Time series monitoring of the influent feed. The target steady state sCOD in the feed (after day 15) was 1000 mg/L. A spike in sCOD on day 41 corresponds to the addition of riboflavin.

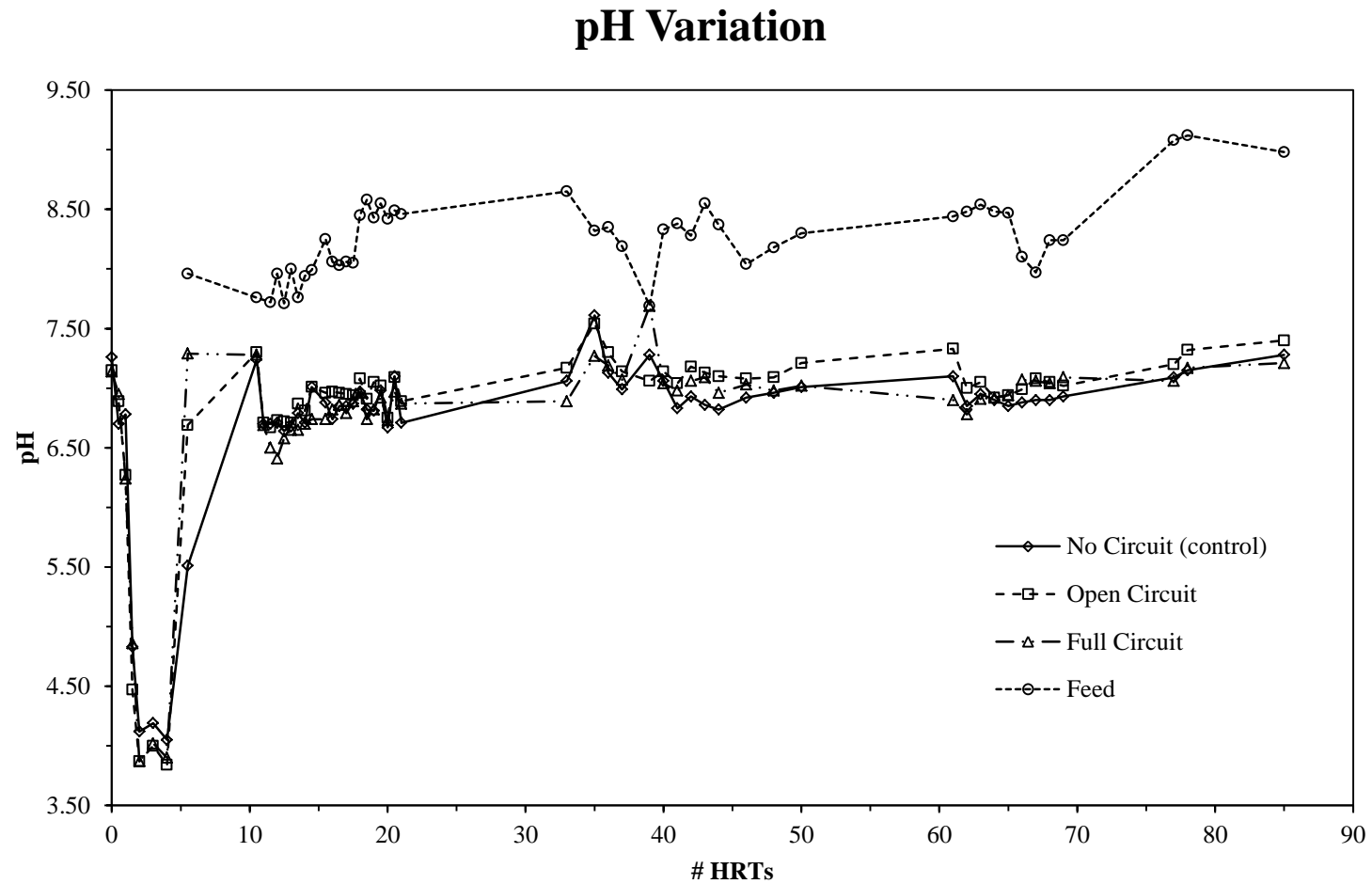


Figure 3.3: Time series monitoring of the bench scale reactors in terms of pH. The control did not contain any carbon cloth. The open circuit had carbon cloth submerged in the liquid and sludge layers. The full circuit had carbon cloth submerged in the sludge layer and extended outside of the reactor into an aerated water bath.

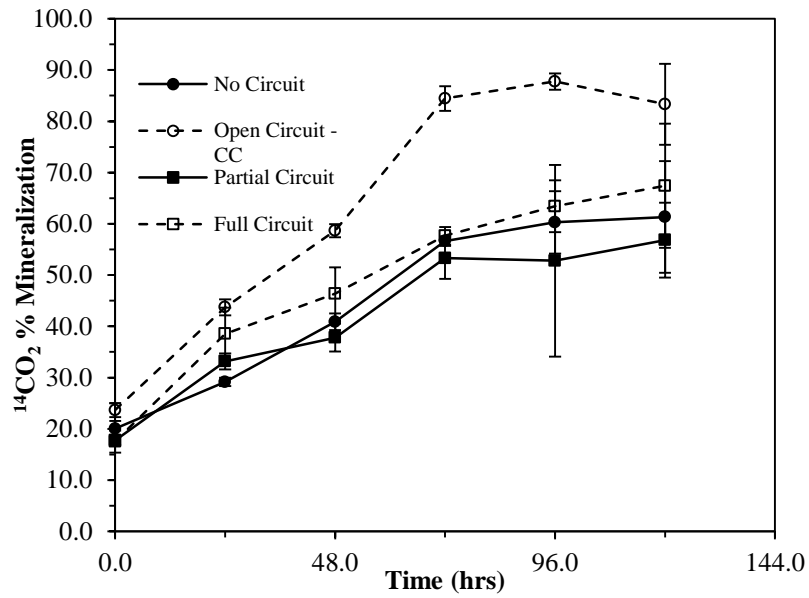


Figure 3.4: Carbon dioxide mineralization results for acetate over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

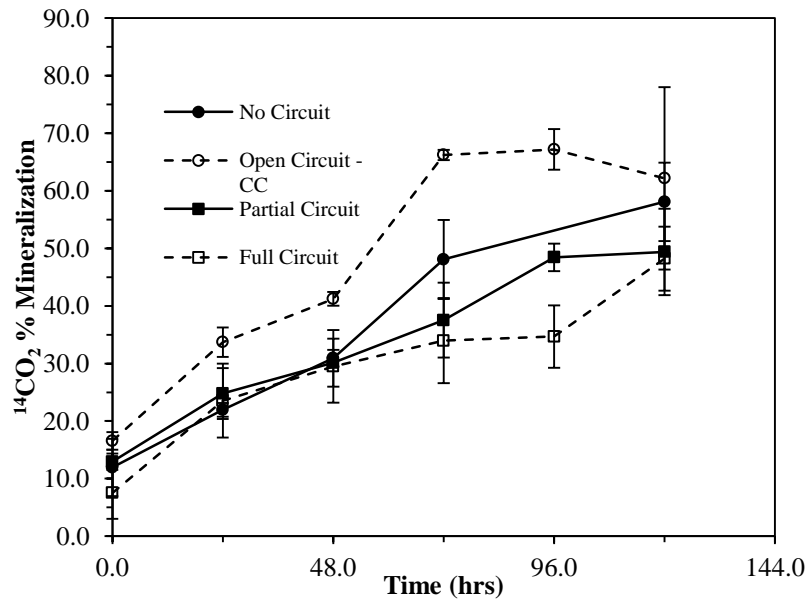


Figure 3.5: Carbon dioxide mineralization results for lactate over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

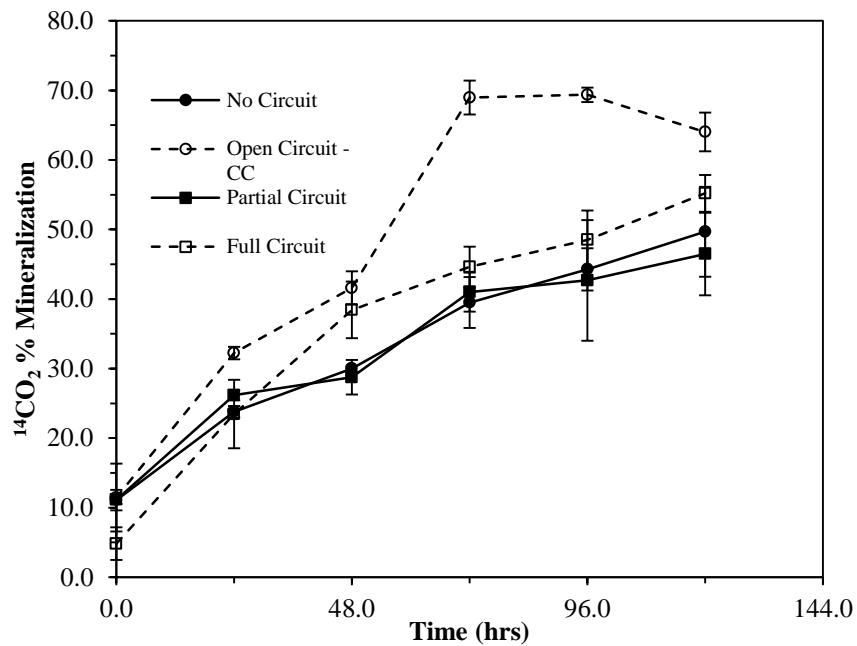


Figure 3.6: Carbon dioxide mineralization results for butyrate over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

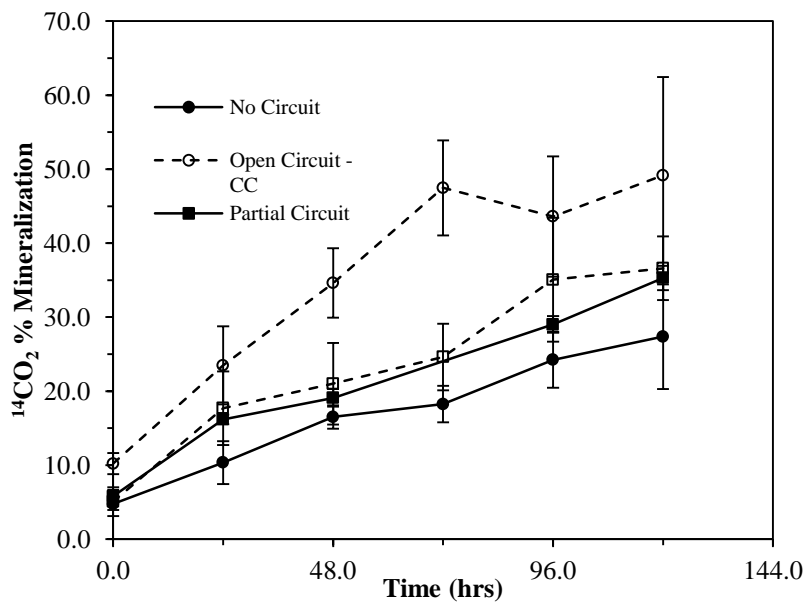


Figure 3.7: Carbon dioxide mineralization results for propionate over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

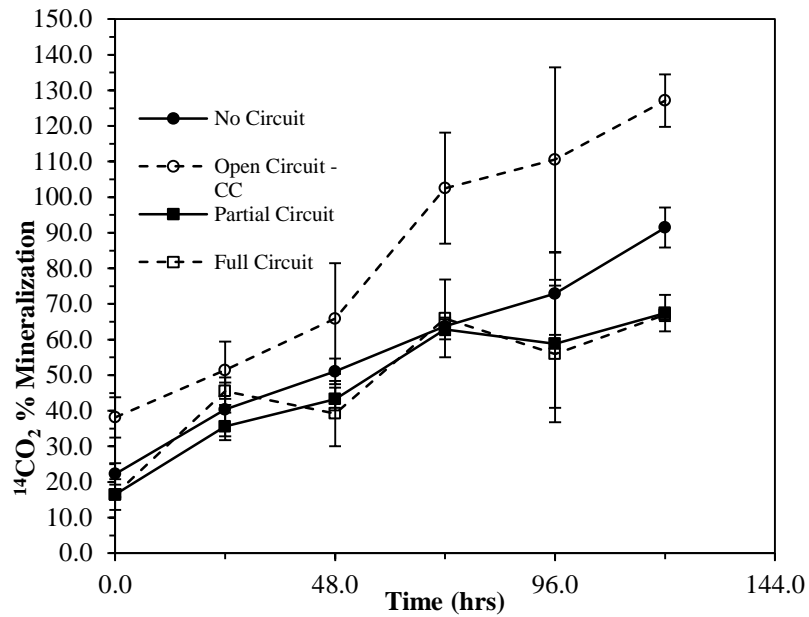


Figure 3.8: Carbon dioxide mineralization results for oleic acid over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

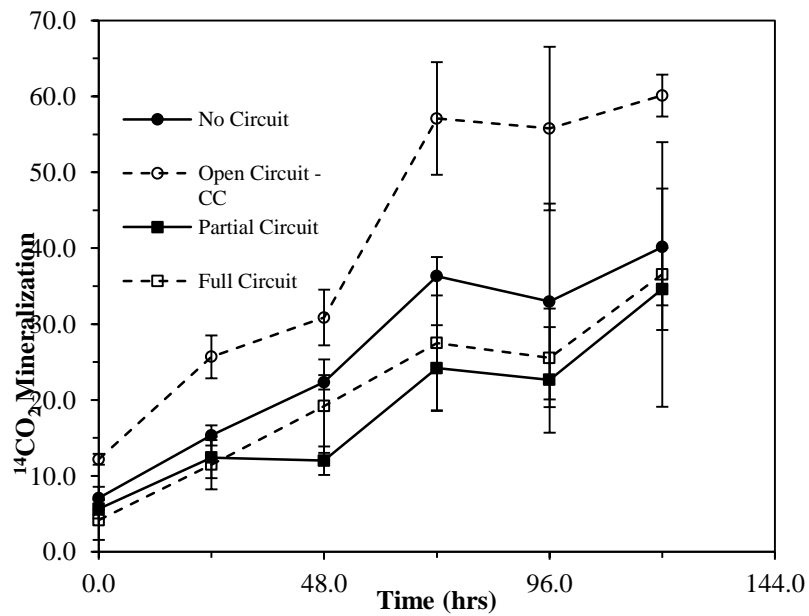


Figure 3.9: Carbon dioxide mineralization results for lactate over a five day period. Error bars represent one standard deviation. Circuit configurations are described in the methods.

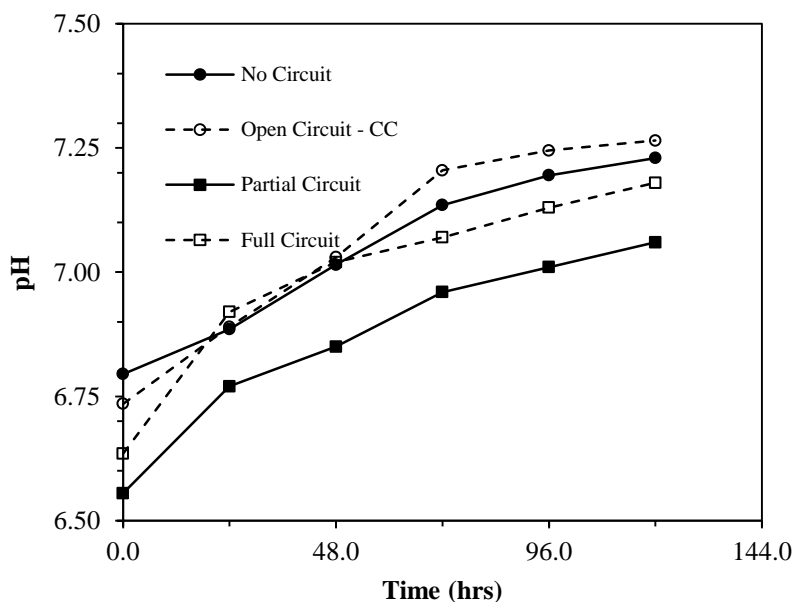


Figure 3.10: pH response of the different circuited series over five days. pH was measured to determine a partition value, or the fraction of carbon dioxide present in the headspace. Data points represent duplicate averages, except for the full circuit. One of the full circuit tubes was invalidated for having an unusually high pH.

APPENDICES

Appendix A
COD Standards

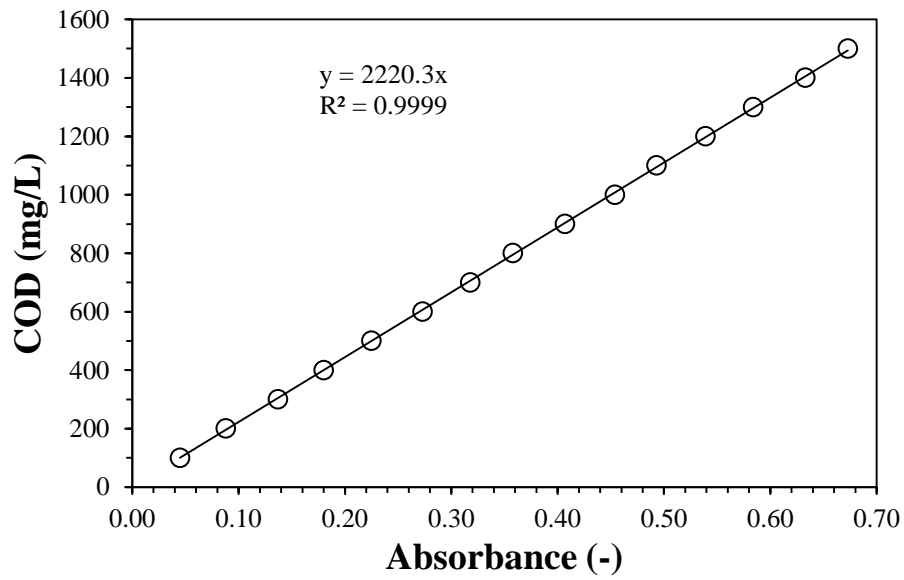


Figure A.1: Standards generated for high range COD vials used in monitoring the bench scale reactors. Standards were prepared using dried potassium hydrogen phthalate and measured at concentrations between 100 and 1500 mg/L at 620 nm.

Appendix B Leak Test Results

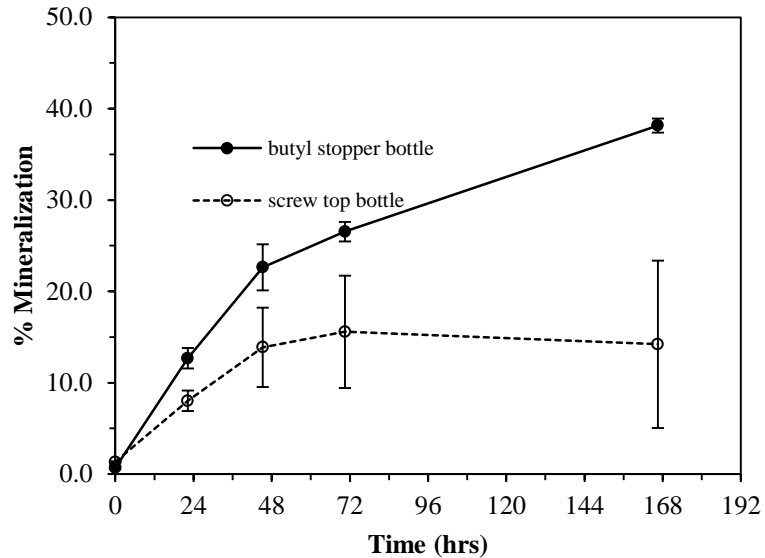


Figure B.1: Results confirming leaks in screw top bottle reactors. Butyl stopper tubes and bottles are used as a reference, since there is a high degree of confidence in their ability to keep an air-tight seal.

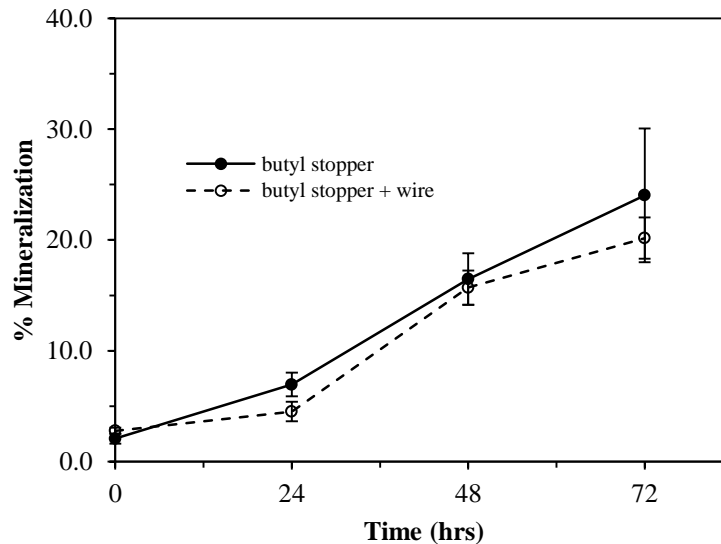


Figure B.2: Results confirming the reliability of the circuited test system. A wire was threaded through a butyl stopper and sealed with an aluminum crimp. Both systems with and without the wire responded similarly.

Appendix C
RS4 Data

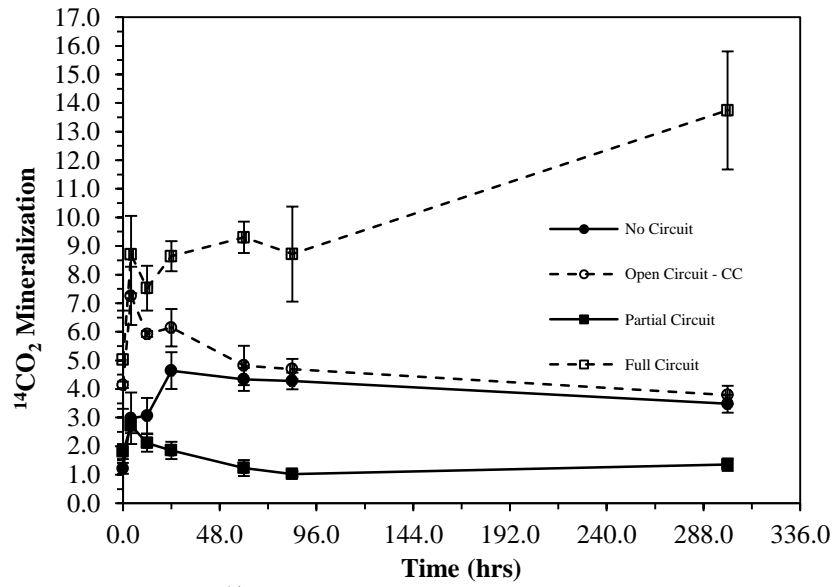


Figure C.1: Mineralization of ¹⁴C starch in terms of carbon dioxide generated for RS4

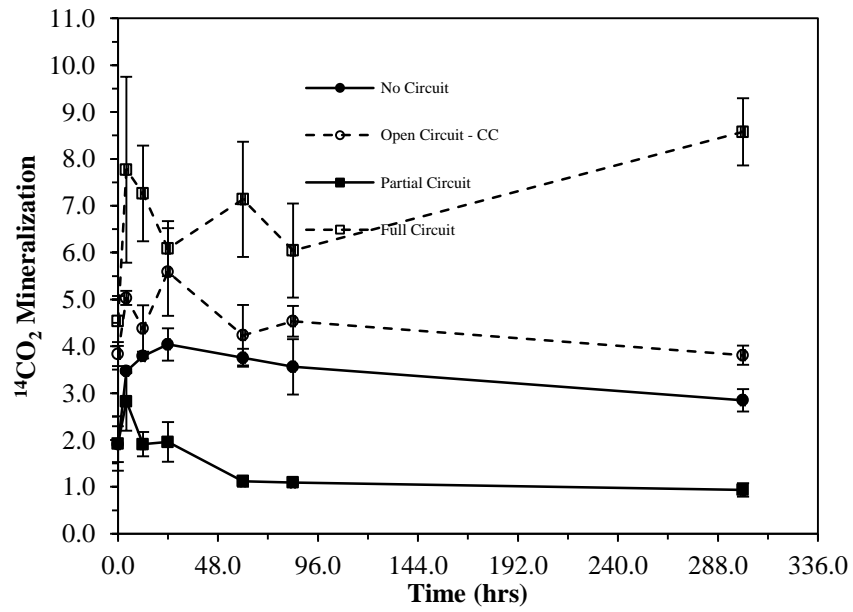


Figure C.2: Mineralization of ^{14}C starch in terms of carbon dioxide generated with $100\mu\text{M}$ of the electron shuttle AQDS added for RS4

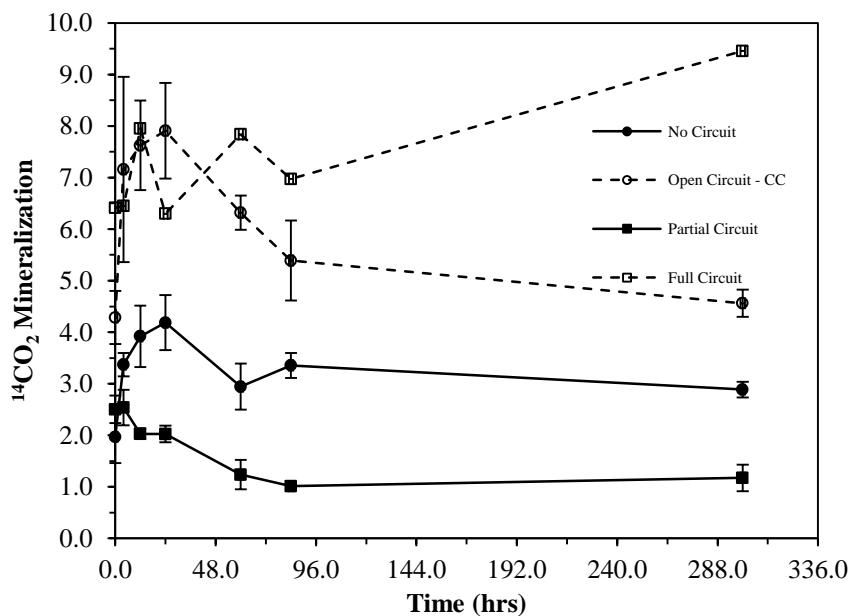


Figure C.3: Mineralization of ^{14}C starch in terms of carbon dioxide generated with $100\mu\text{M}$ of the electron shuttle riboflavin added for RS4

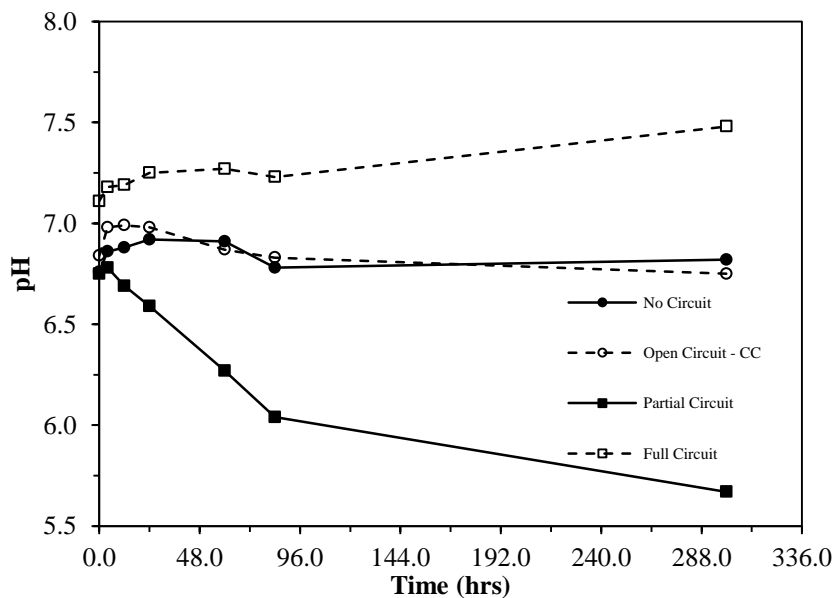


Figure C.46: pH response of the different circuited series over 12.5 days for RS4. pH was measured to determine a partition value, or the fraction of carbon dioxide present in the headspace

Reactor Study 4 (RS4) system description and comments

RS4 had an identical setup to the one described in the materials and method section with three main exceptions: copper welding wire was used instead of stainless steel lockwire, a smaller volume of radiolabeled substrate was added to each reactor (0.1mL instead of 0.2mL), and the initial pH was **not** adjusted before the experiment. While the data presented here (Appendix C) seems to suggest that the full circuit configuration performed the best, the extent of mineralization is quite low. Corrosion was noted on the copper wires at the conclusion of the experiment. This could have impacted the pH and the ability of the wire to transfer electrons. The bigger issue was pH. The final measurement taken for the full circuit setup showed that the pH had risen to 7.48, meaning that the partition (or fraction of CO₂ in the headspace) was 0.07. Responses on the gas proportional counter were less than 100 dpm for two of the full circuit series. This was flirting with background counts for the instrument. Had the pH been lower, the response on the instrument would have been higher. This would improve the confidence of the apparent result, which was not replicated in reactor study five (RS5).

Appendix D Sulfamethoxazole Data

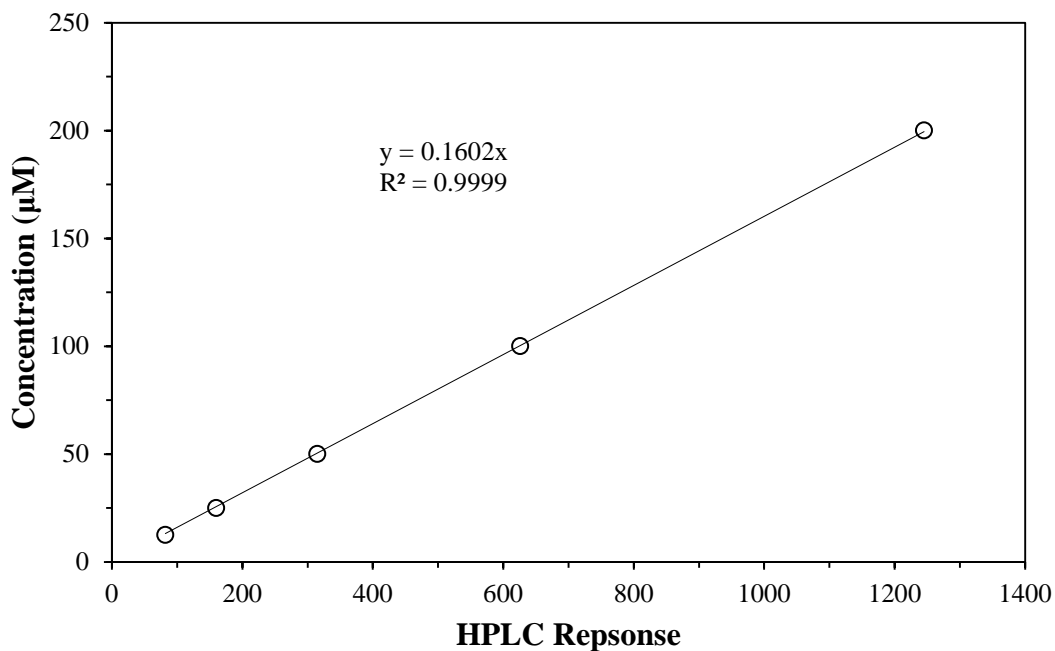


Figure D.1: Standard curve for sulfamethoxazole. Standards were prepared volumetrically from a 10mM stock solution.

SMX Experiments: setup and comments

As mentioned briefly in the discussion, this research originally also included evaluating the persistence of pharmaceutically active compounds (PACs) in septic systems. These compounds range from common food and drink constituents like caffeine to active ingredients in prescription drugs like sulfamethoxazole. Little research has been done to assess the recalcitrance of these compounds in septic systems.

A batch experiment (results in Figure) was setup to evaluate SMX attenuation. A SMX stock solution was prepared at a concentration of 10mM in 100% methanol. A target amendment concentration of 500µM was used. Bottles were setup by diluting raw septic sludge 1:10 in anoxic tap water. SMX was measured using HPLC.

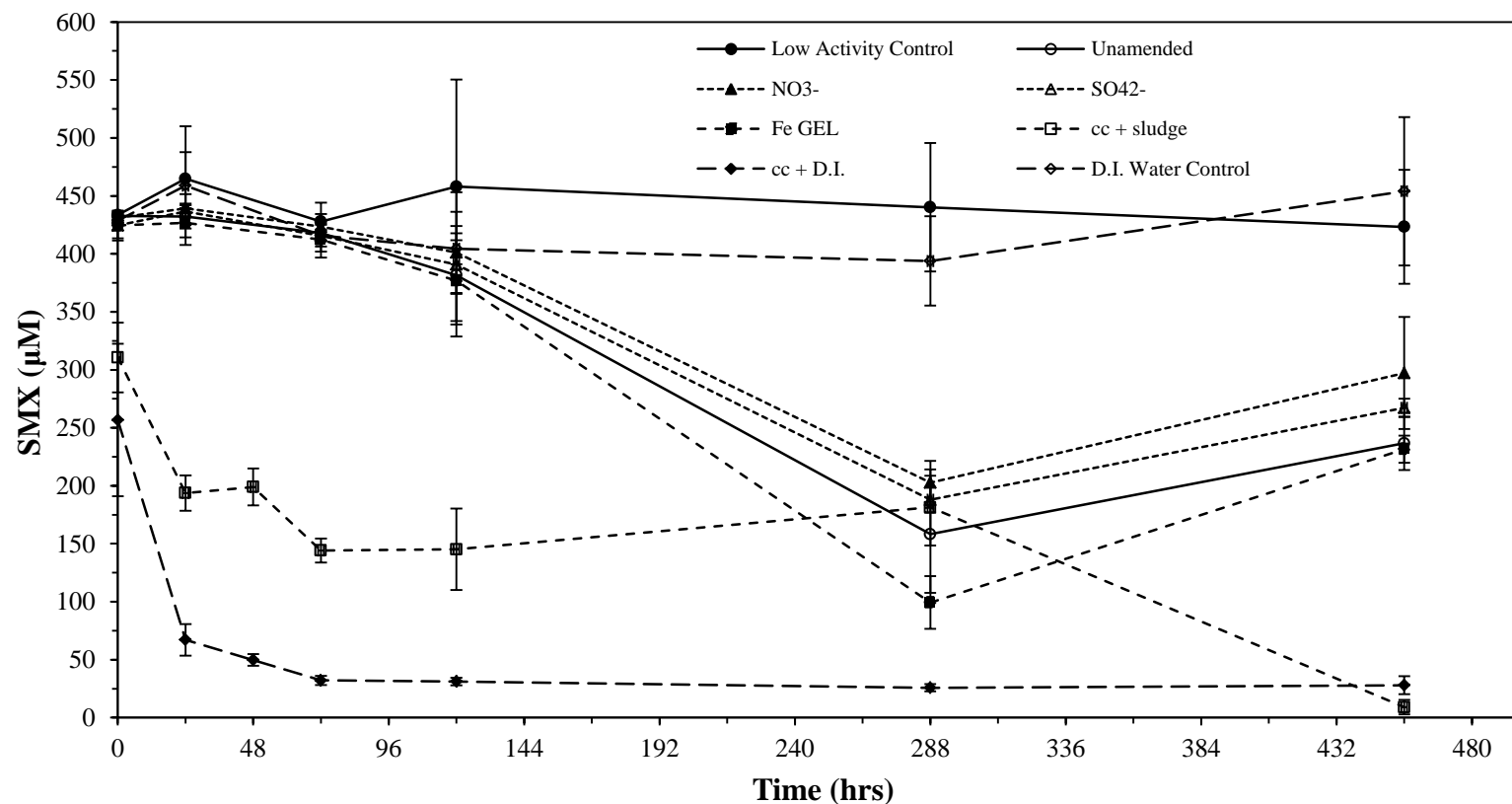


Figure D.2: cc = carbon cloth, D.I. = deionized. Time series attenuation of SMX with different amendments. Low activity controls were kept at 4° C. Kill controls were not used to avoid physically altering the sludge in the autoclave process, which may have changed the systems adsorptive properties. Spikes in SMX concentration after 288 hours are likely analytical artifacts. Split peaking was observed at this time point, and resolving the SMX peak was not possible.

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