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Animal Co-Products as Novel Electron Donors for Biodegradation of Trichlorethylene

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ANIMAL CO-PRODUCTS AS NOVEL ELECTRON DONORS FOR BIODEGRADATION OF TRICHLORETHYLENE

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ABSTRACT

The United States produces approximately 55 billion pounds of animal co-products in a year. These materials are what remains once cattle, chickens, and other animals are slaughtered and the food grade material is processed for consumption. Although a substantial amount of this waste is used in the pet food industry, it leaves thousands of pounds as true waste, without a market. Given the nature of co-products, these materials have high protein and lipid content, and these waste materials have the potential to be used as electron donors for microbial processes. Animal waste co-products used as electron donor for bioremediation could be an inexpensive alternative for the industry, while reducing the amount of waste sent to a landfill.

Batch incubations were used to screen 38 animal co-products, provided by renderers within North America. Batches were constructed using TCE-contaminated aquifer material, and each electron donor (co-product) was added as the sole electron donor. Each animal co-product was compared to five controls containing common electron donors (lactate, acetate + hydrogen, EOS) and a sterile and unamended control. TCE and its degradation products were measured using GC-FID.

The data demonstrate that, of the five controls, lactate was able to facilitate the complete dechlorination of TCE in approximately 45 days. Lactate was the most effective electron donor of the controls and as a result each animal co-product was compared to it. Of the 38 animal co-products, four promoted the dechlorination of TCE to ethene faster than lactate (approximately 35 days) and three promoted the dechlorination of TCE in the same amount of time as lactate. One material, dissolved air flotation sludge (DAF),

dechlorinated TCE to ethene at a 1:1 stoichiometry faster than any commercially available lipid based electron donor (e.g. emulsified vegetable oil). The difference is that the co-product is available for pennies per pound, while commercial electron donors sell for dollars per pound in some cases, and cost is often the limiting factor in site closure.

Future research will include optimizing the concentration of the animal co-products. The goal of future research is to determine the lowest possible concentration of animal co-product that can be used to still achieve complete dechlorination. Future research will also include investigating the use of animal co-products as electron donors for the bioremediation of other contaminants.

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CHAPTER ONE

Introduction

Trichloroethylene (TCE) is a common groundwater pollutant. TCE is a chlorinated ethene that was primarily used as an industrial degreaser (Loffler, Ritalahti, & Zinder, 2013). It was widely used in the twentieth century. Its use began to decrease in the 1970s because of environmental concerns (Bakke et al., 2007). TCE and other chlorinated solvents are colorless, volatile liquids at room temperature. Chlorinated solvents are more dense than water and have a low solubility in water, making them dense non-aqueous phase liquids (DNAPLs). Their stability and widespread use caused chlorinated solvents to become common groundwater pollutants (Alvarez & Illman, 2006; Weatherill, et al., 2018).

Previous disposal methods for TCE included dumping it in the environment. It was thought that TCE would volatilize and then undergo photochemical degradation. Instead, TCE percolates through the ground and contaminates groundwater (Loffler, Ritalahti, & Zinder, 2013). It is estimated that 9% to 34% of the United States water supply is contaminated with TCE (Environmental Protection Agency, 1992).

TCE is a known human carcinogen (International Agency for Research on Cancer, 2014). It is known to cause liver cancer, kidney cancer, and non-Hodgkin's lymphoma (Agency for Toxic Substances and Disease Agency, 2007). Vinyl chloride (VC), a degradation product of TCE, is also a known human carcinogen. VC is known to cause liver angiosarcoma (Kielhorn et al., 2000).

TCE can be biologically degraded by the bacteria in the genus *Dehalococcoides*. An overview of the biodegradation of TCE is shown in Figure 1. The carbon-chlorine bond is reduced by a two electron transfer from *Dehalococcoides.* One chlorine atom is removed and replaced with a hydrogen atom to form cis-dichloroethene (cis-DCE). The reduction of a carbon-chlorine bond on cis-DCE forms vinyl chloride (VC). A final reductive dechlorination step transforms vinyl chloride into ethene (Jugder, et al., 2016). Typically, the degradation products of TCE become less toxic as *Dehalococcoides* removes chlorine atoms. The exception is vinyl chloride, which is more toxic than its parent molecule cis-DCE (Kielhorn et al., 2000).

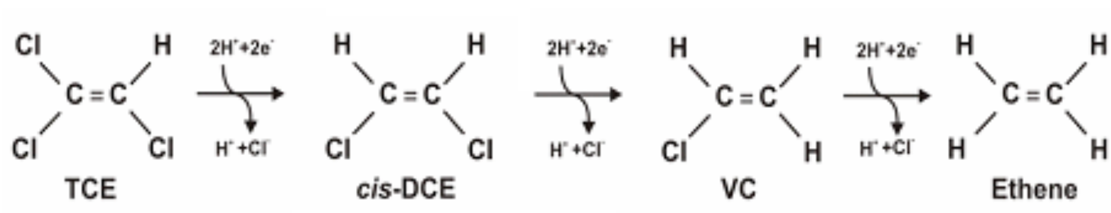

Figure 1: Reductive dechlorination of trichloroethylene

TCE and its degradation products are used as terminal electron acceptors by *Dehalococcoides*. Hydrogen is the sole electron donor for their metabolism. Dehalococcoides relies on fermenters being present to produce hydrogen. Low hydrogen concentrations are required for fermentation to be energetically favorable (Ter Meer et al., 1999). When bioremediation is being used to treat groundwater pollutants, an electron donor is injected into the subsurface. The electron donor is fermented by microorganisms

in the subsurface. Hydrogen is produced as a product of this fermentation process. Dehalococcoides are able to use this hydrogen as an electron donor.

Vitamin B12 is an enzymatic cofactor that facilitates the dechlorination of TCE. *Dehalococcoides* also require vitamin B12 for reductive dechlorination but are unable to produce it. Therefore, communities containing *Dehalococcoides* must contain other microbes that produce vitamin B12. A few examples of these communities are *Desulfovibrio desulfuricans* and *Acetobacterium woodii* (He et al., 2007).

The mechanism for reductive dechlorination is shown in Figure 2. This figure shows the reductive dechlorination of perchloroethylene (PCE) to TCE but the mechanism is the same for each chlorine atom. The reaction begins with a one-electron transfer from the vitamin B12 to PCE. Next a chloride atom is removed from PCE, forming a radical that combines with a hydrogen radical to yield TCE (Holliger et al., 1999). This reaction can only occur in an anoxic environment. If oxygen is present, the electrons on cobalt will transfer to oxygen instead of TCE.

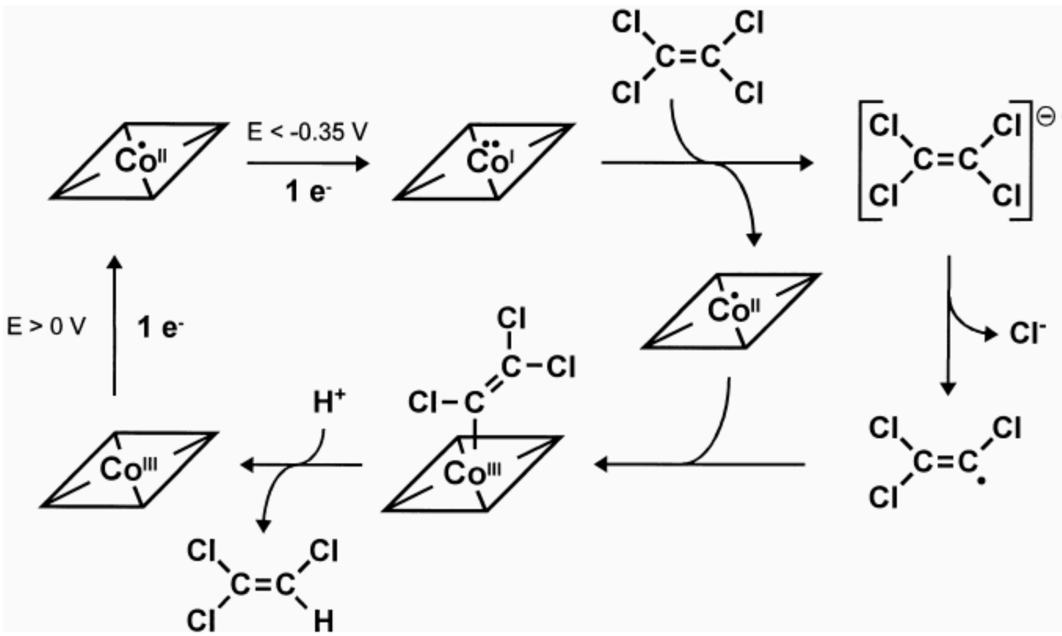

Figure 2: Mechanism for reductive dechlorination of PCE. CoI-III within the rhombus represents the corrinoid portion of vitamin B12 in its respective oxidation state (Holliger et al., 1999).

TCE is a common groundwater pollutant and as a result there are many remediation options to remove it from groundwater. An appropriate remediation method must be determined on a site by site basis. An abiotic method of removing TCE from groundwater uses a permeable reactive barrier (PRB). TCE can be reduced by a PRB that is made of iron (Vogan et al., 1999). Another common remediation practice is to provide contaminated sites with electron donors to stimulate the communities that provide *Dehalococcoides* with necessary nutrients. Commonly used electron donors are lactate, acetate, and emulsified vegetable oils like soybean oil. This method works under the assumption that there are microbes present to dechlorinate TCE. *Dehalococcoides* can be added to a site that does not have any microbes capable of dehalorespiration.

A remedial strategy that is becoming more popular recently is the use of slow release electron donors. These donors are advertised as being able to release a fermentable substrate such as lactate over the course of several years. One drawback of using common electron donors such lactate or acetate is that they are quickly consumed in the subsurface. Multiple amendments over time are often required to completely remediate a contaminated site. Slow release electron donors are able to remediate a site with a single amendment. Emulsified soybean oil is an example of a slow release electron donor.

The focus of this paper is to investigate alternative electron donors for TCE bioremediation. Animal co-products have the potential to serve as novel electron donors for bioremediation. These materials are what remains once cattle, chickens, and other animals are slaughtered and the food grade material is processed for consumption. Figure 3 provides an overview of the meat rendering process. First, animal parts are delivered from a slaughterhouse. These parts are prepared based on what they will be used to produce. Once they have been appropriately prepared, they will be cooked and sterilized. These two steps are an effort to prevent products becoming contaminated with pathogens. After sterilization, the products are then typically separated based on their protein and lipid content.

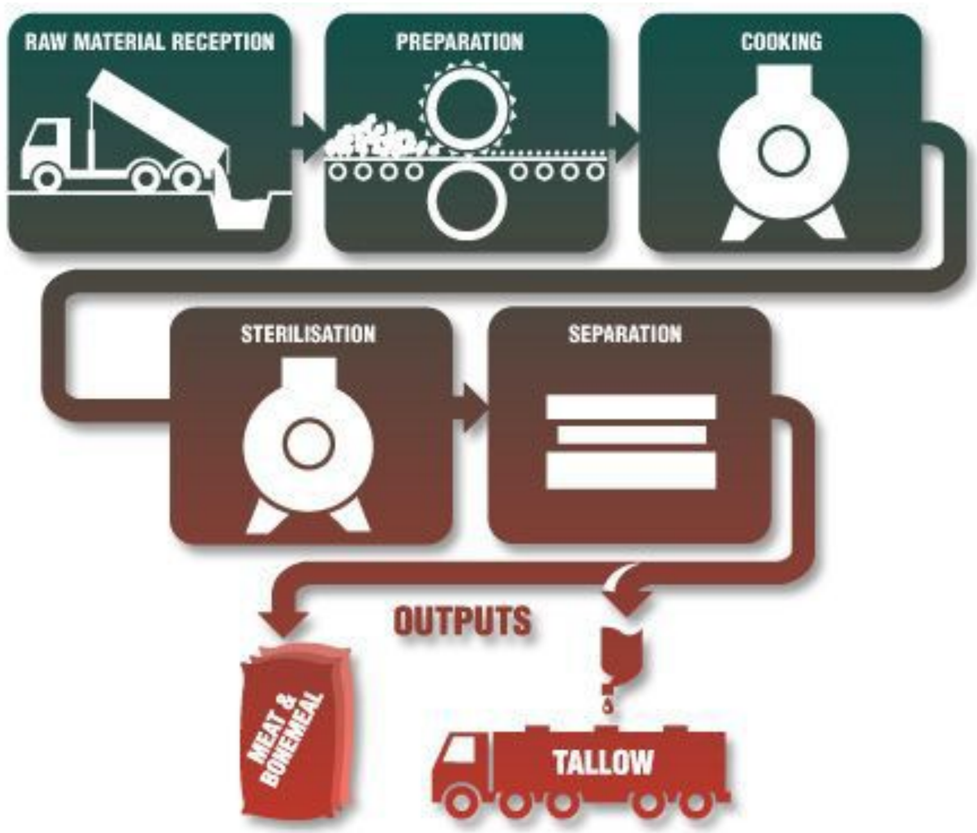

Figure 3: Overview of the animal rendering process (Ulster Farm Byproducts Ltd, 2016).

Historically, many of the animal co-products produced from the rendering process were used as feed for animals. However, there has been an increasing concern over animal co-products that have been contaminated with bovine spongiform encephalopathy (BSE), commonly referred to as mad cow disease. BSE is a prion based disease that is not removed by heat sterilization. Animal co-products that are contaminated with BSE can transmit the disease to animals that ingest the co-products. Therefore, there has been an effort to find alternative used for these animal co-products. These materials have high protein and lipid content, and these waste materials have the potential to be used as

electron donors for microbial processes. Animal waste co-products used as electron donors for bioremediation could be an inexpensive alternative for the industry, while eliminating a waste stream. Animal co-products as electron donors also have a lower potential of spreading BSE compared to using animal co-products as feed. Prions in the environment tend to sorb to sediment. This could pose a health risk if surface sediment becomes contaminated but in the subsurface prions should become relatively immobilized (Saunders et al., 2008).

Hypotheses

The purpose of this project is to determine whether or not animal co-products can be used as an electron donor for in situ bioremediation of TCE. With that in mind, the following hypotheses were made:

1) Animal co-products can facilitate TCE dechlorination.
2) Protein based animal co-products will facilitate TCE dechlorination faster than the lipid based animal co-products.

The first hypothesis is the more critical of the two. In order for animal co-products to be considered successful, they need to facilitate the complete dechlorination of TCE in a similar timeframe to traditionally used electron donors. If the first hypothesis is correct, then the second hypothesis is an important step in determining what makes an animal co-product an effective electron donor.

CHAPTER TWO

Materials and Methods

Sample Collection

Soil and sediment were collected at a site in South Carolina. The exact location of this site cannot be disclosed as it is currently being remediated. Groundwater at this site is contaminated with TCE. Samples were collected in a stream north of the source of the contamination. The stream was fed by groundwater below the source of contamination. Collected sediment and water were stored in an incubator at 30℃.

Electron Donors

Various animal co-products were provided by third party companies. Each co-product and the company that provided it can be found in Table 1. Each co-product was stored on the benchtop.

Table 1: List of each animal co-product provided and the company that provided it. The column "Sample Given Name" refers to the name provided by the supplier and the column "Sample" refers to an abridged name given for convenience.

Sample Given Name	Sample	Supplier
All Beef MBM	All Beef MBM	Darling Ingredients
Blood Meal Jackson	Blood Meal Jackson	Darling Ingredients
Blood Meal Newberry	Blood Meal Newberry	Darling Ingredients
Bone Residue	Bone Residue	Darling Ingredients
Brown Grease Tank D-3	Brown Grease	Baker Commodities Inc.
Centrysis Solids	Centrysis	Baker Commodities Inc.
Chicken Meal	Chicken Meal	Darling Ingredients
Choice White Grease	CWG	Wintzer & Son Co.
Cookie Meal	Cookie Meal	Darling Ingredients
DAF (Water Plant)	DAF	Baker Commodities Inc.

DARPRO 58	DARPRO 58	Darling Ingredients
Feather Meal	Feather Meal (Darling)	Darling Ingredients
Feed GR PM	Feed GR PM	Darling Ingredients
Fines	Fines	Darling Ingredients
Frass	Frass	Darling Ingredients
GrifPet	Grifpet	Darling Ingredients
Kerman MBM	K-MBM	Baker Commodities Inc.
Kerman Tallow Tank F-2	Tallow	Baker Commodities Inc.
Low Pro Meat and Bone Meal #1	Lo Pro 1	Central BiProducts
Low Pro Meat and Bone Meal #2	Lo Pro 2	Central BiProducts
Low Pro Meat and Bone Meal #3	Lo Pro 3	Central BiProducts
Low Pro Meat and Bone Meal #4	Lo Pro 4	Central BiProducts
MBM	MBM	Darling Ingredients
PL, Rendering Plant	PL	Darling Ingredients
PNG (Poultry Nutrient Grease)	PNG	Darling Ingredients
PNM, Poultry Nutrient Meal	PNM	Darling Ingredients
Poultry Byproduct Meal with Ethoxyquin	PBM+E	Darling Ingredients
Poultry Meal	Poultry Meal	Wintzer & Son Co.
True Energy 80/20 blend	TE-80/20	Wintzer & Son Co.
True Energy Formula A	TEFA	Wintzer & Son Co.
True Energy Hog Blend	TEHB	Wintzer & Son Co.
True Energy Vegetable Blend	TEVB	Wintzer & Son Co.
Turkey Meal	Turkey Meal	Darling Ingredients
WAPAK Feather Meal "Hydrolyzed Poultry Feathers"	Feather Meal	Wintzer & Son Co.
"WAPAK" Meat and Bone Meal #5	Wapak Bone Meal	Wintzer & Son Co.
WAPAK Poultry By-Products Meal	Poultry Byproducts	Wintzer & Son Co.

Batch Setup

The batch experiments to measure TCE degradation were set up in 160 mL serum bottles. Each bottle contained 50 g of saturated sediment and an additional 20 mL of water. Once each bottle contained sediment and water, electron donors were added. Each animal co-product was added to achieve a final concentration of 1 g/L of donor in each bottle. Experiments containing co-products were set up in triplicate. The exception to this is the animal co-products from Darling Ingredients. These experiments have one replicate because time on the shared GC-FID in the lab was limited. The serum bottles were sealed with a butyl stopper and the headspace was flushed with nitrogen for six minutes to remove any oxygen present. Approximately 20 µmol of TCE was added to each bottle. The batches were allowed to sit for 48 hours to allow the TCE to reach an equilibrium. After 48 hours an initial measurement was made with a GC-FID. Subsequent measurements were made weekly.

Controls were set up in the same manner as the experimental bottles. Lactate, acetate with hydrogen, and emulsified soybean oil were used as electron donors. The emulsified soybean oil was in the form of Emulsified Oil Substrate (EOS) from EOS Remediation. An unamended control was created with just sediment and water to determine if any degradation would occur without donor. A sterile control was also created to determine if any abiotic processes in this sediment were degrading TCE. The sterile controls were autoclaved three times over three days to kill any microbes capable of forming spores. Each control has six separate bottles.

Chlorinated Solvent Measurement

Trichloroethylene, cis-dichloroethylene, vinyl chloride, ethene, and methane concentrations were measured with a Shimadzu 2014 Gas Chromatograph equipped with a flame ionization detector. A 30 m GS-Q column was used to separate the gases present in the headspace of each batch incubation. The temperature program started at 40℃ for 1.5 minutes and then increased at a rate of 40℃/min for 4 minutes and was held at 200℃ for 2 minutes. A Pressure-Lok syringe was used to inject 0.2 mL of the headspace at atmospheric pressure into the GC-FID.

Interpretation of Data

The efficacy of an animal co-product was determined by how quickly the co-product was able to facilitate the complete dechlorination of TCE. An experiment was considered to be complete dechlorinated when the concentration of VC dropped to zero and there was a rise in ethene concentration. Experiments needed to show a rise in the concentration of VC as the concentration of cis-DCE decreased and then a decrease in VC concentration as the concentration of ethene increased. An experiment could not be considered completely dechlorinated if any chlorinated ethenes were detected.

CHAPTER THREE

Results and Discussion

Controls

The results for the five controls can be seen in Table 2. The data for each control are available in Appendix A. Lactate was the most effective electron donor for the controls. Complete dechlorination of TCE was observed in the lactate controls at 45 days. EOS was the second most effective electron donor. Complete dechlorination was observed in the EOS controls at 78 days. Acetate with hydrogen facilitated the complete dechlorination of TCE at 91 days. Dechlorination was not observed in the sterile and unamended controls.

Table 2**:** Summary of results from controls. Methane concentrations are the final concentration when the batches were discontinued (approximately three months after the batch was made).

Sample Name	Days to Complete Dechlorination	Methane Produced (µmol/bottle)
10 mM Acetate + 15 psi H_2	91	291
1 g/L EOS	78	822
20 mM Lactate	45	283
Sterile	n/a	0.89
Unamended	n/a	8.03

n/a – batch was not able to completely dechlorinate TCE

Animal Co-Product Experiments

The results for the animal co-products can be seen in Table 3. The data for each co-product is available in Appendix B. The time to dechlorination for each animal co-

product was compared to the time to dechlorination for each control. Of the 38 animal co-products that were tested, four were able to facilitate the dechlorination of TCE in 35 days, 10 days faster than the lactate controls. An additional three animal co-products were able to facilitate dechlorination in 45 days, the same time in which lactate promoted complete dechlorination. There were 12 animal co-products that promoted the dechlorination of TCE in 78 days, the same amount of time it took EOS to promote complete dechlorination. Only one animal co-product facilitated dechlorination in 91 days, the same time it took the acetate with hydrogen control to dechlorinate TCE. Complete dechlorination was not observed in the remaining 18 animal co-products.

Table 3: Summary of results from experiments. Methane concentrations are the final concentration when the batches were discontinued (approximately three months after the batch was made).

Sample Name	Days to Complete Dechlorination	Methane Produced (μmol/bottle)
All Beef MBM	n/a	590
Blood Meal Jackson	n/a	107
Blood Meal Newberry	n/a	141
Bone Residue	n/a	3.47
Brown Grease	78	141
Centrysis	n/a	226
Chicken Meal	n/a	107
Cookie Meal	n/a	1614
CWG	79	385
DAF	45	260
DARPRO 58	n/a	116
Feather Meal	35	781
Feather Meal (Darling)	n/a	98
Feed GR PM	n/a	1232
Fines	n/a	42

Frass	n/a	443
GrifPet	n/a	243
K-MBM	45	323
Lo Pro 1	78	276
Lo Pro 2	78	372
Lo Pro 3	91	28
Lo Pro 4	78	453
MBM	n/a	1305
PBM+E	79	324
PL	79	2076
PNG	79	364
PNM (Darling)	n/a	152
PNM	78	268
Poultry Byproducts	78	288
PoultryBy (Darling)	n/a	319
Poultry Meal	n/a	122
Tallow	45	674
TE 80/20	35	358
TEFA	35	1455
TEHB	79	440
TEVB	35	22
Turkey Meal	n/a	112
WAPAK Bone Meal	79	673

n/a – batch was not able to completely dechlorinate TCE

Discussion

The goal of these experiments was to determine if animal co-products can serve as effective electron donors for in situ TCE bioremediation. Animal co-products needed to remove TCE in a comparable timeframe to traditional electron donors. Of the 38 animal co-products, 20 were able to dechlorinate TCE in a similar timeframe to the controls. Each experiment was compared to the lactate control and the EOS control. Soybean oil is currently a commonly used electron donor so even though EOS was not the fastest control, each co-product was compared to EOS to determine an affordable alternative. Most of the co-products tested are waste products from meat rendering. They can serve as a low cost alternative electron donor compared to soybean oil or lactate while also eliminating the amount of waste sent to a landfill.

To demonstrate that animal co-products can serve as a low cost alternative to traditional electron donors, here is an example of a permeable reactive barrier (PRB) containing soybean oil and animal co-products. For this example, assume a PRB that is 50 m long, 3 m wide, and 10 m. This PRB will have a total volume of 1500 m^3. A PRB with this volume would require approximately 1.5 million kg of material to backfill. If 10% of the backfill material is electron donor, this PRB will require 150,000 kg (approximately 165 tons) of electron donor. Soybean oil electron donors can cost between \$0.25 to \$3.00 per pound. With this price range, the electron donor required for this PRB would cost somewhere between \$82,500 and \$990,000. Animal co-products can cost between \$0.30 to \$15.00 per ton. The cost associated with animal co-products as electron donors in this PRB is between \$49.50 to \$2475. The price for the animal co-

products in this example is the price without a markup. The price could be increased to $100 per ton and animal co-products would still be a more cost effective alternative to soybean oil.

The amount of methane produced from bacteria should be addressed when selecting an animal co-product to use as an electron donor. Methane production from remedial actions can have several negative consequences. Methane in the subsurface can cause a vapor intrusion problem in nearby buildings. Large concentrations of methane can also be an explosion hazard, which could be a hazard for workers using injection/monitoring wells onsite as well as a hazard for buildings where vapor intrusion has become an issue. Methane is also a potent greenhouse gas. Its release into the environment can have an impact on global climate change. The amount of methane produced in each experiment is shown in Table 3.

Several experiments actually showed an increase in TCE before a loss of TCE. The data for the animal co-product Blood Meal Jackson in Figure B-3 is an example of this incident. A possible explanation for this initial rise in TCE is that when TCE was added to the experiment, it may have initially adsorbed to natural organic matter in the sediment. Bacteria present in the sediment could have consumed the natural organic matter present, causing TCE to partition into the water phase more to reach a new equilibrium. TCE and its degradation products were measured in the gas phase. Therefore, TCE adsorbed to a solid phase would not be detected by the GC-FID.

Table 3 shows 12 co-products that facilitated the dechlorination of TCE in 78 or 79 days. These animal co-products were: Brown Grease, Lo Pro 1, Lo Pro 2, Lo Pro 4,

PNM, Poultry Byproducts, CWG, PBM+E, PL, PNG, TEHB, and WAPAK Bone Meal. These 12 animal co-products were reported to promote dechlorination in 78/79 days because the GC-FID used to analyze TCE and its degradation products was out of order for a month. The time reported for these animal co-products was the first data point that could be collected once the GC-FID was repaired. It is possible that some of these animal co-products facilitated faster TCE removal. Future research should include additional batches with these animal co-products to determine if degradation did occur more quickly than reported.

The animal co-products from Darling Ingredients were set up as single bottles. The other experiments containing animal co-products were set up in triplicate. The reason that the co-products from Darling Ingredients were set up as single bottles is because they were received several months into this project. The GC-FID used to analyze experiments was shared among several students and at the time there was not enough time to analyze triplicates of the Darling Ingredients experiments. If any of the experiments containing animal co-products from Darling Ingredients showed promising results they would have been remade into triplicates. However, the animal co-products provided by Darling Ingredients were not able to facilitate the complete dechlorination of TCE. Each experiment with co-products from Darling Ingredients stalled at cis-DCE. Their products may contain some additive that inhibits *Dehalococcoides* such as antibiotics. Penicillin and the tetracyclines are commonly used antibiotics in livestock production (Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds, 1980). However, *Dehalococcoides.* have been shown to be resistant to penicillin (Loffler,

Sanford, & Ritalahti, 2005). The dechlorination of TCE to cis-DCE can be carried out by several different bacteria, not just *Dehalococcoides*. It is possible that TCE was converted to cis-DCE by a microorganism besides Dehalococcoidses. Further information about Darling Ingredients' meat rendering process is necessary to determine why these co-products caused TCE degradation to stall at cis-DCE.

It was thought that the protein based animal co-products would promote the dechlorination of TCE quicker than the lipid based co-products. Proteins are able to be broken down by bacteria quicker than lipids (Hanaki, 1981). Table 4 shows each electron donor that was able to dechlorinate TCE. It also shows the percent composition of protein and lipid for each donor. The composition of each co-product was provided by the company that produces the co-product. There are a few problems with drawing conclusions based on the data in Table 4. To start, the compositional data in Table 4 that was provided by the vendors is incomplete. The total percent composition for each co-product does not add up to 100%. In several cases, the total percent composition that was reported is less than 50% of the co-product. Also, the 12 animal co-products that were reported to have promoted dechlorination in 78 or 79 days prevent conclusions from being drawn from this data. A more accurate result for the time required to facilitate complete dechlorination would be required to draw a conclusion. Therefore, no conclusions were made on a co-products ability to promote complete dechlorination and its composition.

Table 4: Percent composition of animal co-products that were able to completely dechlorinate TCE. Note that these percent composition values were provided by the animal co-product vendors and therefore do not always add up to 100%.

Sample Name	Percent Fat	Percent Protein	Total Percent Content	Days to Dechlorinate
TEVB	90	0	90	35
TEFA	90	0	90	35
TE8020	90	0	90	35
Feather Meal	4	86	90	35
Tallow	38	0	38	45
DAF	0	0	0	45
K-MBM	15.04	46.4	61.44	45
PNM	3.96	61.63	65.59	78
Brown Grease	61.8	0	61.8	78
Poultry By-Products	10.5	63	73.5	78
Lo Pro 1	0	46.49	46.49	78
Lo Pro 2	0	43.38	43.38	78
Lo Pro 4	0	45.85	45.85	78
PNG	63.5	0	63.5	79
PL	70.37	0	70.37	79
CWG	92.5	0	92.5	79
TEHB	90	0	90	79
Wapak bone meal	10	57	67	79
PBM + E	0	0	0	79
Lo Pro 3	0	44.12	44.12	91

CHAPTER Five

Conclusions

As an electron donor for TCE bioremediation, animal co-products were an effective alternative to commonly used electron donors such as lactate and soybean oil. Several of the co-products were able to completely dechlorinate TCE in similar or faster times compared to common electron donors. These novel electron donors can serve as a cost effective alternative to commercially available donors. Most of these co-products are waste products so their use as electron donors allow for an economical treatment method while also removing a waste stream from the environment.

There are several areas of future research that can stem from this research. One area of research that needs to be addressed is determining the optimal concentration of animal co-products for bioremediation. Determining whether or not a lower concentration of animal co-product can be used to promote dechlorination could result in a less wasteful, more cost effective remediation strategy. Another topic that should be investigated is the 12 animal co-products that were reported to promote complete dechlorination in 78 or 79 days. It is possible that these co-products were able to promote faster dechlorination. They should be tested again to determine a more accurate dechlorination time. A second topic for future research is why the animal co-products from Darling Ingredients were not able to promote complete dechlorination. Darling Ingredients produces several animal co-products that are similar to co-products that were able to successfully facilitate dechlorination. The reason why these co-products were not able to facilitate dechlorination should be understood before animal co-products are sold as alternative electron donors for bioremediation. It should also be investigated whether

or not animal co-products can be used as an electron donor for the bioremediation of other contaminants. It is possible that animal co-products can facilitate the bioremediation of chromium or explosives in groundwater.

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APPENDICES

Appendix A

TCE Control Data

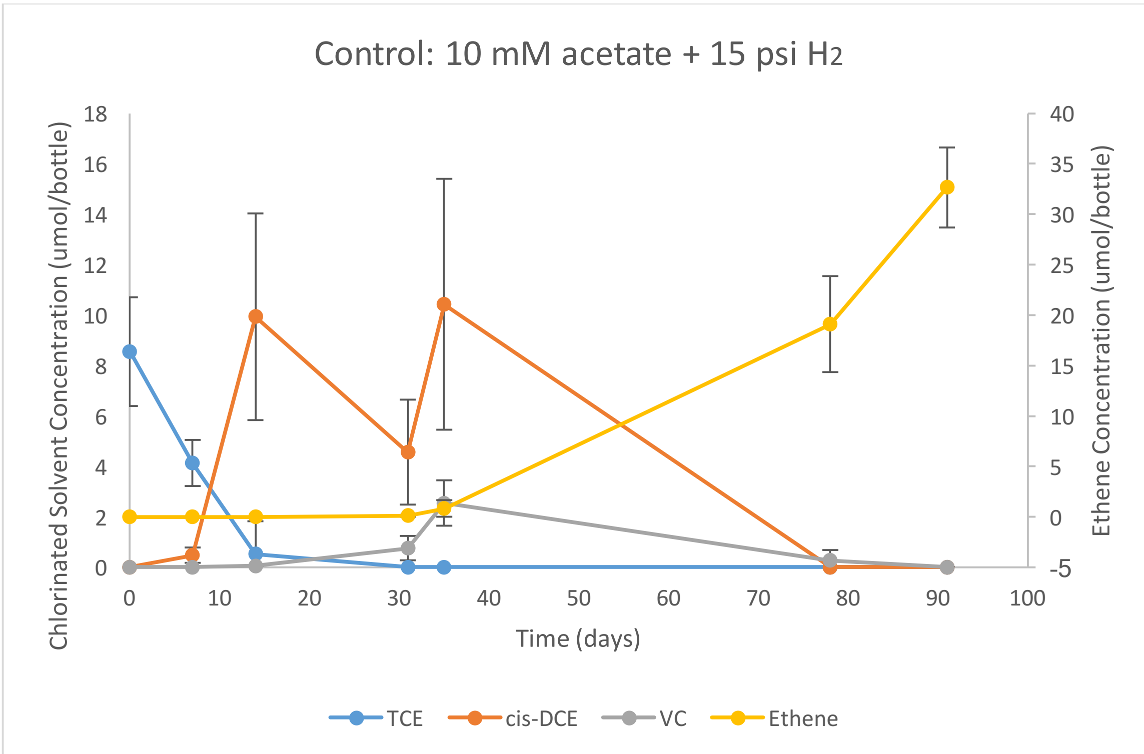

Figure A-1: TCE control with acetate and hydrogen as electron donors. Concentration of TCE and its degradation products over time. Values are the average of 6 bottles. Error bars represent one standard deviation.

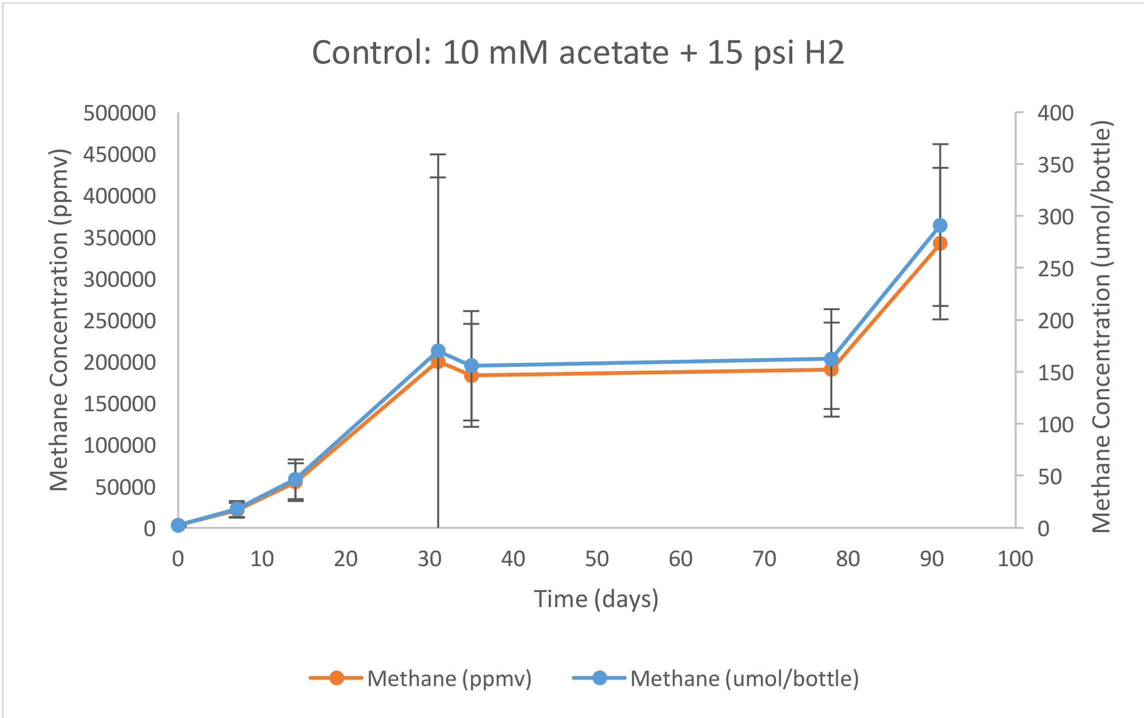

Figure A-2: TCE control with acetate and hydrogen as electron donors. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 6 bottles. Error bars represent one standard deviation.

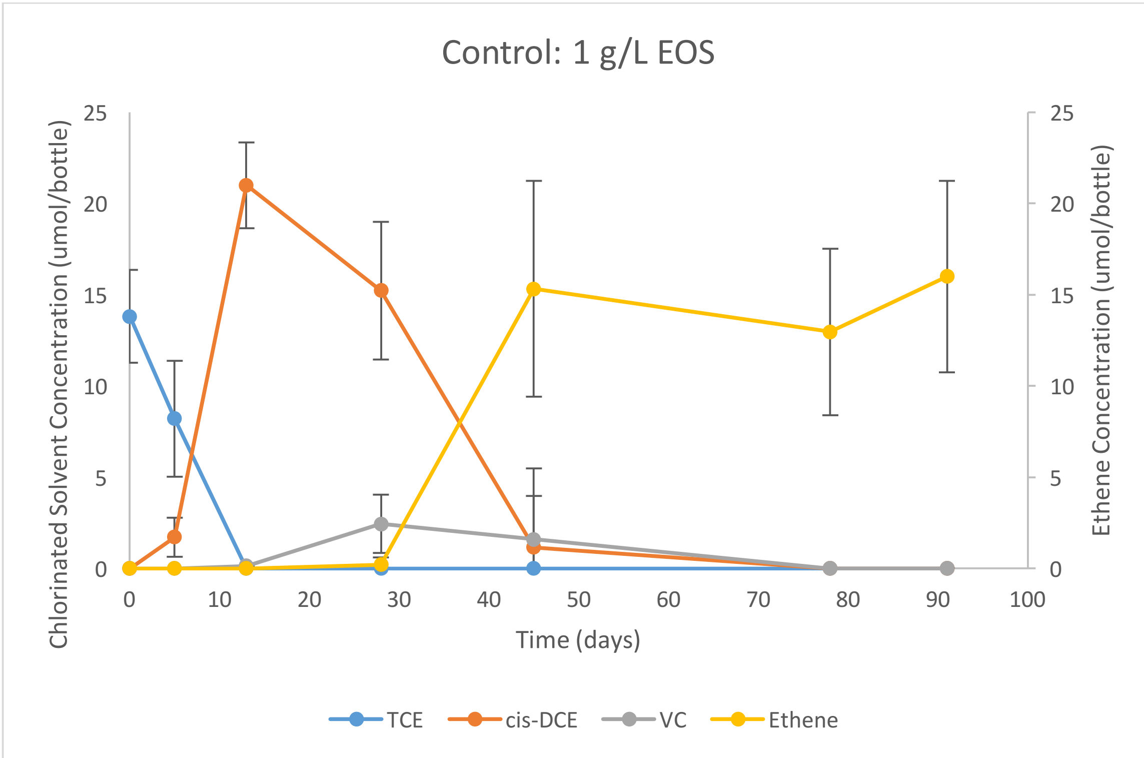

Figure A-3: TCE control with EOS as electron donors. Concentration of TCE and its degradation products over time. Values are the average of 6 bottles. Error bars represent one standard deviation.

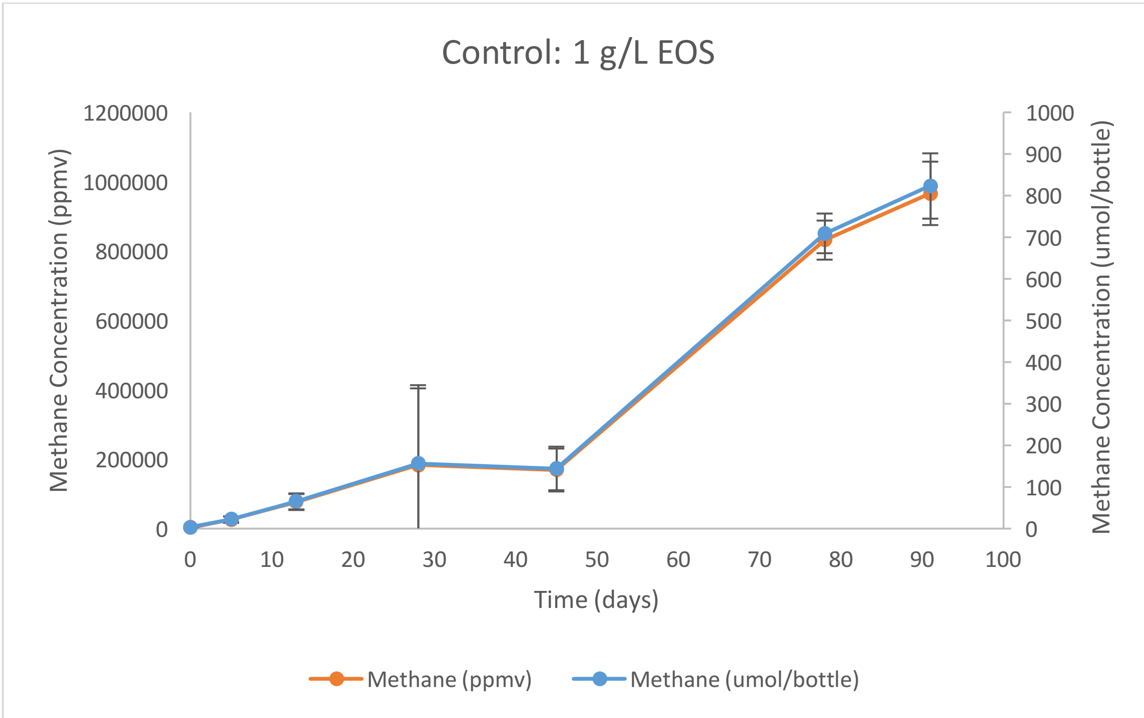

Figure A-4: TCE control with EOS as electron donors . Concentration of methane in μmol/bottle and parts per million by volume. Values are the average of 6 bottles. Error bars represent one standard deviation.

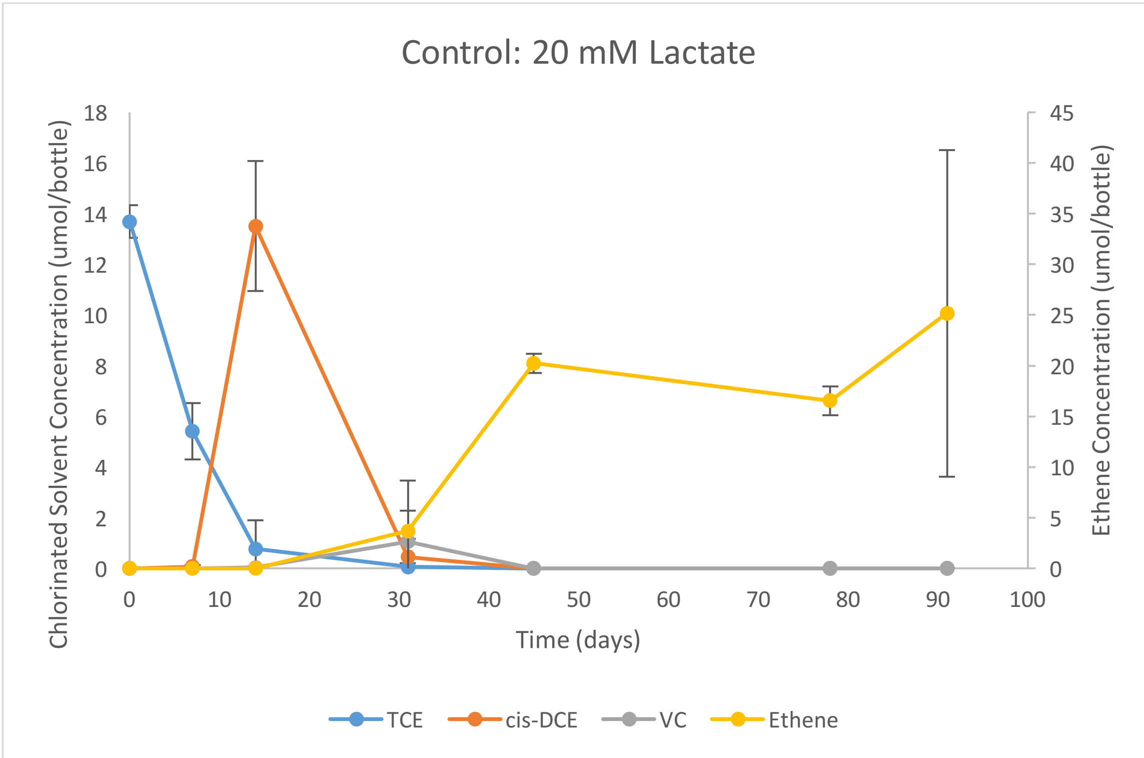

Figure A-5: TCE control with lactate as electron donors. Concentration of TCE and its degradation products over time. Values are the average of 6 bottles. Error bars represent one standard deviation.

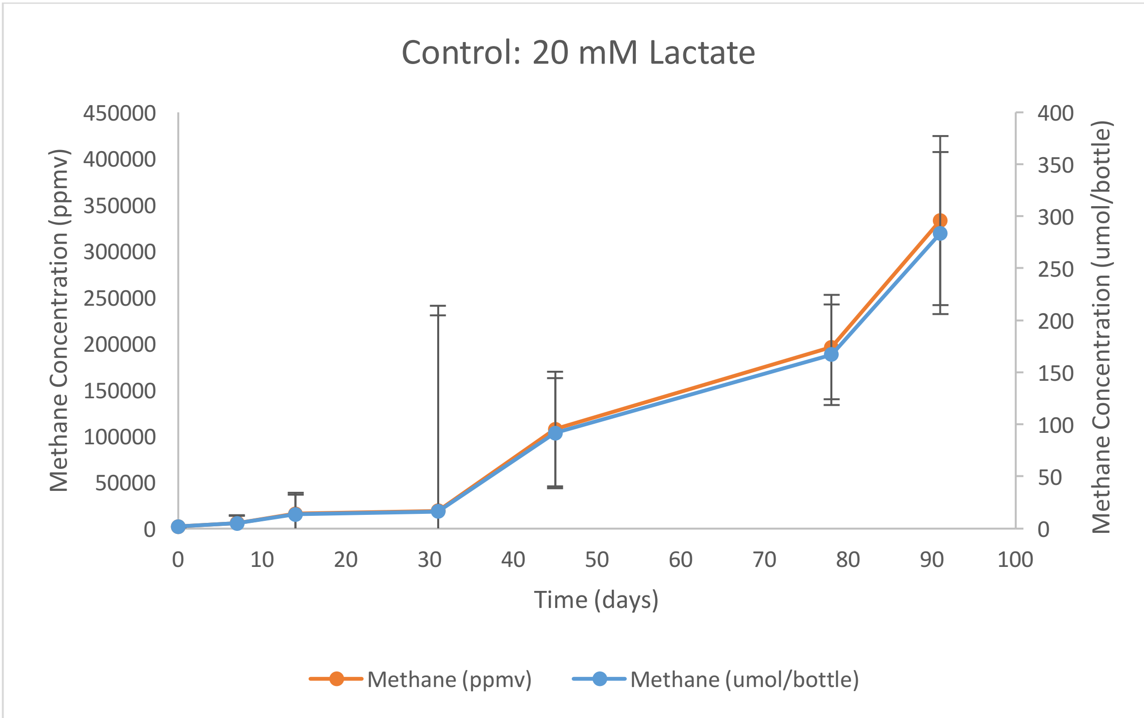

Figure A-6: TCE control with lactate as electron donors. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 6 bottles. Error bars represent one standard deviation.

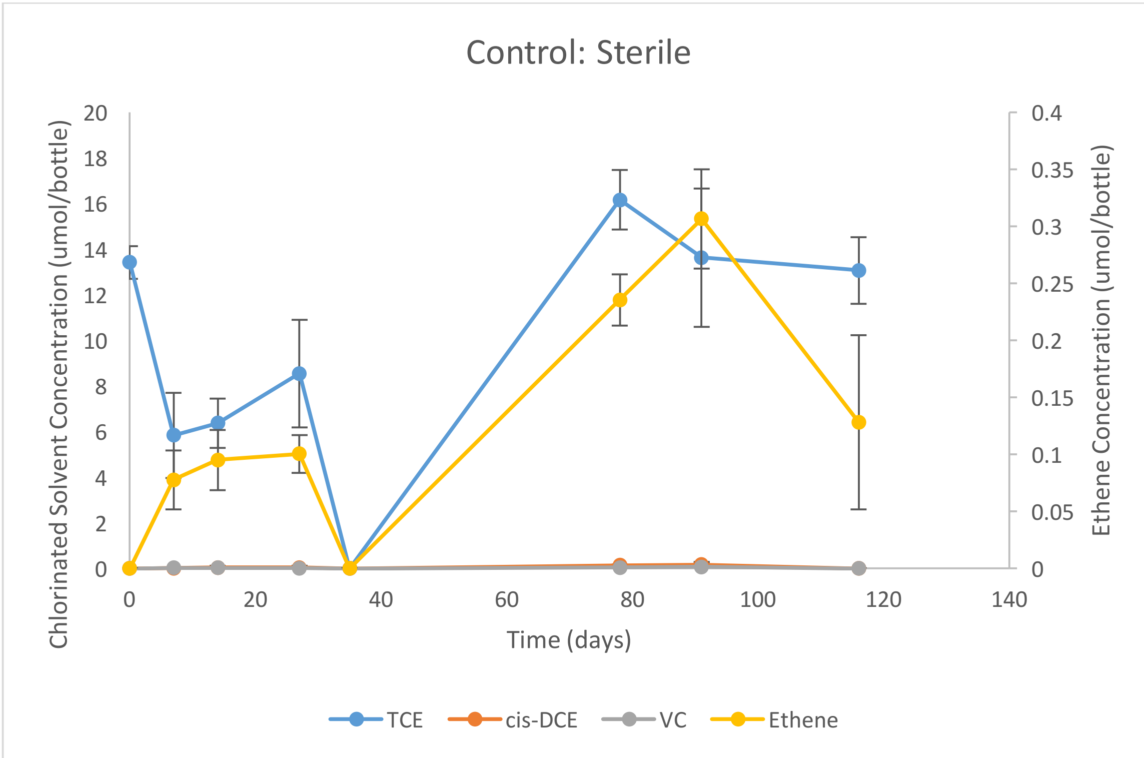

Figure A-7: Sterilized TCE control with no electron donor. Concentration of TCE and its degradation products over time. Values are the average of 6 bottles. Error bars represent one standard deviation.

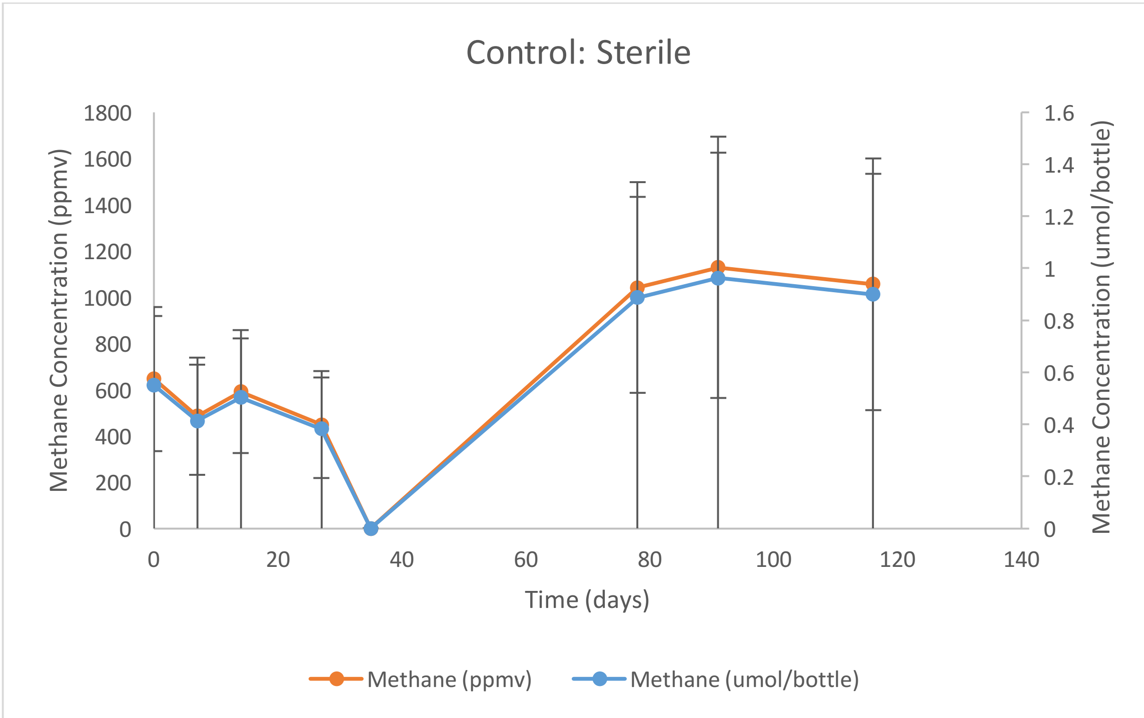

Figure A-8: Sterilized TCE control with no electron donor. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 6 bottles. Error bars represent one standard deviation.

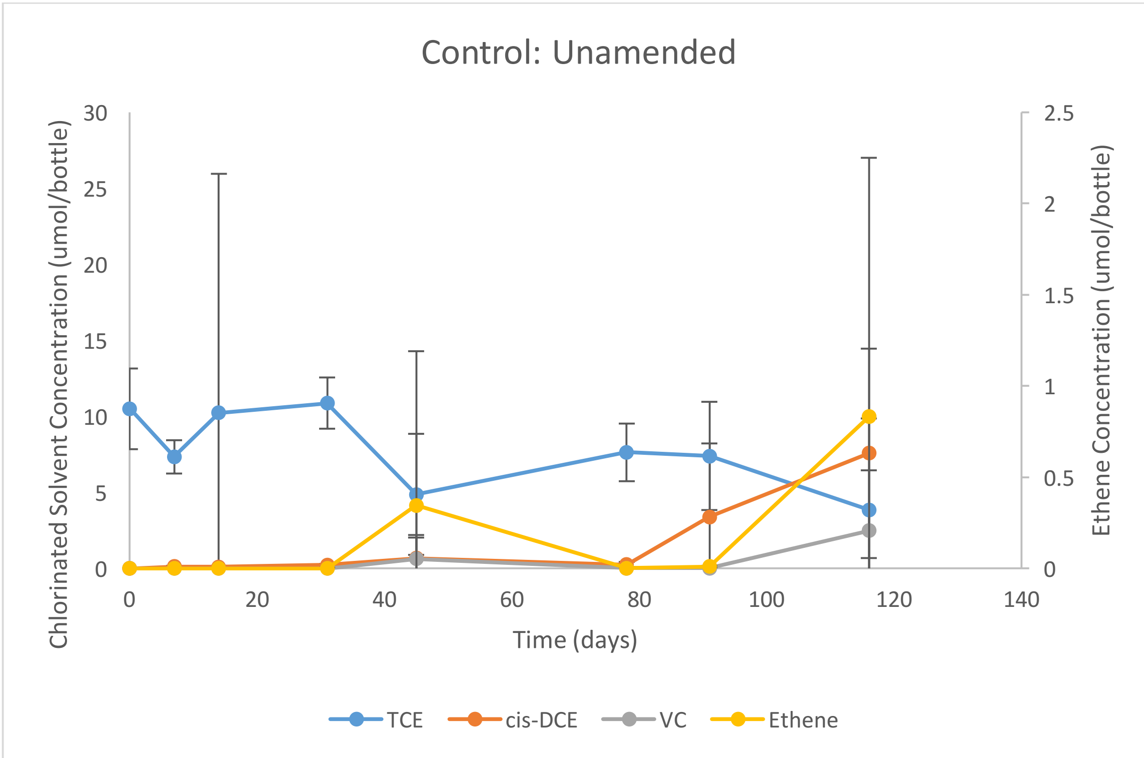

Figure A-9: TCE control with no electron donor. Concentration of TCE and its degradation products over time. Values are the average of 6 bottles. Error bars represent one standard deviation.

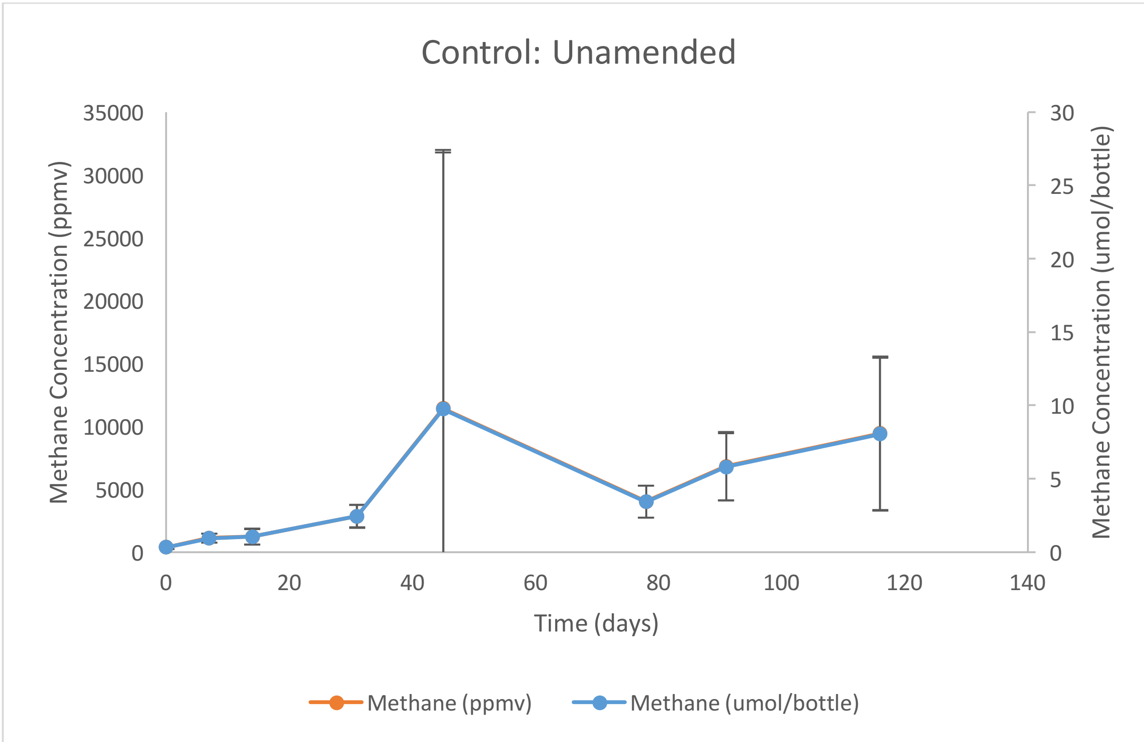

Figure A-10: TCE control with no electron donor. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 6 bottles. Error bars represent one standard deviation.

<u>Appendix B</u>

<u>TCE Experimental Data</u>

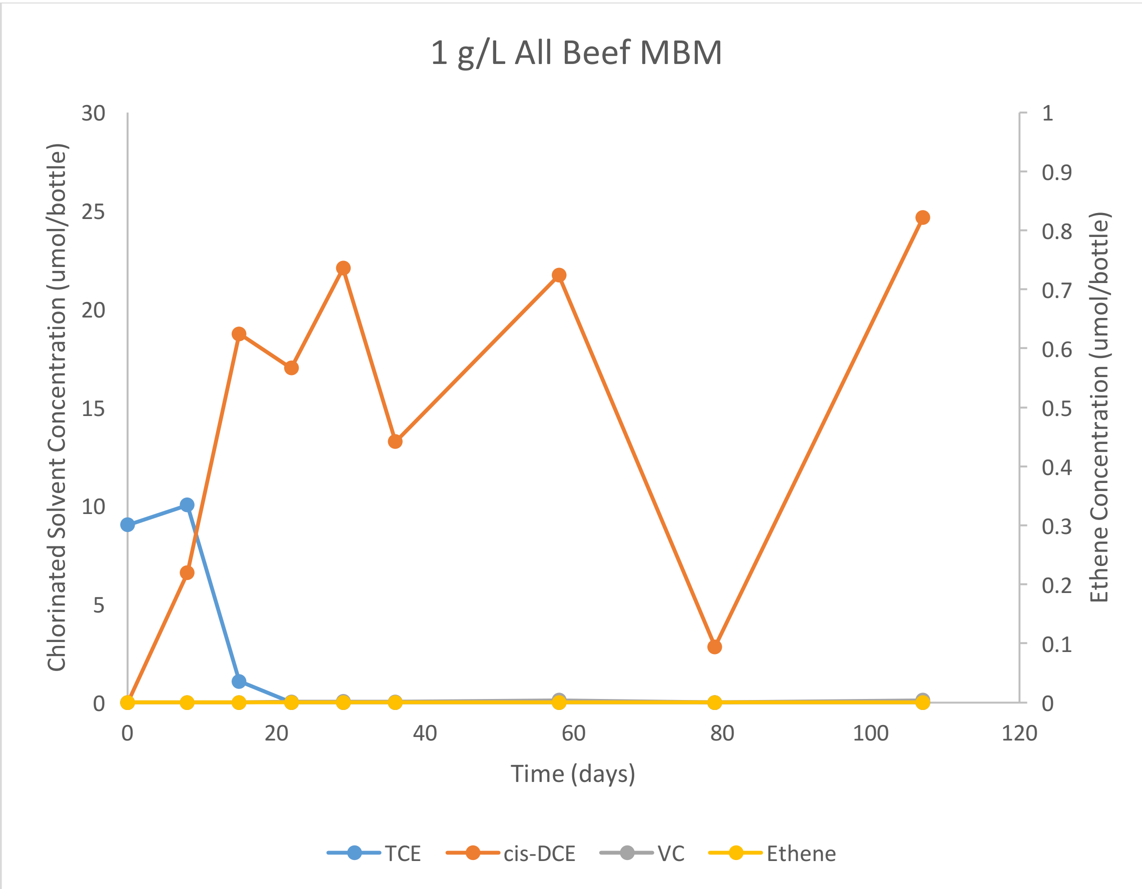

Figure B-1: TCE experiment amended with All Beef MBM. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

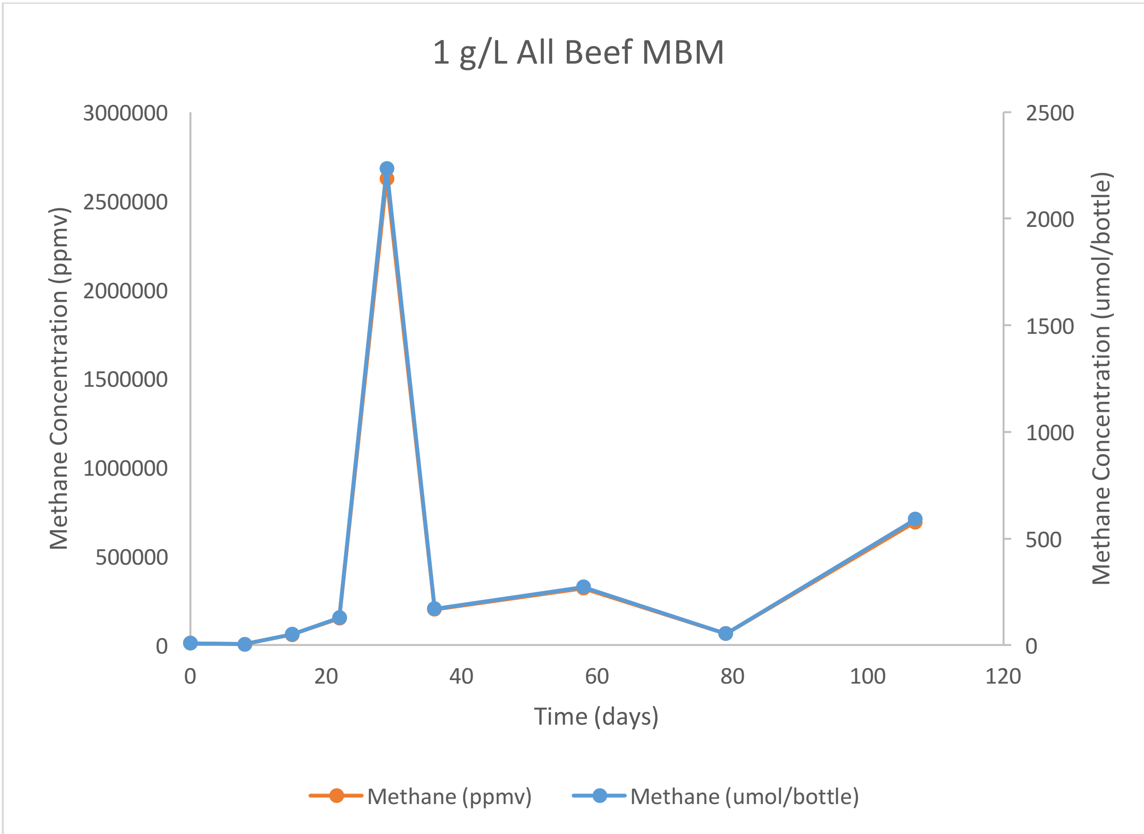

Figure B-2: TCE experiment amended with All Beef MBM. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

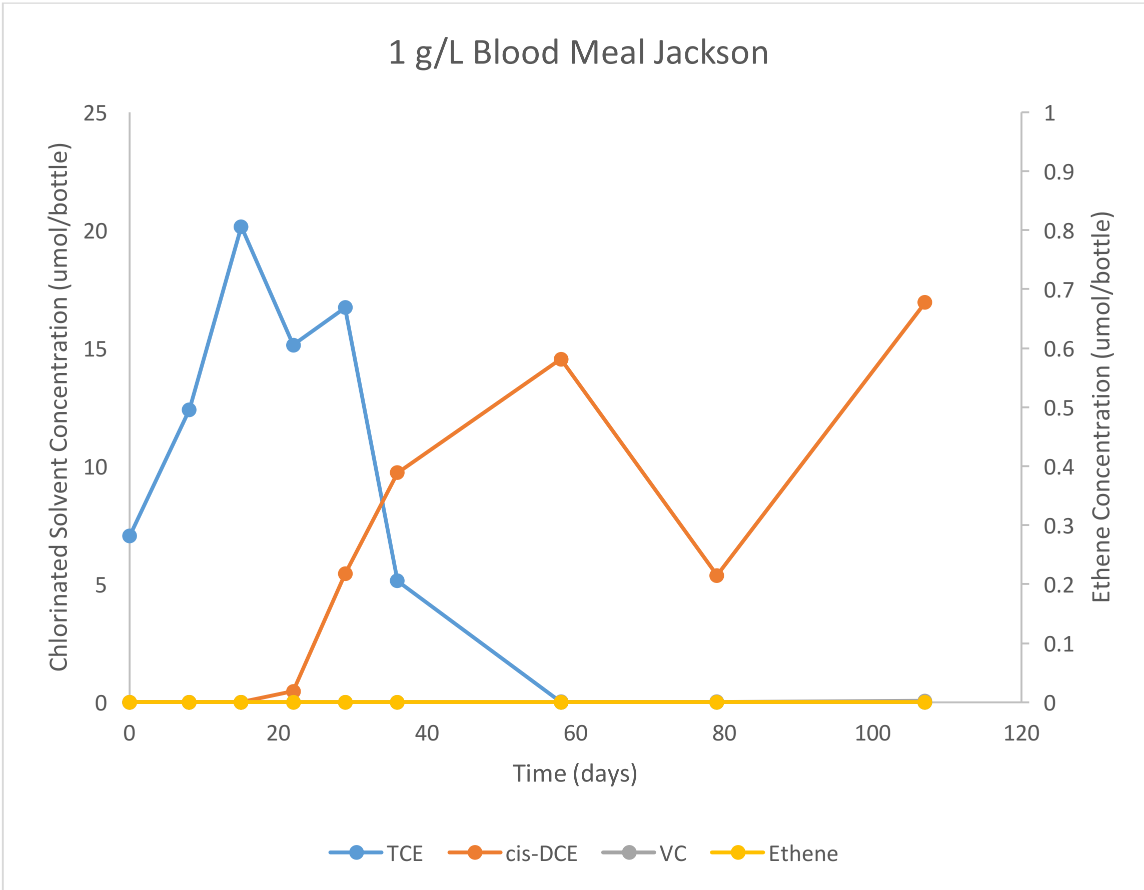

Figure B-3: TCE experiment amended with Blood Meal Jackson. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

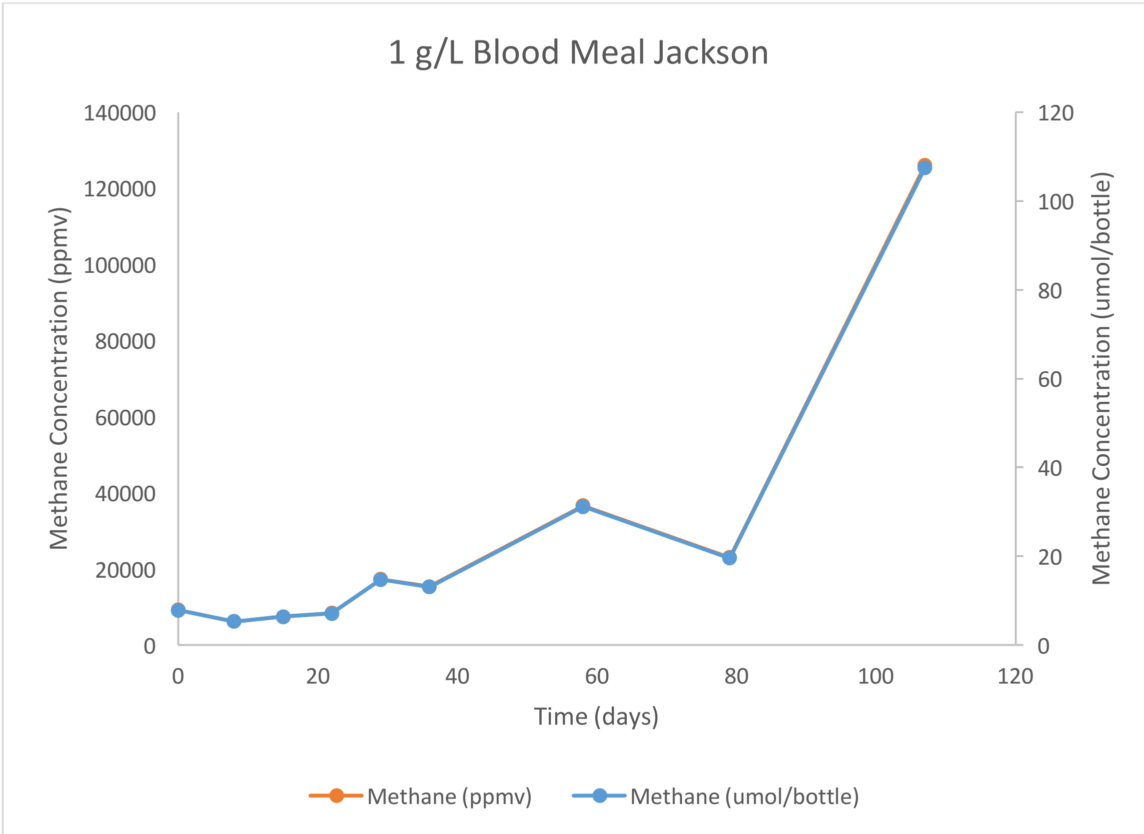

Figure B-4: TCE experiment amended with Blood Meal Jackson. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

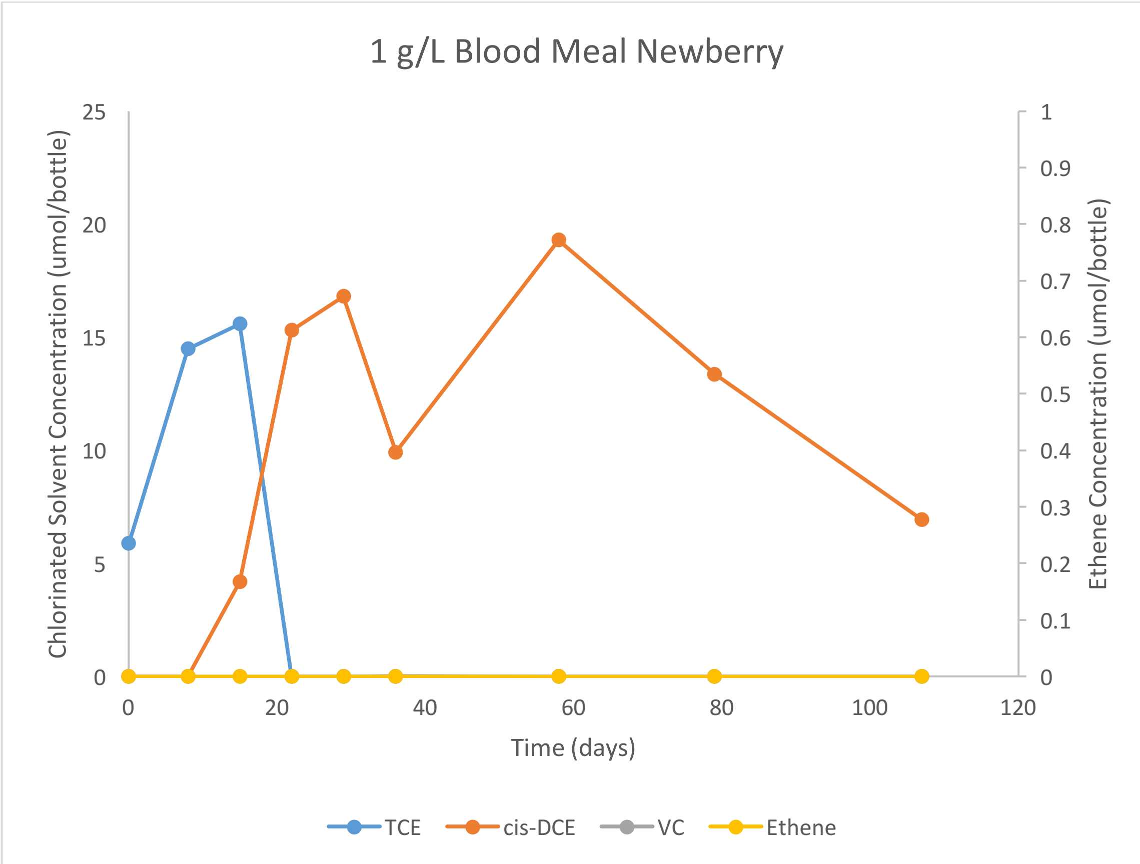

Figure B-5: TCE experiment amended with Blood Meal Newberry. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

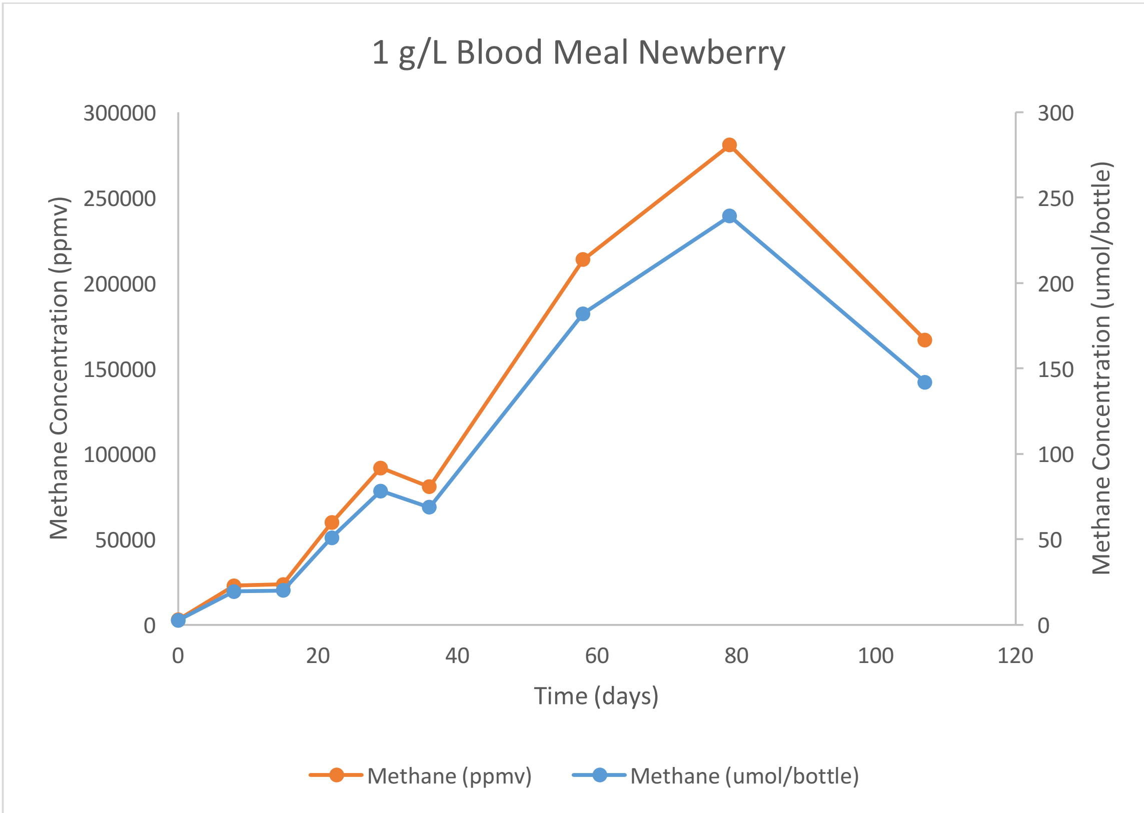

Figure B-6: TCE experiment amended with Blood Meal Newberry. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

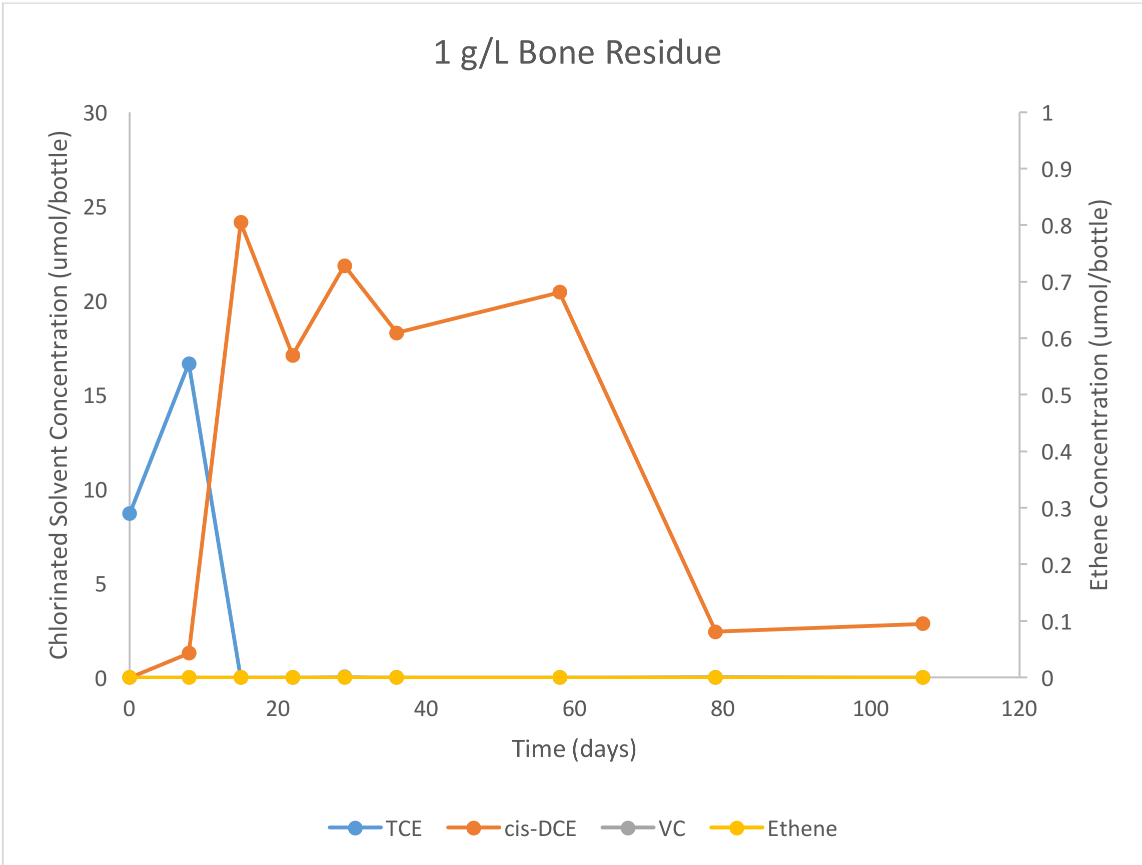

Figure B-7: TCE experiment amended with Bone Residue. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

Figure B-8: TCE experiment amended with Bone Residue. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

Figure B-9: TCE experiment amended with Brown Grease. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

Figure B-10: TCE experiment amended with Brown Grease. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

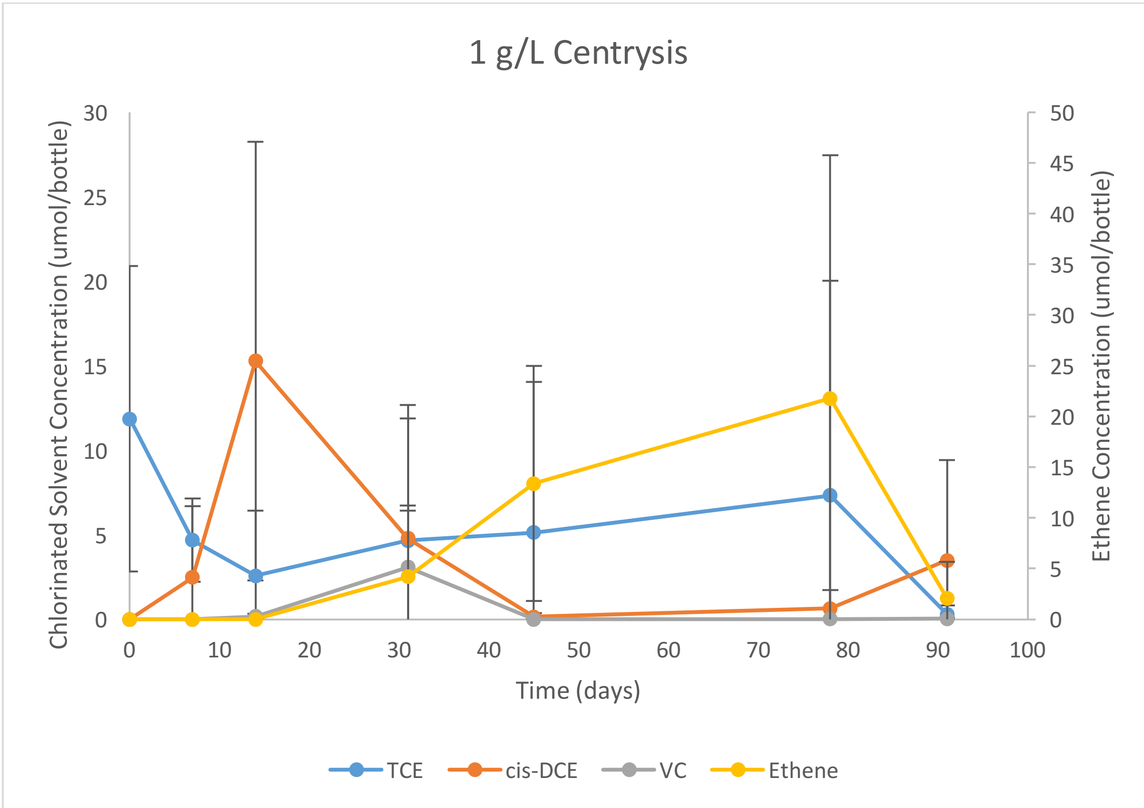

Figure B-11: TCE experiment amended with Centrysis. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

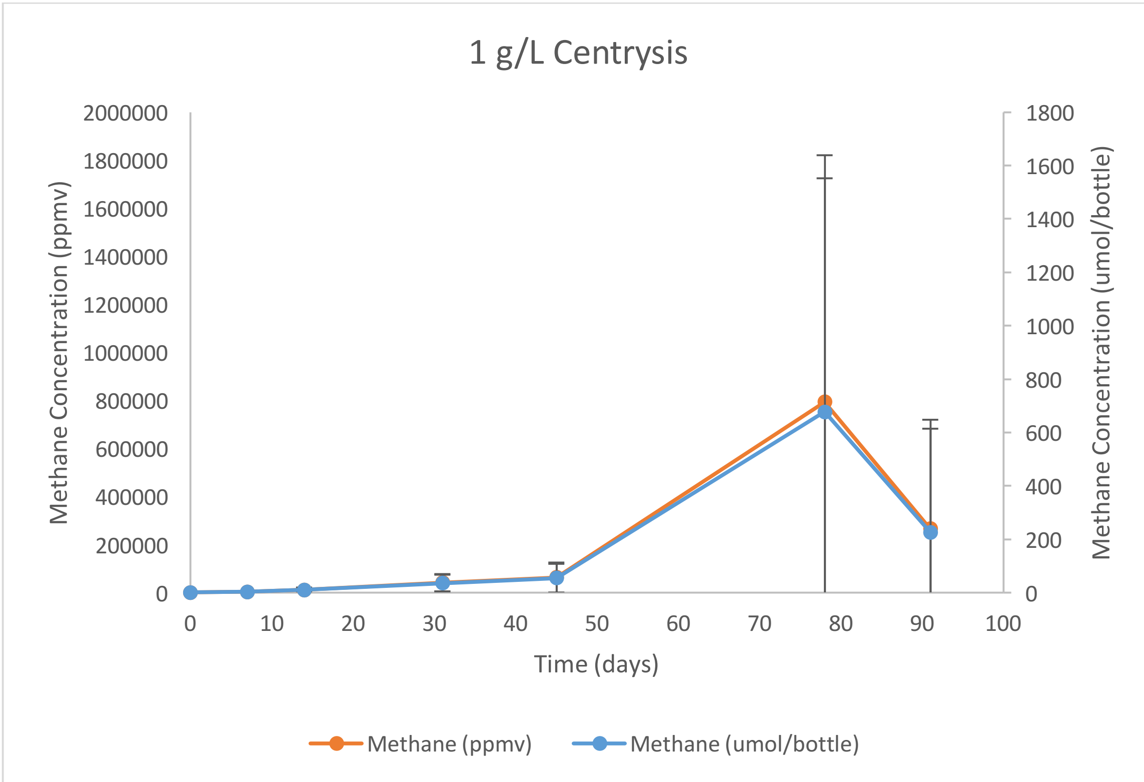

Figure B-12: TCE experiment amended with Centrysis. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

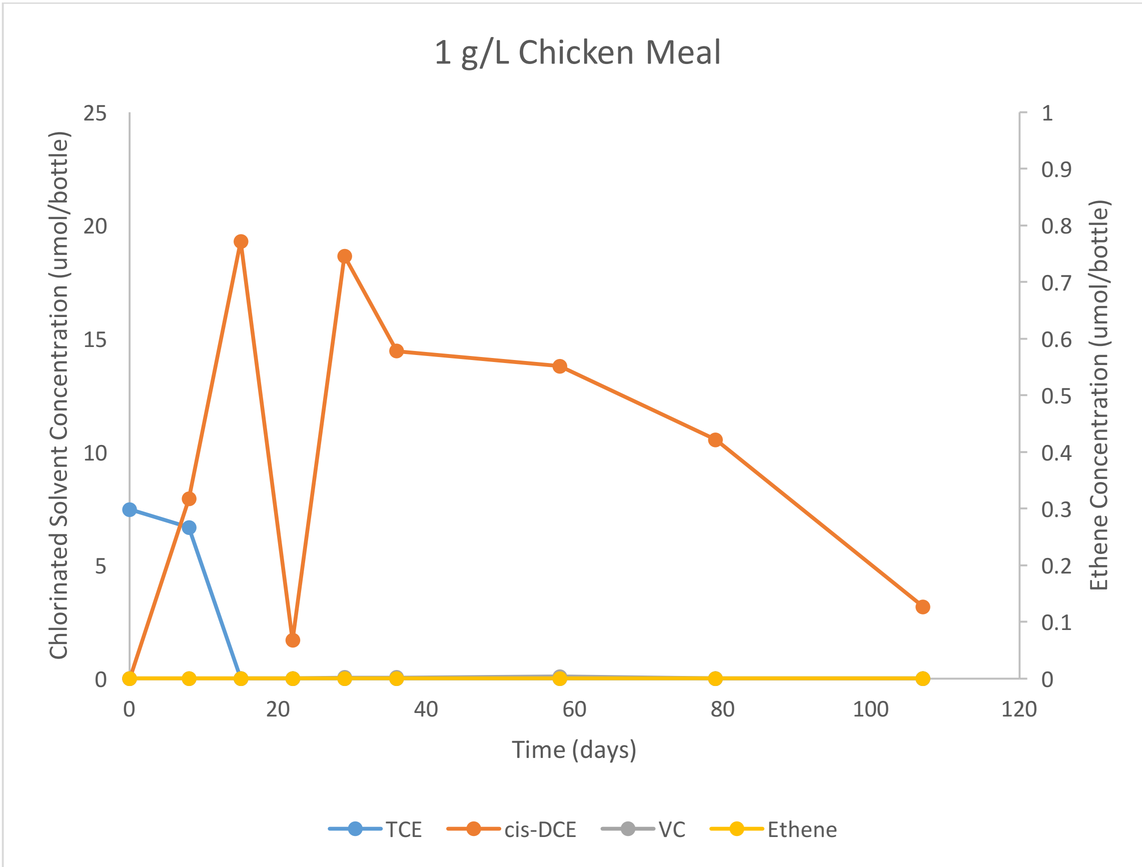

Figure B-13: TCE experiment amended with Chicken Meal. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

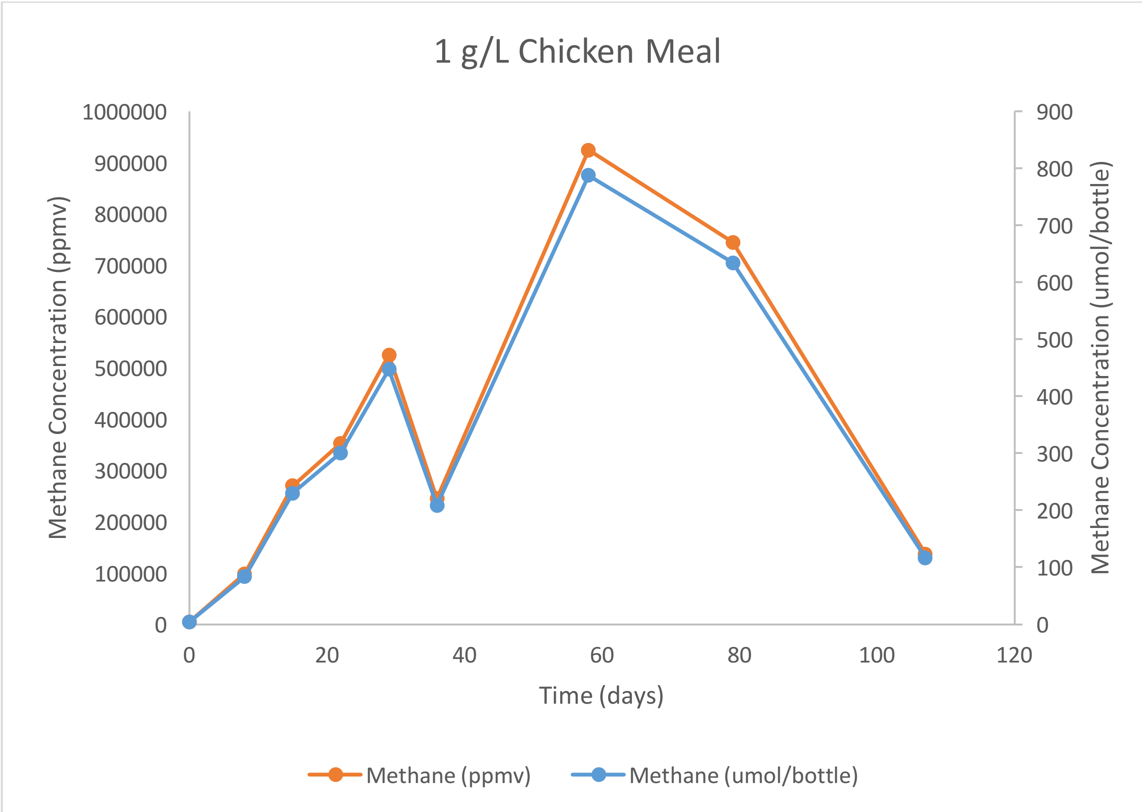

Figure B-14: TCE experiment amended with Chicken Meal. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

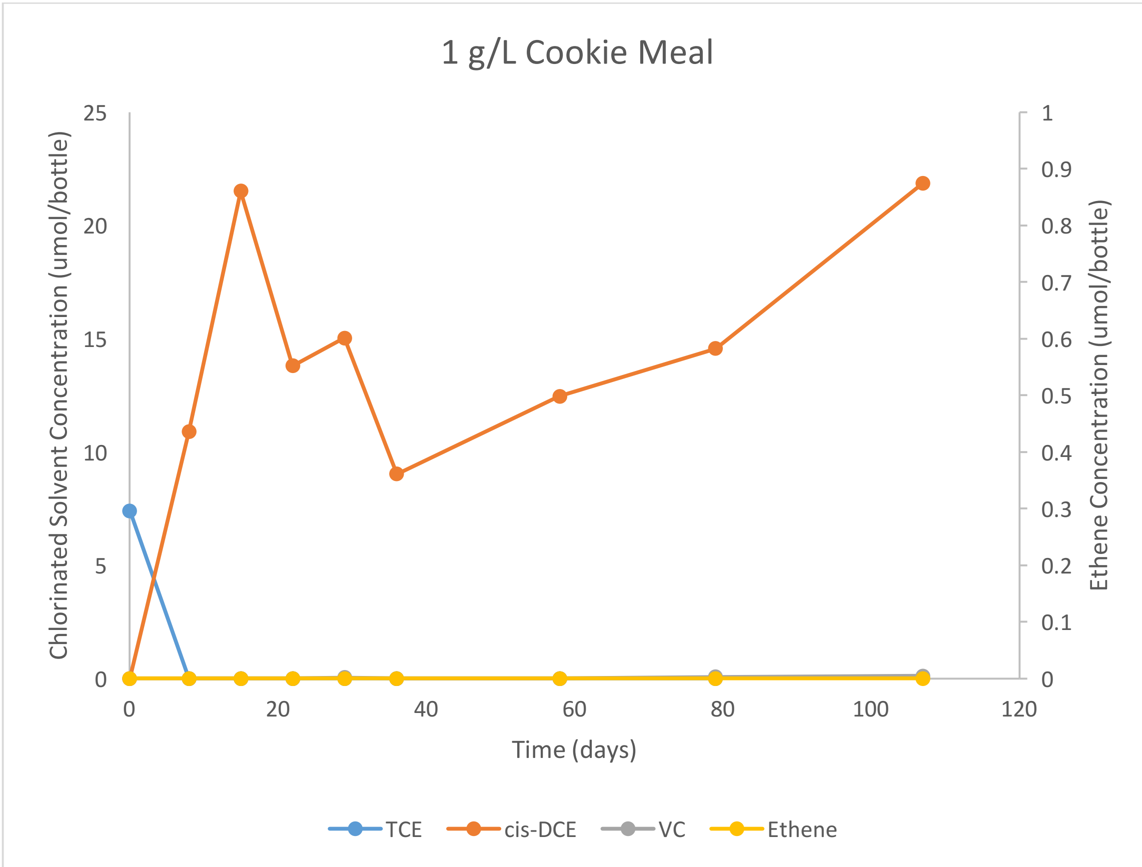

Figure B-15: TCE experiment amended with Cookie Meal. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

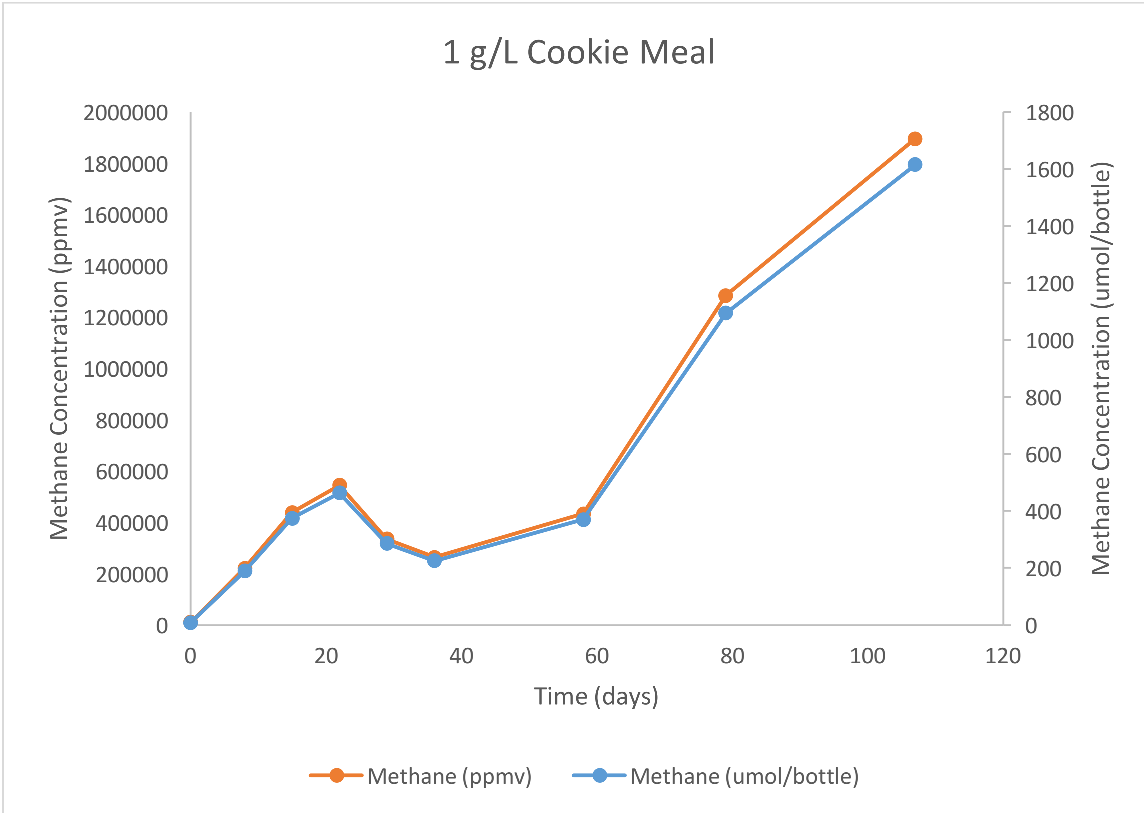

Figure B-16: TCE experiment amended with Cookie Meal. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

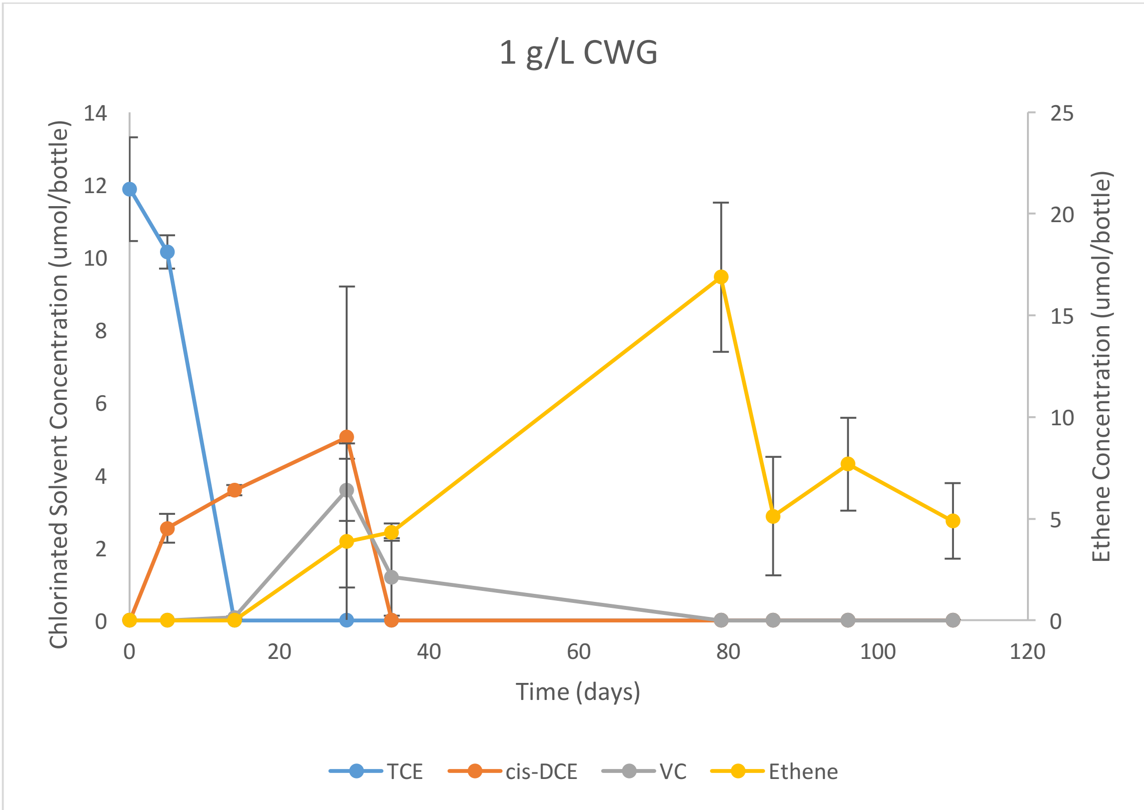

Figure B-17: TCE experiment amended with CWG. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

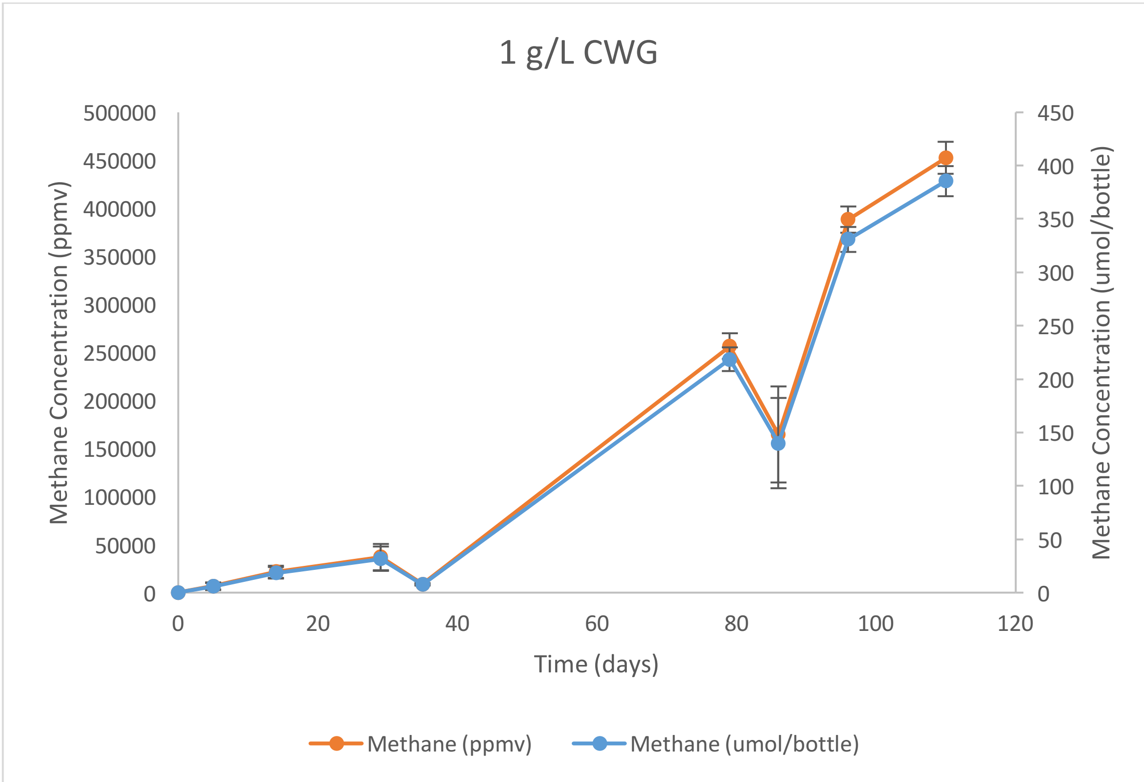

Figure B-18: TCE experiment amended with CWG. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

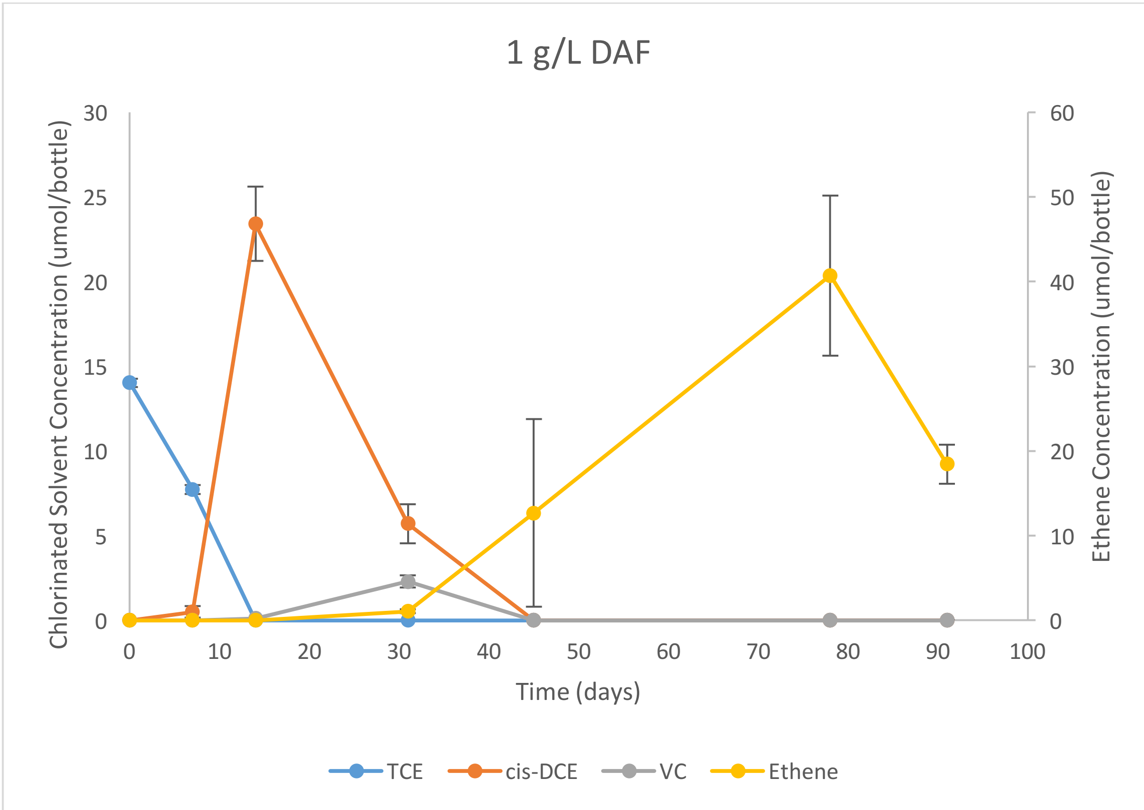

Figure B-19: TCE experiment amended with DAF. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

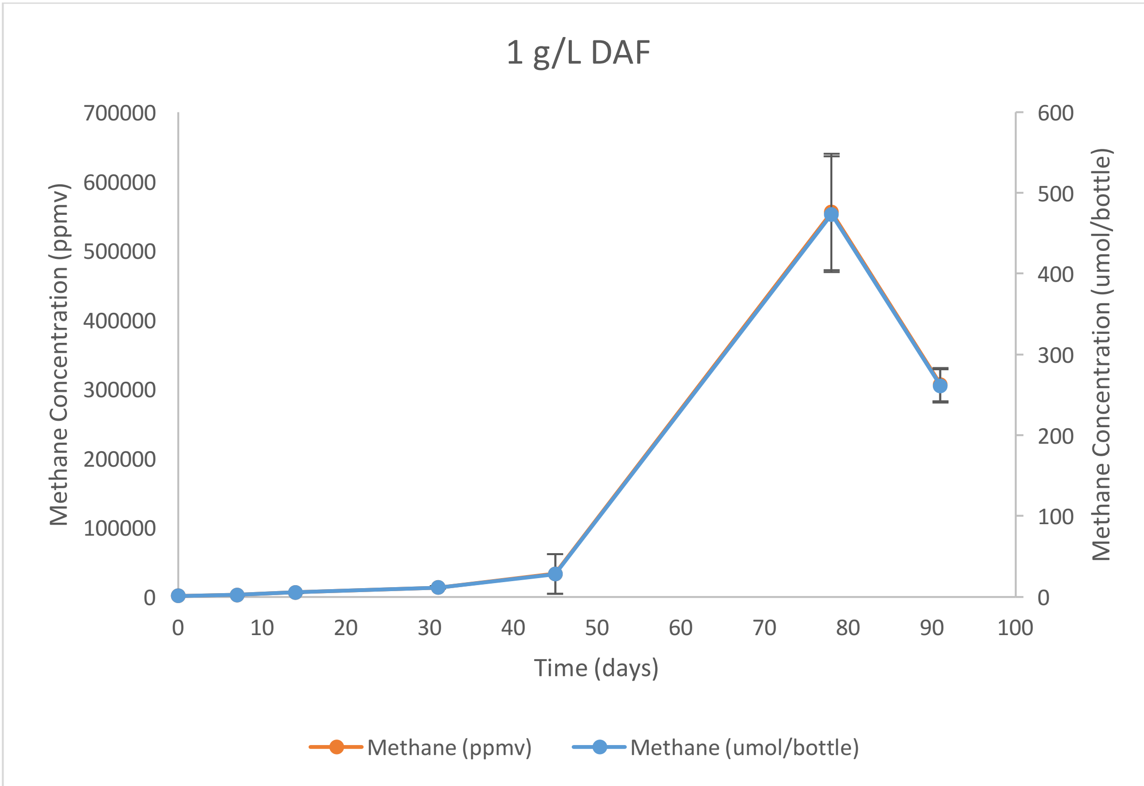

Figure B-20: TCE experiment amended with DAF. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

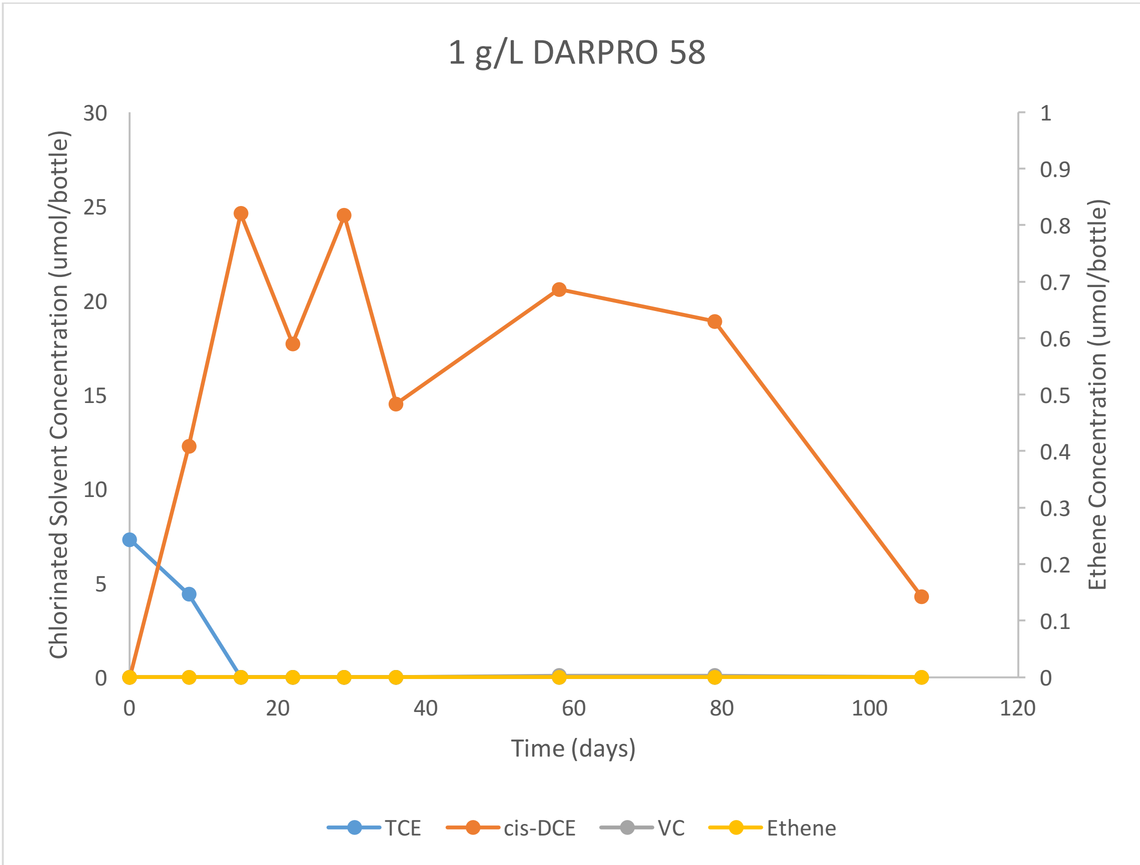

Figure B-21: TCE experiment amended with DARPRO 58. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

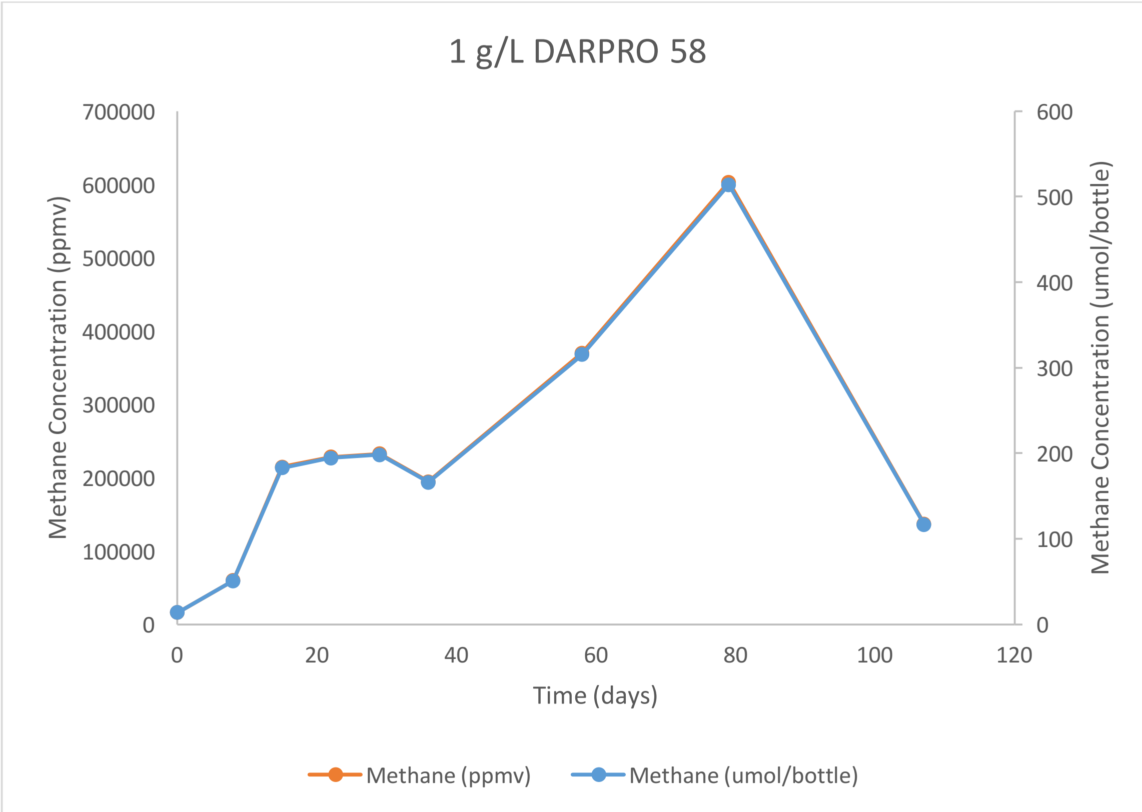

Figure B-22: TCE experiment amended with DARPRO 58. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

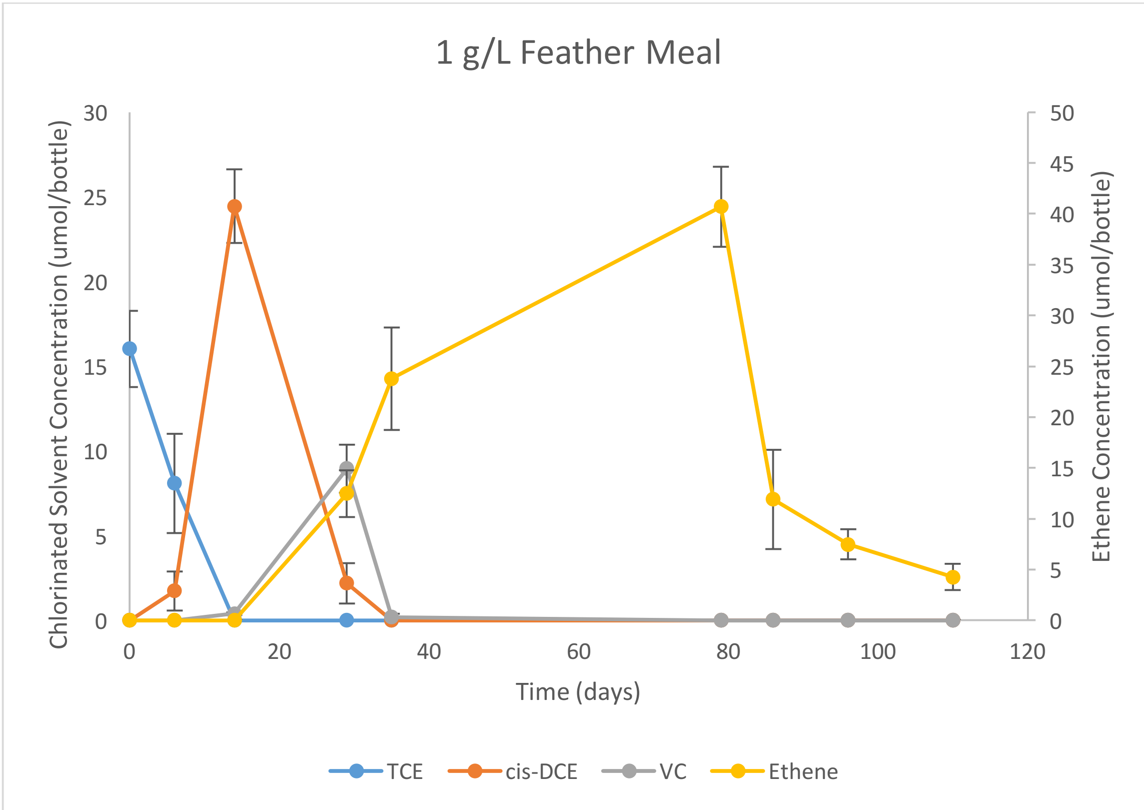

Figure B-23: TCE experiment amended with Feather Meal. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

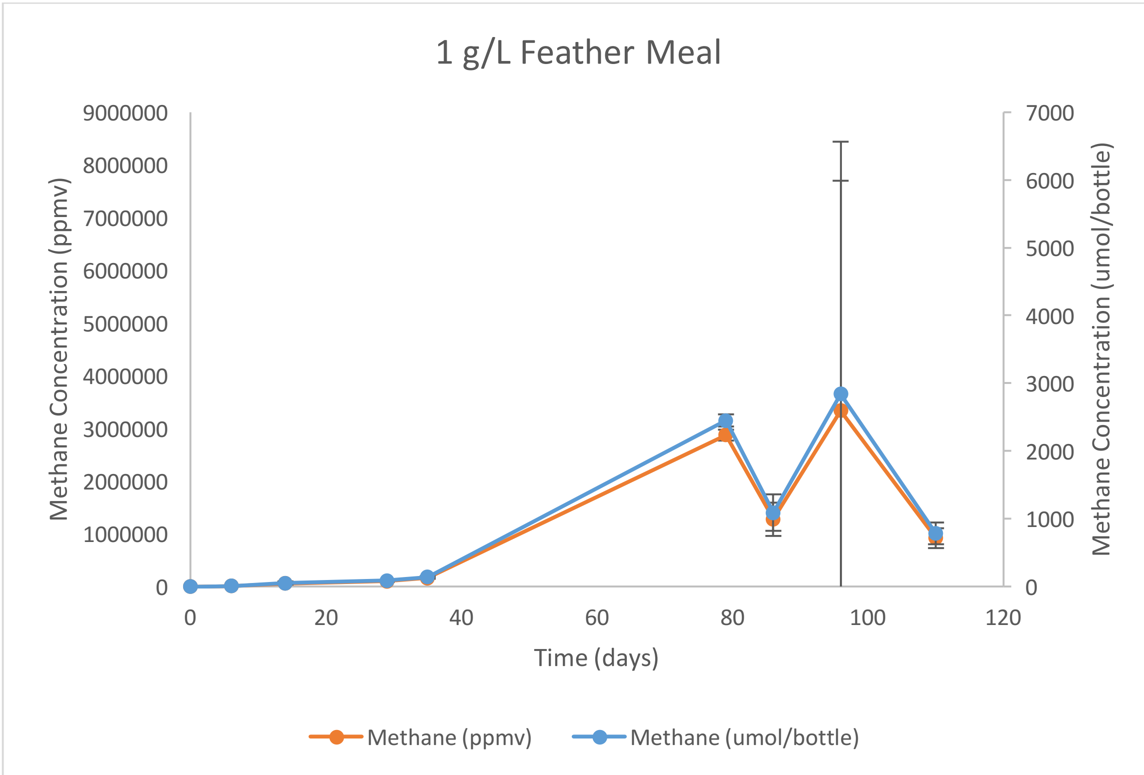

Figure B-24: TCE experiment amended with Feather Meal. Concentration of methane in μmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

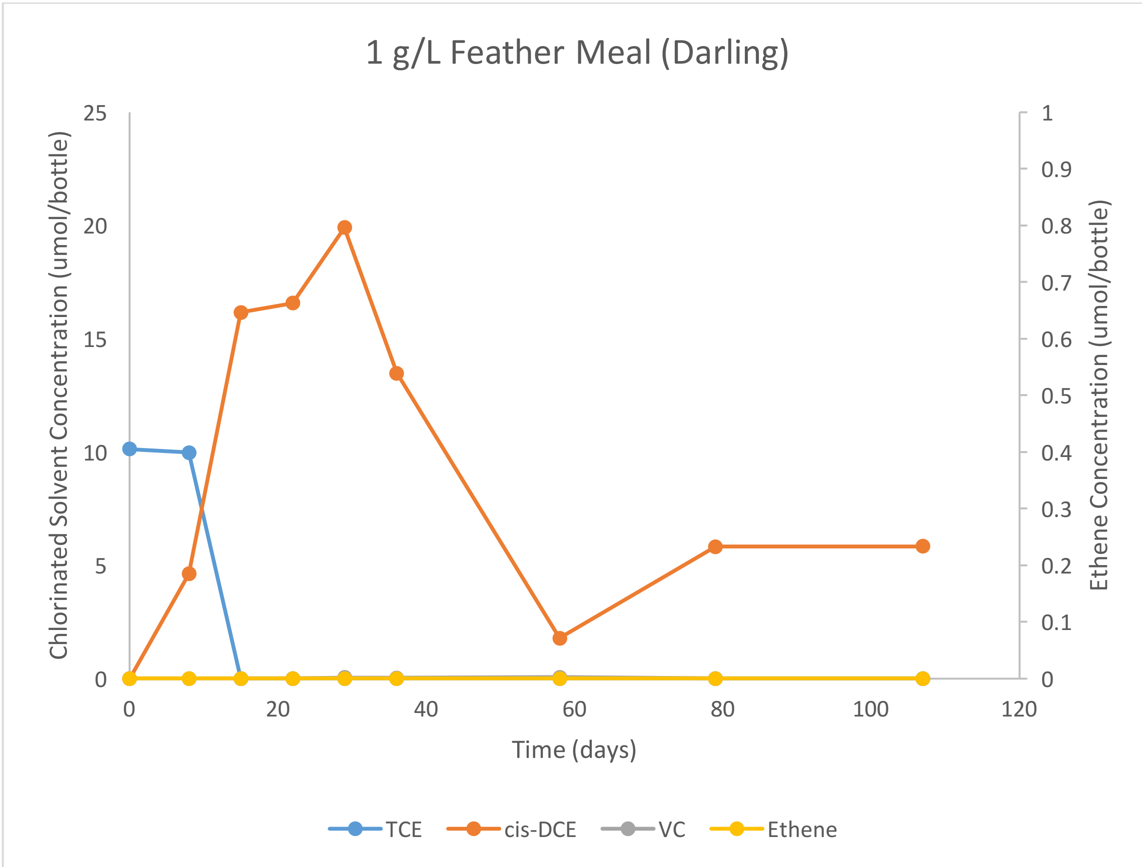

Figure B-25: TCE experiment amended with Feather Meal (Darling). Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

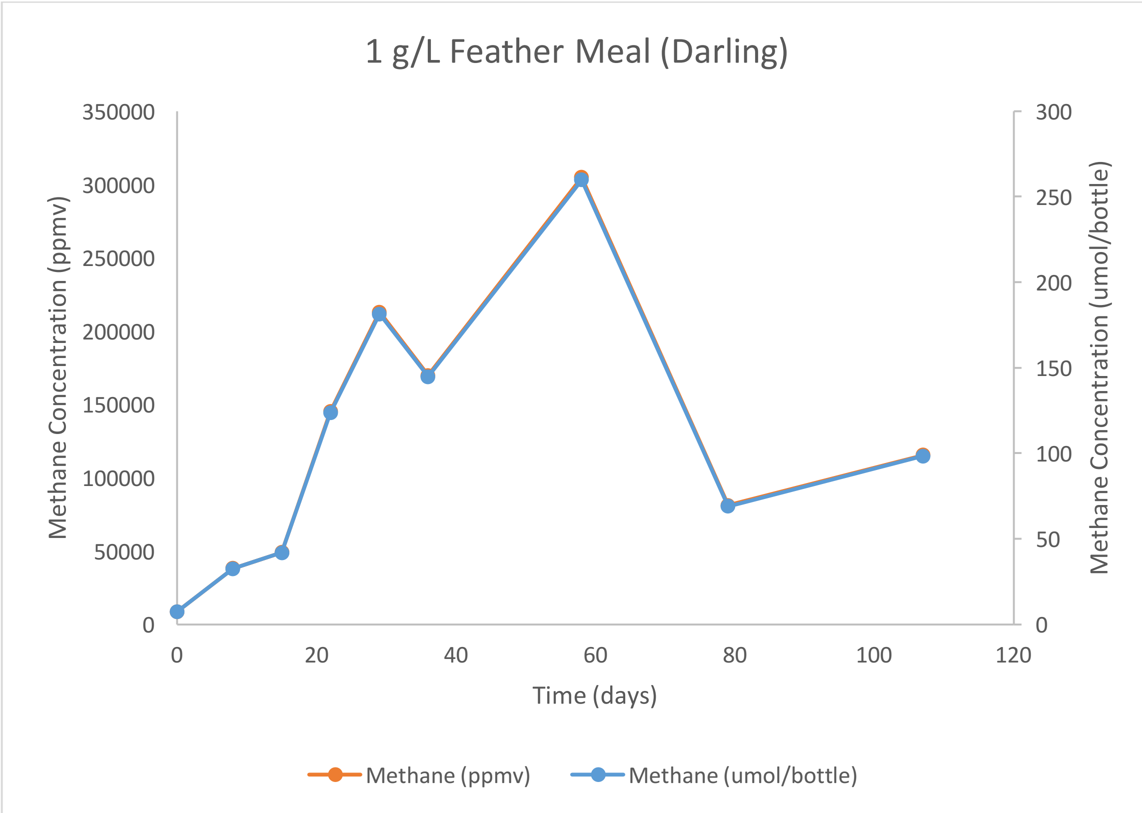

Figure B-26: TCE experiment amended with Feather Meal (Darling). Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

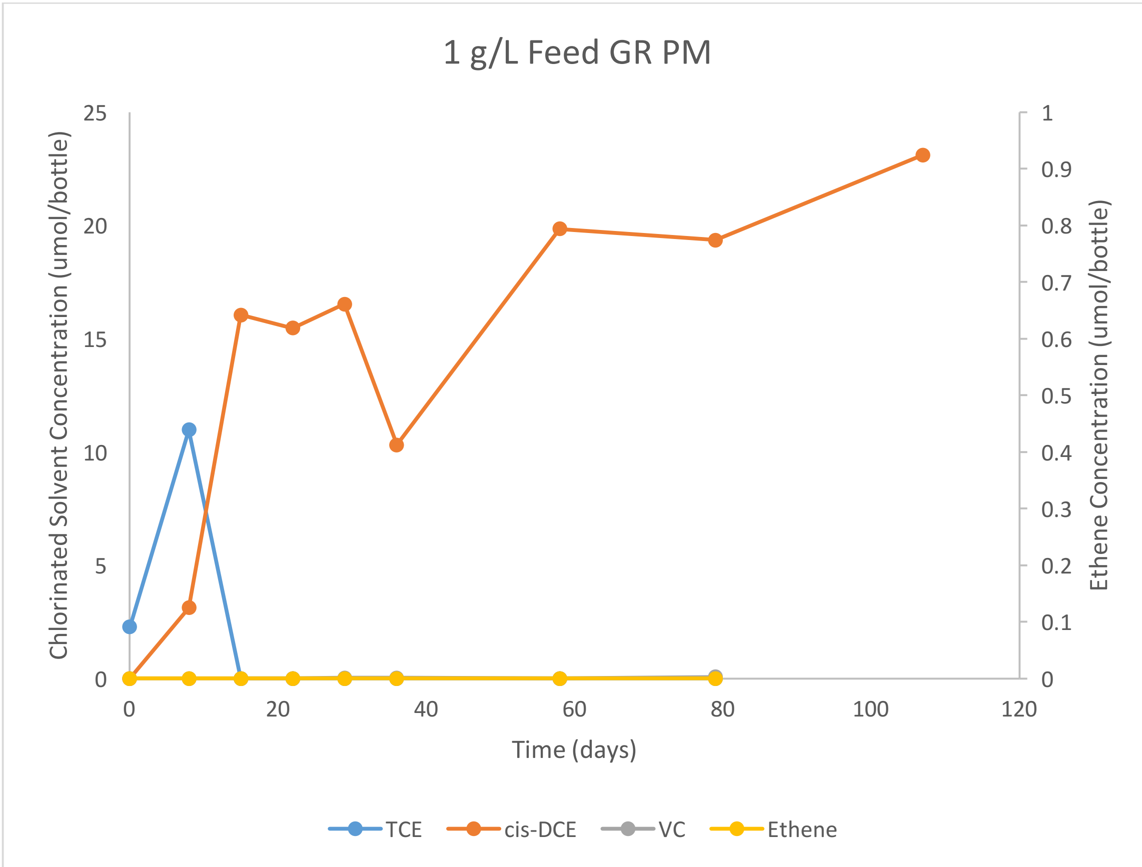

Figure B-27: TCE experiment amended with Feed GR PM. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

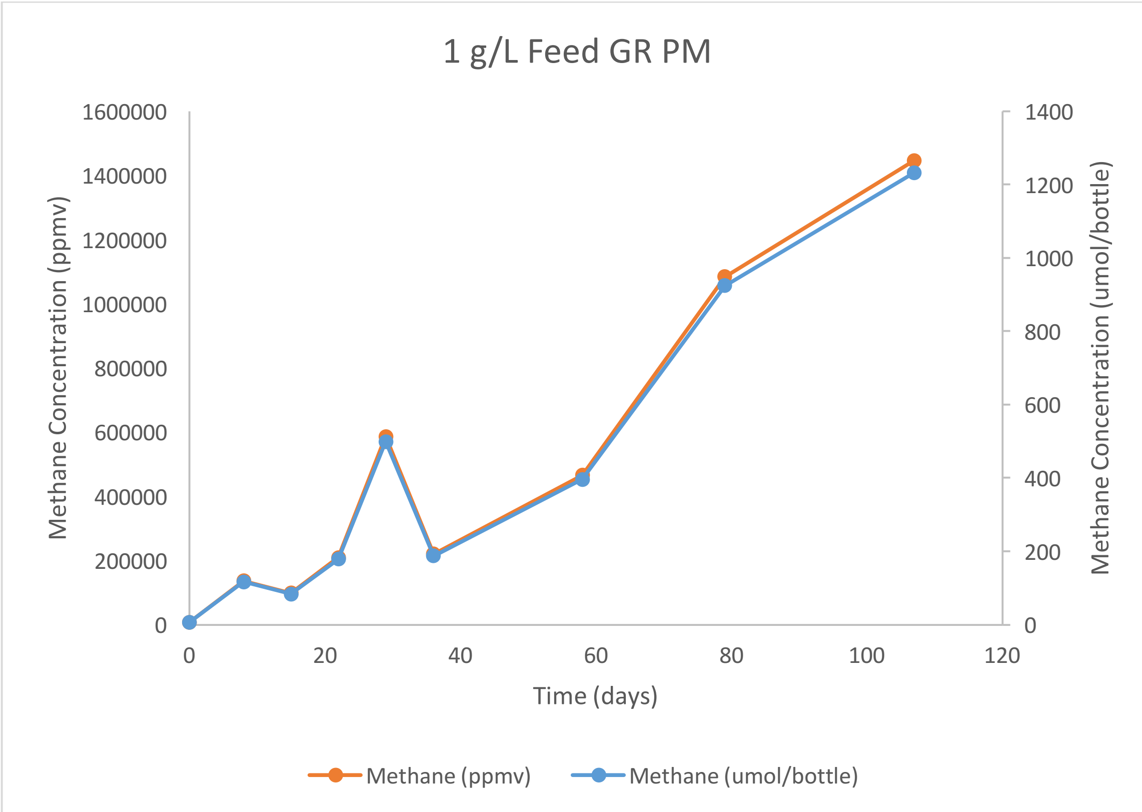

Figure B-28: TCE experiment amended with Feed GR PM. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

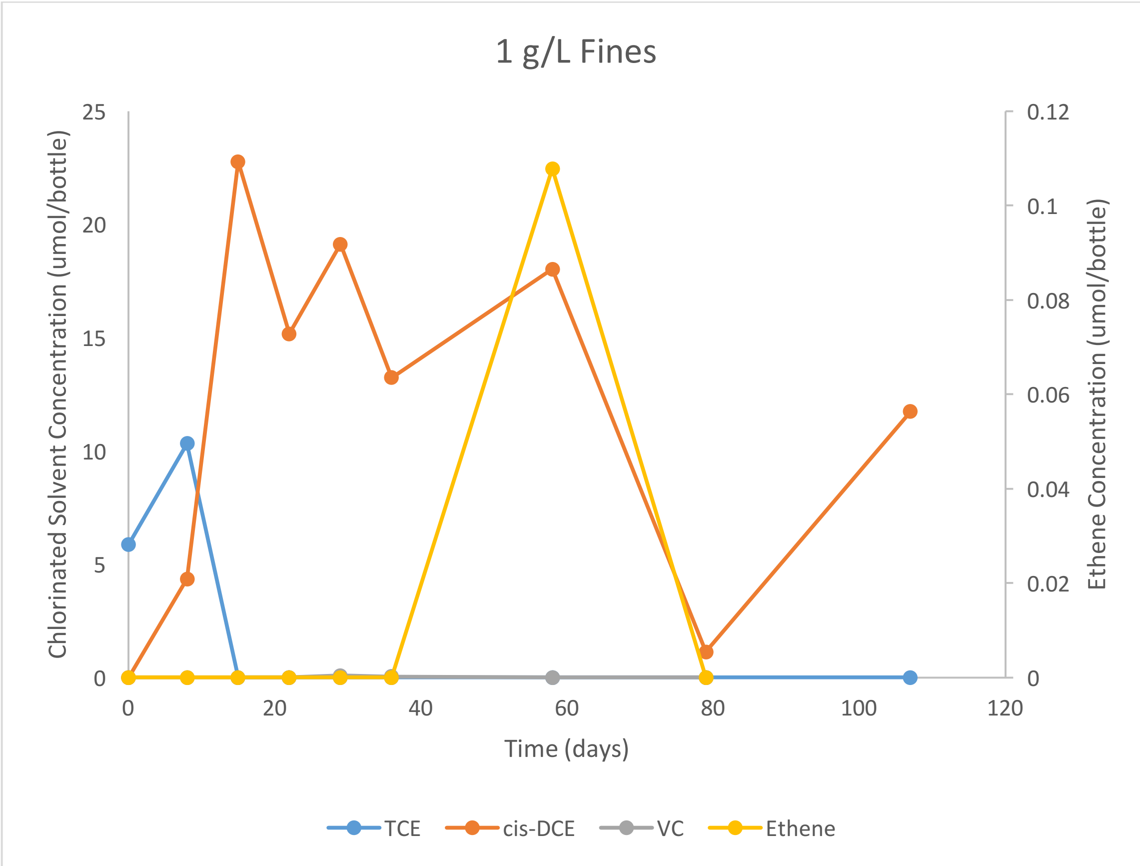

Figure B-29: TCE experiment amended with Fines. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

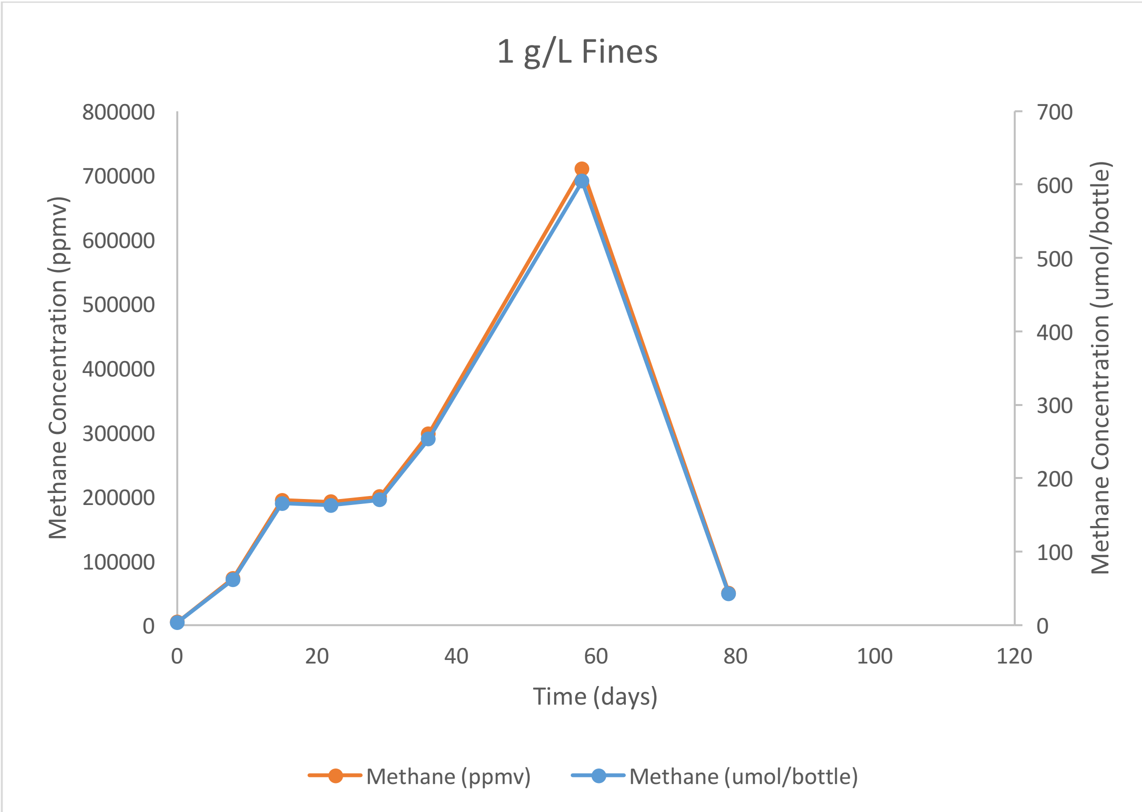

Figure B-30: TCE experiment amended with Fines. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

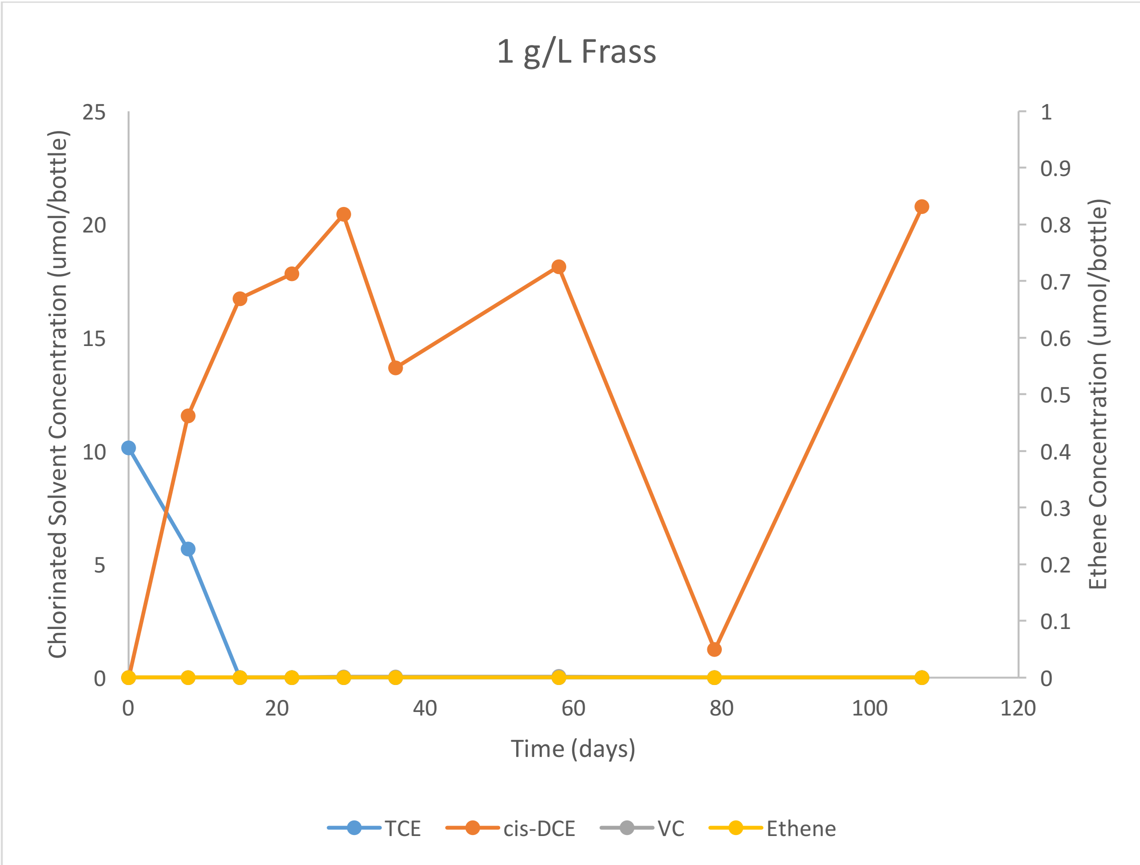

Figure B-31: TCE experiment amended with Frass. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

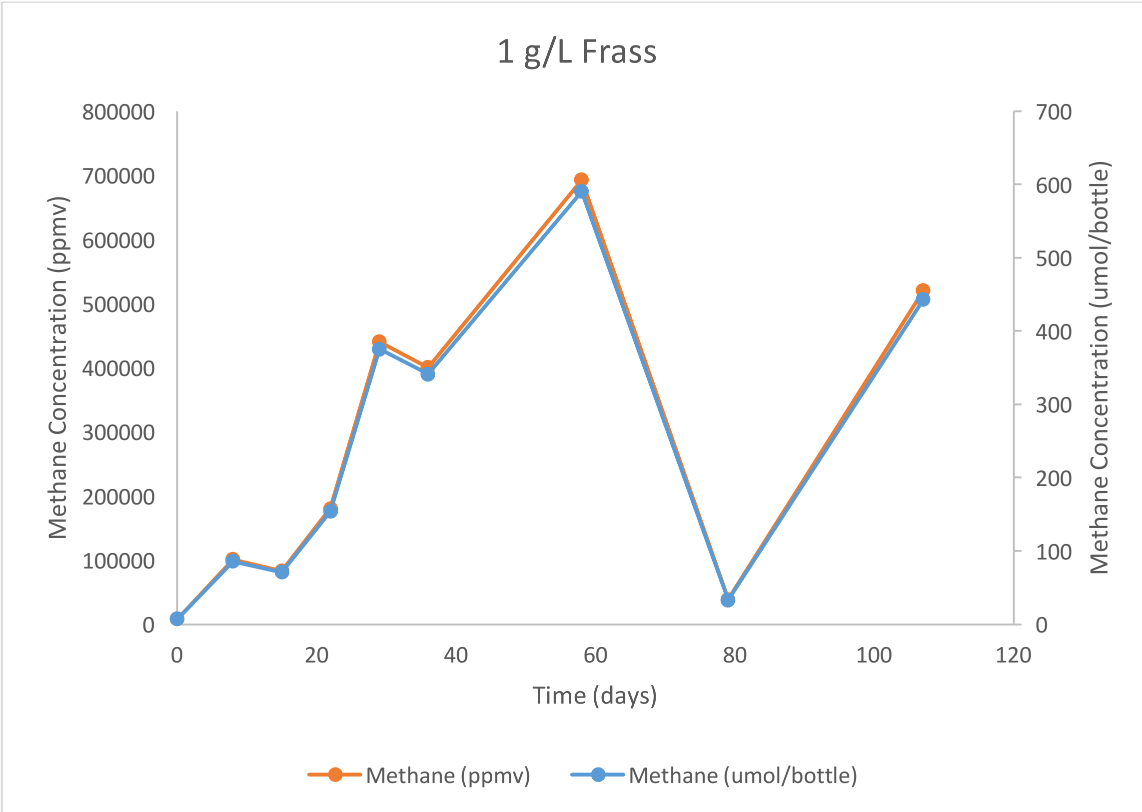

Figure B-32: TCE experiment amended with Frass. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

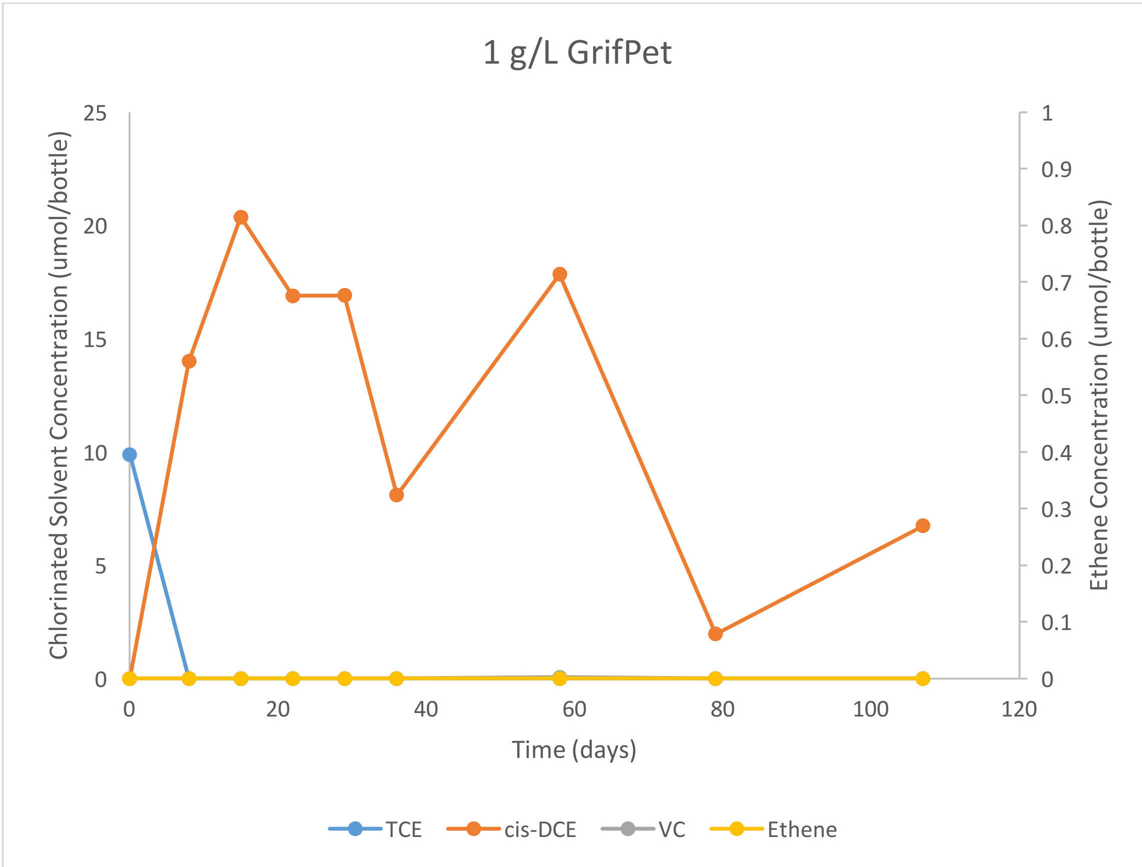

Figure B-33: TCE experiment amended with GrifPet. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

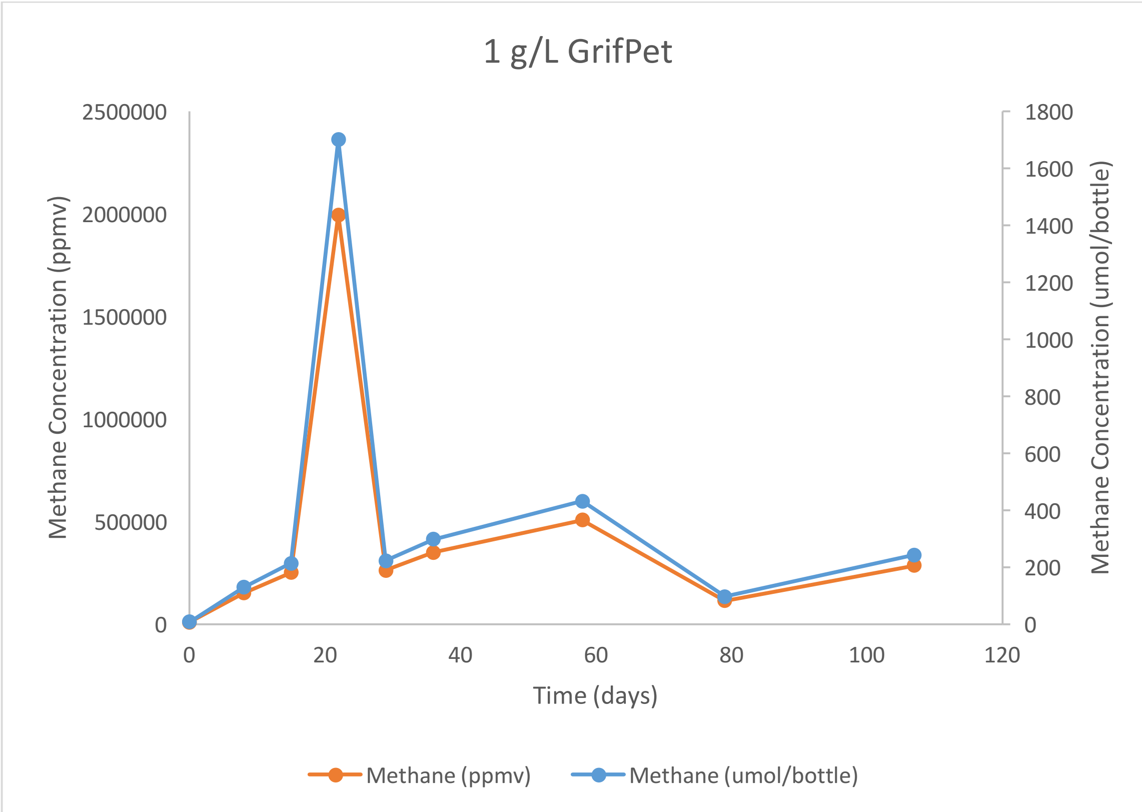

Figure B-34: TCE experiment amended with GrifPet. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

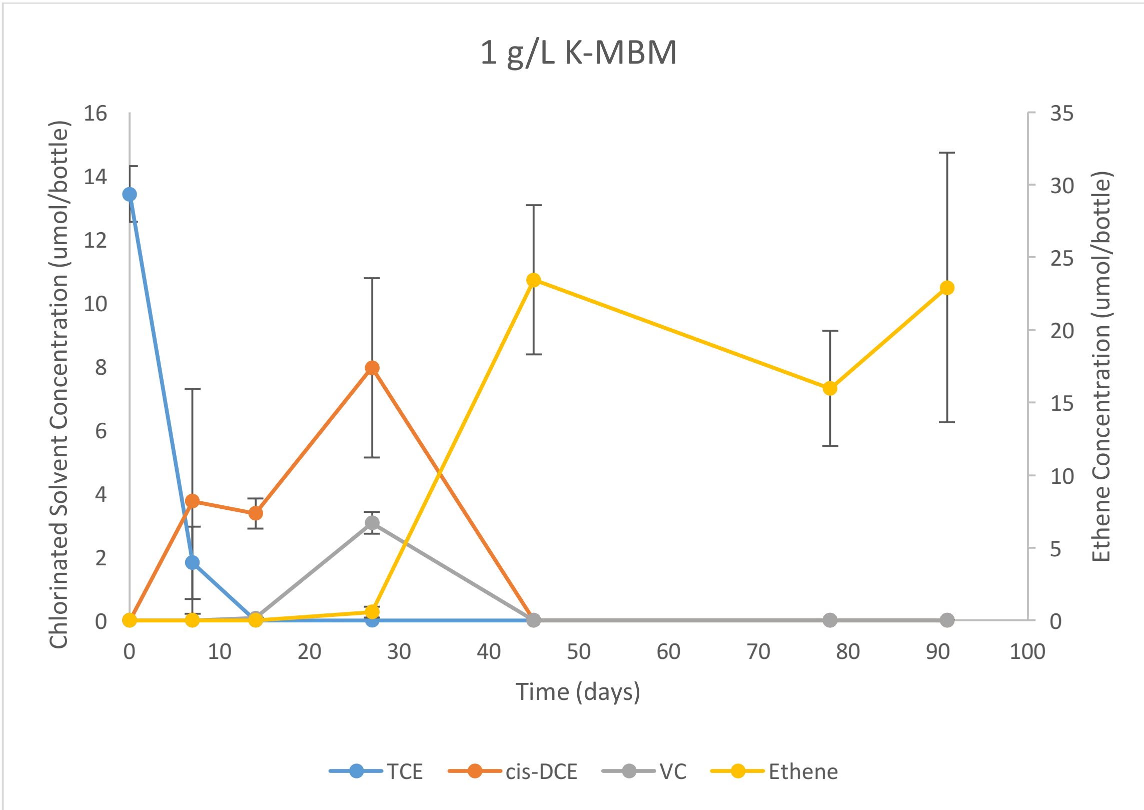

Figure B-35: TCE experiment amended with K-MBM. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

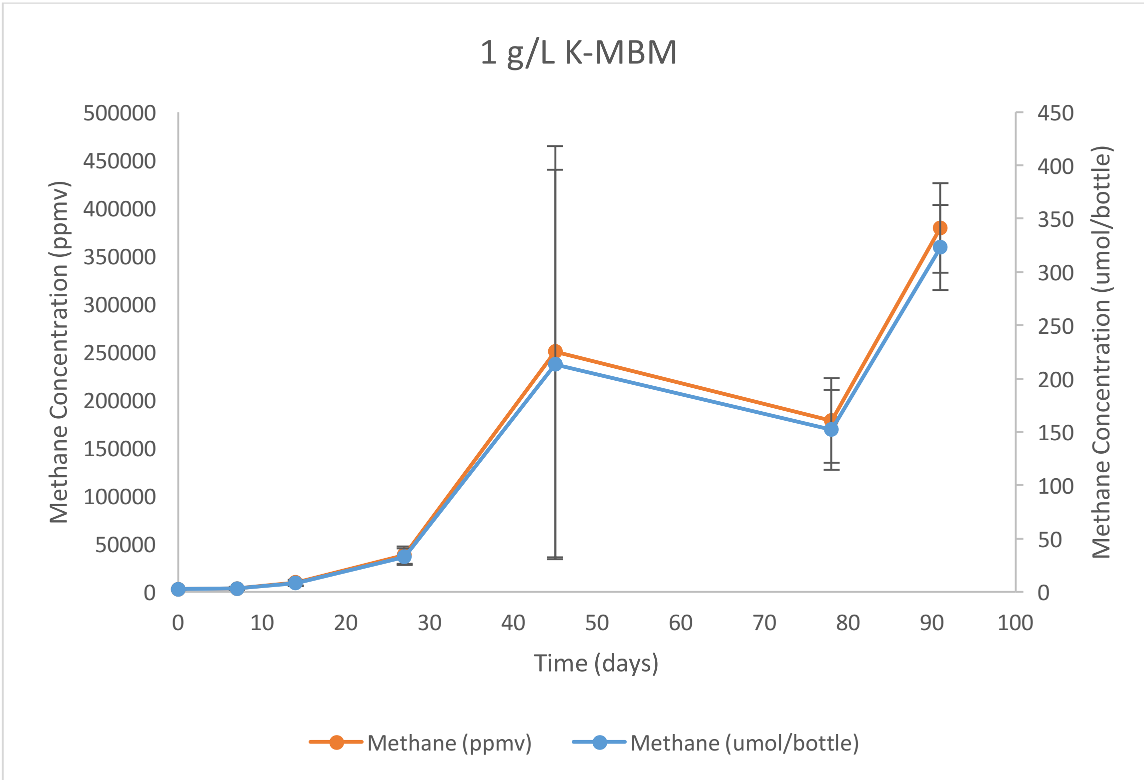

Figure B-36: TCE experiment amended with K-MBM. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

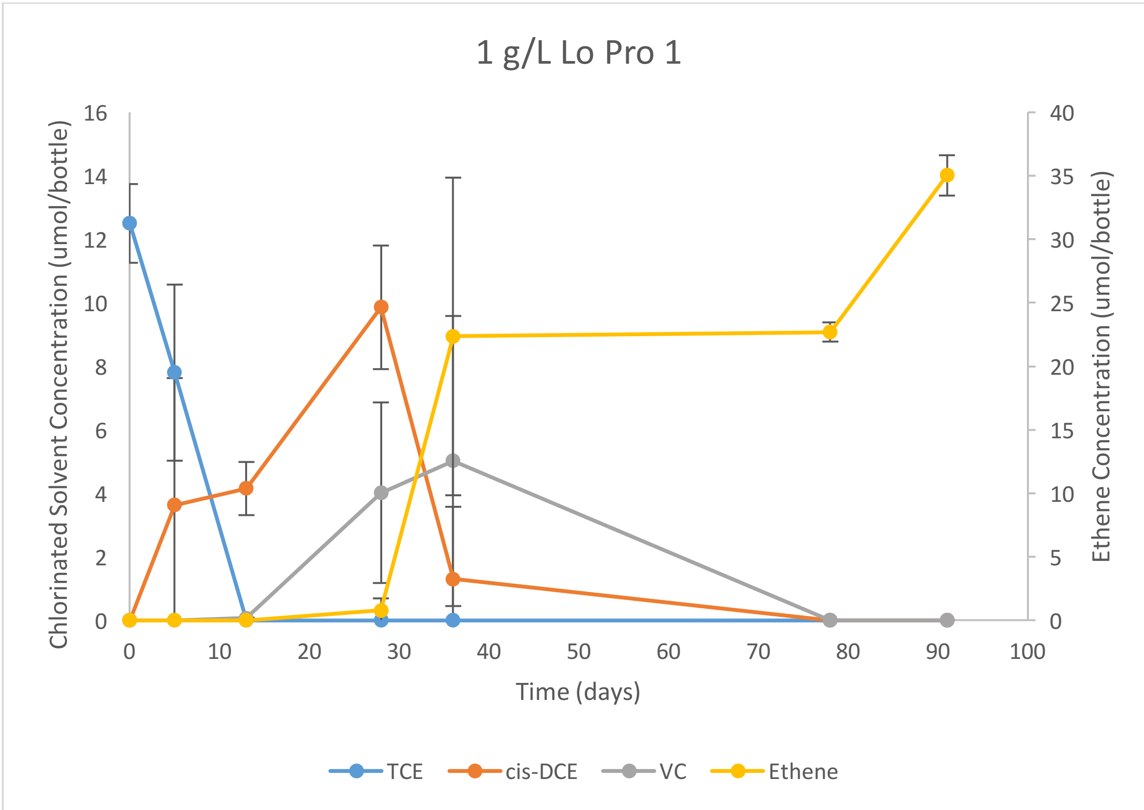

Figure B-37: TCE experiment amended with Lo Pro 1. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

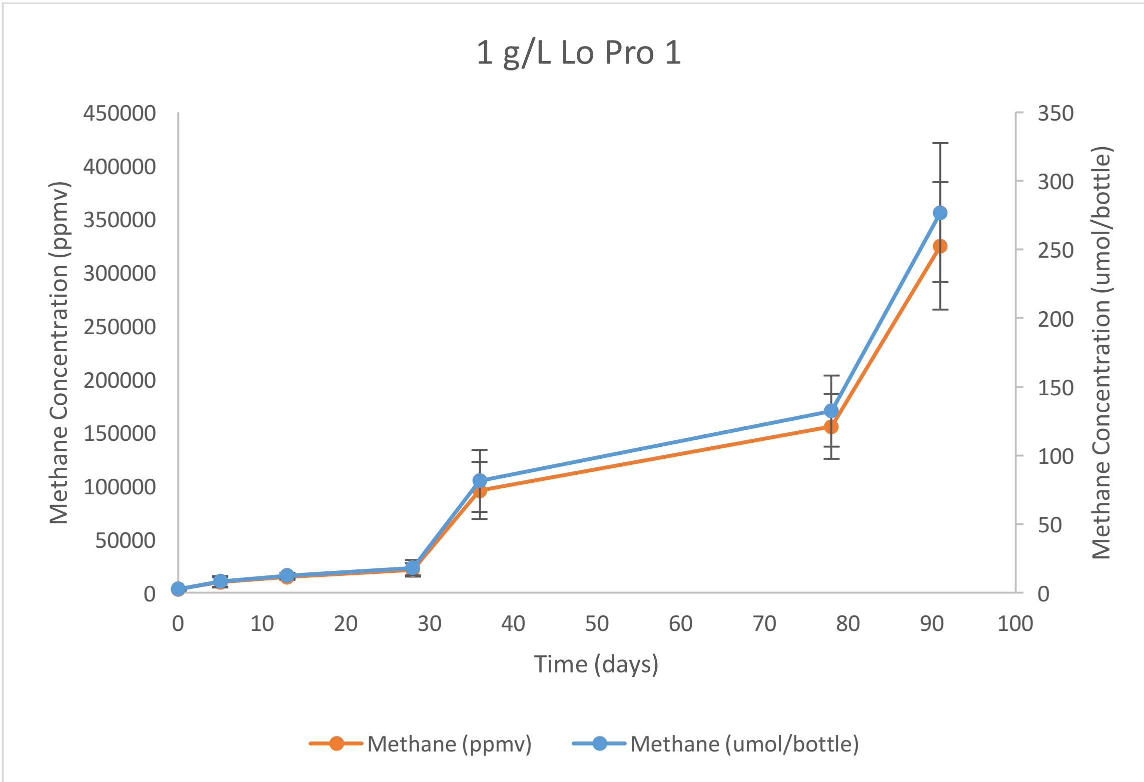

Figure B-38: TCE experiment amended with Lo Pro 1. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

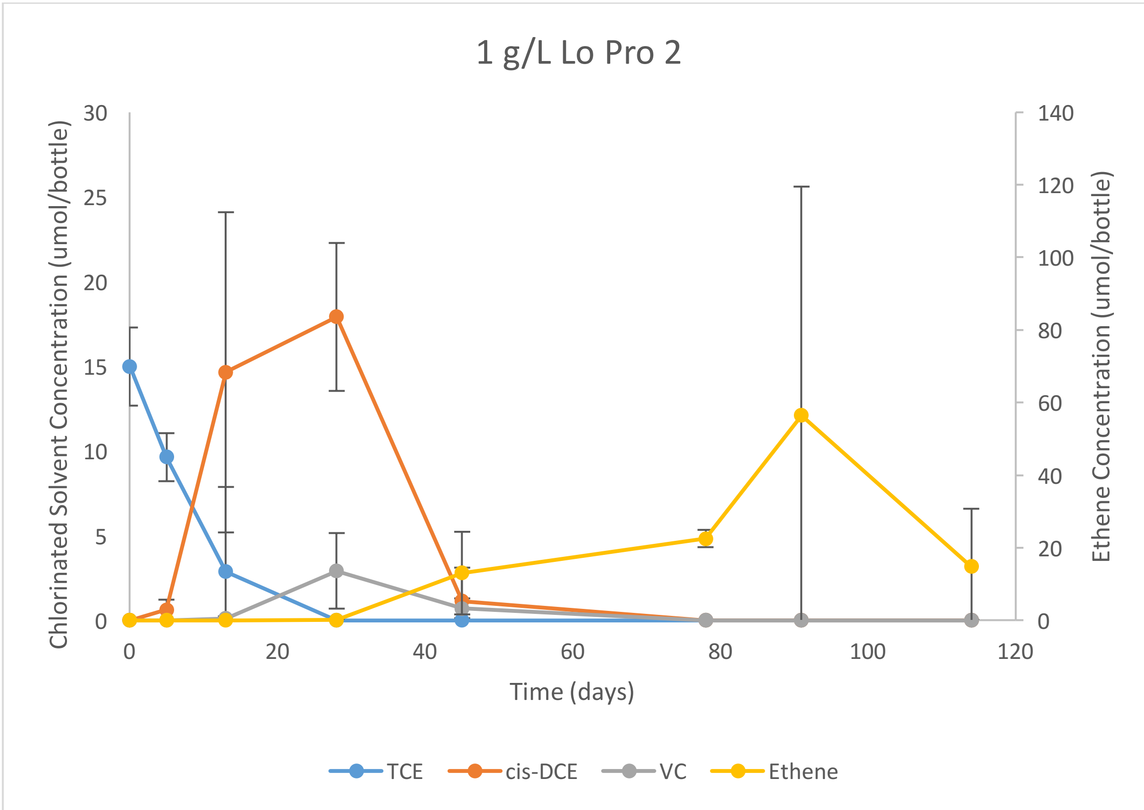

Figure B-39: TCE experiment amended with Lo Pro 2. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

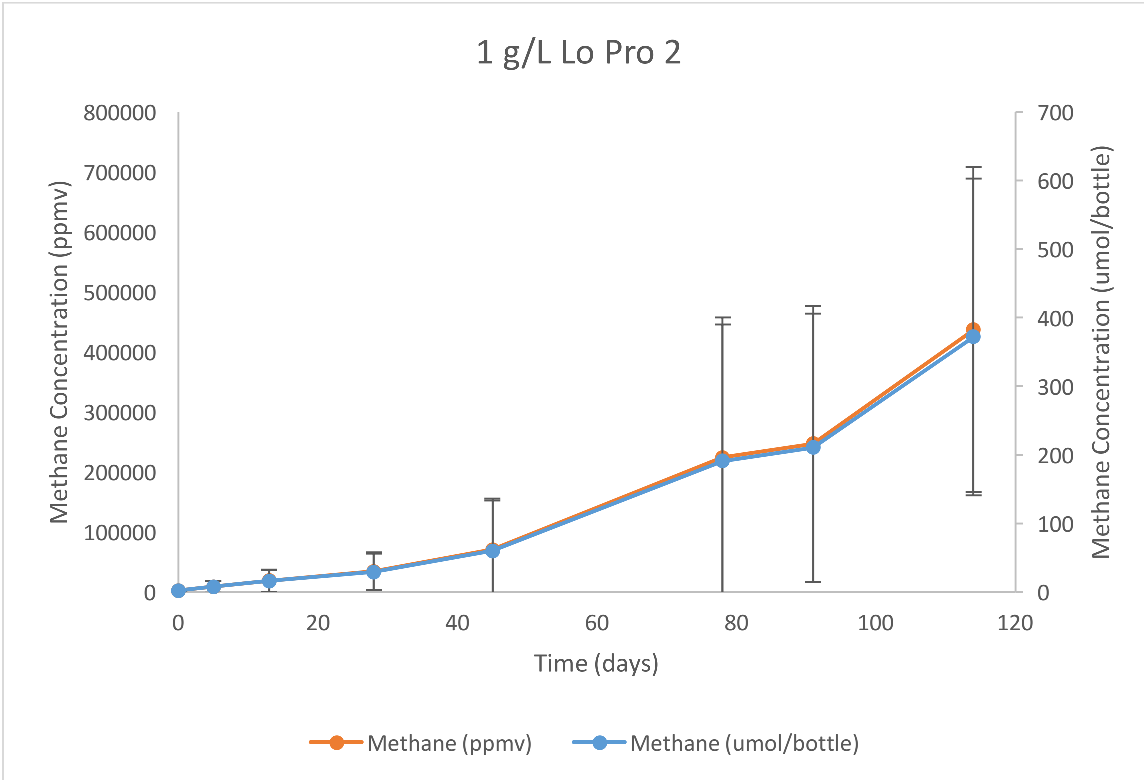

Figure B-40: TCE experiment amended with Lo Pro 2. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

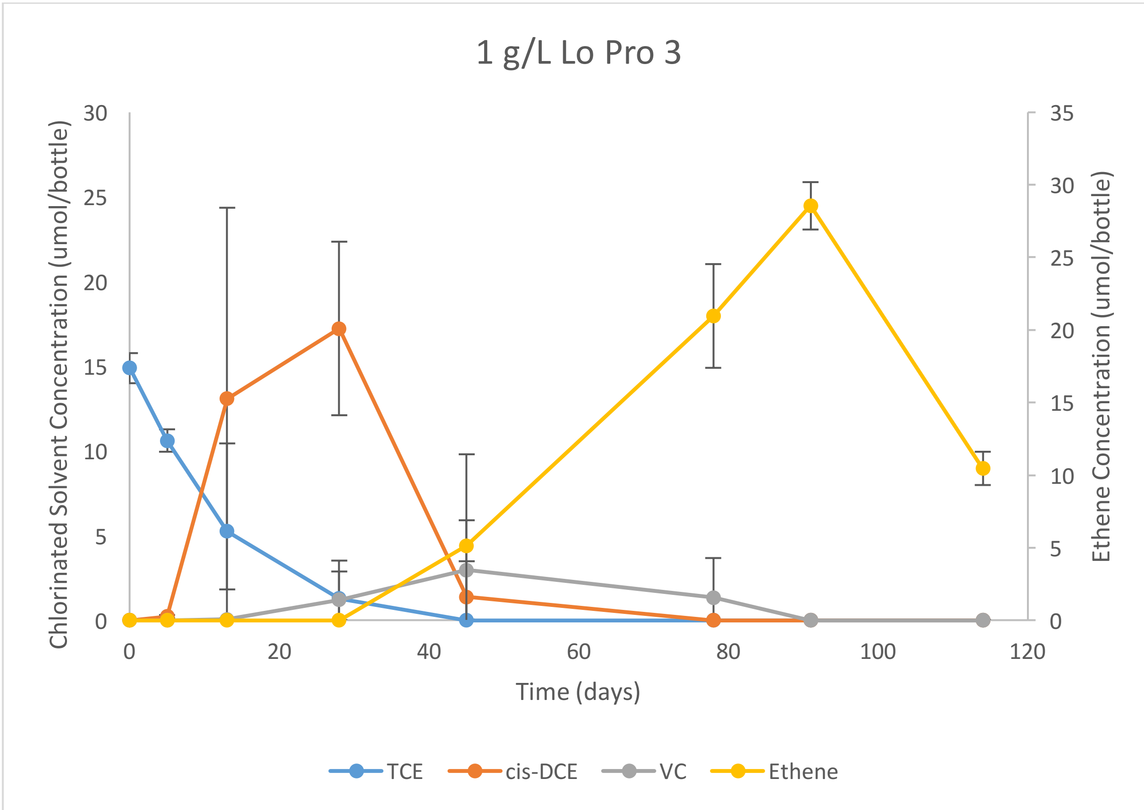

Figure B-41: TCE experiment amended with Lo Pro 3. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

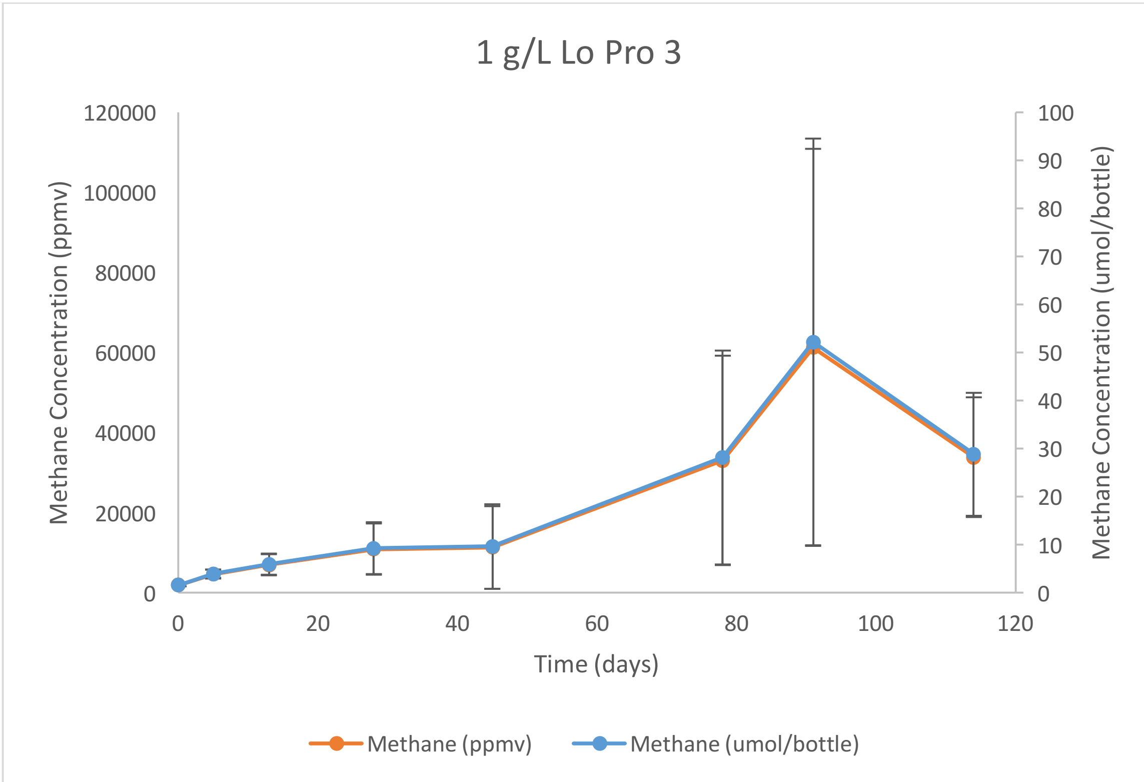

Figure B-42: TCE experiment amended with Lo Pro 3. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

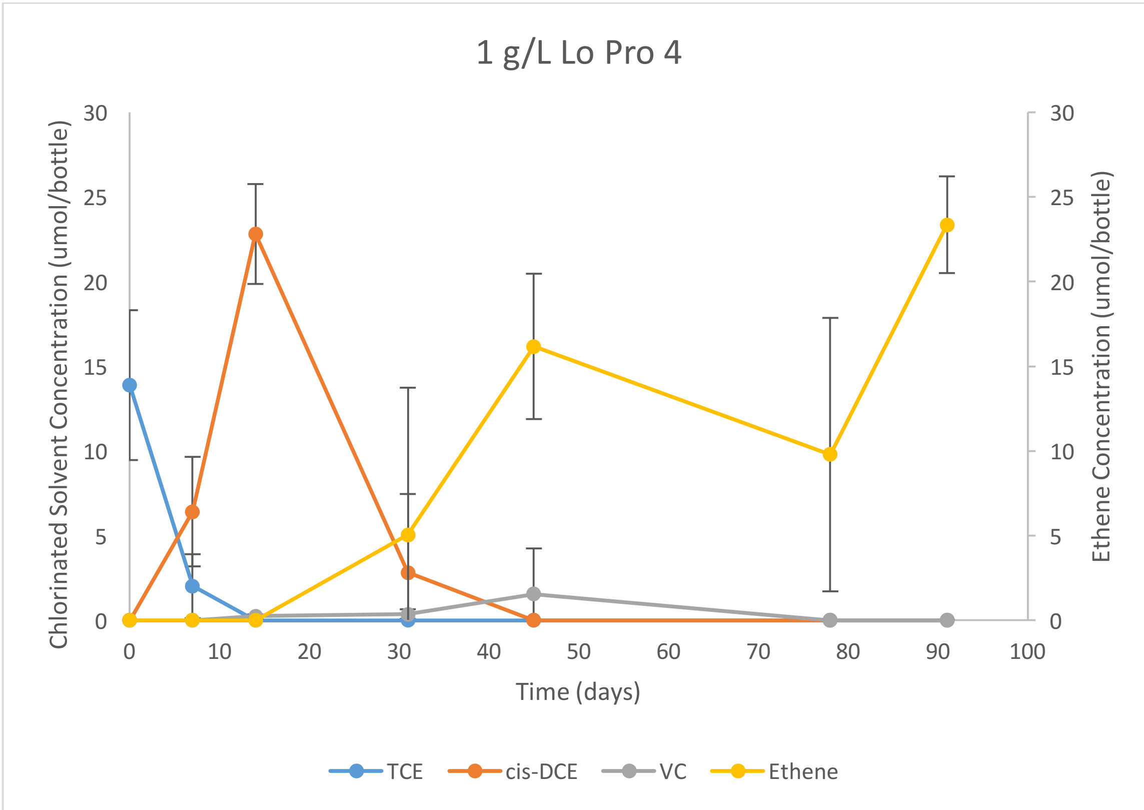

Figure B-43: TCE experiment amended with Lo Pro 4. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

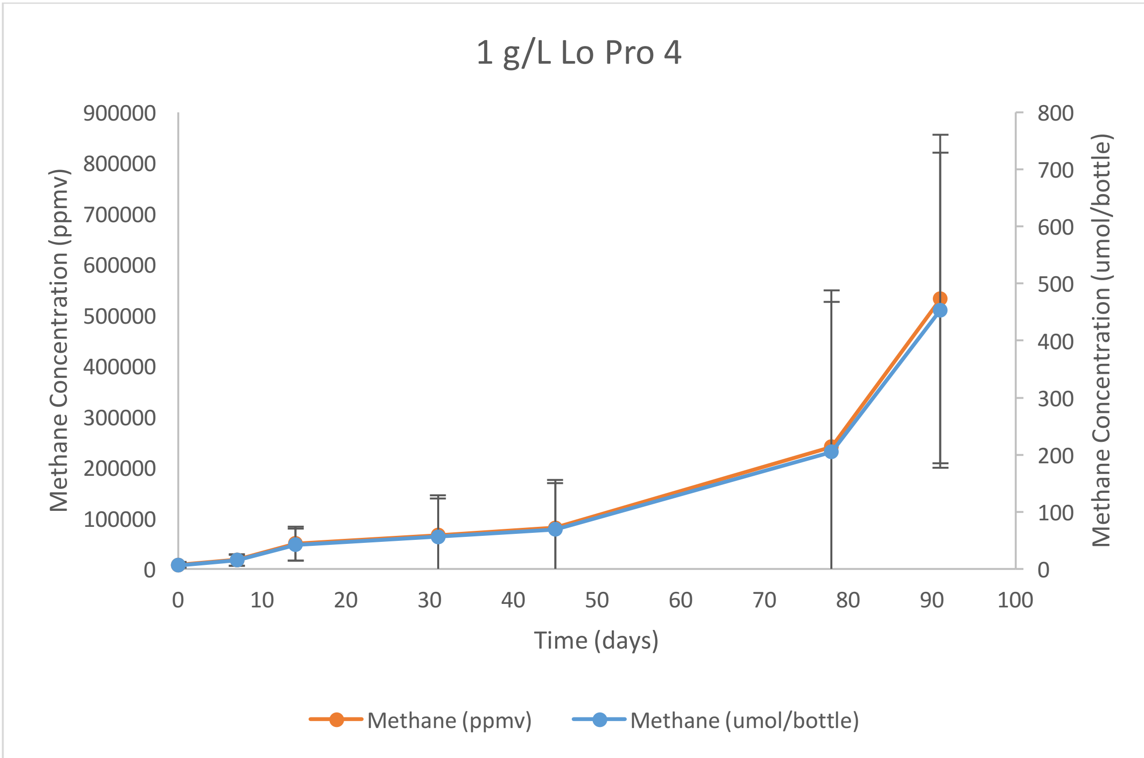

Figure B-44: TCE experiment amended with Lo Pro 4. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

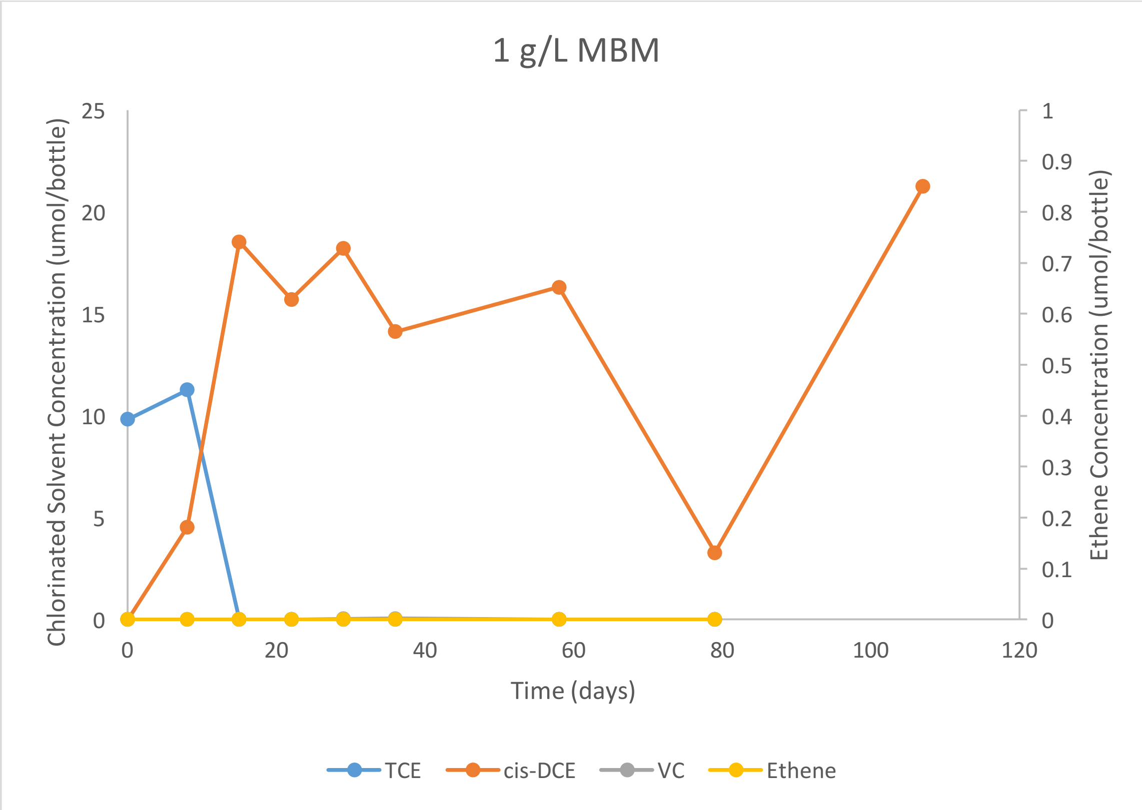

Figure B-45: TCE experiment amended with MBM. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

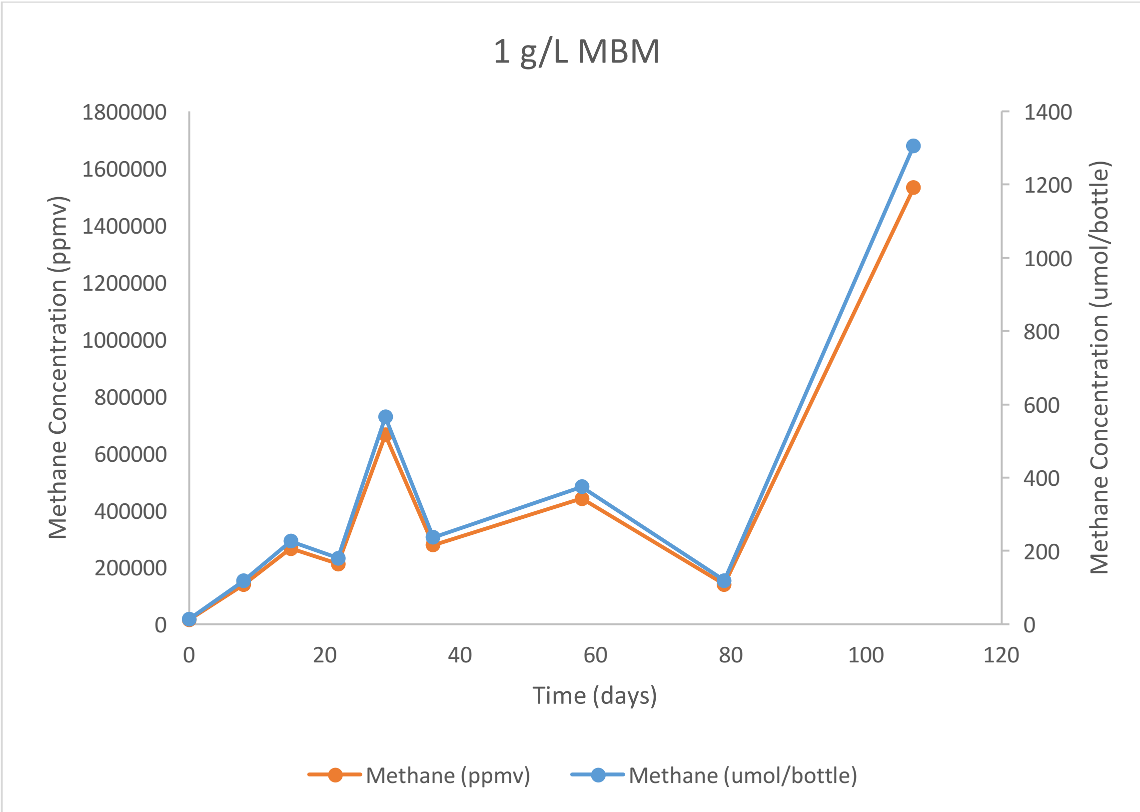

Figure B-46: TCE experiment amended with MBM. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

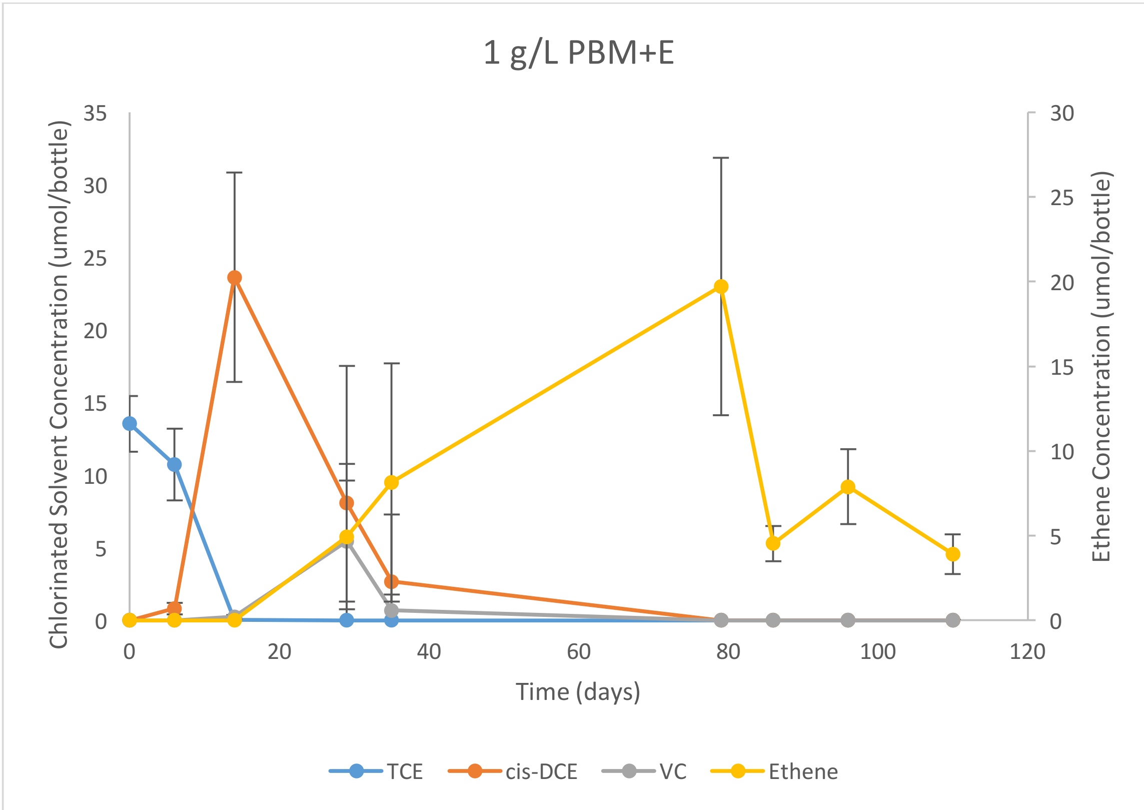

Figure B-47: TCE experiment amended with PBM+E. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

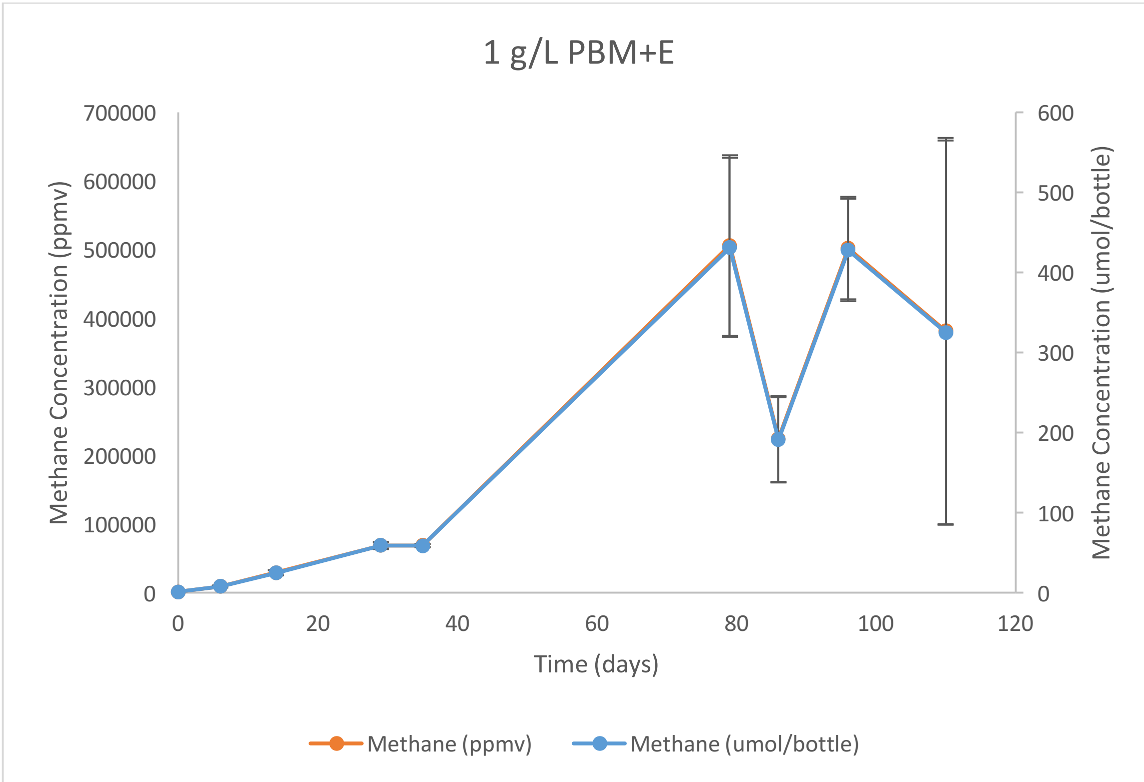

Figure B-48: TCE experiment amended with PBM+E. Concentration of methane in μmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

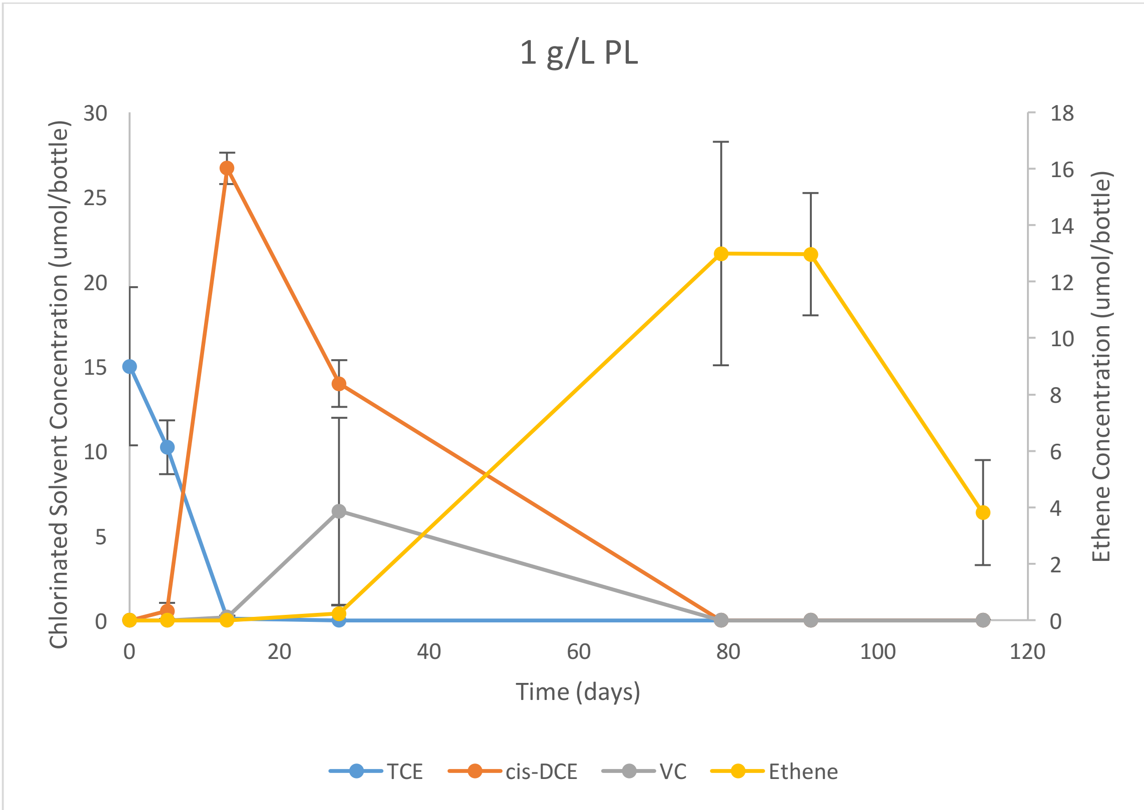

Figure B-49: TCE experiment amended with PL. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

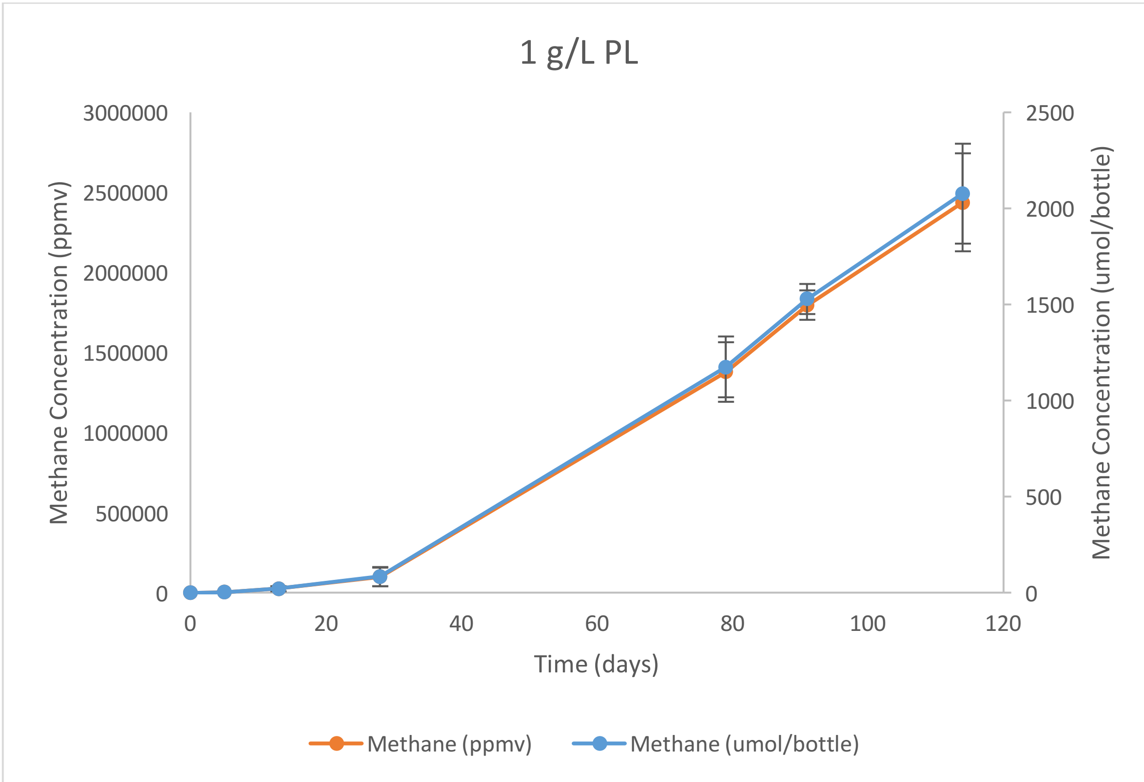

Figure B-50: TCE experiment amended with PL. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

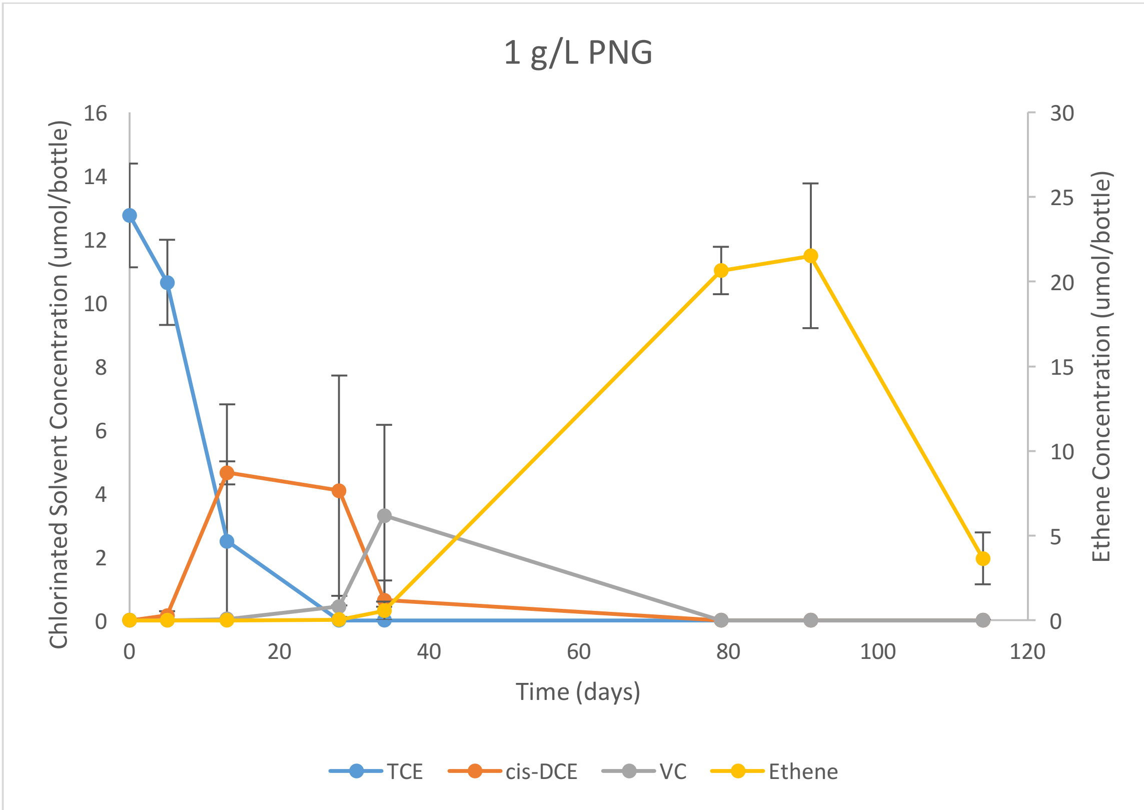

Figure B-51: TCE experiment amended with PNG. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

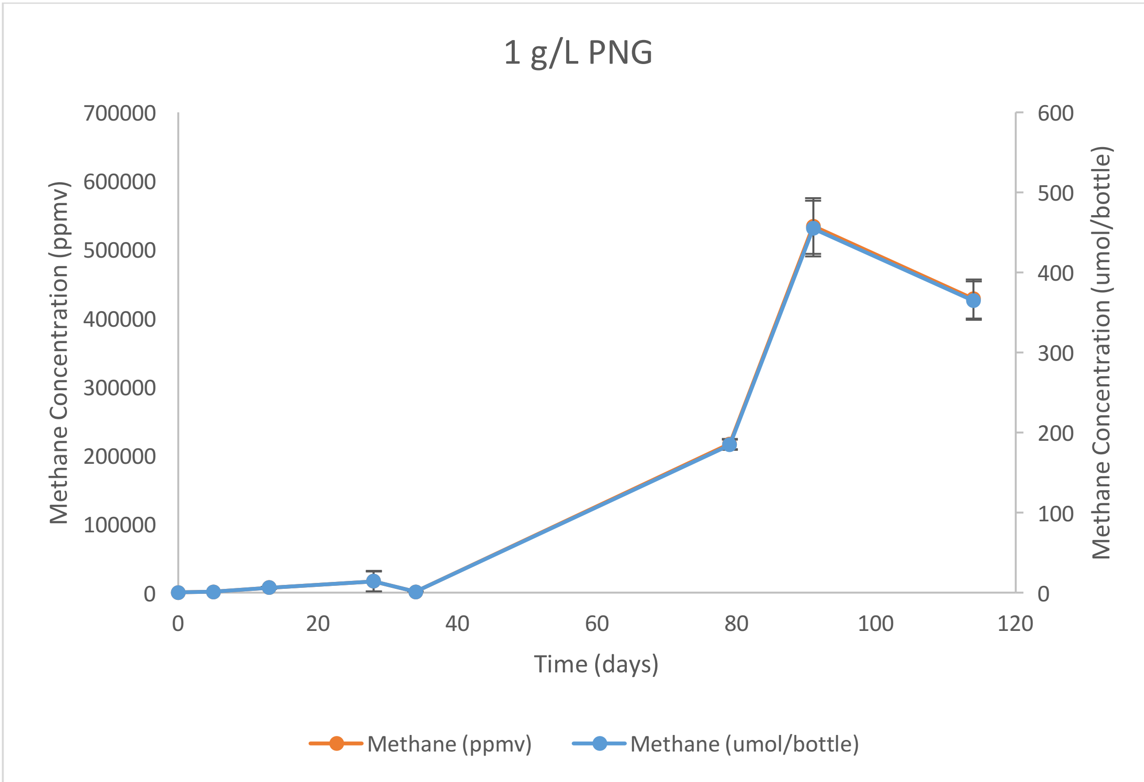

Figure B-52: TCE experiment amended with PNG. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

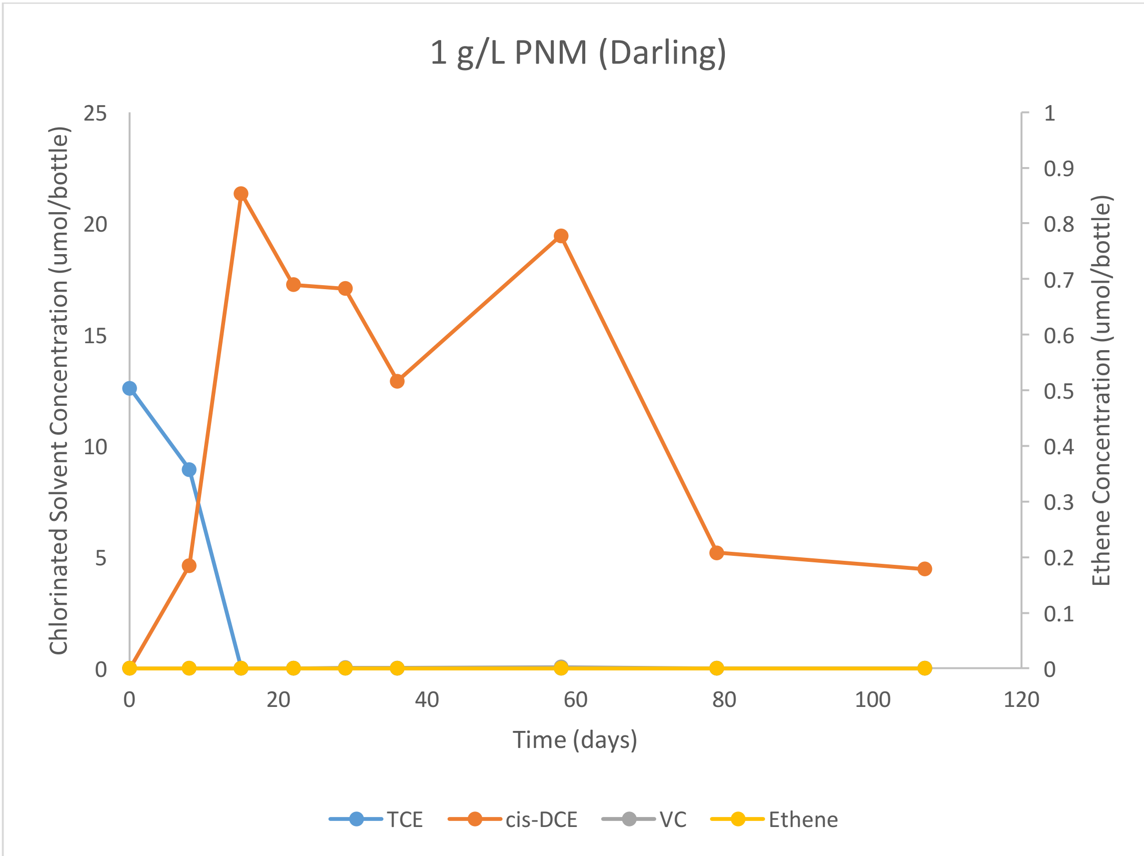

Figure B-53: TCE experiment amended with PNM (Darling). Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

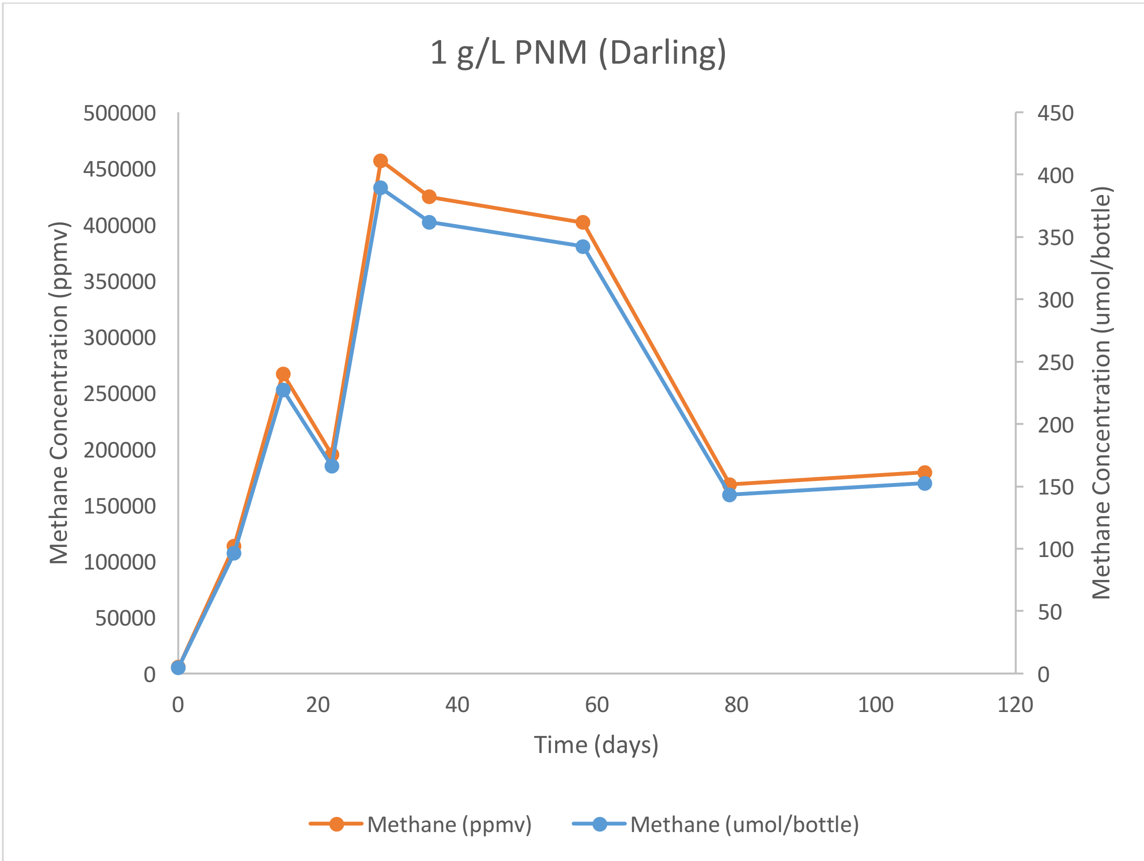

Figure B-54: TCE experiment amended with PNM (Darling). Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

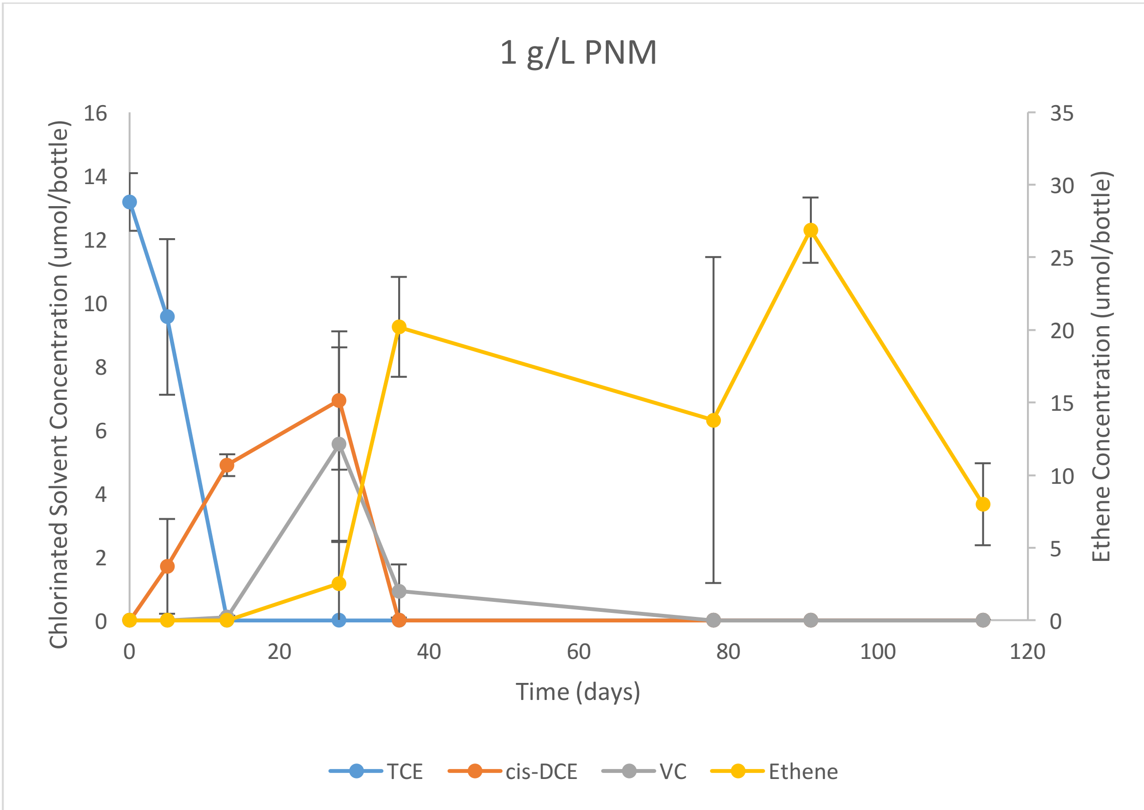

Figure B-55: TCE experiment amended with PNM. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

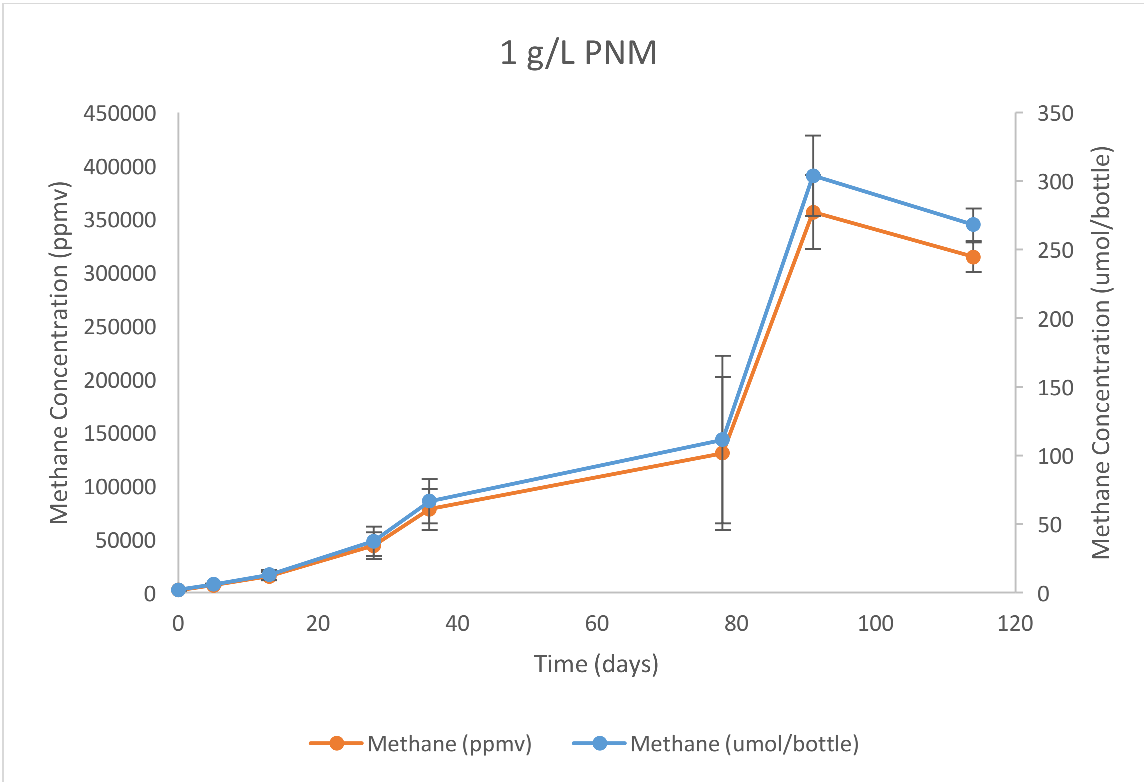

Figure B-56: TCE experiment amended with PNM. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

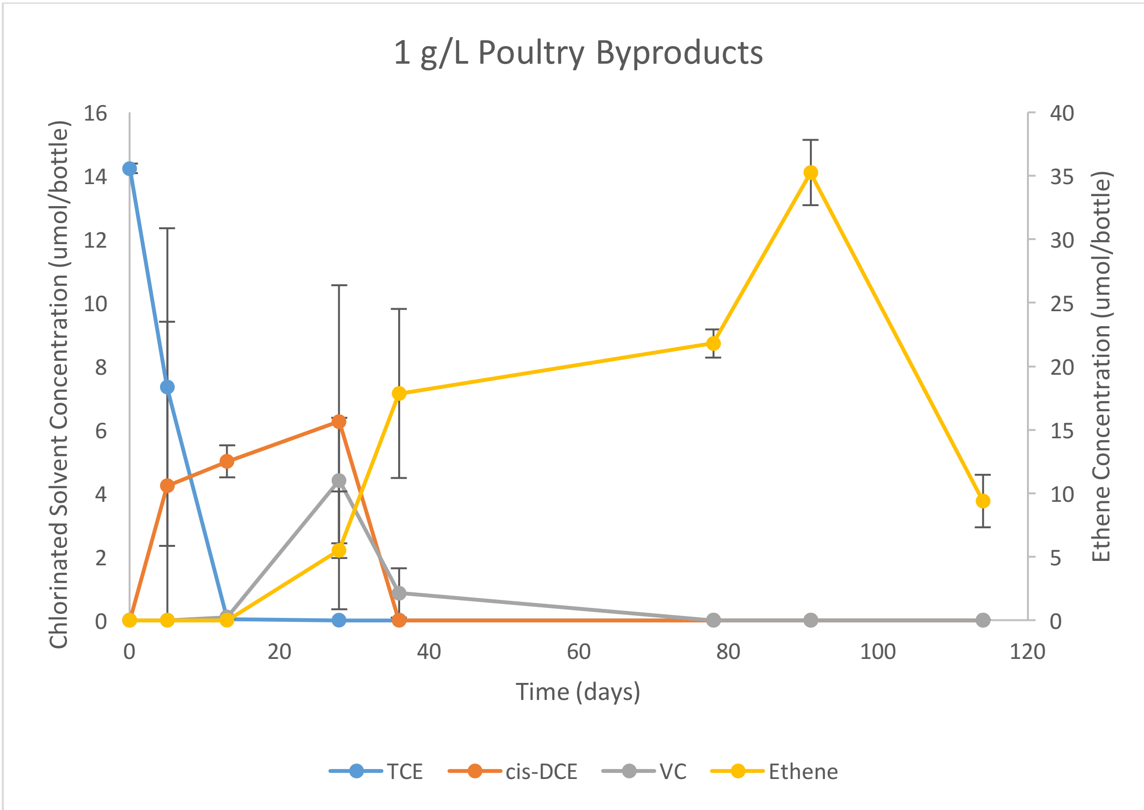

Figure B-57: TCE experiment amended with Poultry Byproducts. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

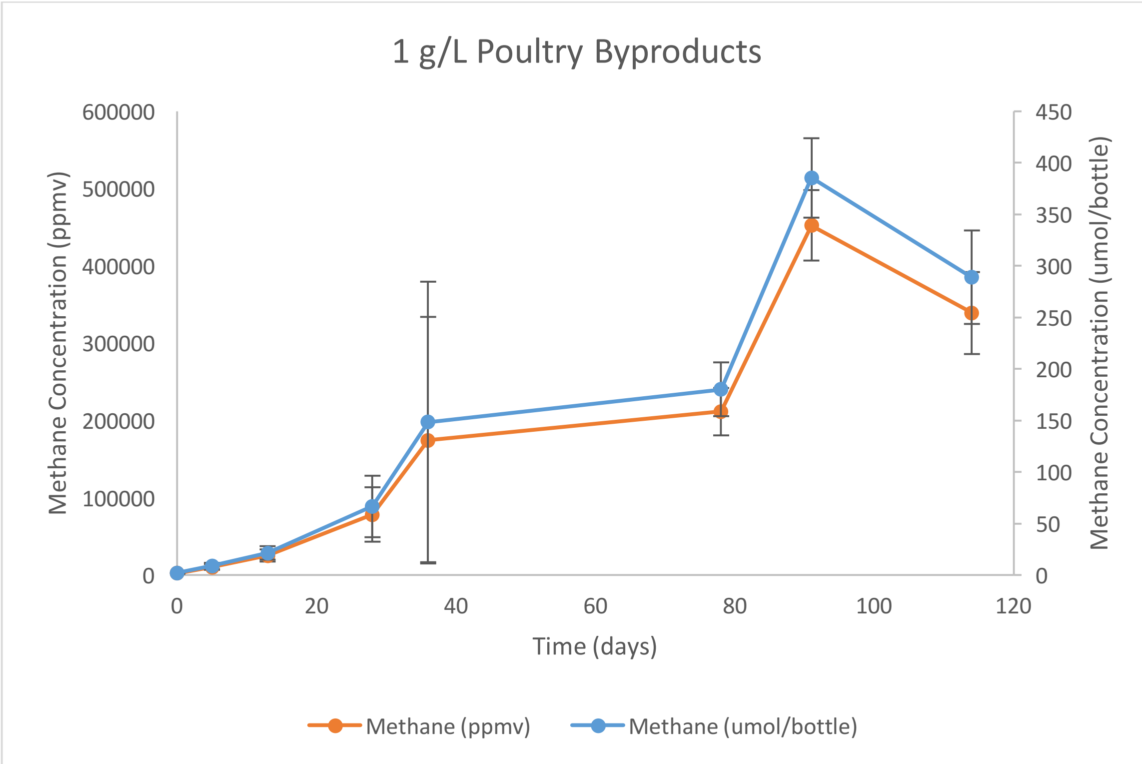

Figure B-58: TCE experiment amended with Poultry Byproducts. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

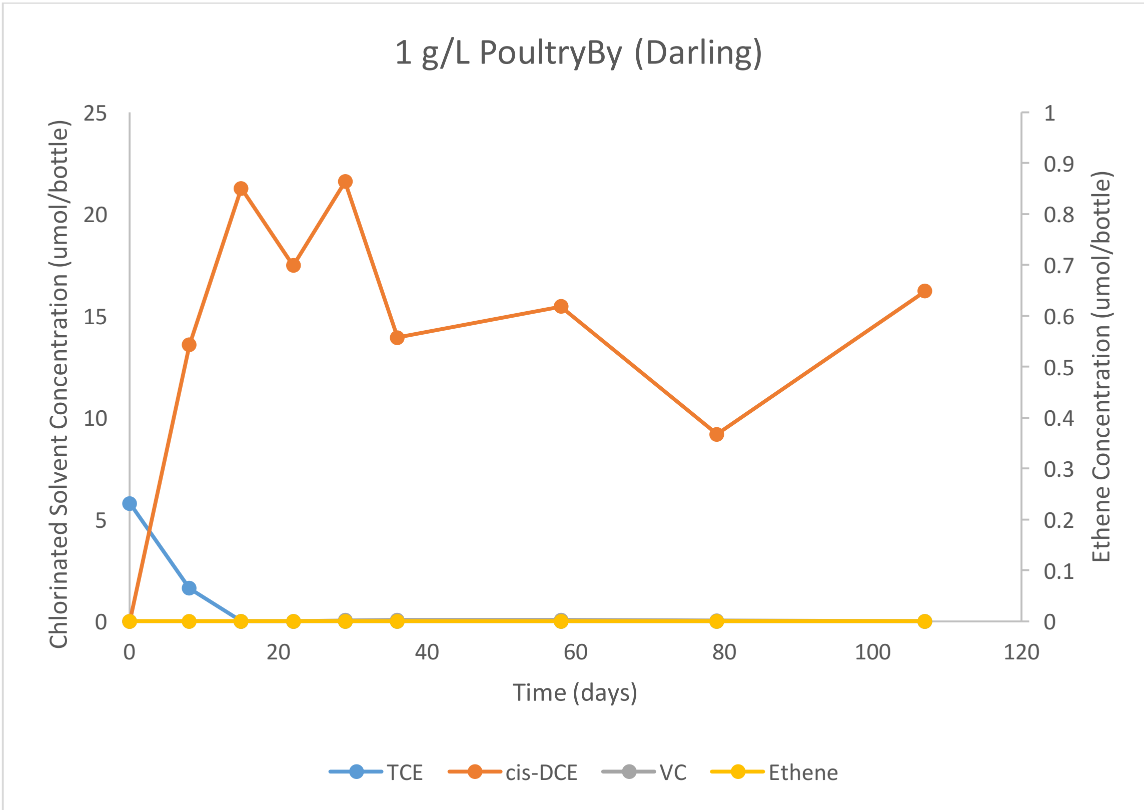

Figure B-59: TCE experiment amended with PoultryBy (Darling). Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

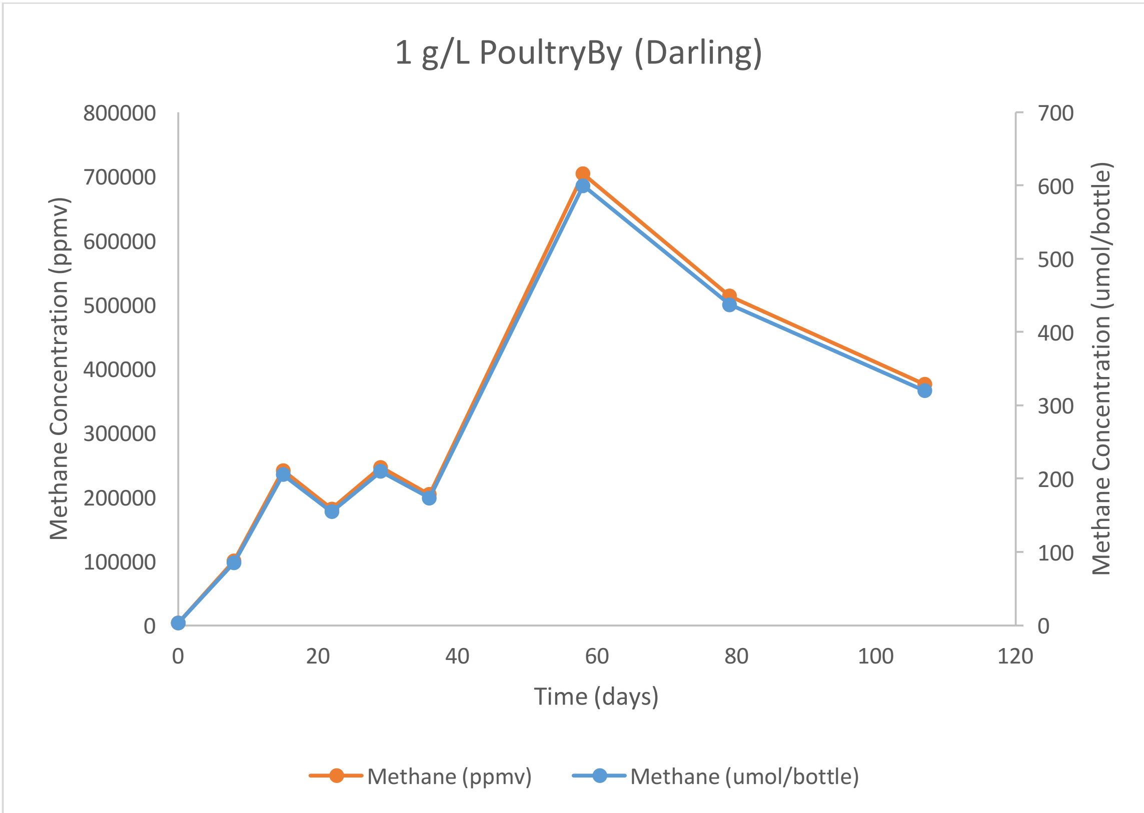

Figure B-60: TCE experiment amended with PoultryBy (Darling). Concentration of methane in μmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

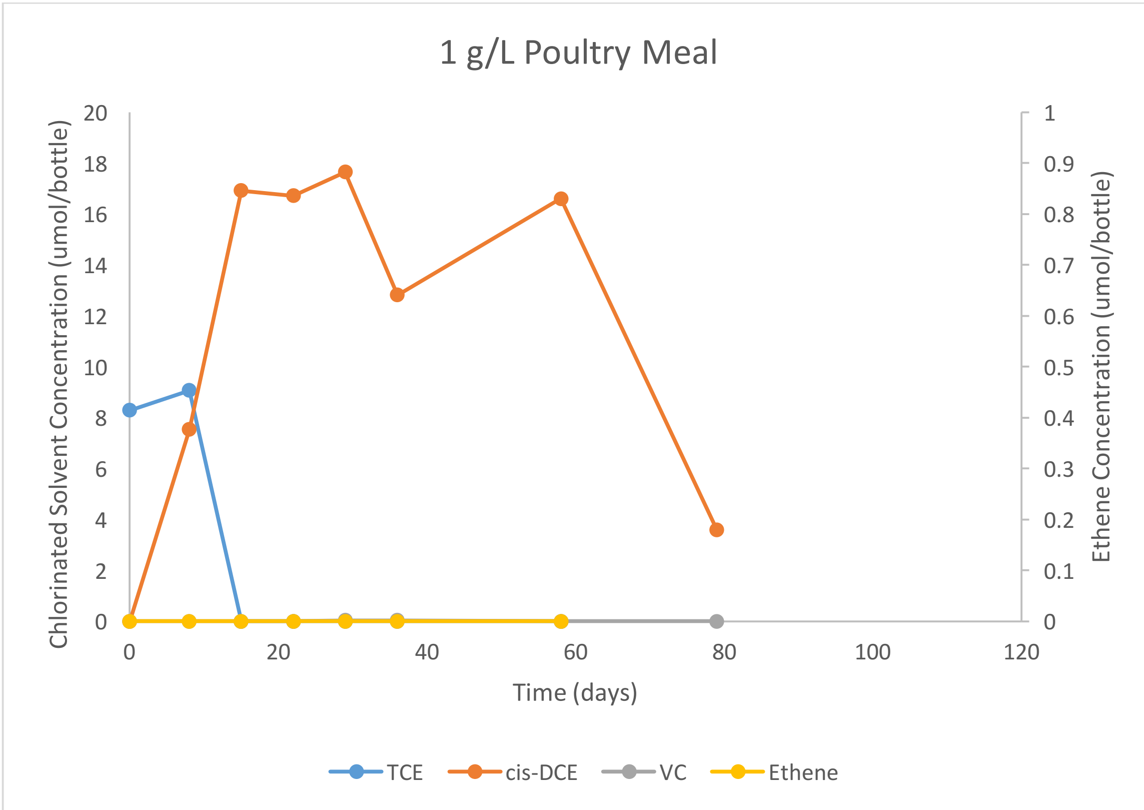

Figure B-61: TCE experiment amended with Poultry Meal. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

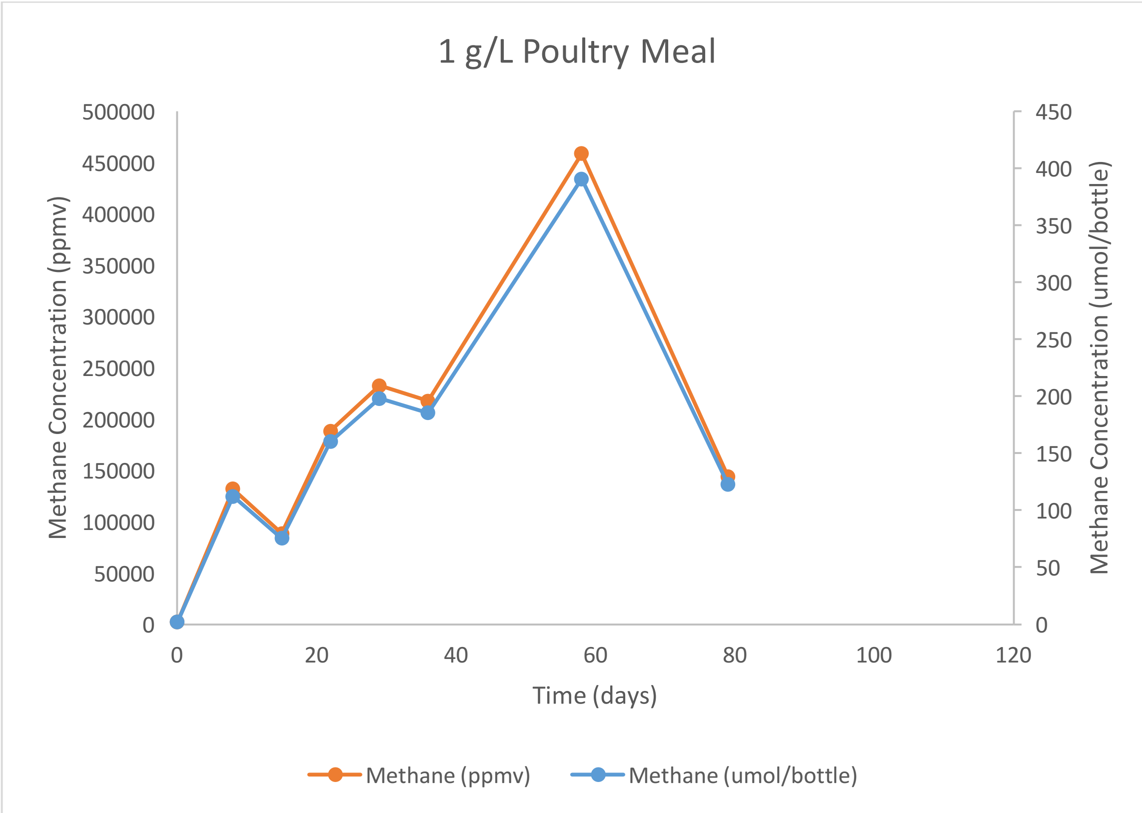

Figure B-62: TCE experiment amended with Poultry Meal. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

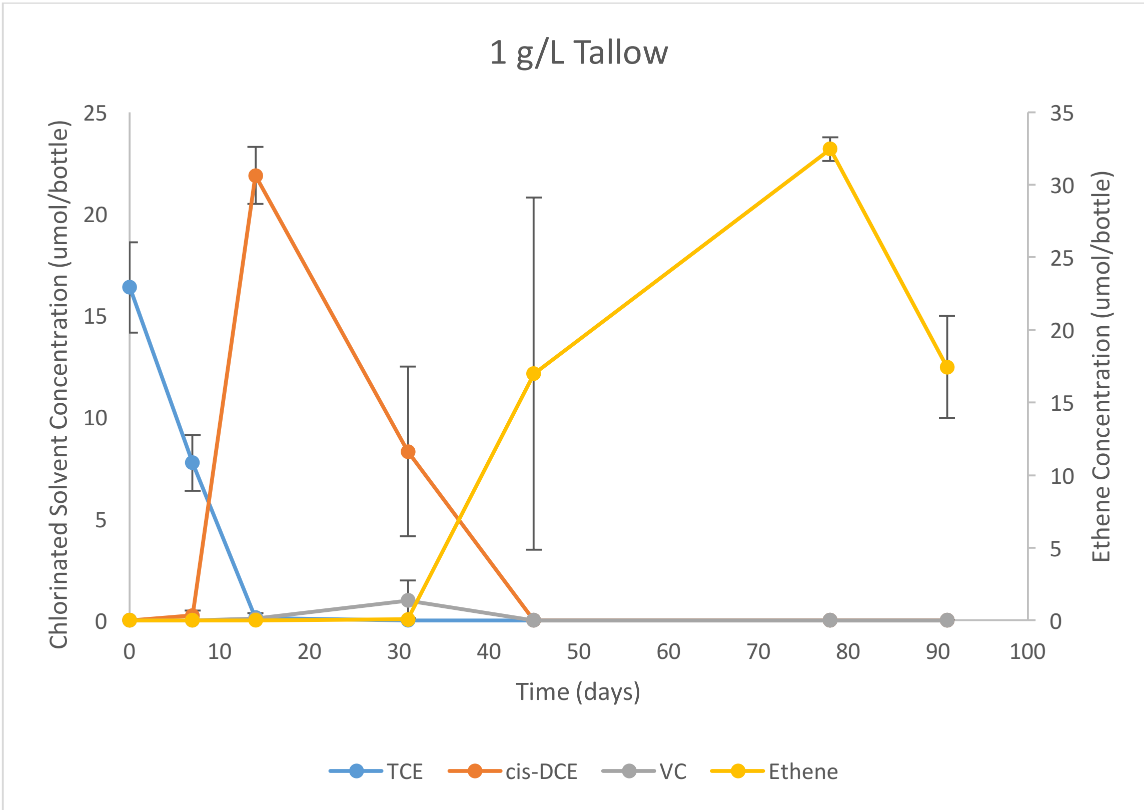

Figure B-63: TCE experiment amended with Tallow. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

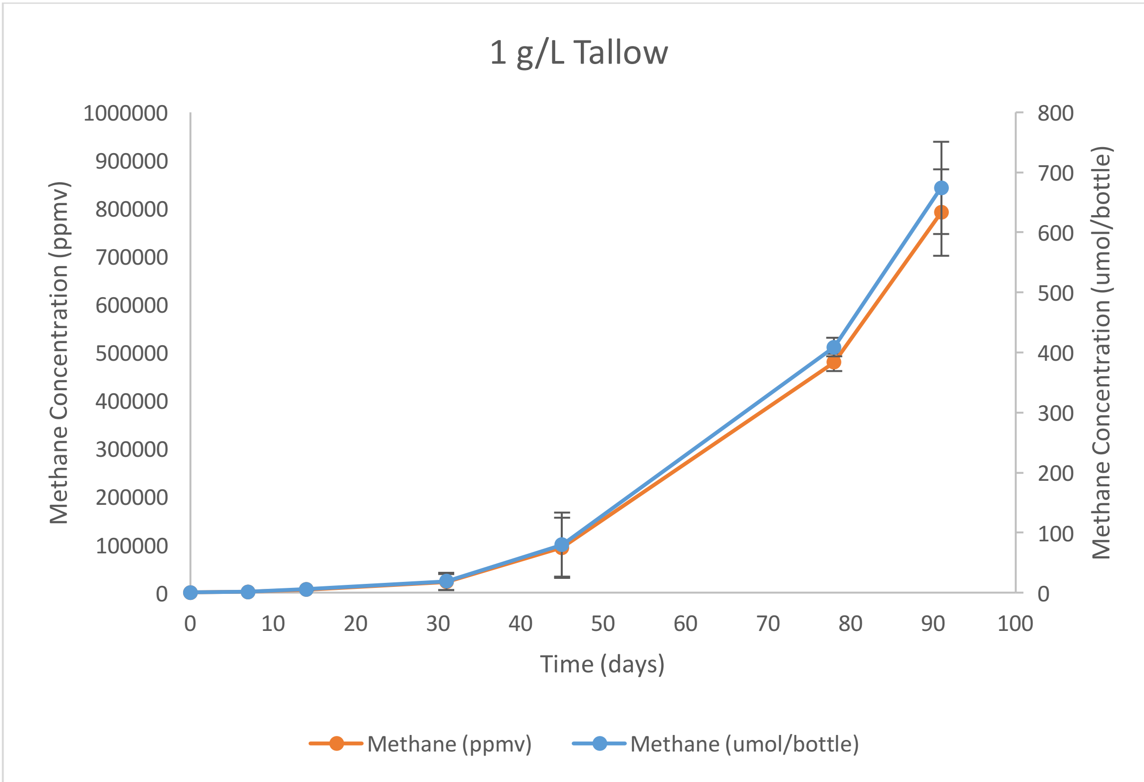

Figure B-64: TCE experiment amended with Tallow. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

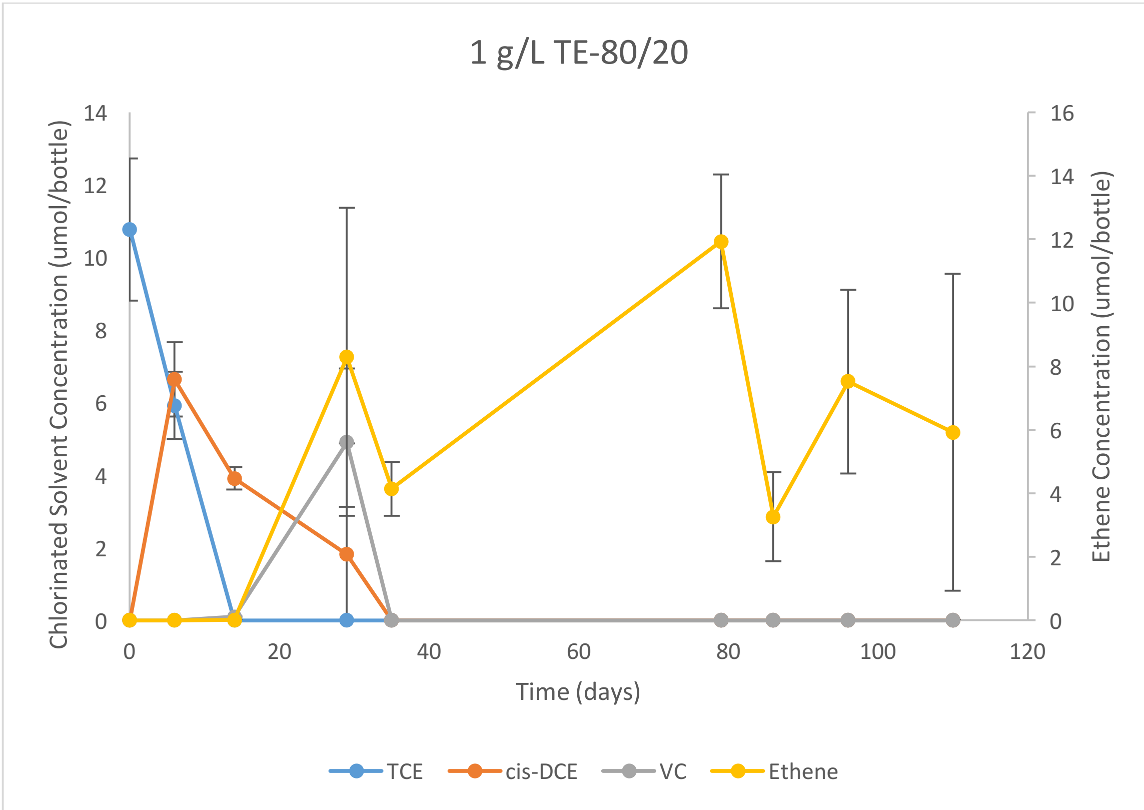

Figure B-65: TCE experiment amended with TE-80/20. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

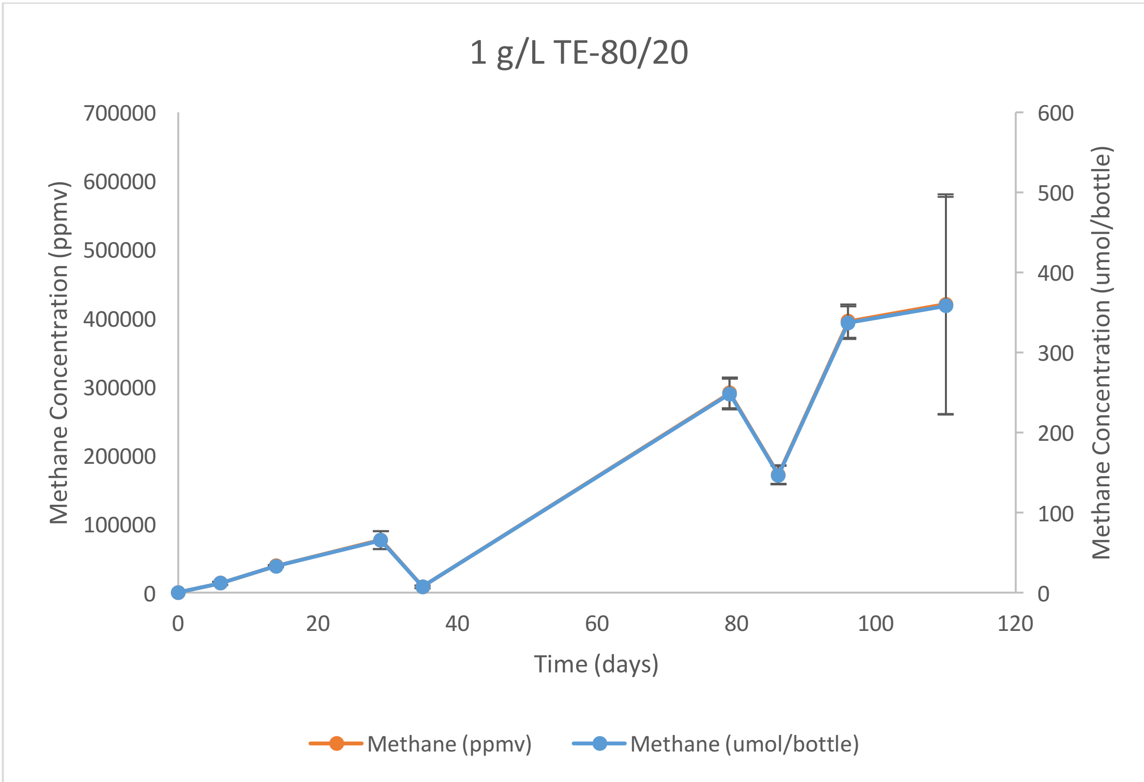

Figure B-66: TCE experiment amended with TE-80/20. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

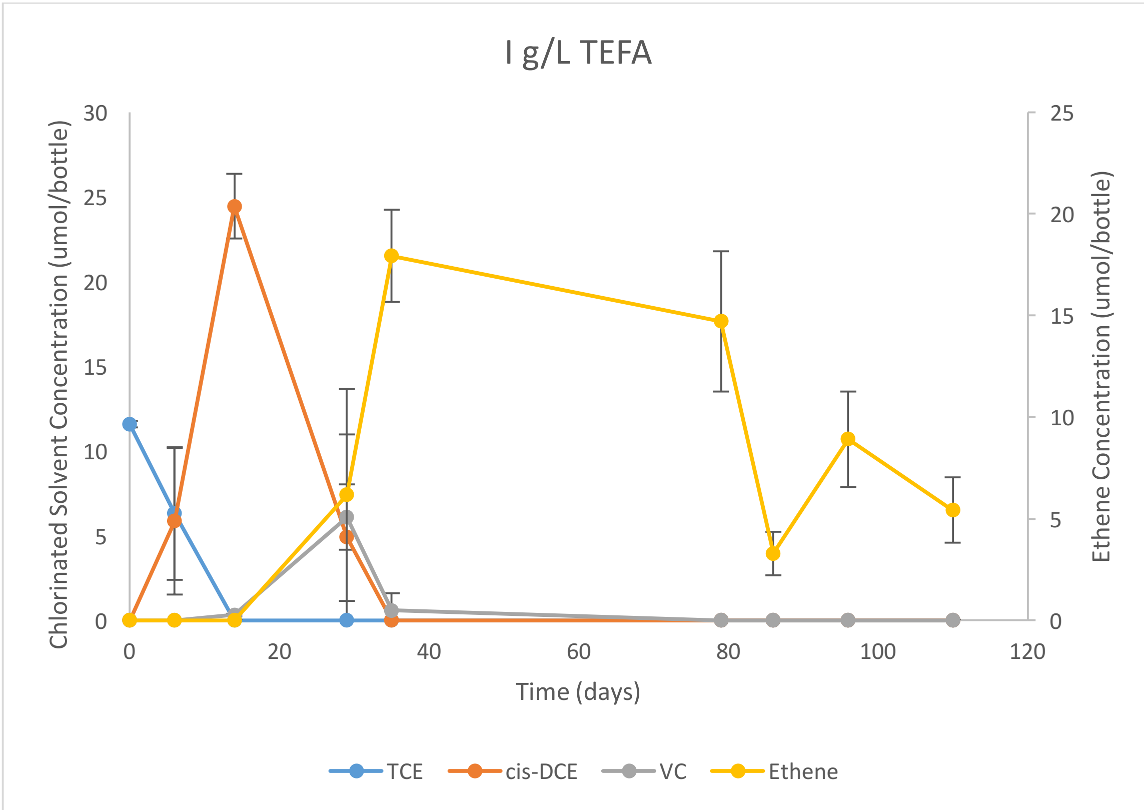

Figure B-67: TCE experiment amended with TEFA. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

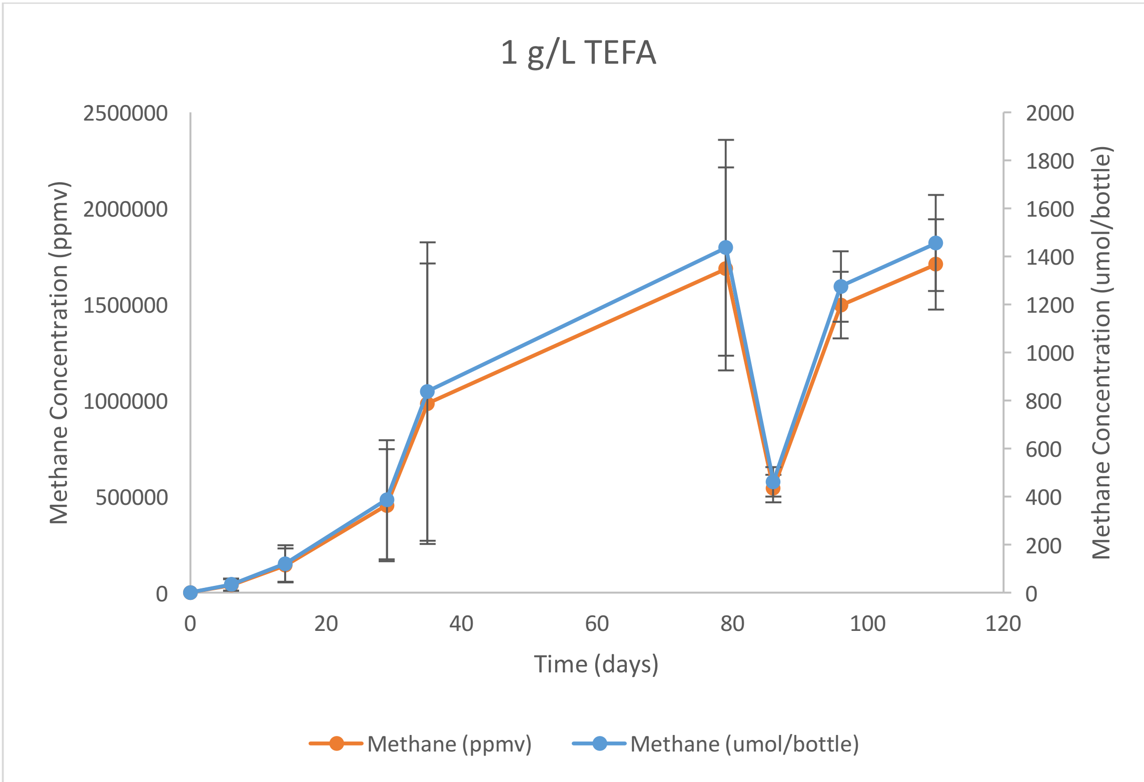

Figure B-68: TCE experiment amended with TEFA. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

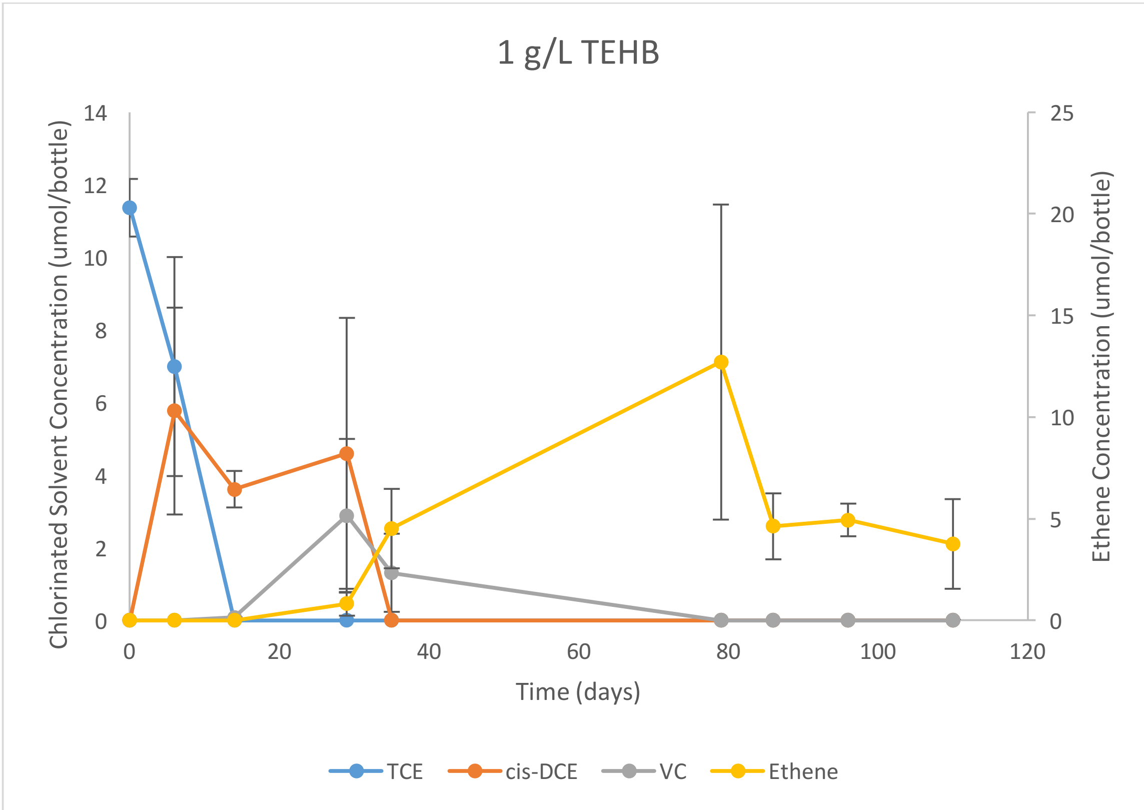

Figure B-69: TCE experiment amended with TEHB. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

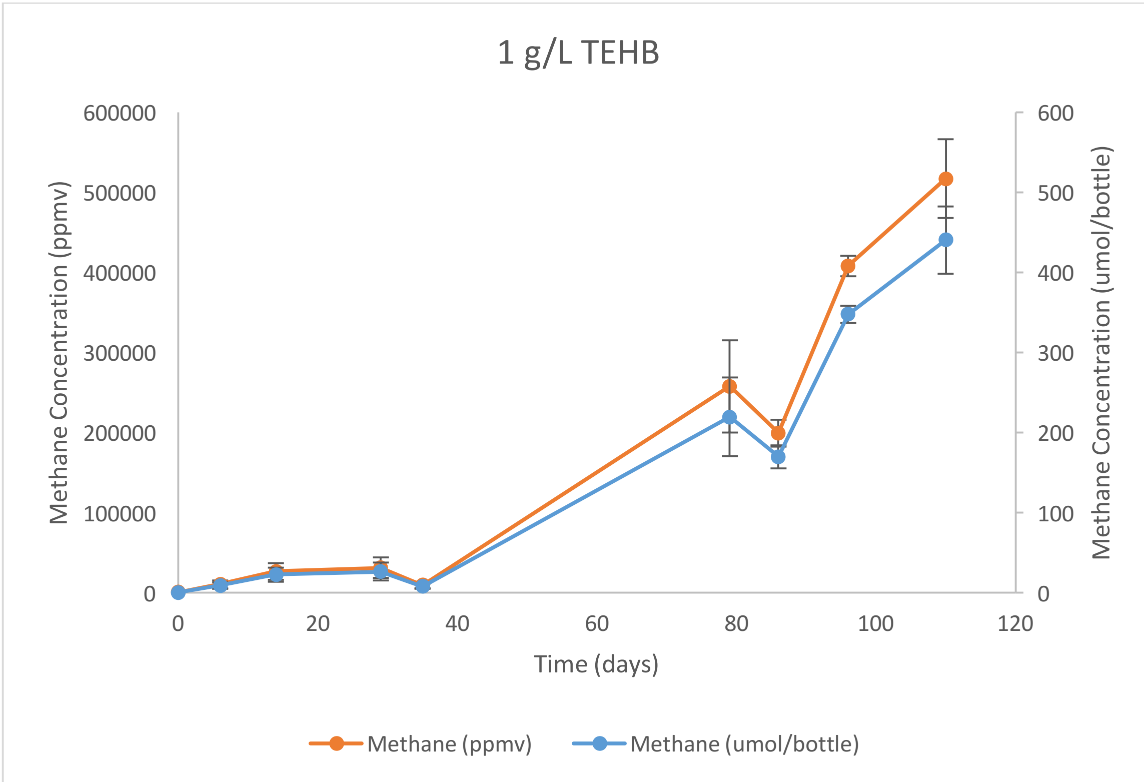

Figure B-70: TCE experiment amended with TEHB. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

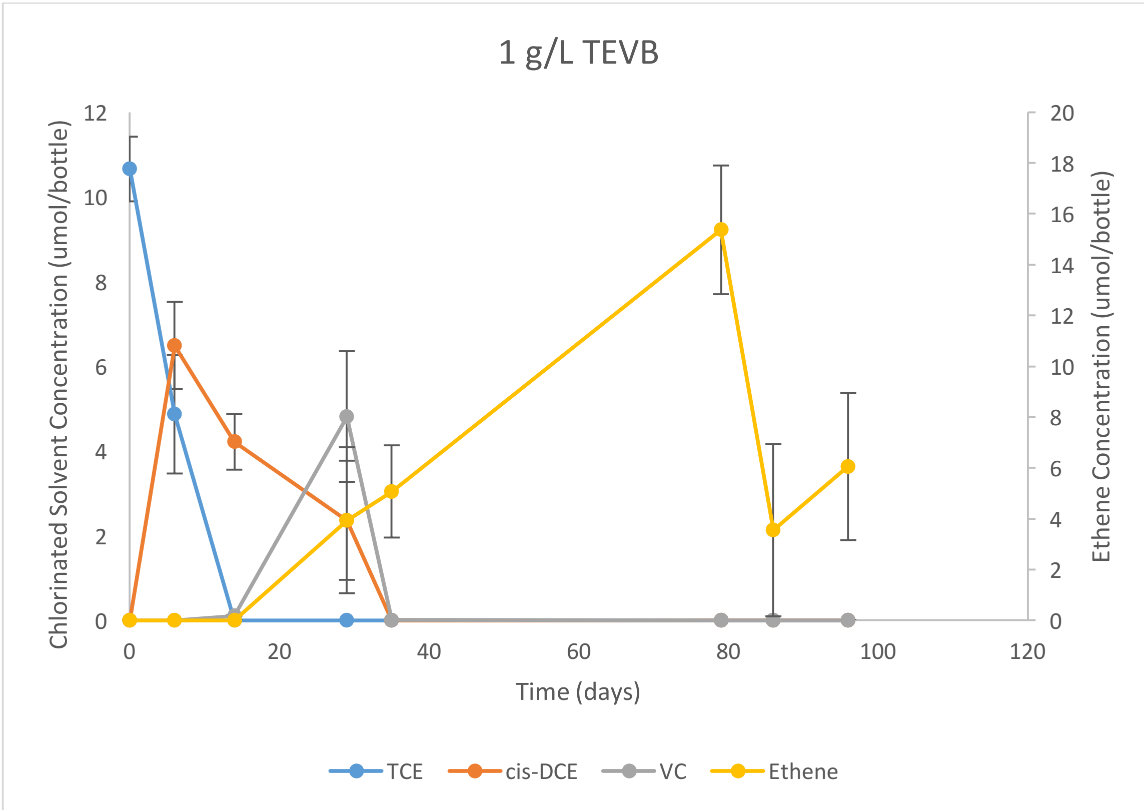

Figure B-71: TCE experiment amended with TEVB. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

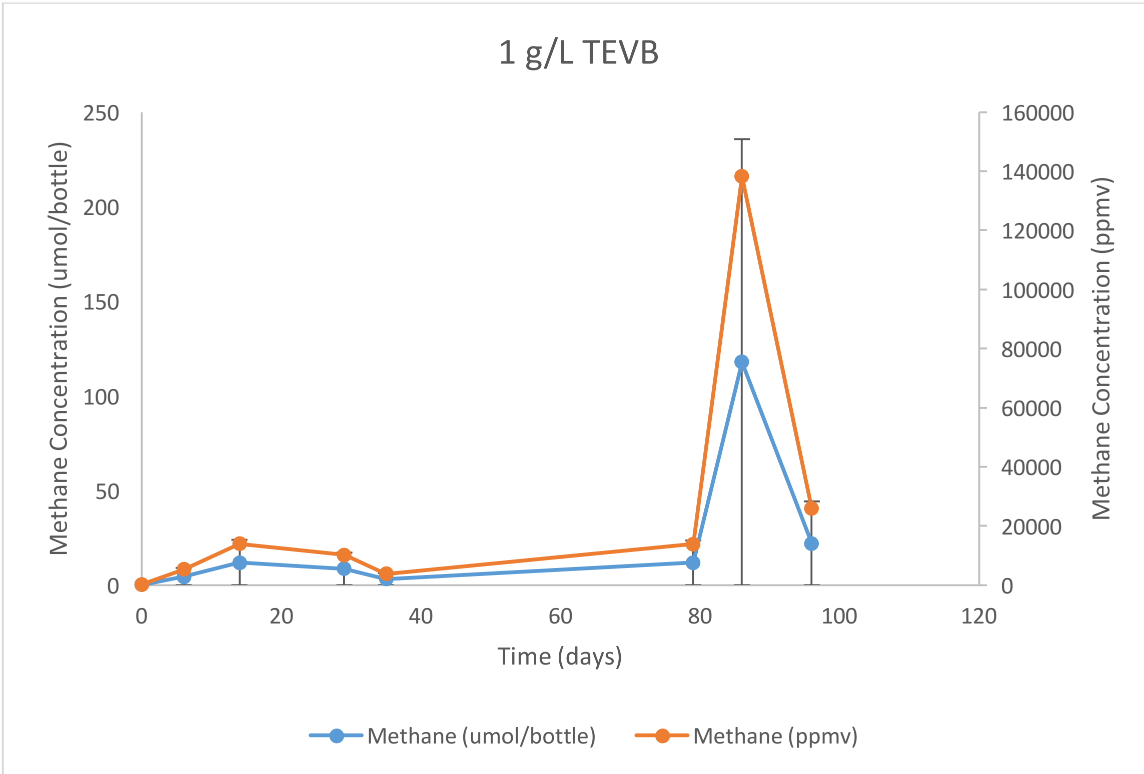

Figure B-72: TCE experiment amended with TEVB. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.

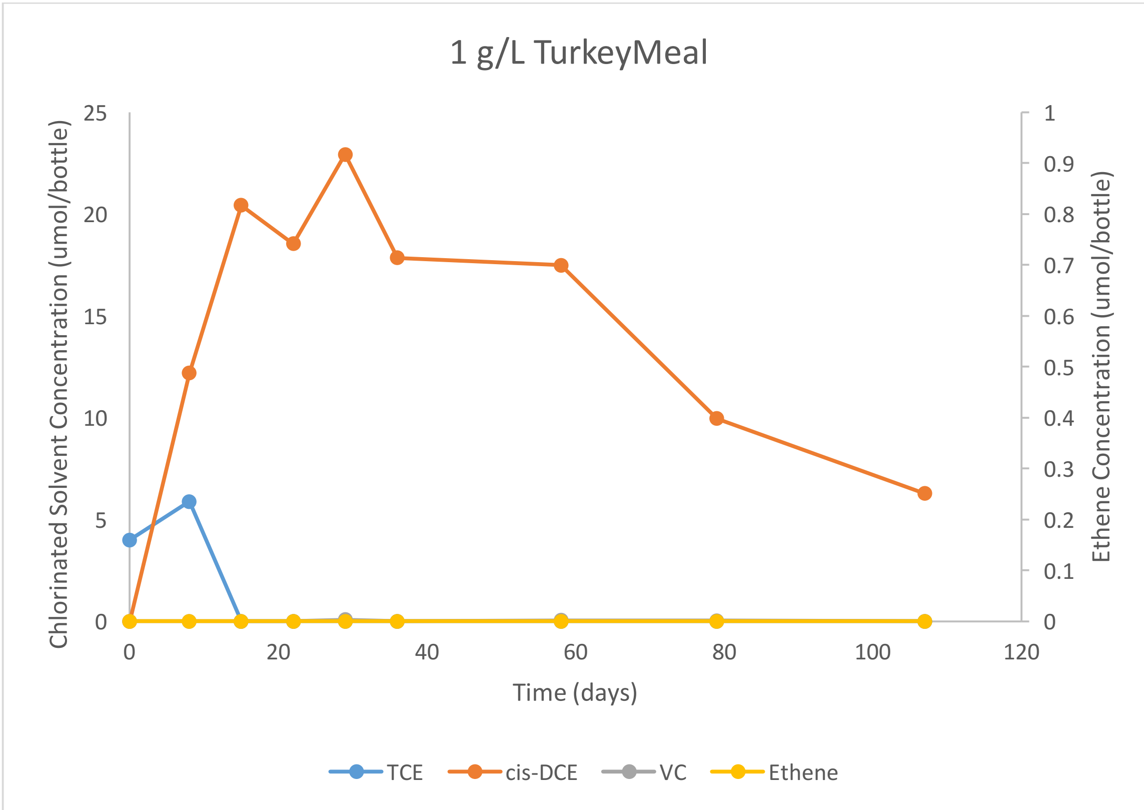

Figure B-73: TCE experiment amended with Turkey Meal. Concentration of TCE and its degradation products over time. Values represent measurements from a single bottle.

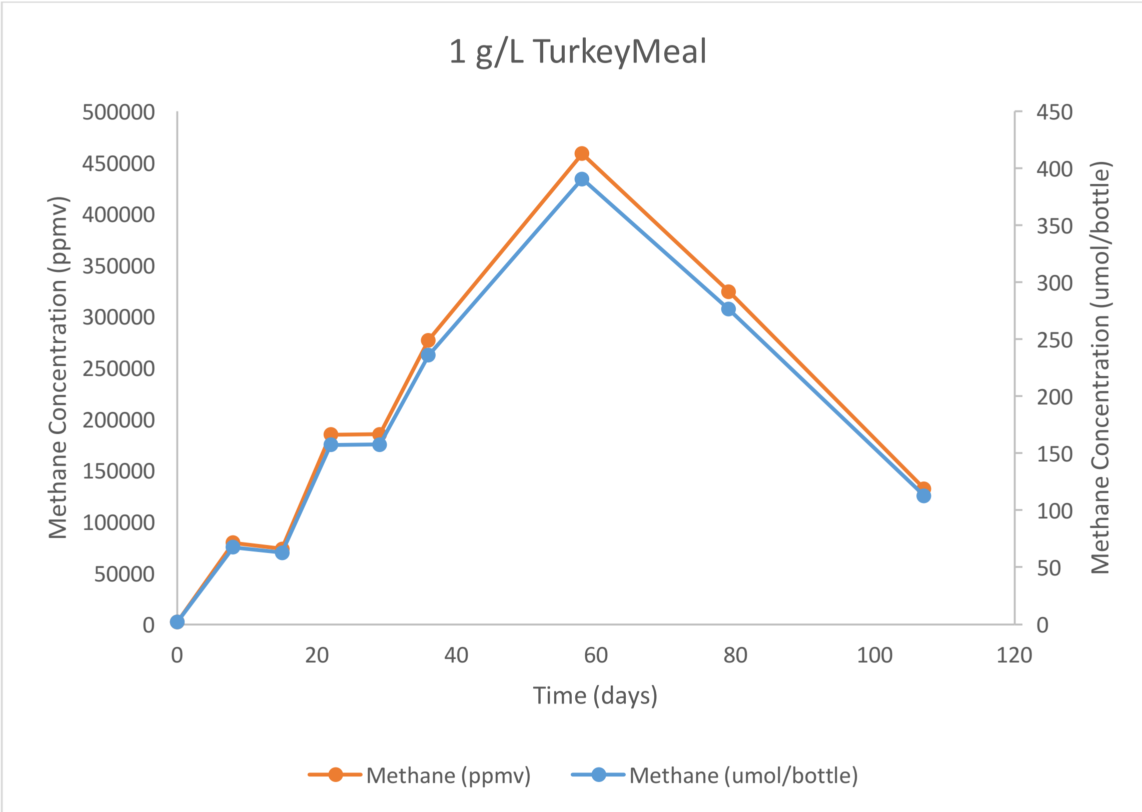

Figure B-74: TCE experiment amended with Turkey Meal. Concentration of methane in µmol/bottle and parts per million by volume. Values represent measurements from a single bottle.

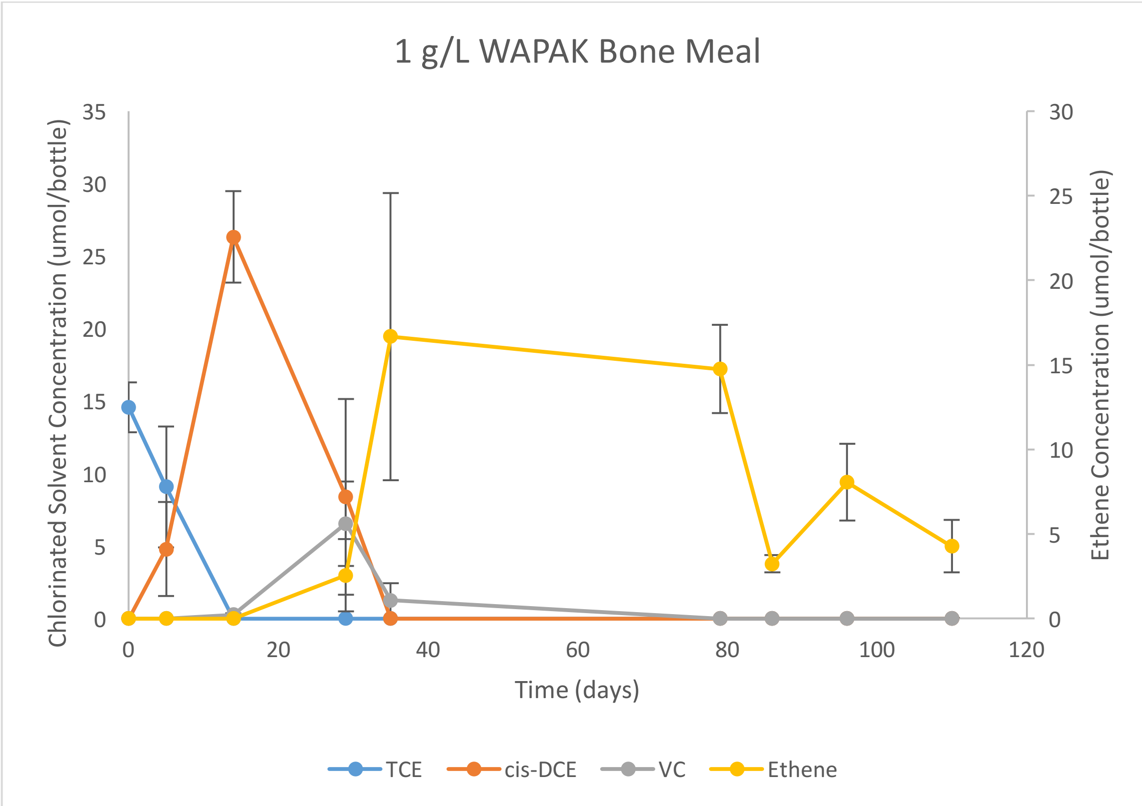

Figure B-75: TCE experiment amended with WAPAK Bone Meal. Concentration of TCE and its degradation products over time. Values are the average of 3 bottles. Error bars represent one standard deviation.

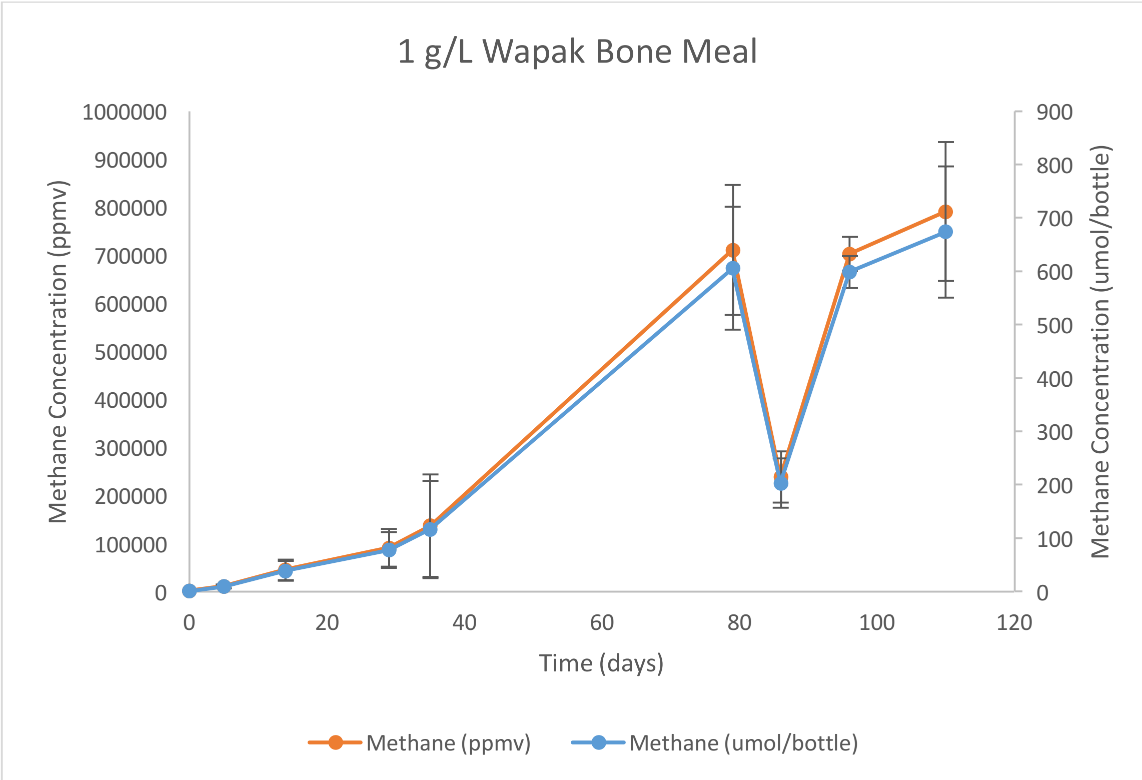

Figure B-76: TCE experiment amended with WAPAK Bone Meal. Concentration of methane in µmol/bottle and parts per million by volume. Values are the average of 3 bottles. Error bars represent one standard deviation.