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Influence of Methane Inhibitors and High Molecular Mass Electron Donors on Chlorinated Solvent Bioremediation

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INFLUENCE OF METHANE INHIBITORS AND HIGH MOLECULAR MASS ELECTRON DONORS ON CHLORINATED SOLVENT BIODEGREDATION

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ABSTRACT

Chlorinated solvent bioremediation encompasses a number of combined microbial and chemical reactions that either oxidize or reduce the contaminant(s) of concern. In the case of trichloroethylene (TCE), many approaches rely on adding electron donors to stimulate chlororespiration, in which cells gain energy to grow by sequentially reducing TCE to ethene. In the recent years, the idea that chlorinated solvents could be reduced by inhibiting methane production to stimulate dechlorination has been put into practice by vendors. The theory is that if methane production is inhibited, electrons will be redirected to chlorinated solvent reduction for complete dechlorination. However, if methanogenesis is inhibited, then microbial activity that is key in reducing chlorinated solvents may, or may not, occur.

The purpose of this research is to evaluate the influence of methane inhibitors on chlorinated solvents by using electron donors in various concentrations in order to evaluate this process. Electron donors include plant-based essential oils, lactate, and statins. The work demonstrates that inhibiting methanogenesis alone may not expedite dechlorination, and the broader impacts on the total microbial community that is central to reducing TCE to ethene are larger than just this single reaction. Additionally, using electron donor amendments at near stoichiometric concentrations will help control methanogenesis while facilitating complete dechlorination.

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1. INTRODUCTION

Chlorinated solvents have been widely manufactured for use in industrial, military, agricultural, and household settings since the beginning of the twentieth century. These chlorinated compounds were produced in such large quantities and so widely used that careless handling and ignorance to health effects and environmental dangers led to extensive groundwater contamination (IRAC 1995, Tiehm and Schmidt 2011). Corrective actions are necessary to regain control over the numerous cases of uncontrolled releases of chlorinated solvents around the world and to meet present day regulations (Löffler, Ritalahti et al. 2013). This class of solvents are a high priority for remediation to limit the adverse health effects on people and on groundwater sources.

Trichloroethylene (TCE) is a volatile organic compound, known human carcinogen (Kielhorn J 2000), and third most prevalent groundwater contaminant in the United States (Barbee 1994, Demarest 2014). The U.S. consumes upwards of 255 million pounds of TCE per year, mostly in industrial and commercial settings as solvent degreasers and dry cleaning spotting agents (EPA 1990, EPA 2014). Inhalation exposure poses the biggest risk to human health, especially in settings where TCE is vaporized and used as a spray onto clothes or machinery. Health risks from exposure to TCE and its daughter products include cancers, nervous system disorders, immune disorders, and birth defects (Duhamel, Mo et al. 2004, Ise, Suto et al. 2007). The Environmental Protection Agency estimates that 300,000 people experience health problems from exposure to TCE each year, (EPA 2014). The majority of these people do not develop cancer; there is a more likely chance that exposure will lead to chronic heart, kidney, or liver problems (Duhamel, Mo et al. 2004,

Ise, Suto et al. 2007, Demarest 2014) As TCE degrades, it loses chlorine atoms to produce dichloroethene (DCE) then vinyl chloride (VC), then ethene. VC is a known human carcinogen with a higher probability of causing cancerous diseases than TCE and is considered to be the greatest threat to human health out of all chlorinated ethenes. (Kielhorn J 2000, Bradley 2003). TCE has a maximum contaminant level (MCL) for drinking water of 5 μg/L, DCE has an MCL of 70 μg/L, and VC has a MCL of 2 μg/L (Bradley 2003, EPA 2014). The prevalence of TCE contamination in groundwater sources and the severity of human health risks has pushed TCE contaminated sites to be a priority for clean-up efforts and to be the focus of remediation techniques such as bioremediation.

Bioremediation encompasses a number of combined microbial and chemical reactions that oxidize or reduce the contaminant of concern, either ex situ or in situ (Azubuike, Chikere et al. 2016). In situ bioremediation techniques involve leaving soils and contaminants in place and putting remediation efforts into the subsurface. This is the cheaper and less invasive option. Ex situ bioremediation involves bringing contaminated soils and groundwater up to the surface and contains them in an area like a soil pile or bioreactor. Bioremediation has become an essential component of TCE remediation due to its effective, reliable nature and its eco-friendly features. It is also cost efficient; bioremediation techniques allow for the use of locally available material and require less intervention than other remediation techniques (Vidali 2009, Tiehm and Schmidt 2011, Azubuike, Chikere et al. 2016). Designing a bioremediation plan requires taking into account pollutant type, cost, location, indigenous microorganisms present, and environmental policy (Frutos, Pérez et al. 2012, Smith, Thavamani et al. 2015, Azubuike,

Chikere et al. 2016). By definition, bioremediation requires the use of microbial processes to remove hazardous pollutants through reactions that are a part of their natural metabolic processes (Vidali 2009). This microbial reductive dechlorination is a promising approach for in situ remediation. In the case of trichloroethylene (TCE), many in situ approaches rely on adding electron donors to stimulate chlororespiration, in which cells gain energy to grow by sequentially reducing TCE to ethene. Chlororespiration drives successful reductive dechlorination in anaerobic aquifer systems by using chloroethenes as electron acceptors and hydrogen as the ultimate electron donor (Löffler, Tiedje et al. 1999, Tiehm and Schmidt 2011).

Indigenous microorganisms present in anaerobic aquifer environments, such as the *Dehalococcoides* (DHC) microbe, have been shown to successfully remediate chlorinated solvents. The discovery of these microbial mechanisms has improved remediation techniques and become an essential component of chlorinated solvent remediation. Specifically, the discovery of DHC prompted the shift to think of chlorinated solvents as primary substrates for microbial metabolism (Maymó-Gatell, Chien et al. 1997, Bradley 2003). DHC is the only naturally present microbe that has shown to be consistently able to maintain growth and energy production to reductively dechlorinate TCE to ethene and are assumed to be widely distributed in anaerobic aquifers (Maymó-Gatell, Chien et al. 1997, Bradley 2003). DHC obtains energy via oxidation of hydrogen gas (a byproduct of TCE reduction) which starts the chlororespiration process and subsequently reduces TCE to ethene. (Azubuike, Chikere et al. 2016, Verma and Jaiswal 2016). This process allows DHC to achieve much higher rates of reductive dechlorination through cometabolic

reductive processes than previously thought could be achieved (Maymó-Gatell, Chien et al. 1997, Gerritse, Drzyzga et al. 1999, Bradley 2003). Incomplete or inefficient reductive dechlorination can be attributed to a lack of sufficient biomass of DHC. Typically, electron donors such as lactate, acetate, or hydrogen are added to the contaminated site to ensure that DHC have enough biomass and energy to continue chlororespiration to completely dechlorinate TCE. Previous studies have shown that acetate, glucose, lactate, hydrogen, and methanol are suitable electron donors (Vogel and McCarthy 1985, Freedman and Gossett 1989, Sewell and Gibson 1991, Wei and Finneran 2013) The combination of DHC and electron donors such as those listed previously have been shown to be successful at remediating TCE and have been widely used in the last couple of decades.

In recent years, the idea that TCE could be reduced by inhibiting methane production to stimulate dechlorination has been put into practice by vendors. The theory is that if methane production is inhibited, electrons will be redirected to chlorinated solvent reduction for complete dechlorination. This is a direct misinterpretation of previous work done by Wei and Finneran (2011, 2013). This theory has gained ground within the industry, despite the fact that a specific pathway for linking complete dechlorination to methanogenic conditions has not been identified and previous studies suggest that the two processes may not be linked (DiStefano, Gossett et al. 1991, Ballapragada, Stensel et al. 1997, Kromann, Ludvigsen et al. 1998, Leahy and Shreve 2000, Duhamel, Mo et al. 2004, Wei and Finneran 2013). If methanogenesis is inhibited, then microbial activity that is key in reducing chlorinated solvents may, or may not, occur. Additionally, the practice of adding an excess of electron donor does not guarantee that it will stimulate faster

dechlorination. In fact, this can promote the growth of methanogens by suppressing DHC metabolic activity and diversity (Doong, Wu et al. 1996, Cabirol, Jacob et al. 1998, Cabirol, Jacob et al. 1998, Kennes, Veiga et al. 1998, Chapelle and Bradley 2000, Skubal, Barcelona et al. 2001, Roling and van Verseveld 2002, Mulligan and Yong 2004, Bennett, Gandhi et al. 2007, Rivett and Thornton 2008, Illman and Alvarez 2009, Perelo 2010). The influence of these widely marketed methane inhibitors on chlorinated solvents has not been thoroughly evaluated and there is no evidence that the vendor products work as marketed. Vendors and consultants will benefit from this data because these products are being marketed with broad claims that suggest that one or two amendments will solve multiple issues such as TCE remediation and controlled methane production. There are also products that sound very similar, such as ELS and EOS. Both are emulsified vegetable oil products and it could easily be assumed that both perform similarly.

The broad objective of this project is to demonstrate that adding high molecular mass electron donors and anti-methanogenic reagents at near stoichiometric concentrations to chlorinated solvent contaminated medium will help control methanogenesis while facilitating complete dechlorination. The broader impacts on the microbial community will also be evaluated, with the aim to demonstrate that inhibiting methanogenesis alone may not expedite dechlorination, and the impacts to the microbial community that are central to reducing TCE are larger than this single reaction. Two more specific objectives include quantifying the percent statin content present within the Red Yeast Rice amendment and evaluating the effectiveness of three of the more popular anti-methanogenic reagents to prevent methanogenesis at various pH levels. Evaluating the capacity of multiple electron

donors and methane inhibitors will allow for remediation scientists to make better informed decisions about the remediation strategies that they employ, as well as utilize optimal concentrations to stimulate dechlorination without promoting excess methanogenesis.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Sediment and groundwater were collected from a contaminated industrial site in South Carolina. All aquifer material was collected from below the water table and kept under anaerobic conditions during collection and transport. Aquifer material was stored in a dark, 30°C incubator until the time of experimental set up.

2.2 BATCH STUDY

A total of eleven electron donors and four anti-methanogenic reagents (AMR) will be tested in batch incubations at varying concentrations and combinations. Electron donors include: lactate, ELS Microemulsion, EHC, BOS100, bone meal, rendered chicken fat, ethyl propionate, BES, Provect-IR, EZVI and EOS. AMRs include Red Yeast Rice (RYR), medical grade lovastatin, natural garlic oil (GO#1) and synthetic garlic oil (GO#2). AMR amended series will be evaluated with and without electron donors at multiple concentrations. Controls with no amendments will also be included. Batch incubation experiments were set up in duplicate.

Aquifer material for all sediment batch experiments came from the same site and was homogenized prior to experiment preparation. Each 125 mL serum bottle contains 50

grams of sediment, with an assumed porosity of 0.3, and 30mL of groundwater. Then, each bottle was sealed with a butyl stopper and flushed with an 80:20 nitrogen and carbon dioxide mix that flowed through a heated copper column to ensure anaerobic conditions (Wei and Finneran 2009, Wei and Finneran 2011, Wei and Finneran 2013). Electron donors, AMRs, and approximately 20-25μmol of neat TCE was added via syringe and needle. Bottles were kept in a room temperature, dark incubator throughout the duration of the experiment. A complete experimental list including electron donor and AMR combinations and concentrations is included in Appendix A, Table A.1. The table is sorted by AMR.

A Shimadzu 2014 gas chromatograph with flame ionization detector (GC-FID) and a 30m by 0.53mm GS-Q Plot column was used to quantify the mass of TCE and dechlorination daughter products in micromoles per bottle. A sample of 0.2mL of headspace will be removed with an airtight gas syringe and injected into the GC. The sample analysis program on the GC has a column flow of 20 mL/minute with a total flow of 50 mL/minute and 92kPa of pressure. The temperature ramp begins at 40°C and holds there for 1.5 minutes. Then, the temperature rises to 200°C at a rate of 40°C every two minutes until 200°C is reached. Once 200°C is reached, it is held for 2 minutes. The total program run time is 7.50 minutes. The injector temperature is held constant at 200°C and the detector temperature is held at 300°C.

Standard curves were made using known concentrations of TCE and its daughter products. Standard curves are generated each time GC-FID sampling occurs. These standard curves are used to create a linear equation (ex: y=mx+b), where the peak area

generated from the chromatograph is substituted for x to convert the peak area to micromoles per bottle. Methane values are recorded in parts per million by volume as well. These values are converted from micromoles per bottle to parts per million by volume via the following equation: (μmol/bottle*0.46*0.08205*298)/0.02. The partitioning coefficient, 0.46, is an estimate and is theoretical ((Lomond and Tong 2011)). The actual experimental values may exceed theoretical maximums. The μmol/bottle value is the most important value when discerning whether methane levels are appropriate or not. This sampling procedure occurs once a week beginning twenty-four hours after the addition of neat TCE to the experimental bottles. The experiments will be tracked until complete dechlorination to ethene is achieved.

2.3 PERCENT STATIN ANALYSIS

The red yeast rice (RYR) compound was analyzed for percent statin content using a ThermoFisher Scientific Ultimate 3000 high-performance liquid chromatograph (HPLC). The red yeast rice dissolution utilizes heat and pH level to break down a sometimes stubborn compound ((Chen, Yang et al. 2013)). RYR was dissolved in solutions that contain either 1% or 10% sodium lauryl sulfate (SLS) and 100 mg/L of RYR at either a neutral pH level or at a low pH level. The solution is heated until it is steaming then the SLS and red yeast rice are added. The solution is allowed to cool and is continually stirred. After the solution cools to room temperature, the pH is adjusted with 1 Molar hydrochloric acid. The solution is filtered with a 2μm filter and bottled prior to HPLC analysis. The deionized water and methanol used as effluent are sonicated for twenty minutes each to

degas before each HPLC analysis. A standard curve was generated using known concentrations of the medical grade lovastatin each time sampling occurred. The HPLC program uses 50% degassed, deionized water and 50% HPLC-grade methanol flushed through the column at a rate of 2 mL/minute with a maximum pressure of 5500 psi. The standard curve data points are used to create a liner equation that is used to substitute the HPLC peak area for X to convert to mg/L. A detailed table of all HPLC experiments conducted is in included in Appendix A, Table A.2.

2.4 pH ADJUSTMENT EXPERIMENTS

The pH adjustment experiments are based on a methane culture taken from the aquifer material experiments in batch two, specifically experiment numbers 241 and 242. These experiments were chosen because they had the largest methane production out of the series of batch experiments. The methane cultures were grown in a freshwater media (Lovley, Giovannoni et al. 1993), prepared as previously described (Wei and Finneran 2009).

Freshwater media was dispensed into two 125mL serum bottles and sealed with a butyl stopper, then flushed with an 80:20 nitrogen and carbon dioxide mix for 20 minutes to ensure anaerobic conditions. Then, the media bottles were autoclaved for 30 minutes. After cooling to room temperature, 1mL of aquifer material was removed from each experiment and added to the freshwater media along with 1mL 40mM lactate via syringe and needle. The two bottles were stored in a 30°C incubator for two weeks to allow for culture growth.

Experimental bottles contained no amendments, 250 mg/L Garlic Oil #1, 250 mg/L Garlic Oil #2, or 300 mg/L RYR. Each amendment was evaluated at pH 2, 4, and 7 for a total of 36 experimental bottles. Experimental bottles contain 96mL of freshwater media prepared as described above for pH 7 experiments. For pH 2 and pH 4 experiments, the freshwater media was prepared with 10mM citrate buffer instead of 10mM bicarbonate buffer. RYR was added to applicable experiments, then each bottle was sealed with a butyl stopper and flushed with nitrogen that flowed through a heated copper column for 20 minutes to ensure anaerobic conditions. Media bottles were autoclaved for 20 minutes then allowed to cool to room temperature. Both types of garlic oil amendments were added via syringe and needle. Next, 1mL of 40mM lactate and 3mL of methane culture were added to each bottle with syringe and needle. Bottles were allowed to equilibrate for 24 hours, then tested on the GC-FID using the same program and method as described for the Batch Incubations. Data was recorded in μmol/bottle and parts per million by volume using the same standard curve and conversion equations described above. Sampling occurred once a week for 5 weeks. An experimental list is included in Appendix A, Table A.3.

3. RESULTS AND DISCUSSION

3.1 METHANE PRODUCTION: HOW ELECTRON DONORS INCREASE METHANE

Data indicate that most experiments that do not have added AMR have methane issues, especially if complete dechlorination to ethene is achieved by week 15. One possible explanation for this is that as *Dehalococcoides* reduce TCE to ethene, hydrogen is produced as a byproduct. Methanogens use this hydrogen to produce methane and this

methanogenesis occurs after all dechlorinating processes have taken place because methanogenesis is a terminal electron accepting process in anaerobic aquifers(Lovley and Goodwin 1988). For the purposes of this project, the target methane level is at or below 20 μmol/bottle, or approximately 12,000 PPMV.

In the control experiment with no amendments (Figures B.1, B.2), dechlorination was not achieved within 20 weeks and cis-DCE was still present. Ethene and methane levels were low. Lactate (Figures B.15, B.16), a common electron donor and proven to facilitate dechlorination (Gerritse, Drzyzga et al. 1999, He, Ritalahti et al. 2003), performed as expected. Data indicate that Ethyl Propionate (EP) amended experiments (Figures B.3, B.4) facilitated complete dechlorination to ethene, however there was a large spike in methane level after TCE was removed. The data suggests that EP is a powerful driver of dechlorination, achieving complete dechlorination to ethene in between eight and ten weeks. However, methane levels were consistently above 1,000 μmol/bottle which is extremely high. The same trend was seen in experiments containing Provect-IR and EP (Figures B.9, B.10), EZVI and EP (Figures B.13, B.14), lactate and EP (Figures B.17, B.18), ELS and EP (Figures B.23, B.24), EOS and EP (Figures B.29, B.30), EHC and EP (Figures B.35, B.36), and BOS100 and EP (Figures B.39, B.40).

Carbon-based, zero valent iron (ZVI) amendments do not consistently facilitate complete dechlorination. The data showed that BES (Figures B.5, B.6), Provect-IR (Figures B.7, B.8), EHC (Figures B.33, B.34), and BOS100 (Figures B.37, B.38) amended experiments all contained levels of cis-DCE and VC above 5 μmol/bottle after 20 weeks with little, if any, ethene production. Methane concentrations remained low, but this is

expected since dechlorination was not complete. However, data indicate that EZVI (Figures B.11, B.12) amended experiments did achieve complete dechlorination. BES and EHC amended experiments were tested with other electron donors such as EP, lactate, EOS, and ELS (Figures B.19, B.20, B.25, B.26, B.31, B.32) the data shows that they had mixed results. EHC and EP amended experimental data show complete dechlorination, but methane values were over 100 μmol/bottle. This is the only EHC amended experiment without any AMR to completely dechlorinate. Overall, the only ZVI experiment that could be considered successful based on the data presented is EZVI.

ELS and EOS are two emulsified vegetable oil products intended serve as electron donors to facilitate dechlorination. ELS stands for emulsified lecithin substrate and is an emulsion of fats and oils. EOS stands for emulsified oil substrate and is soybean oil blended with vitamins. The data shows that ELS amended experiments (Figures B.21, B.22) do not reach dechlorination. TCE remains present, though levels do decrease some. Even when combined with other electron donors, the data indicate that ELS is not capable of completely reducing TCE to ethene. EOS amended experiments (Figures B.27, B.28) performed very differently than the ELS amended experiments. The data indicate that EOS amended experiments reach complete dechlorination to ethene in a very short period of time but had methane production continues at an uncontrolled rate.

A series of 155 preliminary experiments were run in the Spring of 2017. These aquifer material experiments were set up and analyzed the same way as the batch study experiments. They covered a wide range of concentrations and combinations of amendments, including high concentrations of AMRs. After these experiments ran for

approximately four months, the data was inconsistent. Some experiments showed little to no dechlorination across replicates while others achieved complete dechlorination in a reasonable time. This preliminary study helped identify which concentrations of amendments were too low to facilitate complete dechlorination and which were too high and caused excess methane generation. Some combinations of amendments were not compatible with each other and resulted in some experiments only reducing to cis-DCE or VC, even after multiple months. The experiments from this set that performed the best were the concentrations in the midrange, reinforcing the idea that adding excess amendment does not equal faster or more complete dechlorination. Taking into account the stoichiometry of the amendments and aquifer material plays a big role in facilitating complete dechlorination.

Ultimately, this set of preliminary experiments helped guide decisions about which concentrations and combinations to focus on in the batch study, especially for the AMRs. The concentrations and combinations that showed promise in the preliminary study were used in the batch study experiments. A full list of all experiments from the preliminary run is included in **Appendix E**. The table is sorted by AMR.

A series of 147 bioaugmentation experiments were set up in the Spring of 2017 and run for four months. The bioaugmentation culture SDC-9, a *Dehalococcoides* culture, was grown in anaerobic conditions using vendor suggested media recipes and methods. The culture was used with the same amendments as all other experiments. However, the data from these experiments was wildly inconsistent and there were no obvious data trends within experiment replicates. Some experiments saw some dechlorination, some saw no

dechlorination, and no experiment set showed complete, stoichiometric dechlorination. There was speculation about if the culture was growing well enough within the experiment bottles to stay alive or growing well enough to have an effect on dechlorination. The SDC-9 culture has inconsistency issues that stem from its limited capacity for solid metabolism and its delicate nature. SDC-9 bioaugmentation cultures are not a stable, strong culture even with added amendments and using this culture for bioremediation is a weak remediation option. For these reasons, bioaugmentation experiments were not continued after this preliminary stage. A complete list of all experiments used in the bioaugmentation cultures is included in **Appendix F**, sorted by AMR.

3.2 HOW AMRS WORK AND IN-SITU APPLICATIONS

Methanogens use excess hydrogen and CO2 or acetate that is naturally present in the aquifers or produced as a byproduct of microbial activity. As electron donors continue to supply energy to *Dehalococcoides*, they also supply energy to methanogens. When using electron donors in the subsurface, the amount added must be carefully controlled because adding excess donor can promote growth of methanogens and suppress *Dehalococcoides* activity (Wei and Finneran 2013). The widely marketed products, like statins and plant-based oils, claiming to inhibit methanogenesis have not been evaluated and there is no evidence that they work as described. Inhibiting methanogenesis alone may not stimulate dechlorination, as microbial processes are more complex than this one reaction. Additionally, a specific pathway linking dechlorination to methanogenic conditions has not been identified despite both processes occurring simultaneously in anaerobic aquifers.

Statins are common in medications, supplements, and cow feed and aim to reduce methane production within intestinal systems by reducing cholesterol levels. Monacolin K is the statin in Lovastatin which is commonly used in medications such as Lipitor and Red Yeast Rice (RYR), a health supplement and cow feed additive. The statins work by targeting Ether-linked lipids, which are essential for Archaea survival. Inhibiting ether-linked lipids from forming reduces cholesterol, which prevents the synthesis of the cell wall. Statins can inhibit cellular processes in methanogens without negatively affecting *Dehalococcoides* because *Dehalococcoides* cell walls are made up of ester-linked lipids, which remain unaffected by the statin (Gottlieb, Wacher et al. 2016). Garlic Oil is another commonly used AMR in cattle to prevent methane production within their digestive tract. Natural and synthetic garlic oil have been used to change the composition of and decrease the abundance of methanogen communities. The garlic oils work the same way as the statins by targeting cell wall synthesis. Garlic oil can also alter the amount of electron donors such as acetate and ethyl propionate present in the intestinal tract (Patra and Yu 2012). Both the change to the methanogen community and amount of electron donor available can prevent methane production. Vendors have hypothesized that the success of these AMR products in humans and cows could mean success in aquifer systems, however there is no data indicating that this is true.

Potential in-situ applications of these AMR products for chlorinated solvent remediation are limited to a few applications. For RYR, this includes a slurry that could be poured down a well since it is a dry powder that is not easily dissolvable. If garlic oil were used in a slurry and introduced to the subsurface via a well, it may not distribute throughout

an aquifer well. The garlic oil could be used as a permeable reactive barrier due to its viscosity and weight. Garlic Oil could also have applications beyond chlorinated solvent remediation. Septic systems and landfills could benefit from using garlic oil to help control methanogenesis. Application for a septic system would be straightforward; pour the product down a drain or toilet inside a home and it will flow to the septic tank. For a landfill, the garlic oil could either be used as a reactive barrier installed during construction or introduced via a well later on.

3.3 INFLUENCE OF RYR ON METHANE PRODUCTION

Red Yeast Rice (RYR) is marketed as a cheap way to introduce a statin into the aquifer. As the name suggests, there is yeast in this amendment but the primary role of the amendment is to prevent methanogenesis. In experiments with RYR alone (Figures B.49, B.50), data indicate that dechlorination is achieved at a quick rate, but methane levels are in excess of 900 μmol/bottle. ELS and RYR amended experiments (Figures B.57, B.58) indicate that ELS is a lackluster amendment with slow dechlorination rates even with RYR added. The addition of RYR causes the methane levels to reach higher methane levels than ELS alone. The data indicate that for experiments containing Lactate and RYR (Figures B.55, B.56), EOS and RYR (Figures B.59, B.60), and BOS100 and RYR (B.63, B.64) amended experiments, complete dechlorination was achieved at a quick rate but methane levels are higher than if each amendment was used without RYR. The data suggests that RYR is a more powerful electron donor than it is methane inhibitor, no matter which electron donor it is paired with. Additionally, it shows that methane levels are consistently

higher when an electron donor is paired with RYR than when it is used alone. Dechlorination rates are also as fast or faster than electron donor alone, again indicating that RYR is a powerful electron donor.

One reason why RYR is not exhibiting the anti-methanogenic properties as expected is that there may not be enough statin to make a difference or the pH may need to be so low to activate the statin that dechlorination does not take place. HPLC analysis of two types of RYR at different pH levels was conducted to determine what percent statin was present in the RYR. There were issues with trying to get the RYR into solution, even with heating up the solution and dropping the pH to 2, then sonicating the solution, there was still large amounts of RYR that did not dissolve. That was the most aggressive approach to dissolution, and milder approaches of adding 1% SLS and heating the solution barely dissolved the RYR. Once the solution was analyzed, the data indicate that concentrations were well below 0.1 percent for both types of RYR. There was little difference between analysis sets with different dissolution techniques. Data tables for each analysis are included in **Appendix C**. This is simply not enough statin content to inhibit methanogenesis, especially given that yeast is a major component in the RYR.

The data indicate that pH is clearly a factor in if the RYR product will control methanogenesis or not. In both the lower pH level experiments, the data shows that there was minimal change from initial methane concentrations in week 0 and concentrations in week 4 in pH 2 adjusted series (Figures D.10, D.11, D.12). Changes over time are an average of 0.05 μmol/bottle increase each week for 4 weeks. Experiments adjusted to pH 4 (Figures D.13, D.14, D.15) show data that is very similar to pH 2 experiments. Change

over time is an average of 0.1 μmol/bottle per week. The data shows that pH 7 experiments (Figure D.16, D.17, D.18) are drastically different than both low pH experiment sets. There is clearly no methane inhibition, concentrations rise as high as 100 μmol/bottle. Methane concentrations are averaging an increase of 20 μmol/bottle per week with continued, steady increase. The data indicate that a low pH environment will allow RYR to prevent methanogenesis, however dechlorination may not be active at these low pH levels. The data from batch study and pH adjustment studies indicate that there is not enough statin within the RYR to allow it to be an effective AMR, but it is a successful electron donor due to the yeast content.

3.4 INFLUENCE OF GARLIC OIL ON METHANE PRODUCTION

Natural Garlic Oil (GO#1) is considered a methane inhibitor and the idea that it could be used in conjunction with RYR to help keep methane levels low despite yeast being present does not prove itself in this set of experiments. The data indicate that the addition of GO#1 provides inconsistent results among replicates. In experiments containing just RYR and GO#1, Experiment 166 (Figure B.65) achieved complete dechlorination with methane levels lower than RYR alone, but still in excess of 200 μmol/bottle. In experiment 167 (Figure B.66), cis-DCE and VC are still present. In experiments containing Provect-IR, RYR, and GO#1 (Figures B.67, B.68) and experiments containing lactate, RYR, and GO#1 (Figures B.71 B.72), the data shows complete dechlorination occurred but at a slower rate than if Provect-IR or lactate were used alone. Both sets had lower methane concentrations than if Provect-IR or lactate were used alone, but an excess of 100

μmol/bottle of methane is still high. EZVI, RYR, and GO#1 amended experiments (Figures B.69, B.70) showed mixed results among replicates. Experiment 194 had no dechlorination at all, TCE levels remain unchanged. However, in Experient 195 there was complete dechlorination with methane production in concentrations between 40 and 70 μmol/bottle. Both experiments showed methane levels lower than EZVI alone amended experiments, but EZVI alone facilitated complete dechlorination. Experiments containing ELS (Figures B.73, B.74), EOS (Figures B.75, B.76), EHC (Figures B.77, B.78), or BOS100 (Figures B.79, B.80) as an electron donor in conjunction with RYR and GO#1 all fail to reach complete dechlorination. In all of these experiments, cis-DCE and VC concentrations are persistent. The data shows that EOS has been successful at dechlorination in every other combination besides this one, and BOS100 saw a significant decrease in performance. EHC and ELS continue to be weak products, even with RYR and GO#1 present. Overall, the data indicate that the addition of GO#1 to experiments helped bring down methane concentrations below what experiments containing only electron donor and RYR, but methane concentrations were still high enough to be a problem. Additionally, there were consistency issues among replicates and a higher number of experiments did not reach complete dechlorination.

The Natural Garlic Oil used alone yielded similar results to when it was used with RYR. The data was inconsistent, with some experiments performing well and others in the same replicate set barely dechlorinating at all. Even though the data indicate that GO#1 used alone yields lower concentrations of methane overall, there are numerous instances of incomplete dechlorination. Experiments containing GO#1 alone have mixed results.

Experiment 164 (Figure B.81) did not achieve complete dechlorination and still has 5 μmol/bottle of both cis-DCE and VC. Experiment 165 (Figure B.82) did achieve complete dechlorinating with one methane spike that returned to acceptable levels within one week. Provect-IR and GO#1 (Figures B.83, B.84), EZVI and GO#1 (Figures B.85, B.86), ELS and GO#1 (Figure B.89, B.90), EHC and GO#1 (Figures B.93, B.94), and BOS100 and GO#1 (Figures B.95, B.96) amended experiments do not reach complete dechlorination and there is still a presence of 5-7 μmol/bottle of cis-DCE and VC in each experiment replicate. This is similar to how these ZVI amendments and ELS preformed when used alone, no amendment of this type promotes dechlorination past cis-DCE, with or without an AMR. Lactate and GO#1 amended experiments (Figures B.87, B.88) was the most successful amendment combination in this category. The data shows that there was complete dechlorination with relatively low methane levels outside of one short peak in experiment 204. EOS and GO#1 (Figures B.91, B.92) had mixed results. Data from Experiment 236 shows a complete reduction of TCE to ethene, however methane levels were near 180 μmol/bottle while data from Experiment 237 contains 25 μmol/bottle of cis-DCE and 10 μmol/bottle of VC after 20 weeks with methane levels around 55 μmol/bottle.

Garlic Oil #2 (GO#2) is a synthetic garlic oil. The data indicate that Garlic Oil #2 is not any better or worse of an AMR than GO#1 and results are equally as inconsistent for both types of garlic oil, regardless of the type of electron donor paired with it. While the data shows that overall methane levels are still lower than when RYR is present, the lack of consistent dechlorination to ethene is a problem. In experiments using only GO#2 (Figures B.97, B.98) the data shows that dechlorination is not fully achieved, there is still

a presence of 5-7 μmol/bottle of both cis-DCE and VC. Methane levels remain relatively constant and low. Experiments amended with Provect-IR and GO#2 (Figures B.99, B.100), EZVI and GO#2 (Figures B.101, B.102), and BOS100 and GO#2 (Figures B.111 and B.112), there was still a significant presence of cis-DCE and VC with not much methane generation. These data are similar to the results with GO#1. ZVI-type amendments and garlic oil-type amendments do not seem work well together, with the exception of ECH ang GO#2 (Figures B.109, B.110). This replicate set achieved complete dechlorination with minimal methane. However, given the inconsistency issues and poor dechlorination potential that data from experiments containing EHC has shown alone and with other electron donors, more testing would be needed to ensure that this is an effective combination. The data indicate that lactate and GO#2 amended experiments (Figures B.103, B.104) were the most effective combination in this subset. There was complete dechlorination with minimal methane production. EOS and GO#2 amended experiments (Figures B.107, B.108) yielded similar data to the lactate experiments, but methane values were in the low 30s μmol/bottle which is above target limits. In some situations, this concentration may be okay and these experiments show the lowest methane concentration out of any other experiment containing EOS. ELS amended experiments (Figures B.105, B.106) remained unable to reduce TCE to ethene and showed no improvement over ELS with other AMRs or alone.

The data indicate that pH is not a limiting factor for garlic oils. GO#1 at pH 2 (Figures D.19, D.20, D.21) had very little methane generation, concentrations stayed below 1 μmol/bottle. The data shows that pH 4 experiments (Figures D.22, D.23, D.24) and pH

7 experiments (Figures D.25, D.26, D.27) all followed the similar trend as experiments at pH 2. For all nine garlic oil pH adjustments, there was very little methane generation. pH does not appear to be a factor in methane inhibition but GO#1 did not allow for complete dechlorination in the batch study. The data shows that GO #2 experiments were very similar to GO#1 experiments and had little variation among pH levels. pH 2 (Figures D. 28, D.29, D.30), pH 4 (Figures D. 31, D.32, D.33), and pH 7 (Figures D.34, D.35, D.36) all maintained low and relatively constant methane concentrations. The methane concentrations never reached 1 μmol/bottle in any of the nine experiments. Overall, both types of garlic oil seem to inhibit methane production at a range of pH levels. However, the data shows that garlic oil does slow down dechlorination rates significantly, so garlic oil may be better suited for applications outside of chlorinated solvent bioremediation. Other potential applications could include septic systems or landfills.

3.5 OTHER AMRS: ACTIVITY AND RESULTS

Lovastatin is a medical grade methane inhibitor that is most commonly used in human digestive systems to relieve symptoms of chronic digestive issues. The data indicate that the high performance of statins in this setting do not mirror the performance of statins in an aquifer, most likely due to the mild pH of aquifers. In experiments amended with only Lovastatin (Figures B.41, B.42), dechlorination was not achieved and cis-DCE persisted in high concentrations, with little methane production. Experiments with Lovastatin and lactate (Figures B.43, B.44) performed similarly, high concentrations of cis-DCE still persisted however there was one big spike in methane concentration that

dropped back down after a week. In ELS and Lovastatin (Figures B.45, B.46) amended experiments, the pattern of incomplete dechlorination persists. The only experiments containing Lovastatin that exhibited complete dechlorination were experiments containing both EOS and Lovastatin (Figures B.47, B.48). However, methane production in these experiments were in excess of 3,000 μmol/bottle, with concentrations as high as 5,000 μmol/bottle. These are some of the highest methane values seen in the entire set of Batch Study Experiments. Overall, experiments containing Lovastatin struggled to reach complete dechlorination to ethene. It may be that an aquifer with mild pH levels is not a good environment for Lovastatin to promote dechlorination and prevent methanogenesis.

4. CONCLUSIONS

Experiments containing lactate and oil-based electron donors may be effective at stimulating dechlorination, if added at low concentrations. The experiments at 40mM concentrations of lactate and EOS showed promise. EOS promoted the fastest dechlorination of all electron donor amendments. ELS was not a successful amendment, it did not promote complete dechlorination and more often than not, still had cis-DCE and VC gasses present even after 20 weeks. EOS was shown to be a better choice for emulsified vegetable oil amendments and finding a way to keep methane production down would make EOS a viable bioremediation solution. EP also has potential for biodegradation solutions but faces similar methane issues as EOS. For batch study experiments, the development of an AMR that would allow EOS and EP to continue to be powerful reducers while keeping methane production down would be the best-case option.

Garlic oil-based AMRs did inhibit methanogenesis, although it varied amongst treatments. There was almost no consistency among replicates and both types of garlic oil struggled to promote complete dechlorination even with added electron donors that were highly successful alone. In most instances, experiments with either type of garlic oil added showed slower and more incomplete dechlorination than electron donor alone. Additionally, ZVI-type amendments preformed much more poorly when the garlic oils were added. Most electron donors seem to work fine, although sometimes slowly, on their own and the addition of RYR or GO greatly increased the amount of methane or halted dechlorination when there were still significant levels of cis-DCE or VC.

Some AMRs may serve better as electron donors, specifically when paired with lactate or oils. RYR experiments had great dechlorination but uncontrolled methane production. Yeast in the RYR really drives dechlorination and consequently, methane production. In some cases, methanogenesis increased due to the other materials present with statins, most notably yeast. Overall, experiments containing RYR demonstrate faster dechlorination but more methane is produced than if electron donor was used alone. No experiment saw methane controlled at a level below the target or at a level below what the electron donor only experiments had. The yeast within the product begins to ferment and because so much of the RYR product is yeast, the tiny percentage of statin is not enough statin to suppress methane production.

The statin is less effective than marketed because of a misreported statin content in the red yeast rice carrier. There is less than 0.1% statin in the RYR, which is not enough to prevent methanogenesis. Vendor reported statin content is 0.4% according to their Material

Safety Data Sheet. However, there may be a correlation between pH and ability to inhibit methane. Low pH conditions could promote methane inhibition, but lower pH environments decrease the chances for dechlorination. pH 2 and pH 4 experiments show substantial methane inhibition compared to pH 7 experiments and to the batch study aquifer material experiments. Although the statins do work better in low pH environments, that may not be the most practical choice for bioremediation applications.

Methanogenesis generally proceeded after complete dechlorination with RYR, which is unique to have such sequential methane production. The common trend with methane production is to see a very large spike of a few hundred to a few thousand μmol/bottle in one to two weeks, then the methane levels falling after that. This sequential methane production could be due to the fact that methanogens present in the soil do not utilize TCE, so once the DHC use all of the chlorinated solvents, the electron donor that is left is utilized by the methanogens. Excess methane is produced, and spikes in methane levels are observed. This is a natural process and, in some field situations, may not be a cause for concern as long as methane levels do not remain elevated. This may not be a process related to dechlorination but is a process that is important to the overall health of the microbial community in the soils.

Overall, the lack of statin content sufficient enough to prevent methanogenesis is likely the reason that RYR does not inhibit methanogenesis, given that in the batch study there were no instances of RYR working to both facilitate dechlorination and control methane concentrations and further analysis of RYR suggests that there is hardly any statin present. There were no instances of an AMR inhibiting methane and driving

dechlorination. In fact, AMRs slowed down or prevented complete dechlorination. Methane concentrations continue to be an issue in all categories of experiments and the faster TCE is reduced to ethene, the faster the methane levels seem to rise. Further research is needed to study the effect of pH on the performance of statins and the effects of both garlic oils to determine if there is any situation where their performance would be consistent.

APPENDICES

Appendix A

Experimental Lists

Table A.1: Experimental List for Batch Study Experiments

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
156	Unamended			
157	Unamended			
162	EP	250 mg/L		
163	EP	250 mg/L		
170			BES	10 mg/L
171			BES	10 mg/L
172	Provect-IR	100 mg/L		
173	Provect-IR	100 mg/L		
178	Provect-IR, EP	100 mg/L, 250 mg/L		
179	Provect-IR, EP	100 mg/L, 250 mg/L		
184	EZVI	100 mg/L		
185	EZVI	100 mg/L		

190	EZVI, EP	100 mg/L, 250 mg/L		
191	EZVI, EP	100 mg/L, 250 mg/L		
196	Lactate	40mM		
197	Lactate	40mM		
202	Lactate, EP	40mM, 250 mg/L		
203	Lactate, EP	40mM, 250 mg/L		
210	Lactate	40mM	BES	10 mg/L
211	Lactate	40mM	BES	10 mg/L
212	ELS	40mM		
213	ELS	40mM		
218	ELS, EP	40mM, 250 mg/L		
219	ELS, EP	40mM, 250 mg/L		
226	ELS	40mM	BES	10 mg/L
227	ELS	40mM	BES	10 mg/L
228	EOS	40mM		
229	EOS	40mM		
234	EOS, EP	40mM, 250 mg/L		
235	EOS, EP	40mM, 250 mg/L		

242	EOS	40mM	BES	10 mg/L
243	ELS	40mM	BES	10 mg/L
244	EHC	100 mg/L		
245	EHC	100 mg/L		
250	EHC, EP	100 mg/L, 250 mg/L		
251	EHC, EP	100 mg/L, 250 mg/L		
256	BOS100	100 mg/L		
257	BOS100	100 mg/L		
262	BOS100, EP	100 mg/L, 250 mg/L		
263	BOS100, EP	100 mg/L, 250 mg/L		
168			Lovastatin	50 mg/L
160			Lovastatin	50 mg/L
208	Lactate	40mM	Lovastatin	50 mg/L
209	Lactate	40mM	Lovastatin	50 mg/L
224	ELS	40mM	Lovastatin	50 mg/L
225	ELS	40mM	Lovastatin	50 mg/L

240	EOS	40mM	Lovastatin	50 mg/L
241	EOS	40mM	Lovastatin	50 mg/L
158			RYR	250 mg/L
159			RYR	250 mg/L
174	Provect-IR	100 mg/L	RYR	250 mg/L
175	Provect-IR	100 mg/L	RYR	250 mg/L
186	EZVI	100 mg/L	RYR	250 mg/L
187	EZVI	100 mg/L	RYR	250 mg/L
198	Lactate	40mM	RYR	250 mg/L
199	Lactate	40mM	RYR	250 mg/L
214	ELS	40mM	RYR	250 mg/L
215	ELS	40mM	RYR	250 mg/L
230	EOS	40mM	RYR	250 mg/L
231	EOS	40mM	RYR	250 mg/L
246	EHC	100 mg/L	RYR	250 mg/L
247	EHC	100 mg/L	RYR	250 mg/L
258	BOS100	100 mg/L	RYR	250 mg/L
259	BOS100	100 mg/L	RYR	250 mg/L
166			RYR, GO#1	250 mg/L, 250 mg/L

167			RYR, GO#1	250 mg/L, 250 mg/L
182	Provect-IR	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
183	Provect-IR	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
194	EZVI	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
195	EZVI	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
206	Lactate	40mM	RYR, GO#1	250 mg/L, 250 mg/L
207	Lactate	40mM	RYR, GO#1	250 mg/L, 250 mg/L
222	ELS	40mM	RYR, GO#1	250 mg/L, 250 mg/L
223	ELS	40mM	RYR, GO#1	250 mg/L, 250 mg/L
238	EOS	40mM	RYR, GO#1	250 mg/L, 250 mg/L

239	EOS	40mM	RYR, GO#1	250 mg/L, 250 mg/L
254	EHC	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
255	EHC	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
266	BOS100	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
267	BOS100	100 mg/L	RYR, GO#1	250 mg/L, 250 mg/L
164			GO#1	250 mg/L
165			GO#1	250 mg/L
180	Provect-IR	100 mg/L	GO#1	250 mg/L
181	Provect-IR	100 mg/L	GO#1	250 mg/L
192	EZVI	100 mg/L	GO#1	250 mg/L
193	EZVI	100 mg/L	GO#1	250 mg/L
204	Lactate	40mM	GO#1	250 mg/L
205	Lactate	40mM	GO#1	250 mg/L
220	ELS	40mM	GO#1	250 mg/L
221	ELS	40mM	GO#1	250 mg/L

236	EOS	40mM	GO#1	250 mg/L
237	EOS	40mM	GO#1	250 mg/L
252	EHC	100 mg/L	GO#1	250 mg/L
253	EHC	100 mg/L	GO#1	250 mg/L
264	BOS100	100 mg/L	GO#1	250 mg/L
265	BOS100	100 mg/L	GO#1	250 mg/L
160			GO#2	250 mg/L
161			GO#2	250 mg/L
176	Provect-IR	100 mg/L	GO#2	250 mg/L
177	Provect-IR	100 mg/L	GO#2	250 mg/L
188	EZVI	100 mg/L	GO#2	250 mg/L
189	EZVI	100 mg/L	GO#2	250 mg/L
200	Lactate	40mM	GO#2	250 mg/L
201	Lactate	40mM	GO#2	250 mg/L
216	ELS	40mM	GO#2	250 mg/L
217	ELS	40mM	GO#2	250 mg/L
232	EOS	40mM	GO#2	250 mg/L
233	EOS	40mM	GO#2	250 mg/L
248	EHC	100 mg/L	GO#2	250 mg/L
249	EHC	100 mg/L	GO#2	250 mg/L

260	BOS100	100 mg/L	GO#2	250 mg/L
261	BOS100	100 mg/L	GO#2	250 mg/L

Table A.1: The complete list of experiments included in batch study experiments with aquifer material experiments sorted by AMR.

Table A.2: Experimental List for pH Adjustment Experiments

Bottle Number	AMR	pH
1	None	7
2	None	7
3	None	7
4	250 mg/L GO#1	2
5	250 mg/L GO#1	2
6	250 mg/L GO#1	2
7	250 mg/L GO#1	4
8	250 mg/L GO#1	4
9	250 mg/L GO#1	4
10	250 mg/L GO#1	7
11	250 mg/L GO#1	7
12	250 mg/L GO#1	7
13	250 mg/L GO#2	2
14	250 mg/L GO#2	2
15	250 mg/L GO#2	2
16	250 mg/L GO#2	4
17	250 mg/L GO#2	4
18	250 mg/L GO#2	4

19	250 mg/L GO#2	7
20	250 mg/L GO#2	7
21	250 mg/L GO#2	7
22	300 mg/L RYR	2
23	300 mg/L RYR	2
24	300 mg/L RYR	2
25	300 mg/L RYR	4
26	300 mg/L RYR	4
27	300 mg/L RYR	4
28	300 mg/L RYR	7
29	300 mg/L RYR	7
30	300 mg/L RYR	7
31	None	4
32	None	4
33	None	4
34	None	2
35	None	2
36	None	2

Table A.2: The complete list of experiments included in the pH adjustment Experiments.

Appendix B

Batch Study Experimental Data

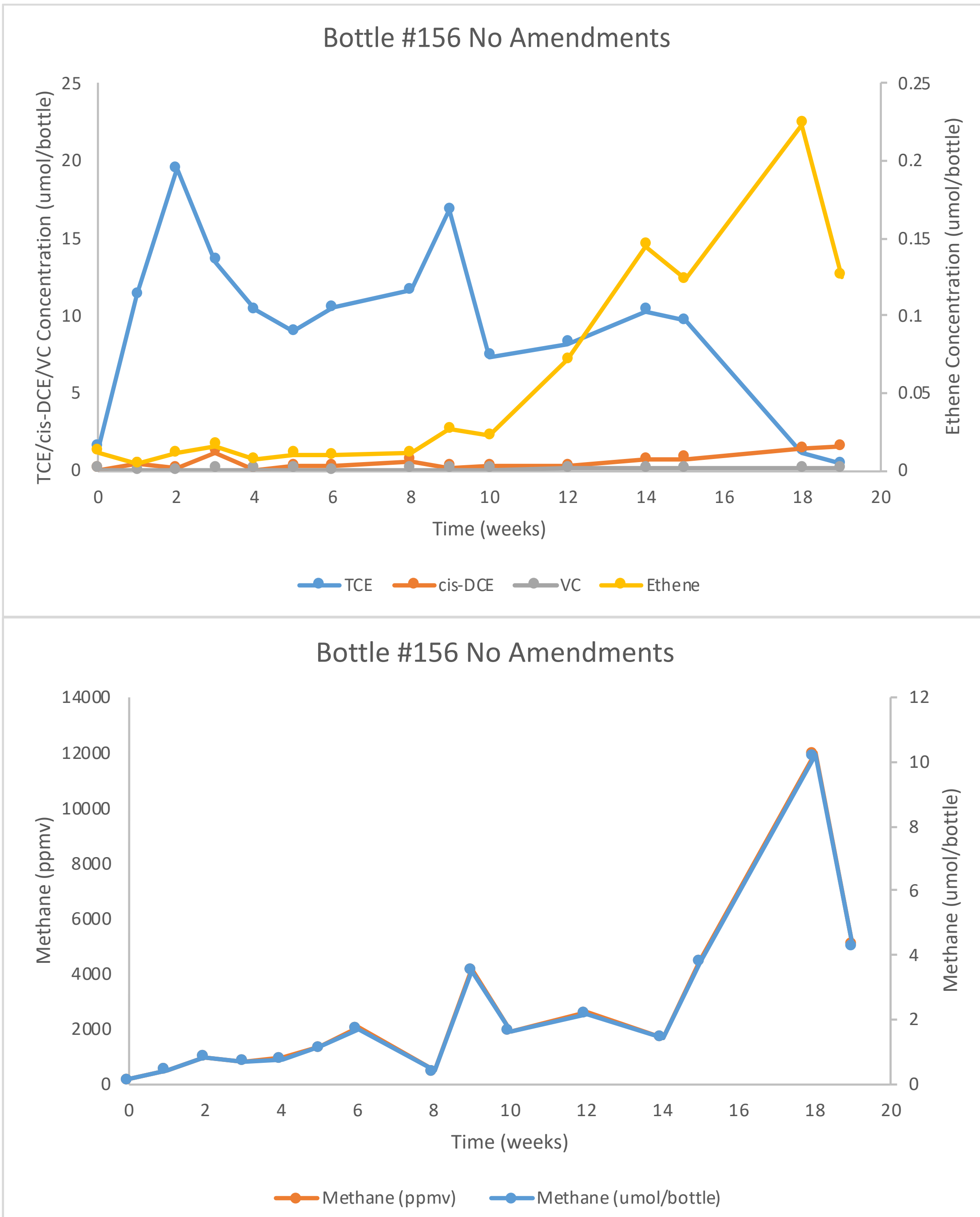


Figure B.1: TCE reduction and methane reduction for aquifer material with no amendments. Plot indicates replicate one of two.

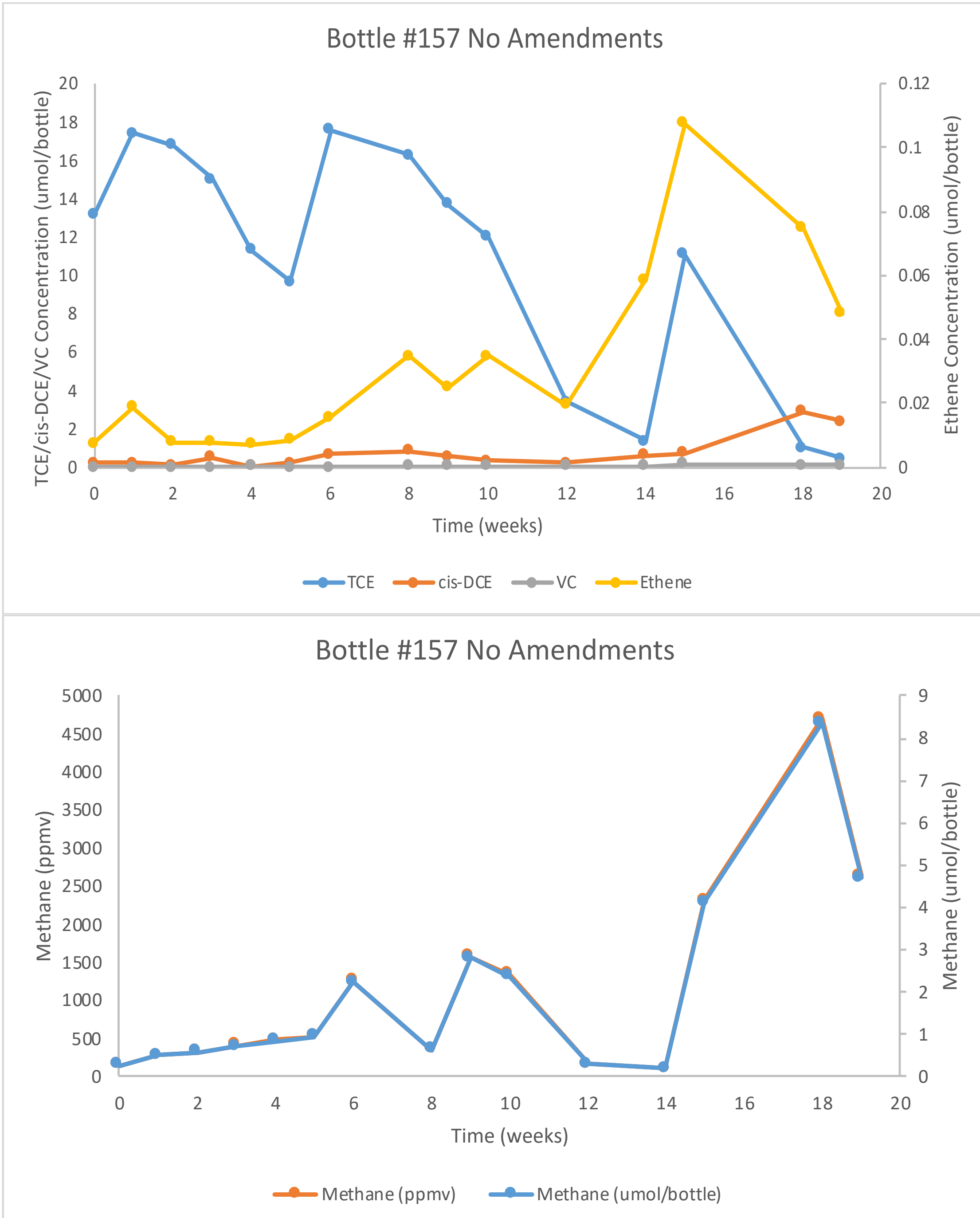


Figure B.2: TCE reduction and methane reduction for aquifer material with no amendments. Plot indicates replicate two of two.

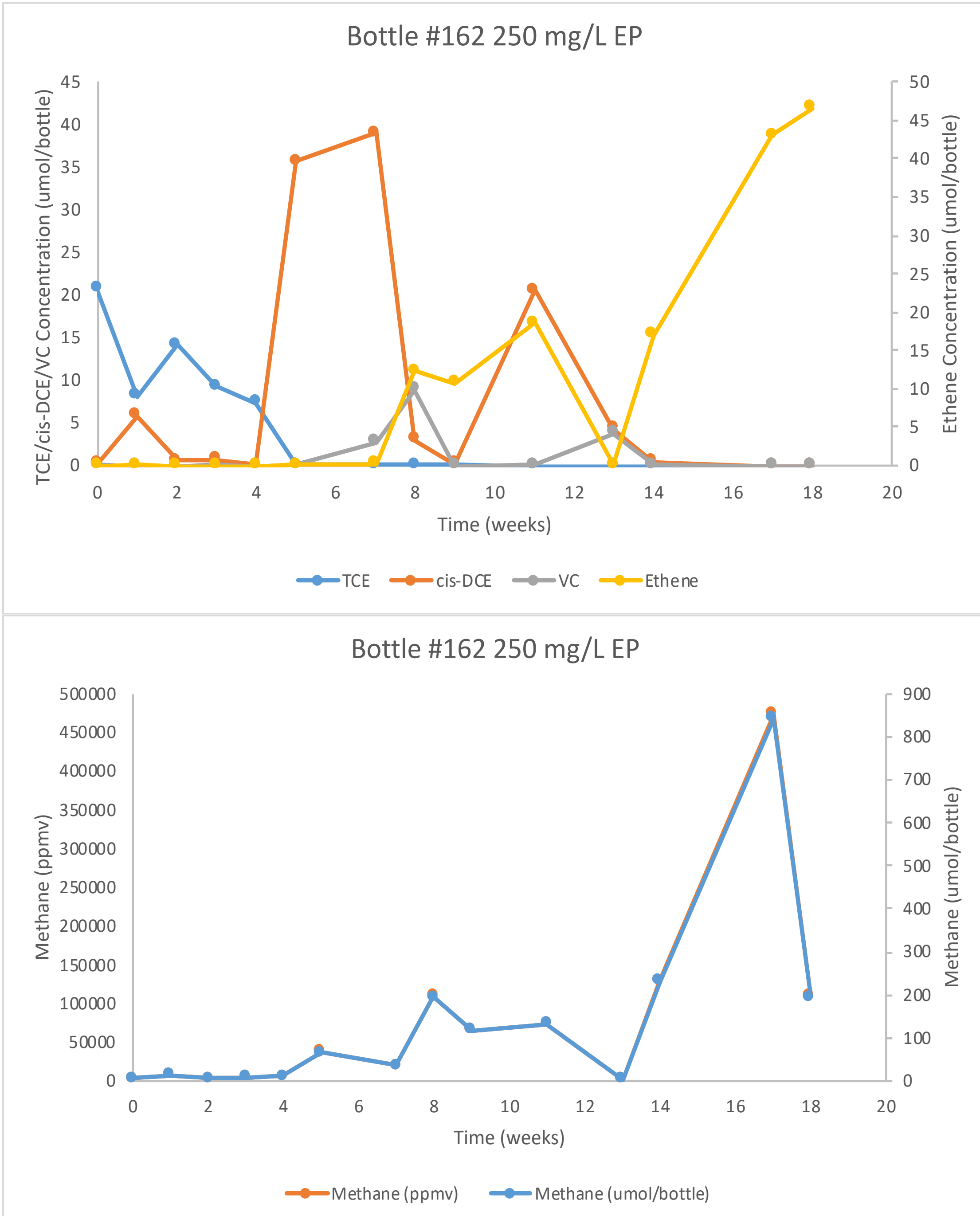


Figure B.3: TCE reduction and methane reduction for aquifer material with 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

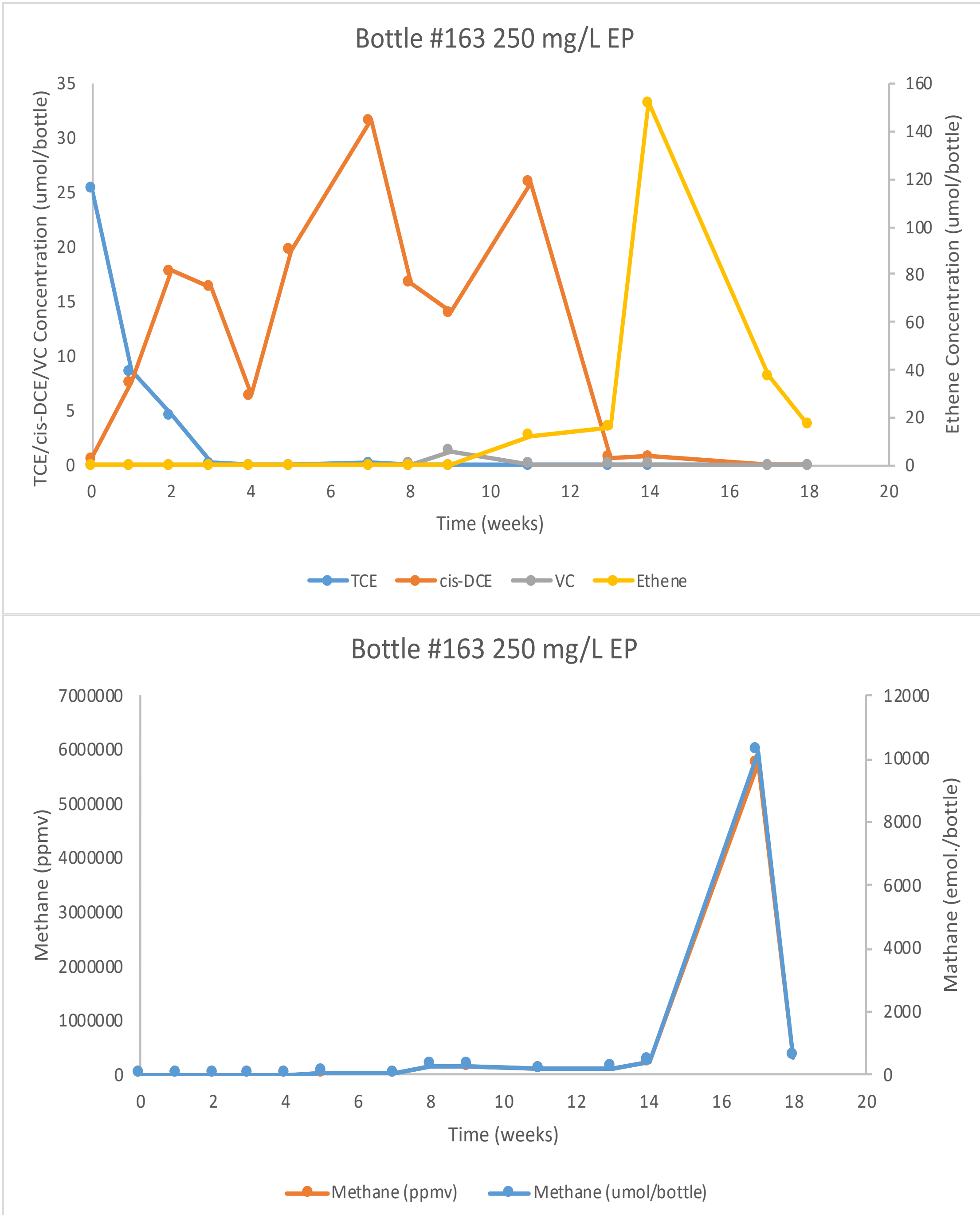


Figure B.4: TCE reduction and methane reduction for aquifer material with 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

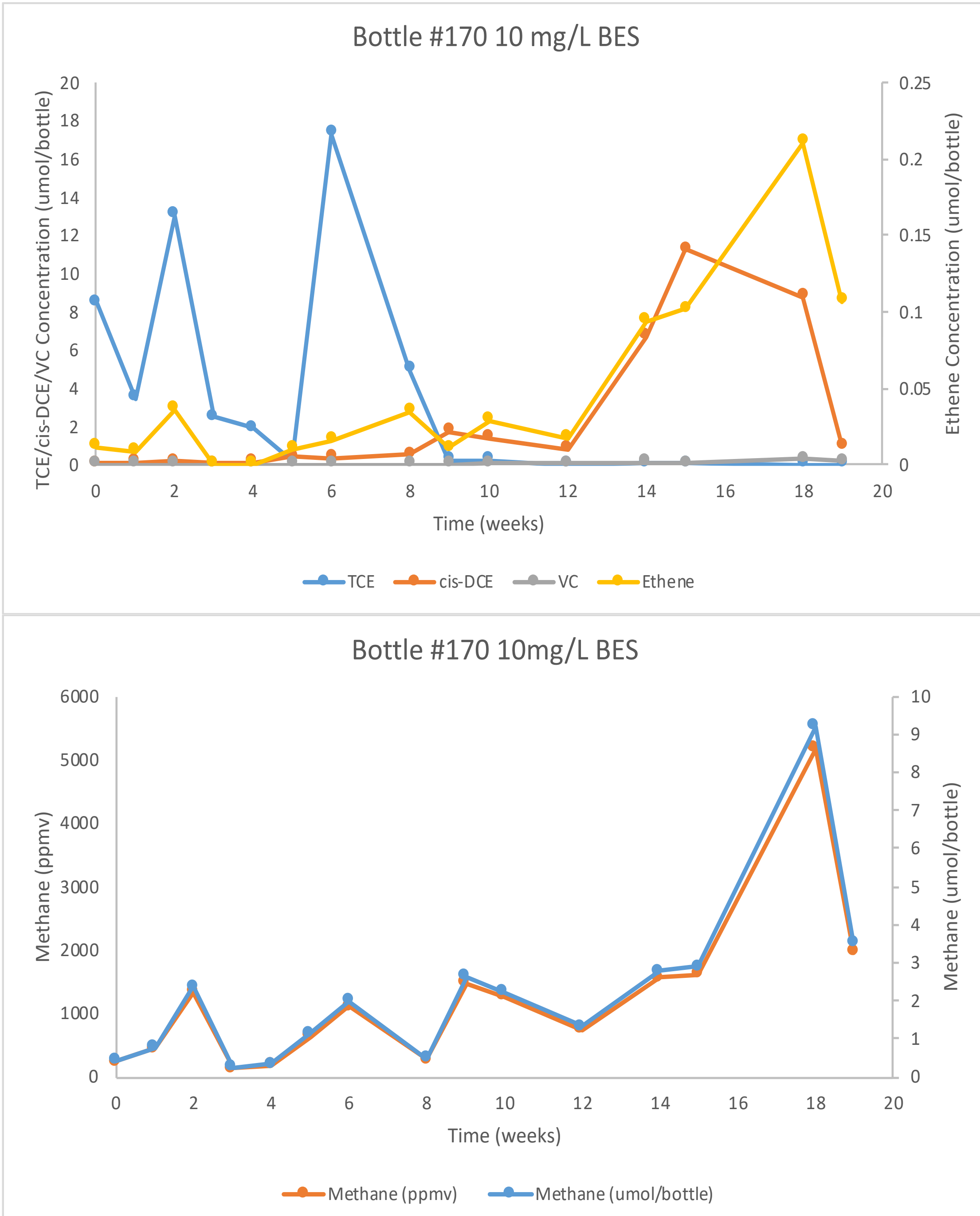


Figure B.5: TCE reduction and methane reduction for aquifer material with 10 mg/L BES. Plot indicates replicate one of two.

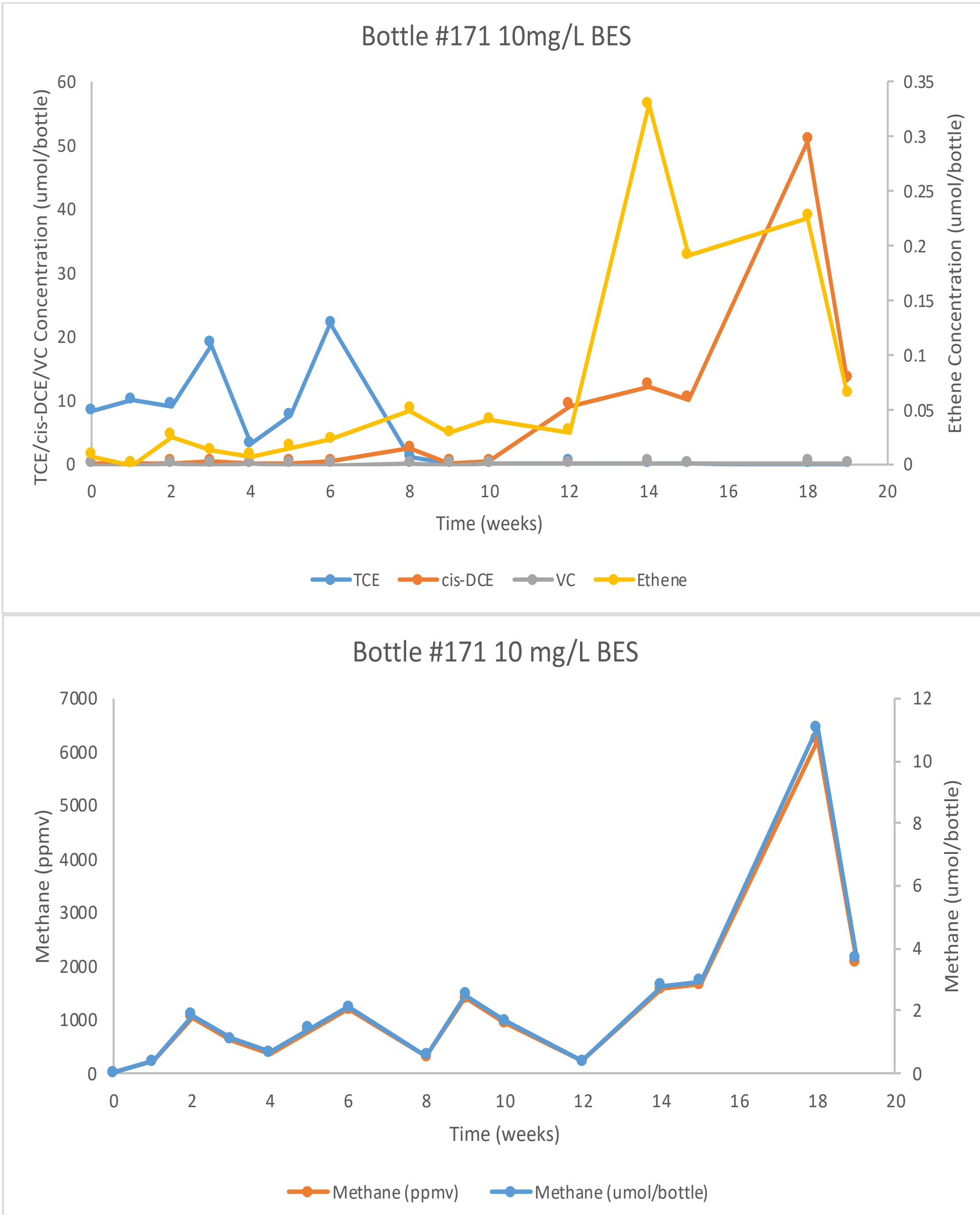


Figure B.6: TCE reduction and methane reduction for aquifer material with 10 mg/L BES. Plot indicates replicate two of two.

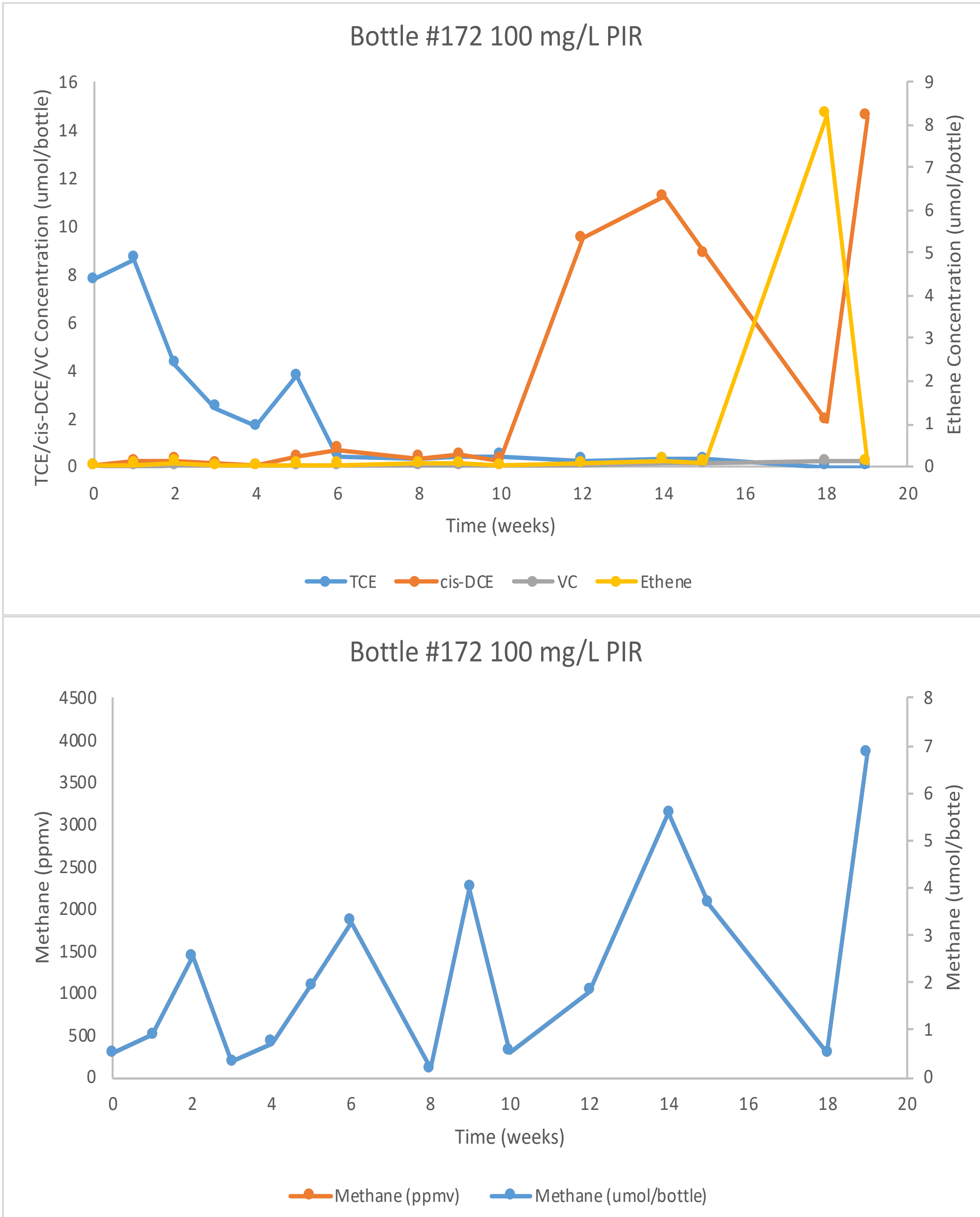
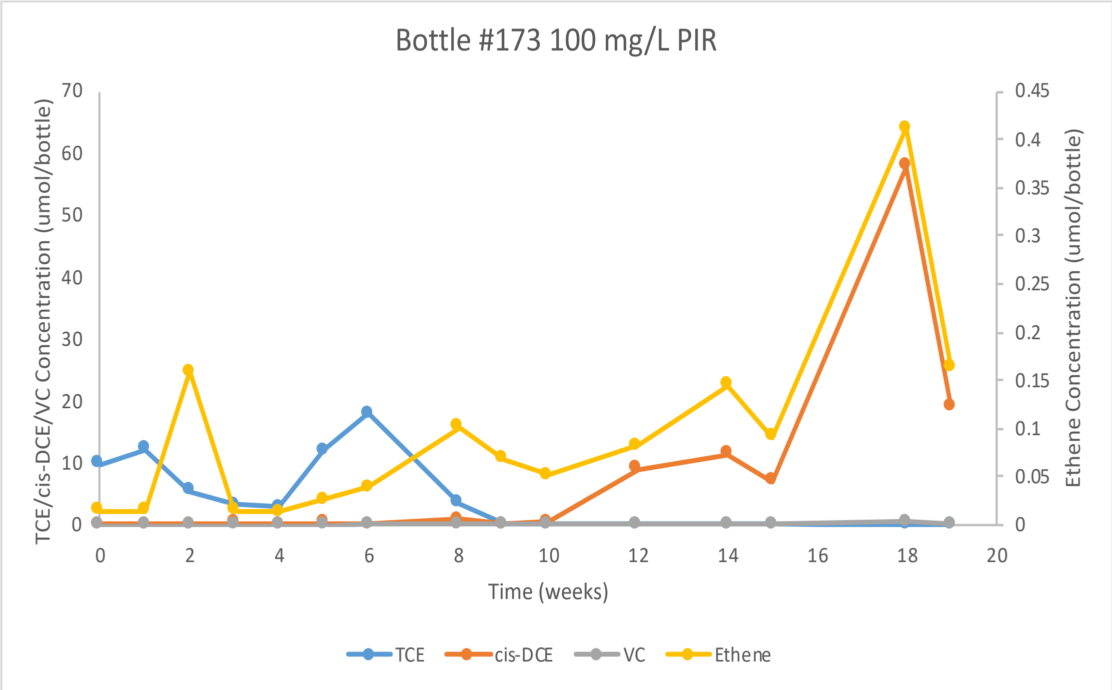


Figure B.7: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR. Plot indicates replicate one of two.



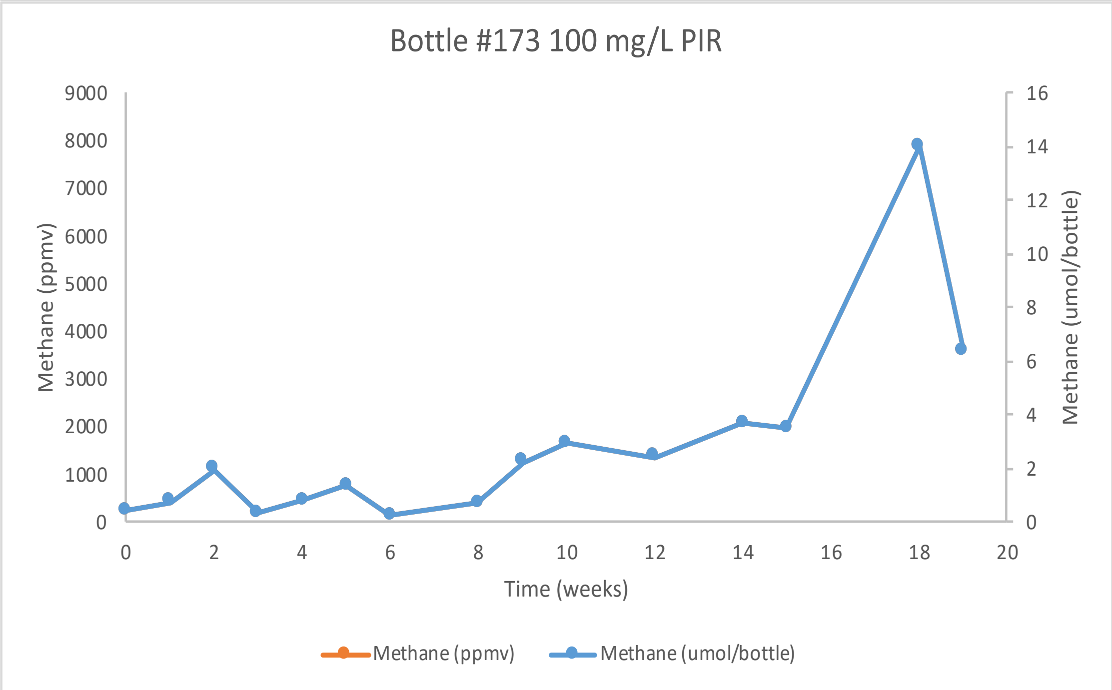


Figure B.8: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR. Plot indicates replicate two of two.

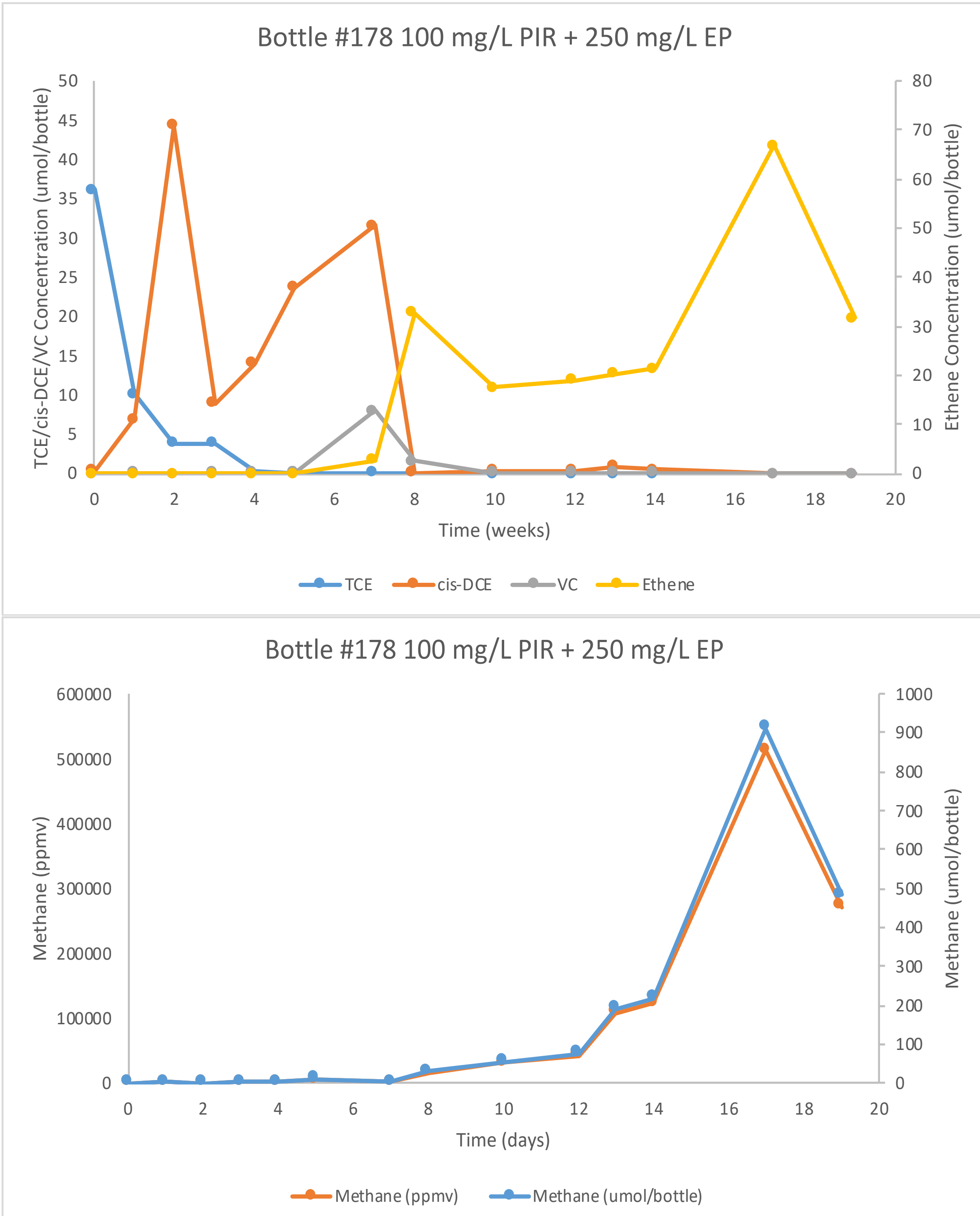


Figure B.9: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

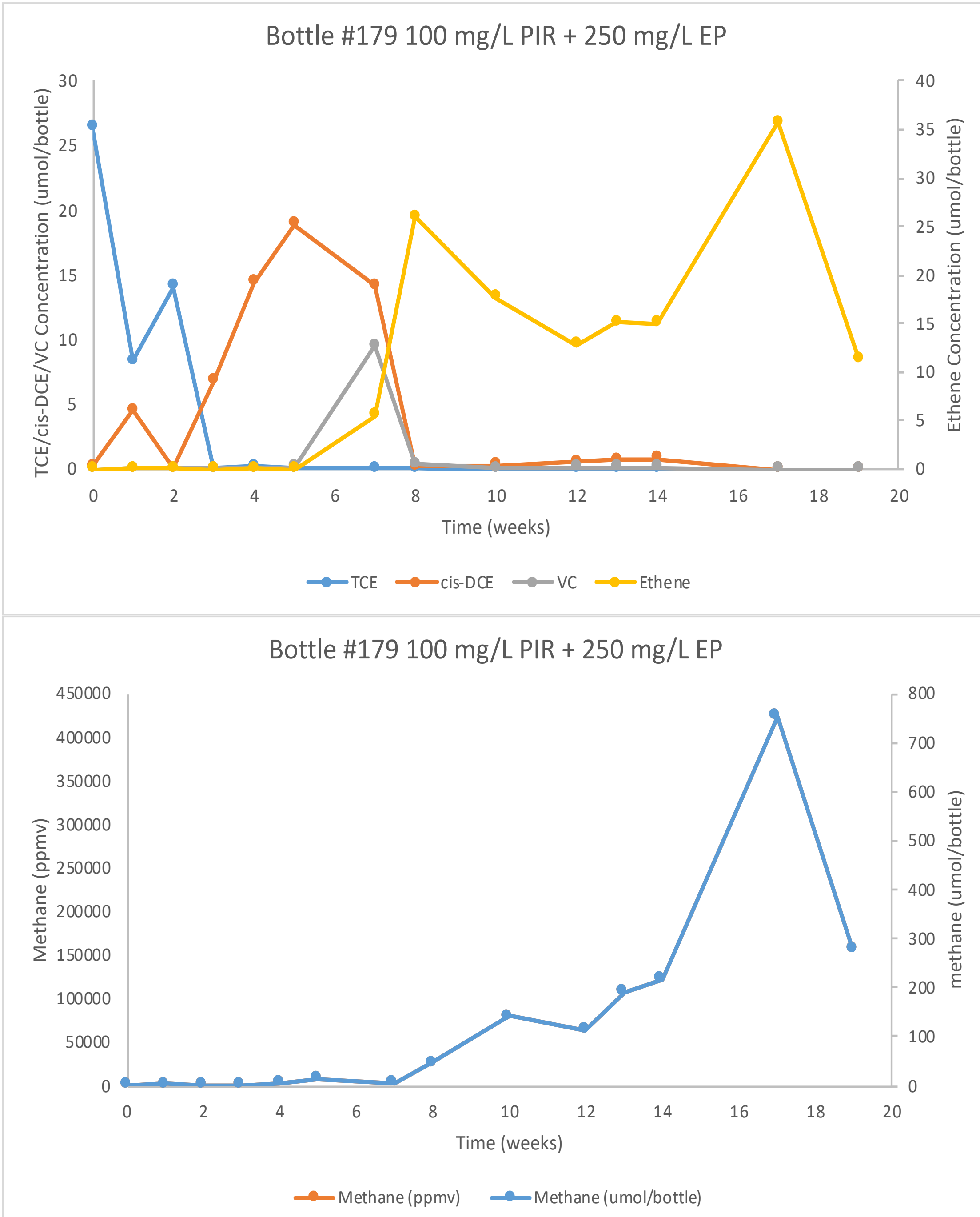


Figure B.10: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

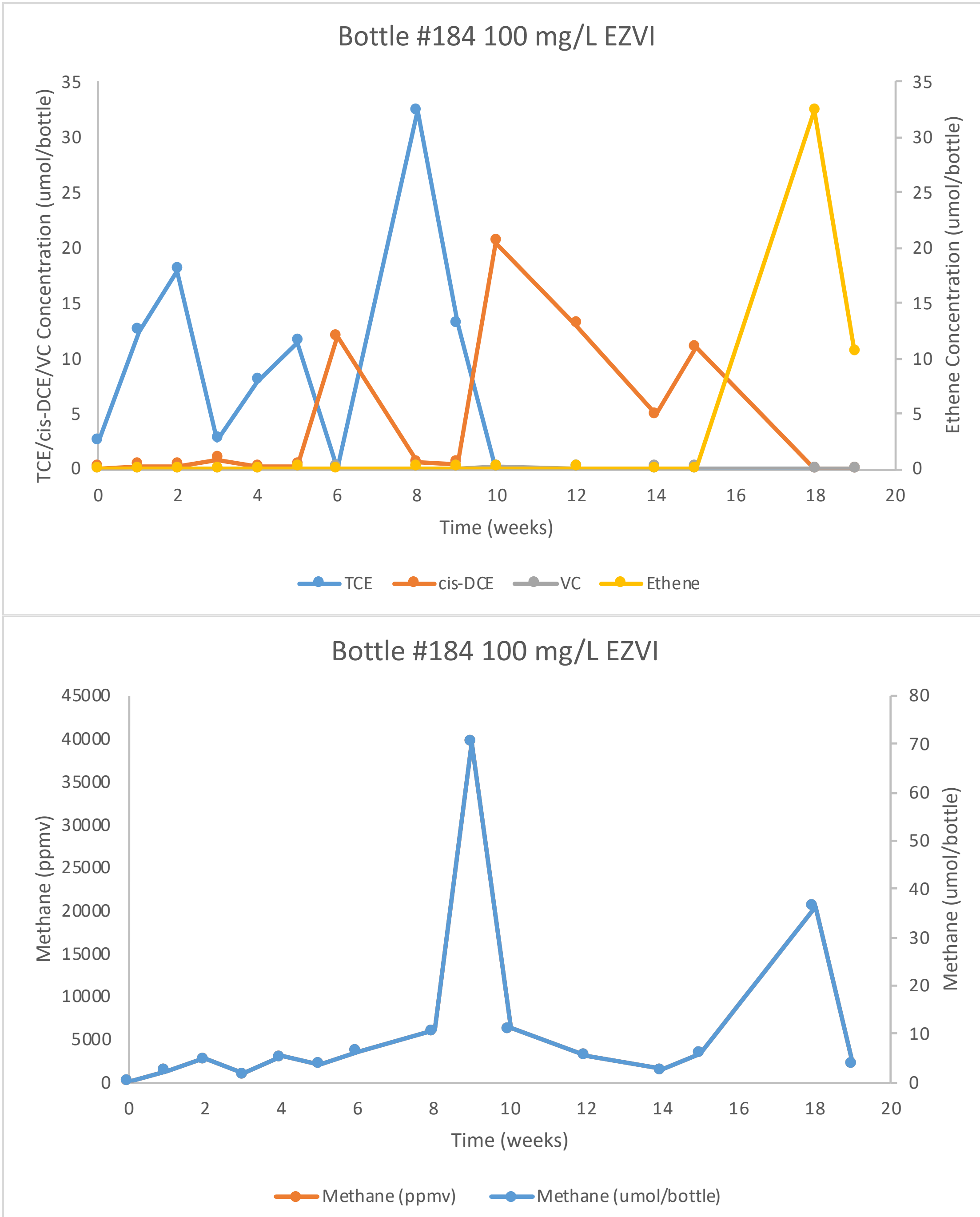


Figure B.11: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI. Plot indicates replicate one of two.

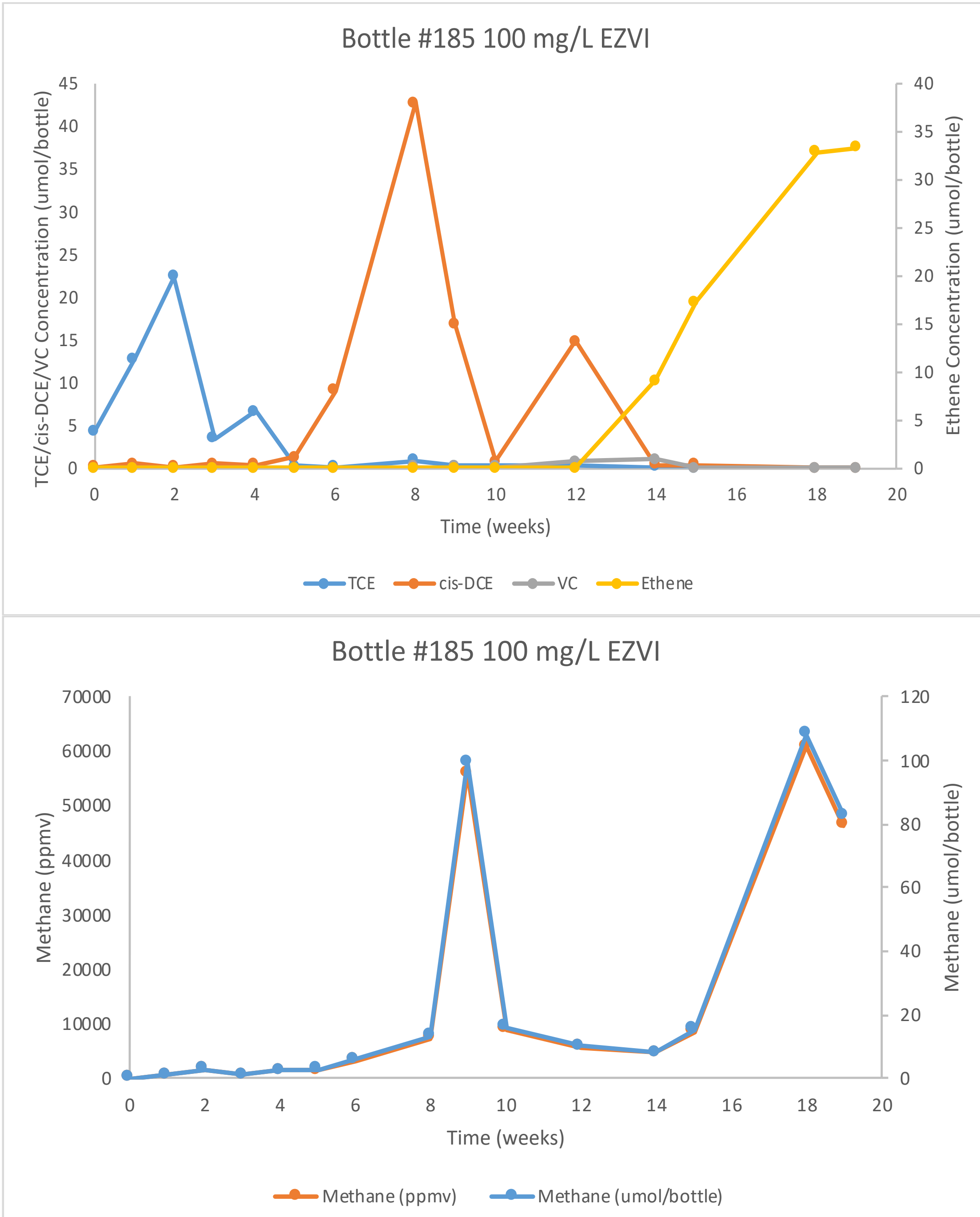
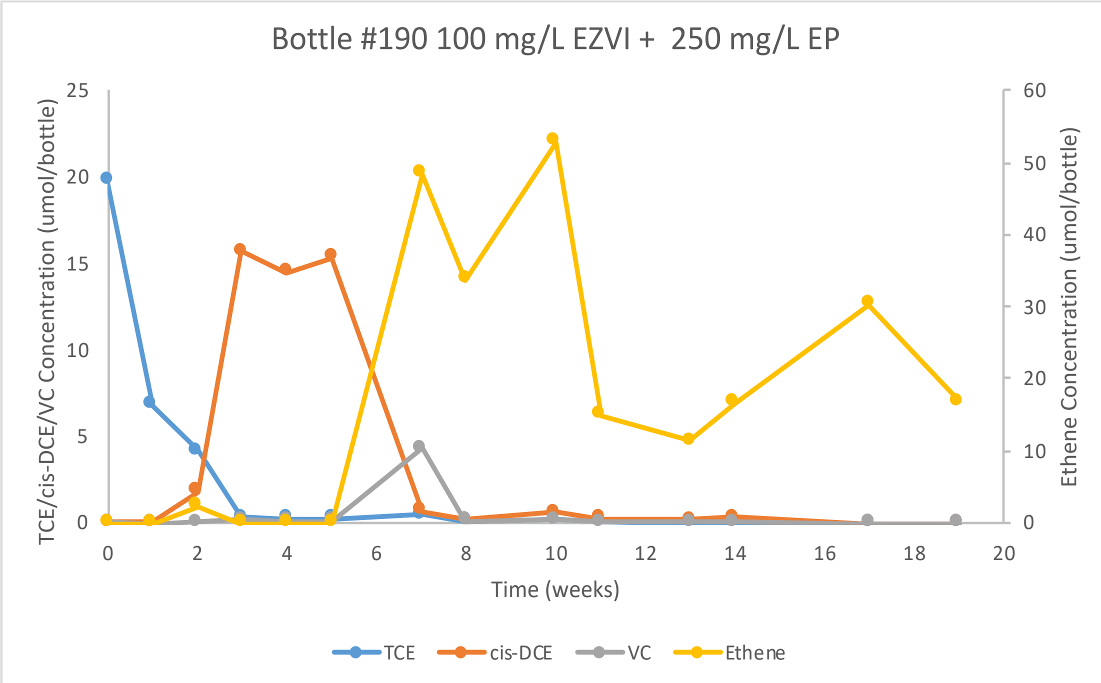


Figure B.12: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI. Plot indicates replicate two of two.



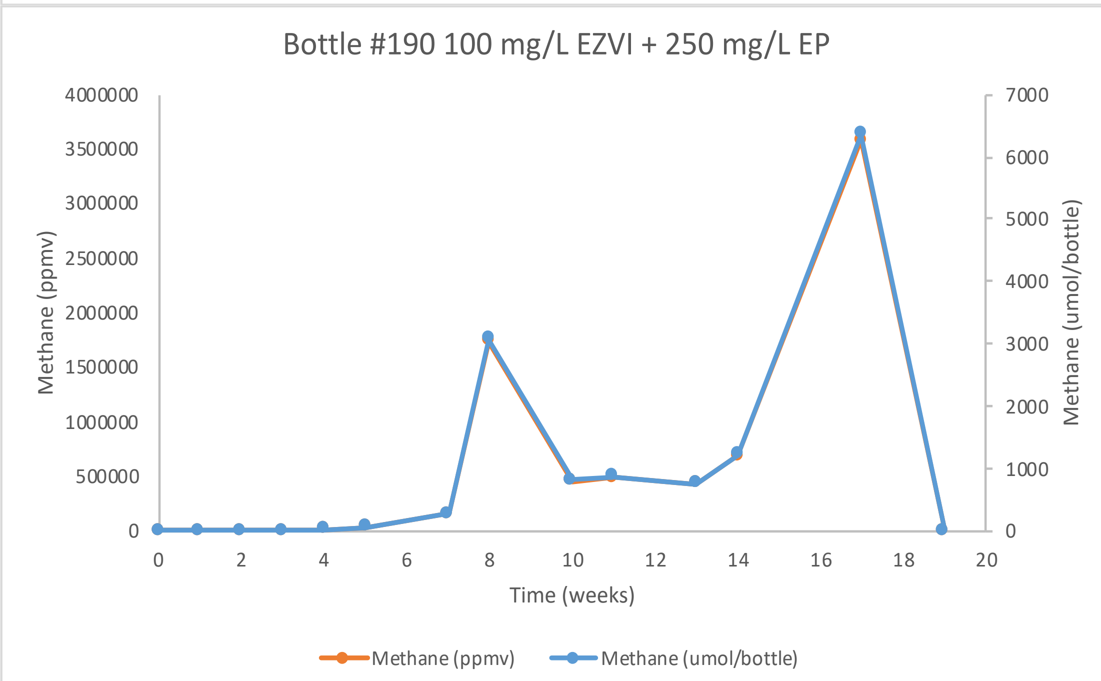


Figure B.13: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

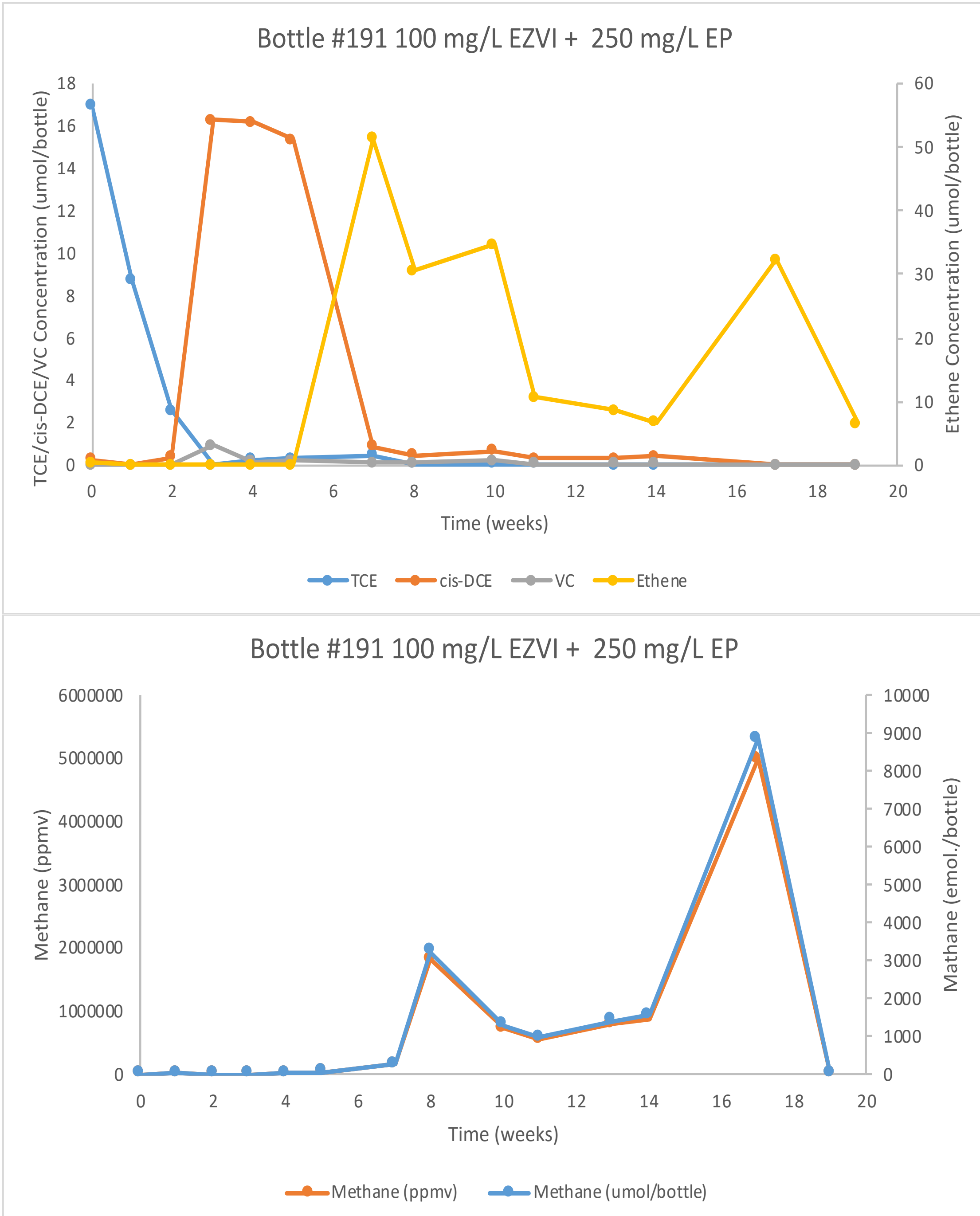


Figure B.14: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

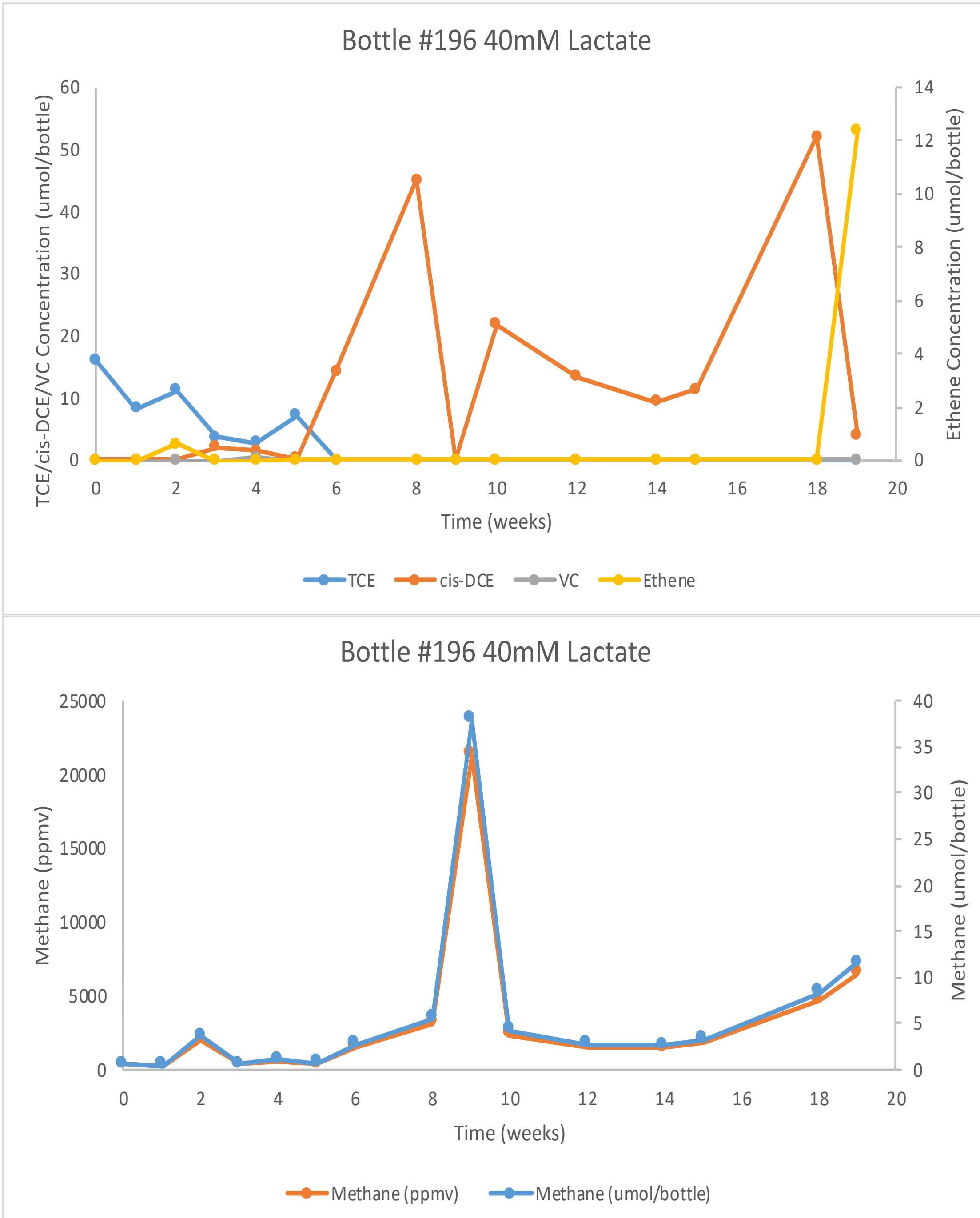
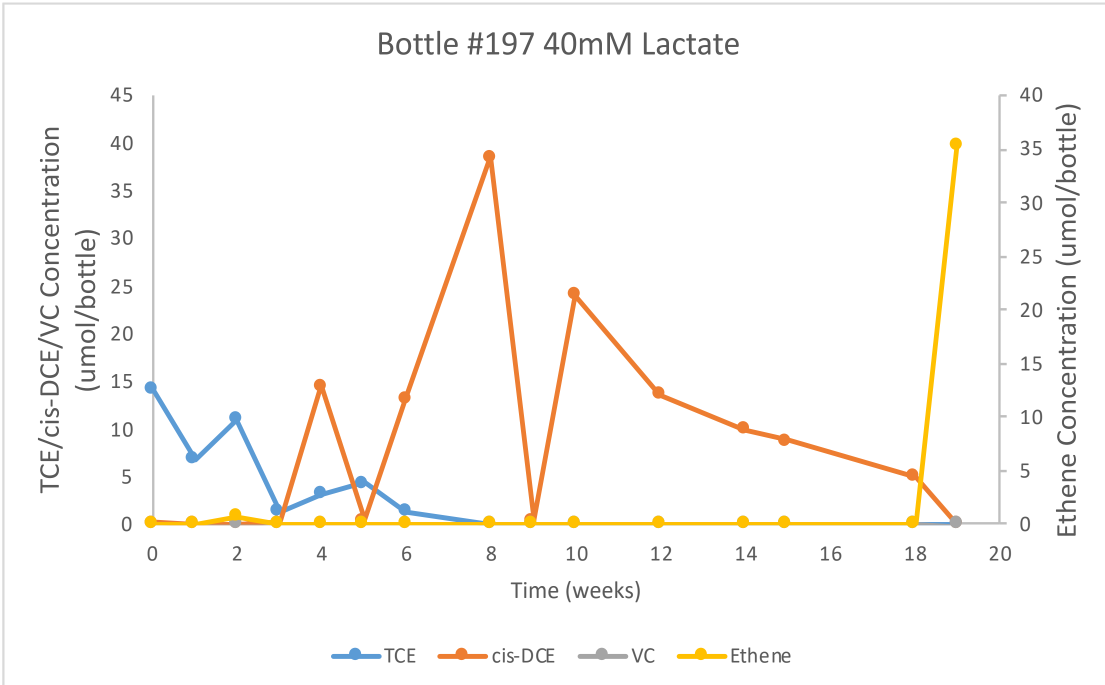


Figure B.15: TCE reduction and methane reduction for aquifer material with 40mM Lactate. Plot indicates replicate one of two.



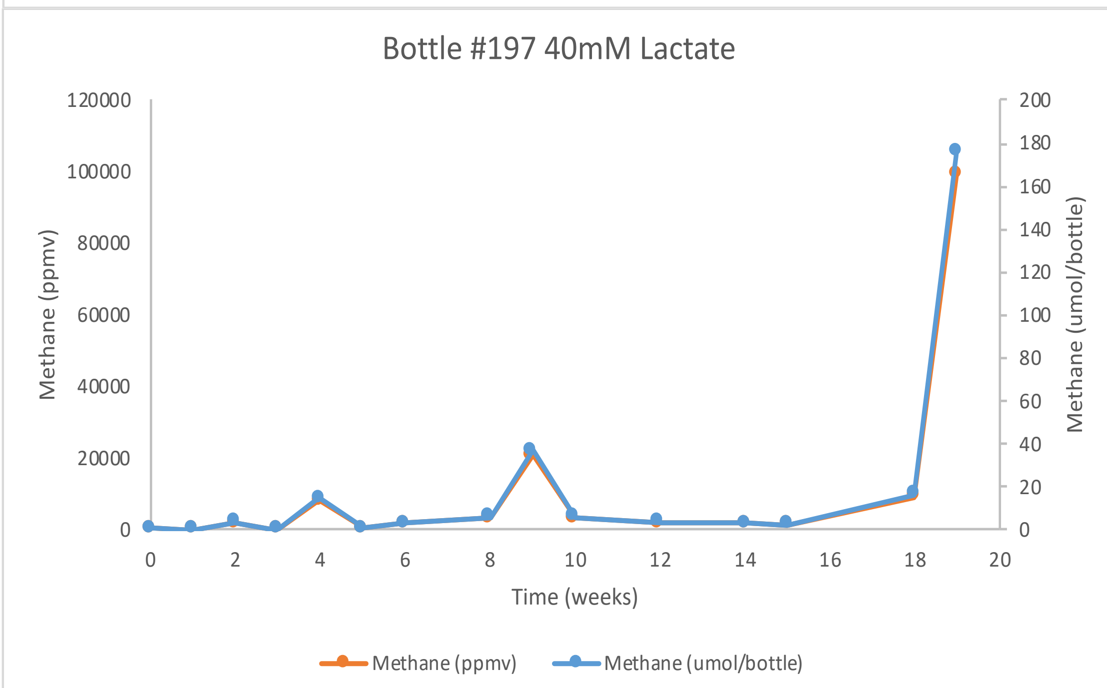


Figure B.16: TCE reduction and methane reduction for aquifer material with 40mM Lactate. Plot indicates replicate two of two.

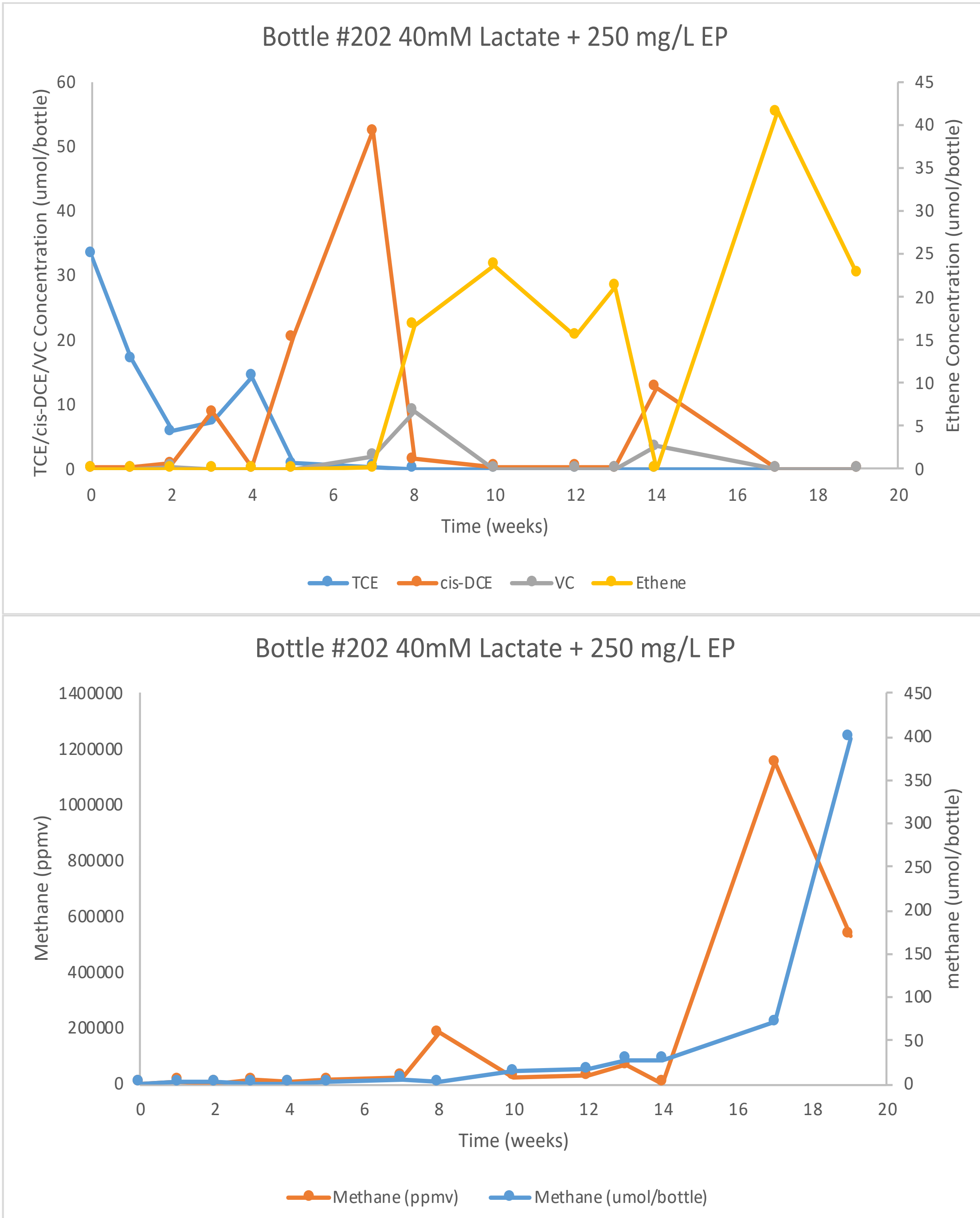
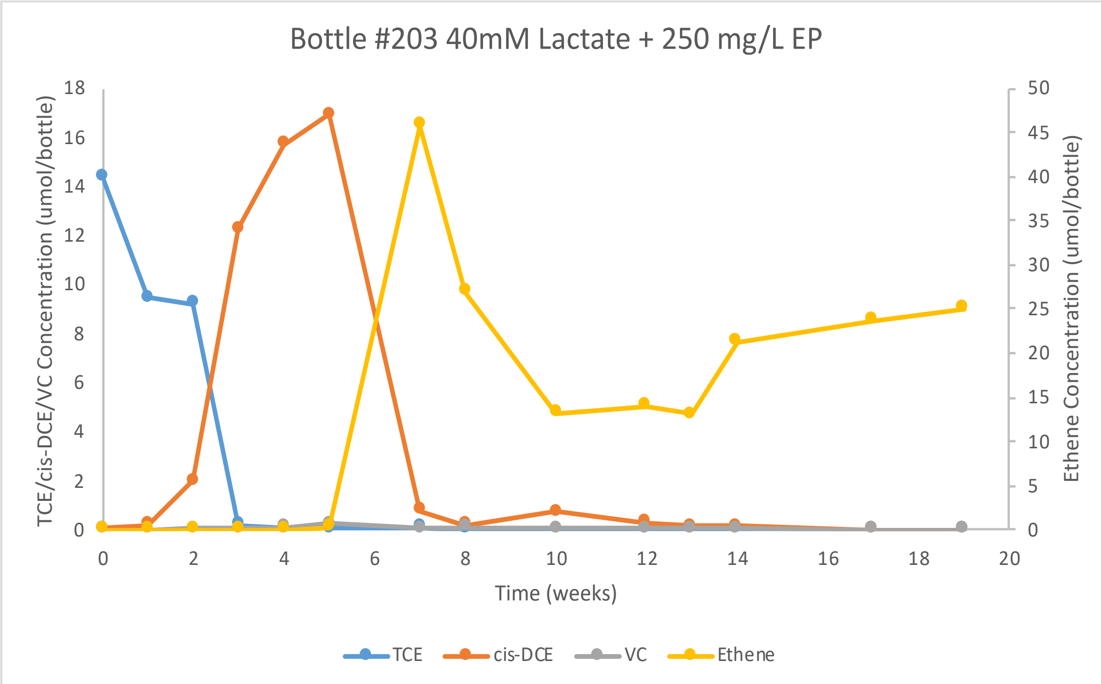


Figure B.17: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.



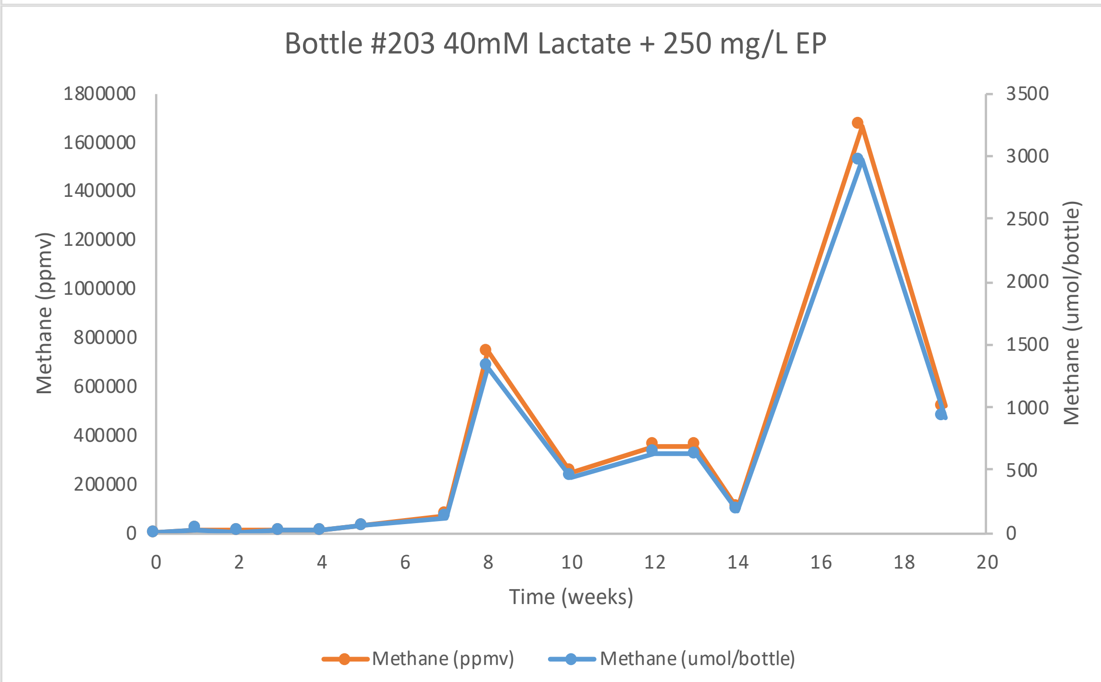


Figure B.18: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

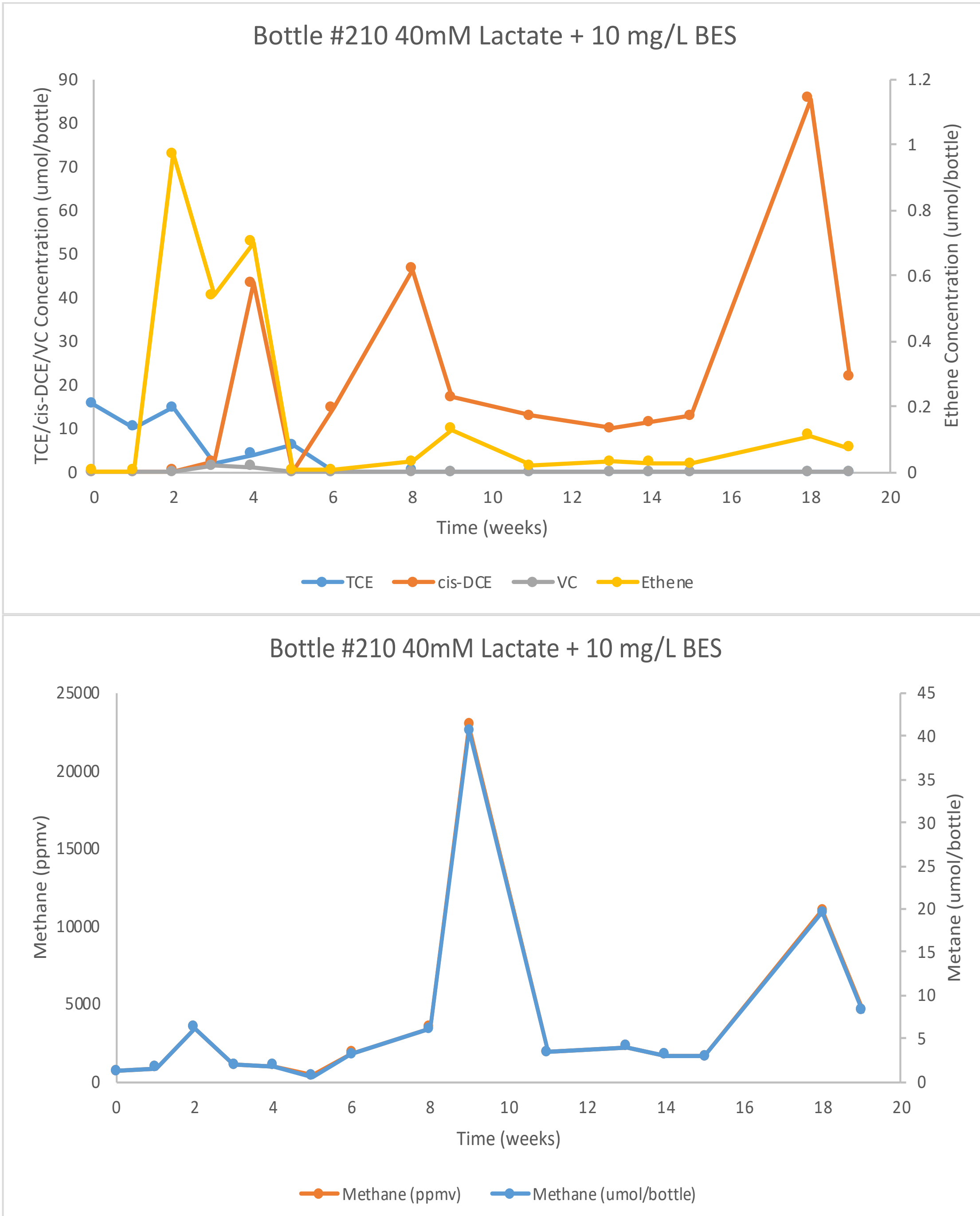
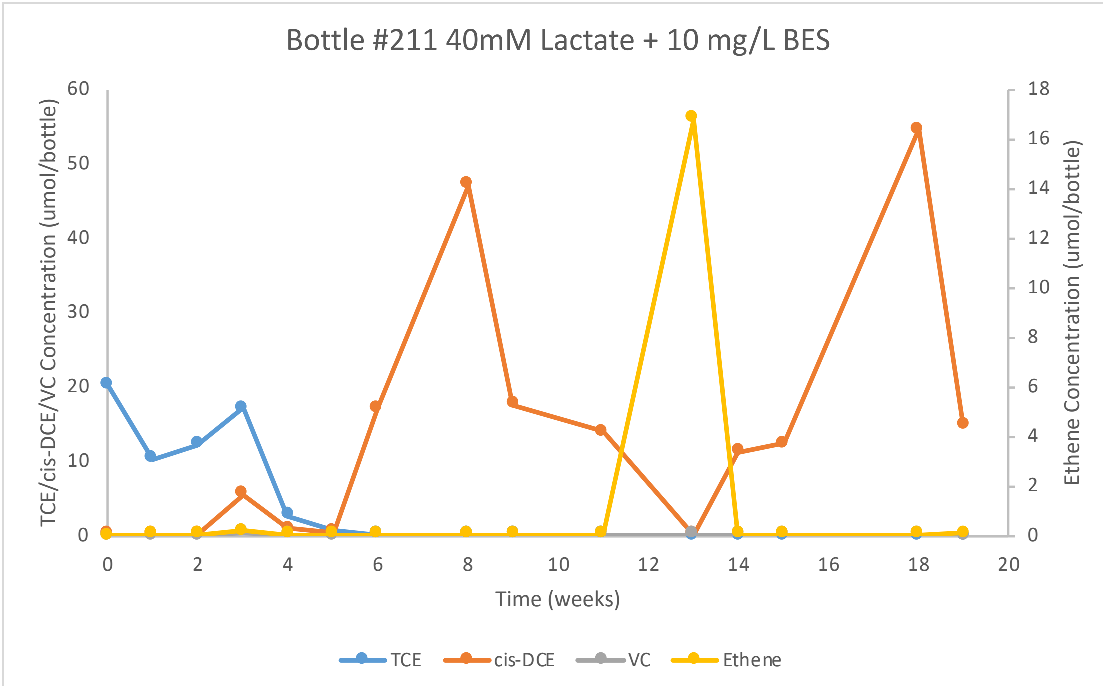


Figure B.19: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 10 mg/L BES. Plot indicates replicate one of two.



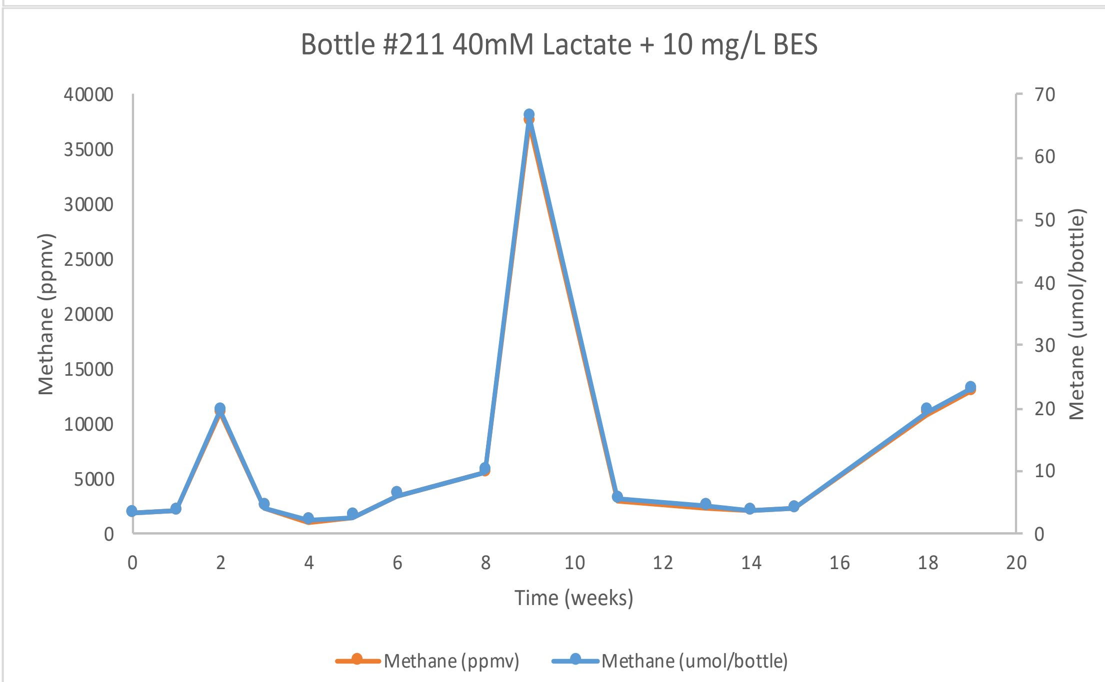


Figure B.20: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 10 mg/L BES. Plot indicates replicate two of two.

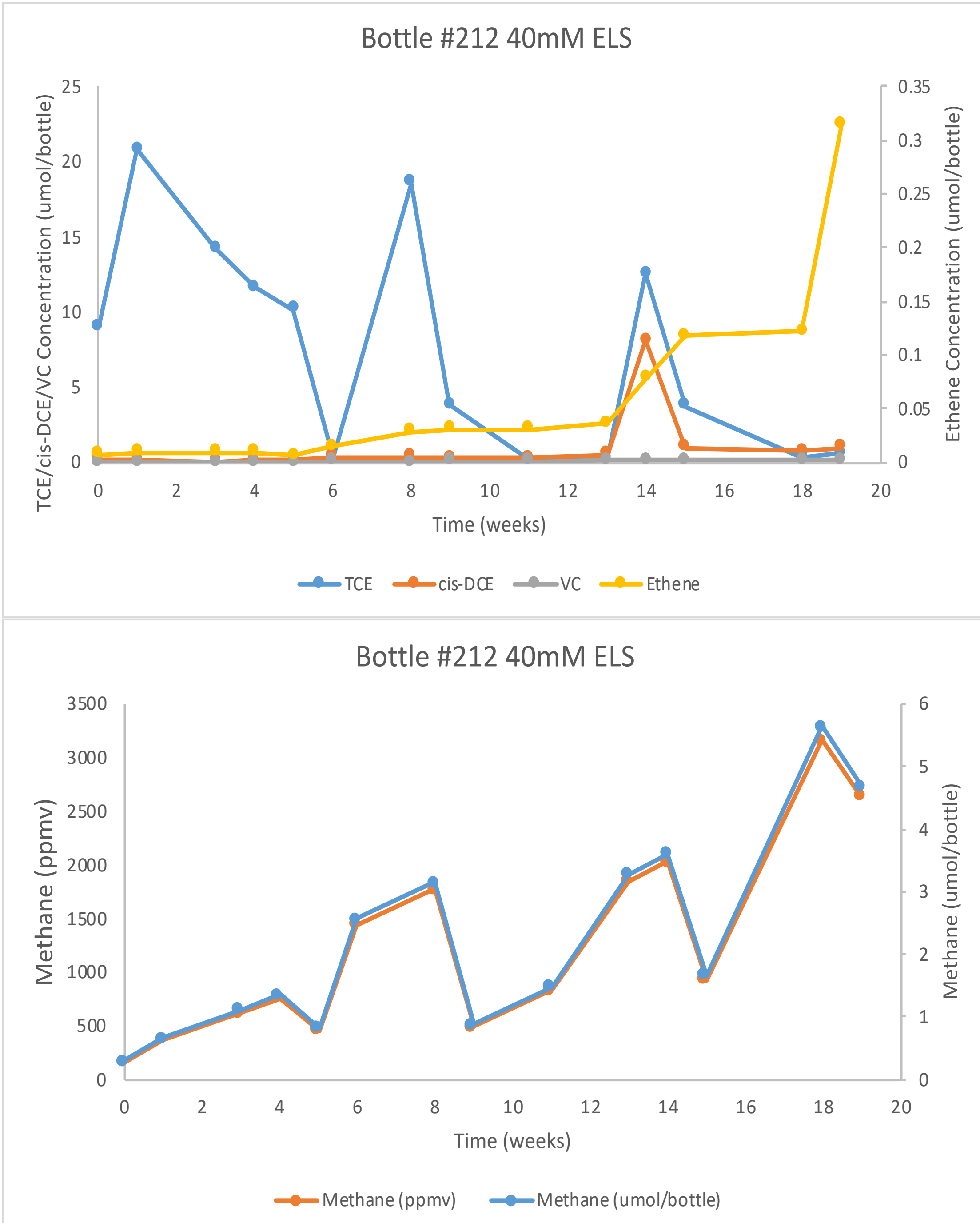
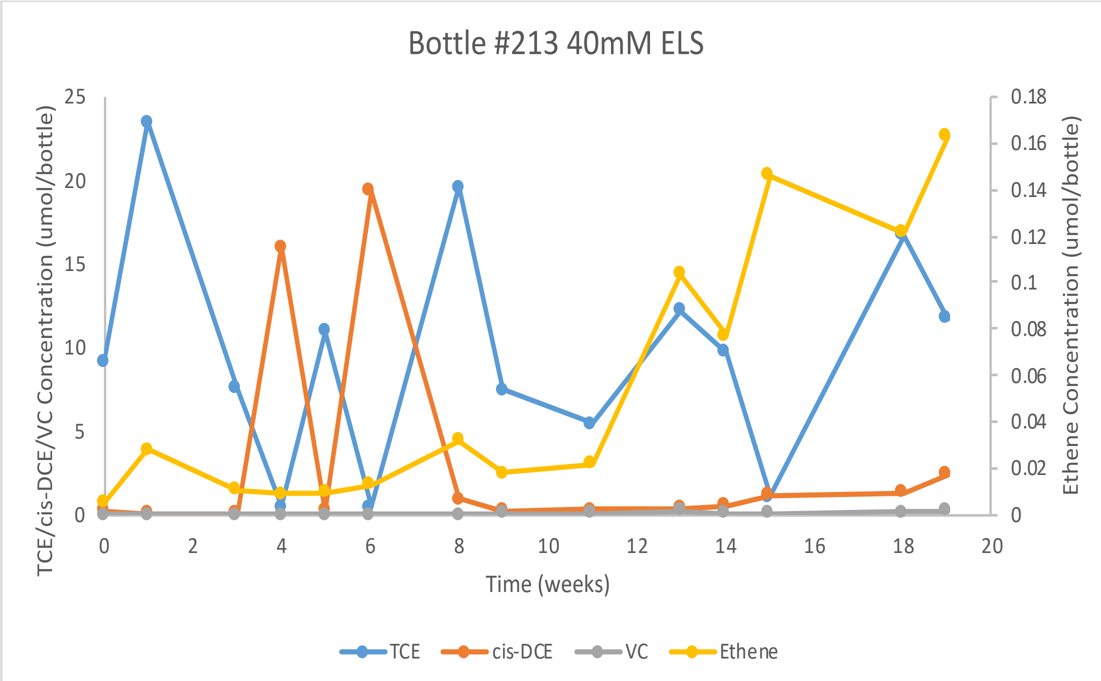


Figure B.21: TCE reduction and methane reduction for aquifer material with 40mM ELS. Plot indicates replicate one of two.



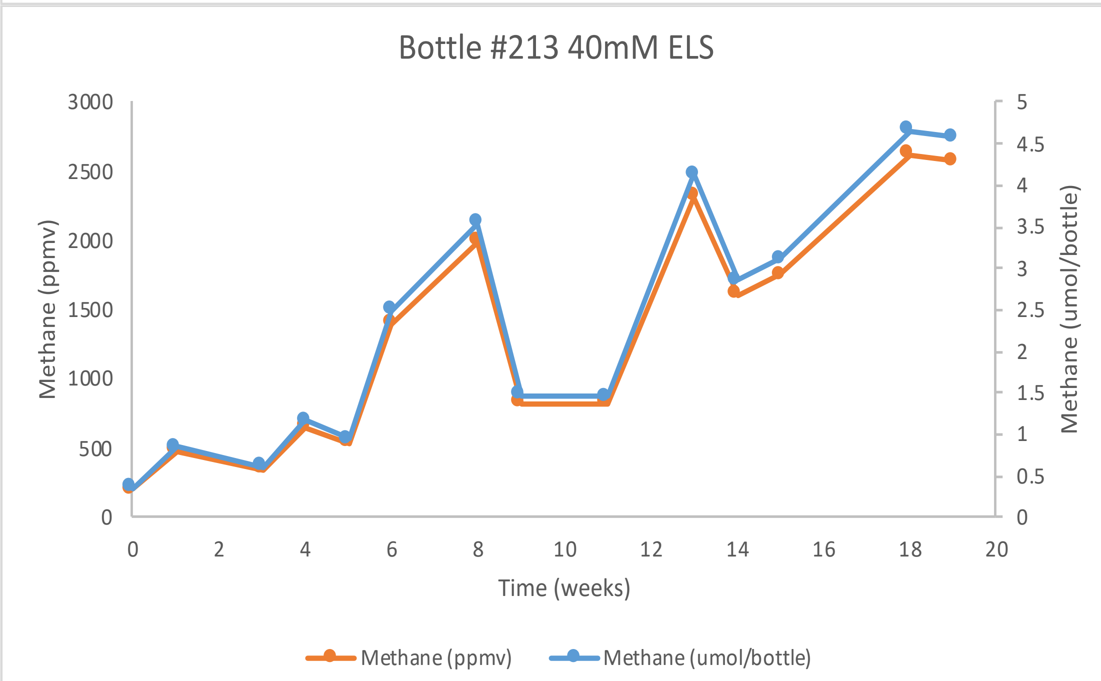


Figure B.22: TCE reduction and methane reduction for aquifer material with 40mM ELS. Plot indicates replicate two of two.

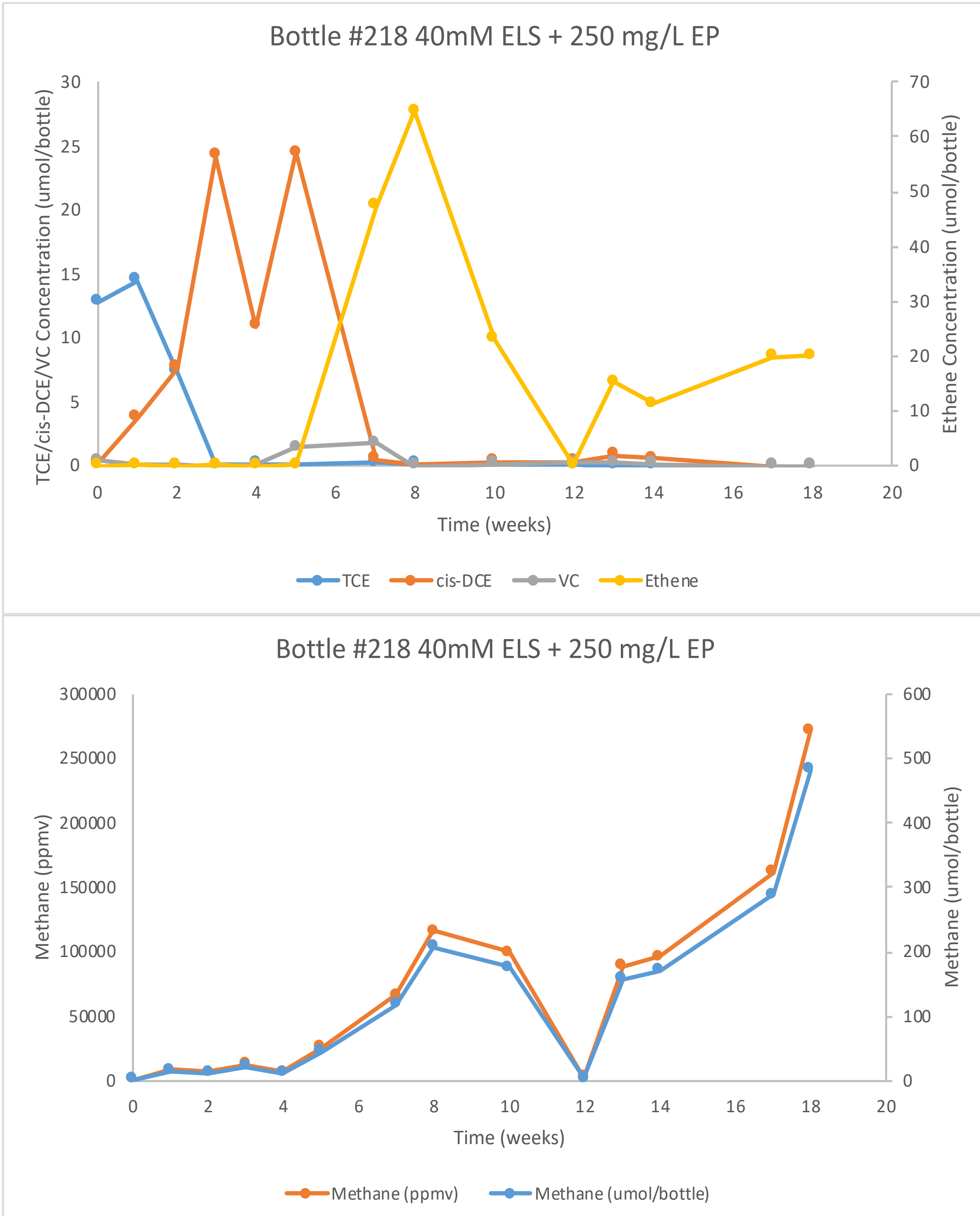


Figure B.23: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L ELS. Plot indicates replicate one of two.

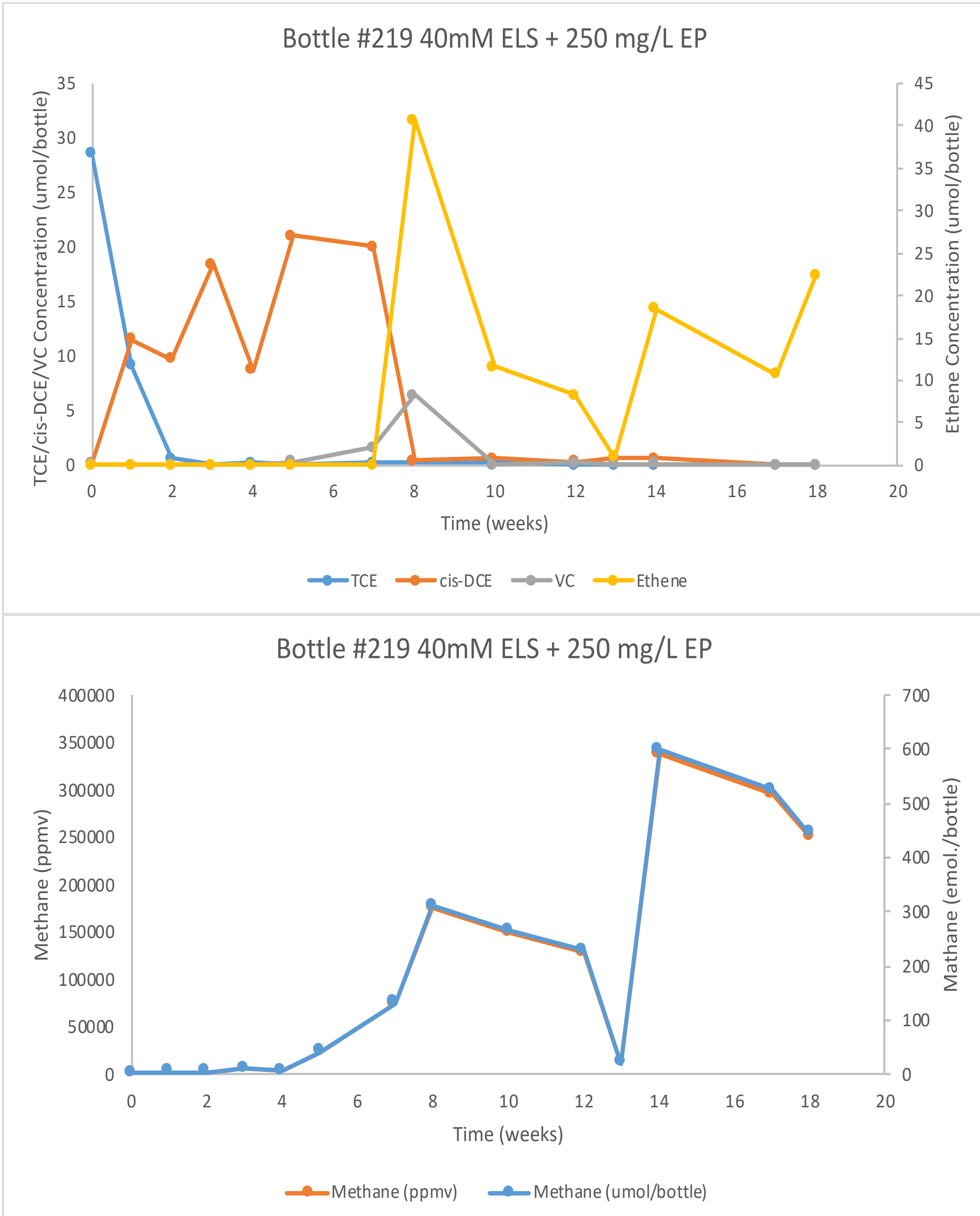


Figure B.24: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L ELS. Plot indicates replicate two of two.

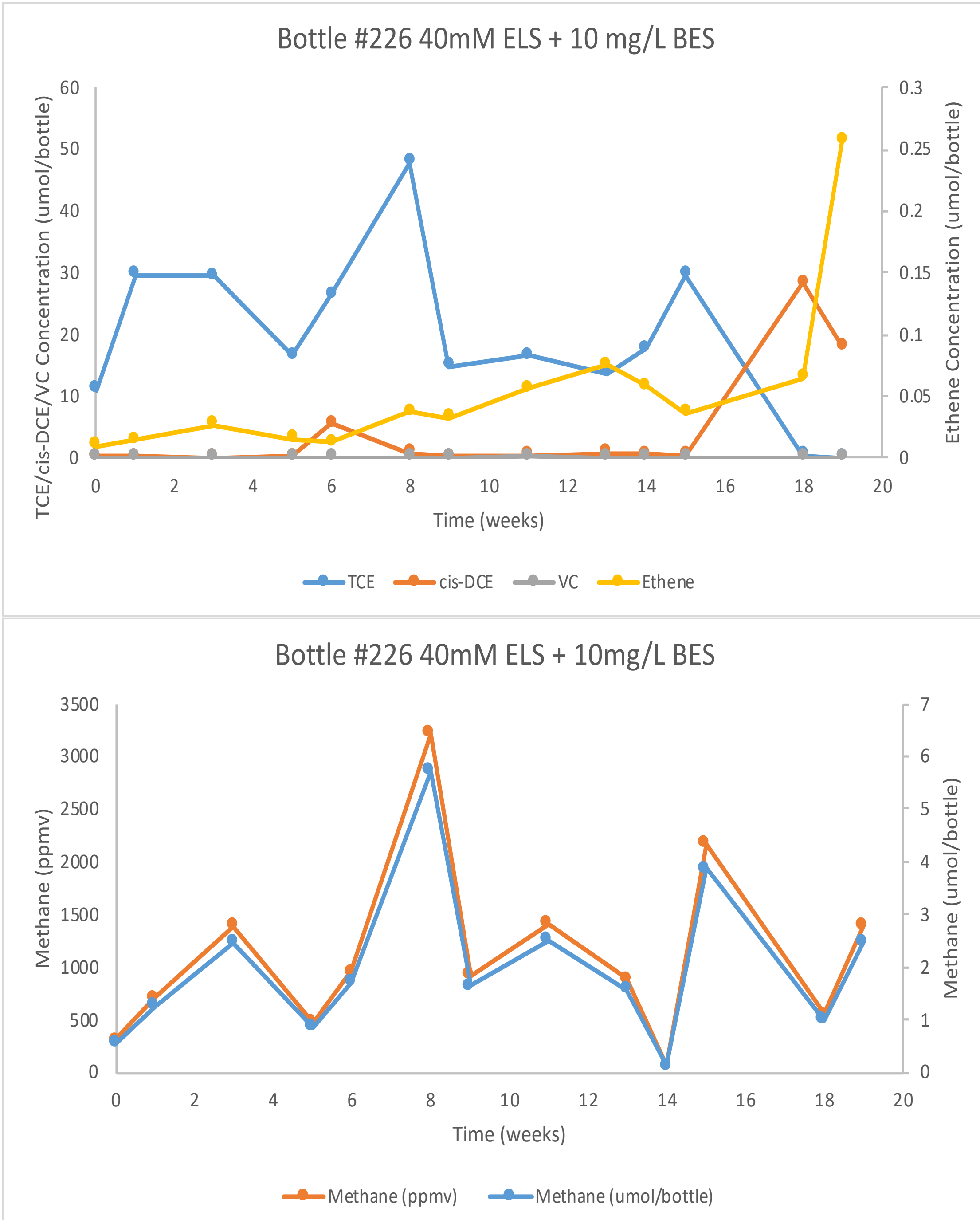
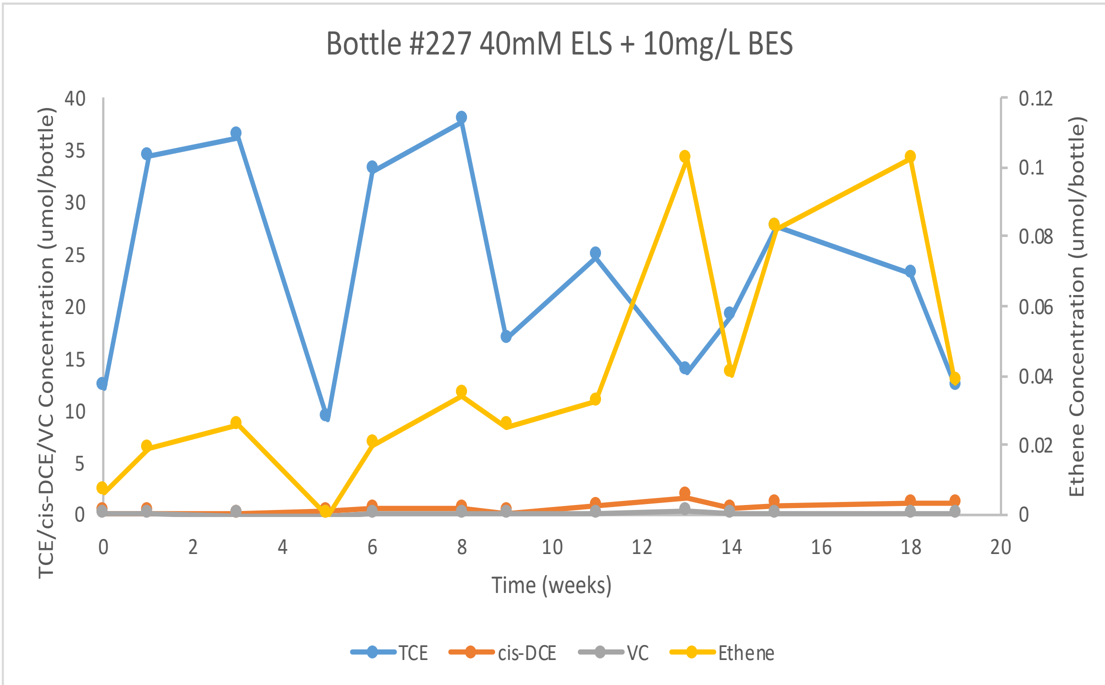


Figure B.25: TCE reduction and methane reduction for aquifer material with 40mM ELS and 10 mg/L BES. Plot indicates replicate one of two.



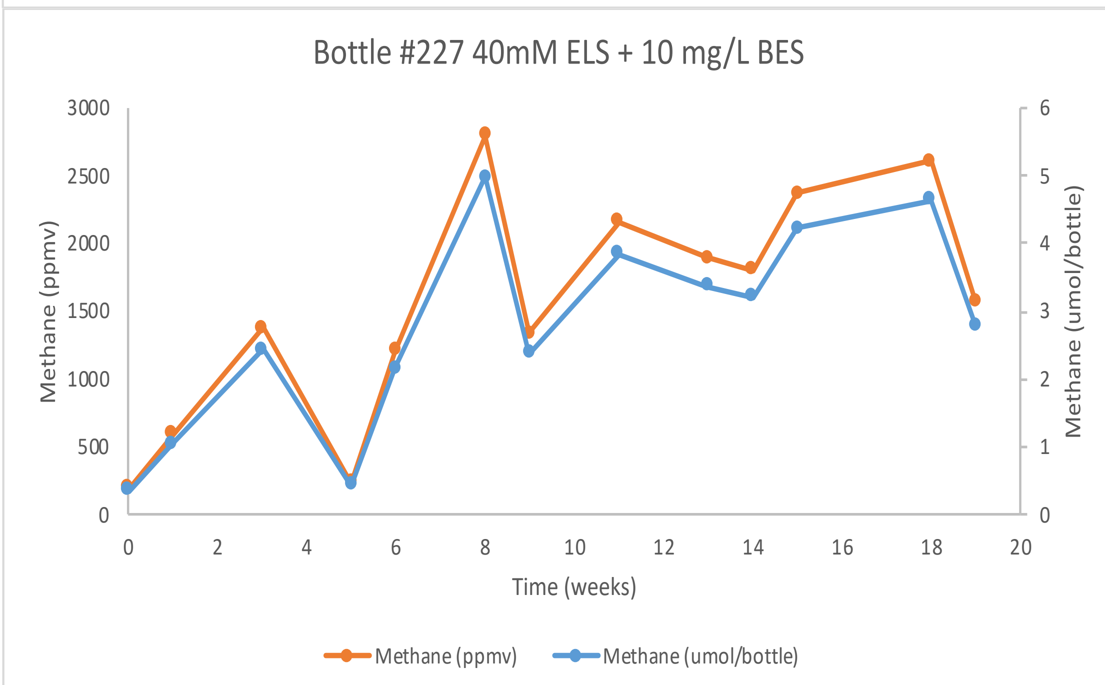


Figure B.26: TCE reduction and methane reduction for aquifer material with 40mM ELS and 10 mg/L BES. Plot indicates replicate two of two.

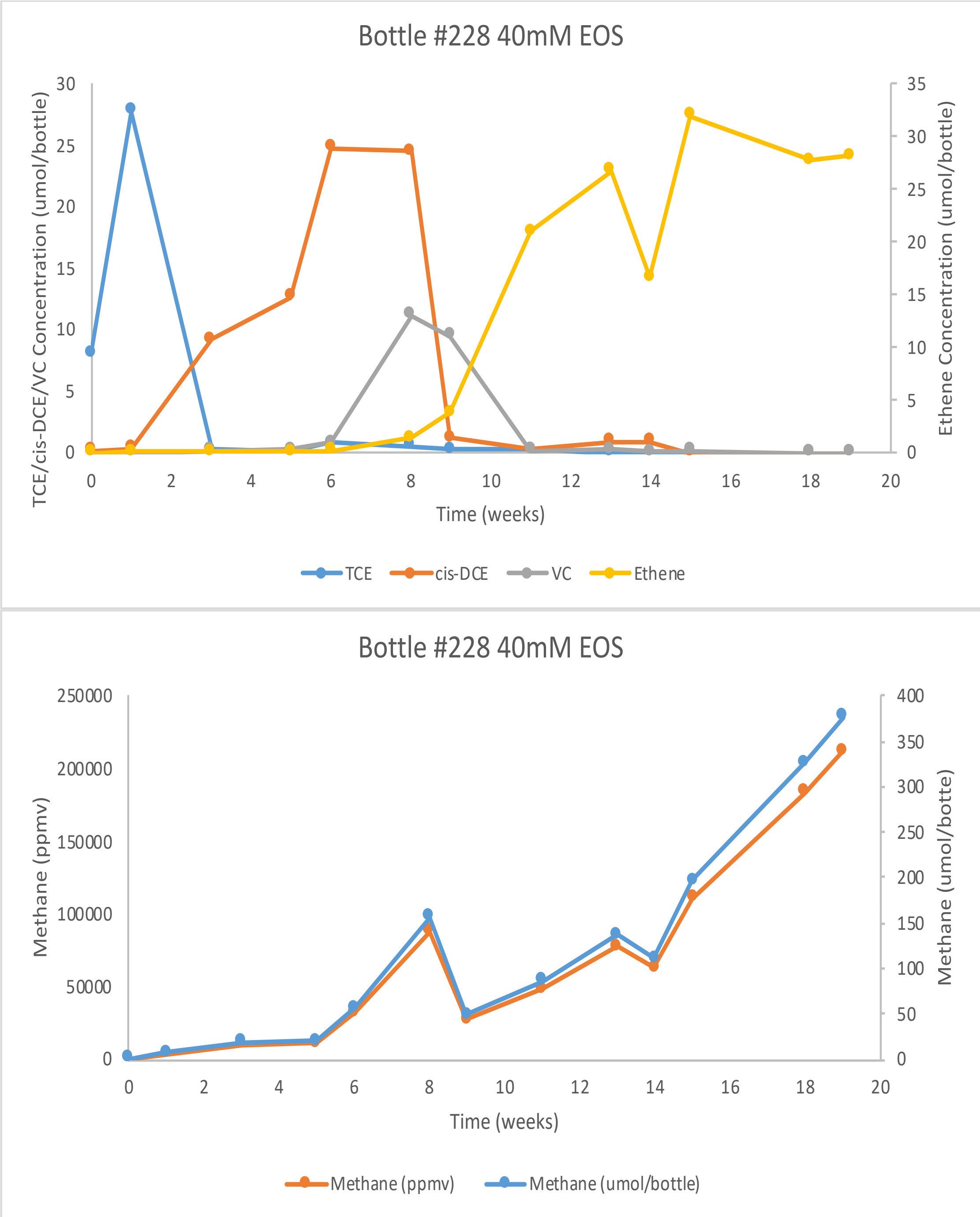
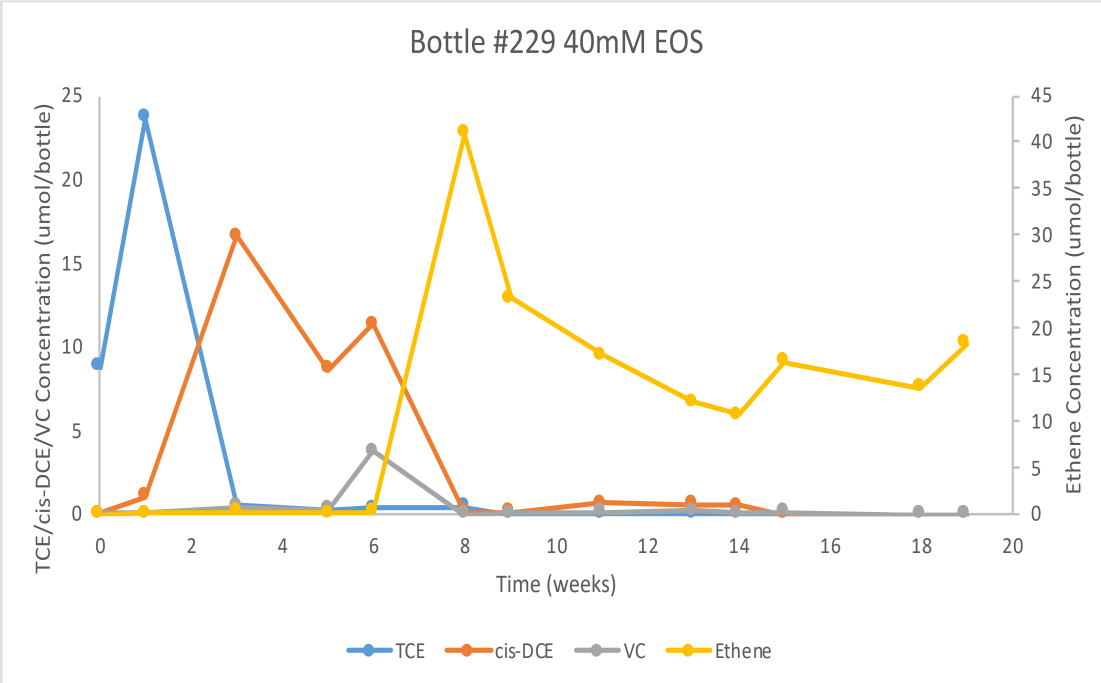


Figure B.27: TCE reduction and methane reduction for aquifer material with 40mM EOS. Plot indicates replicate one of two.



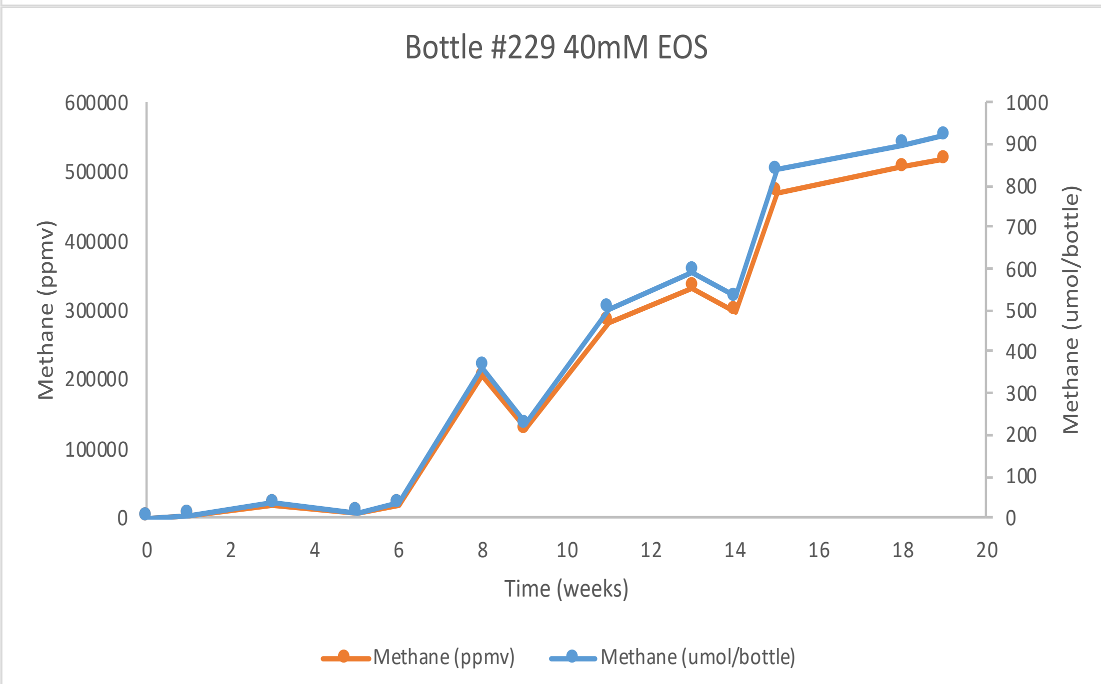


Figure B.28: TCE reduction and methane reduction for aquifer material with 40mM EOS. Plot indicates replicate two of two.

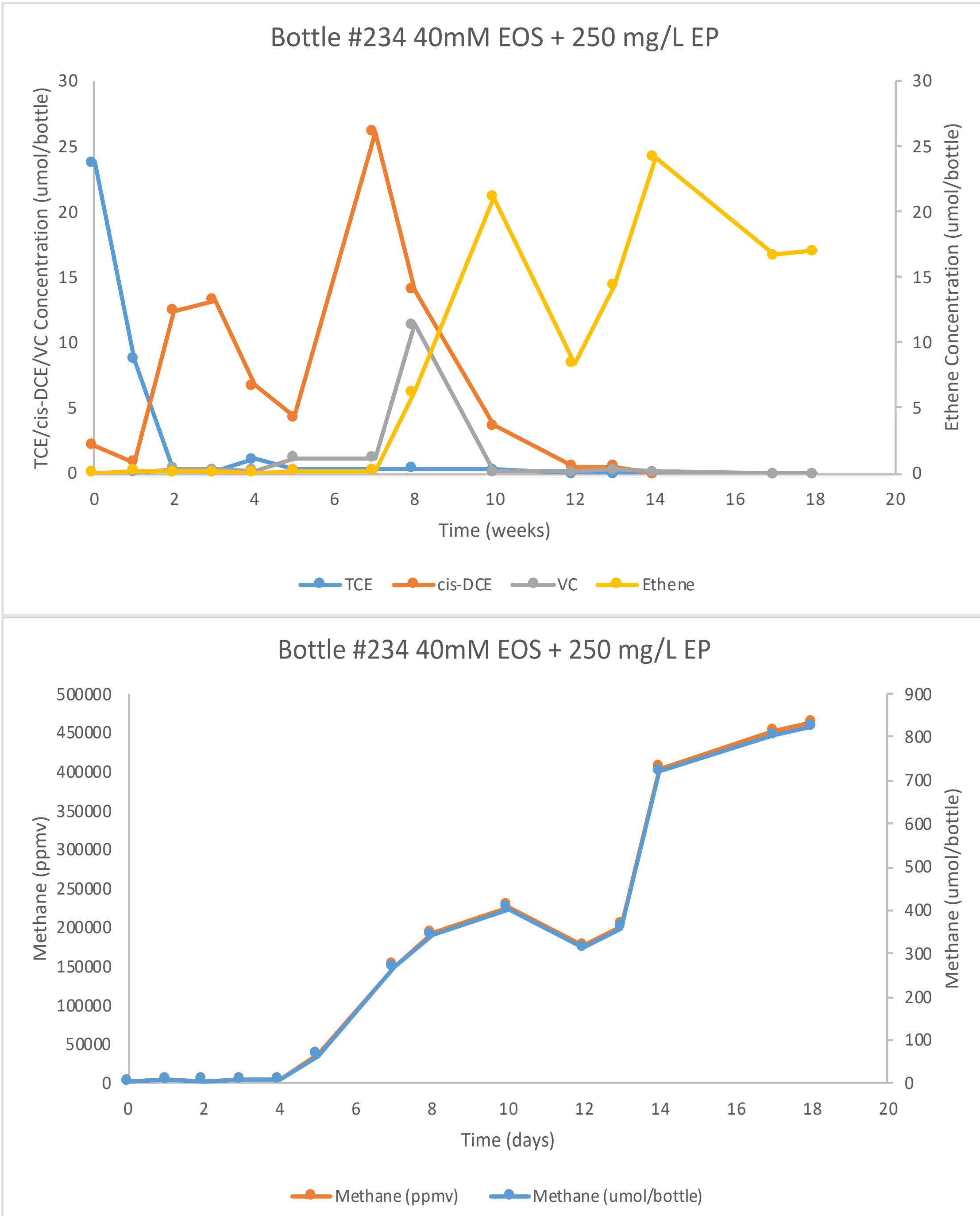


Figure B.29: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

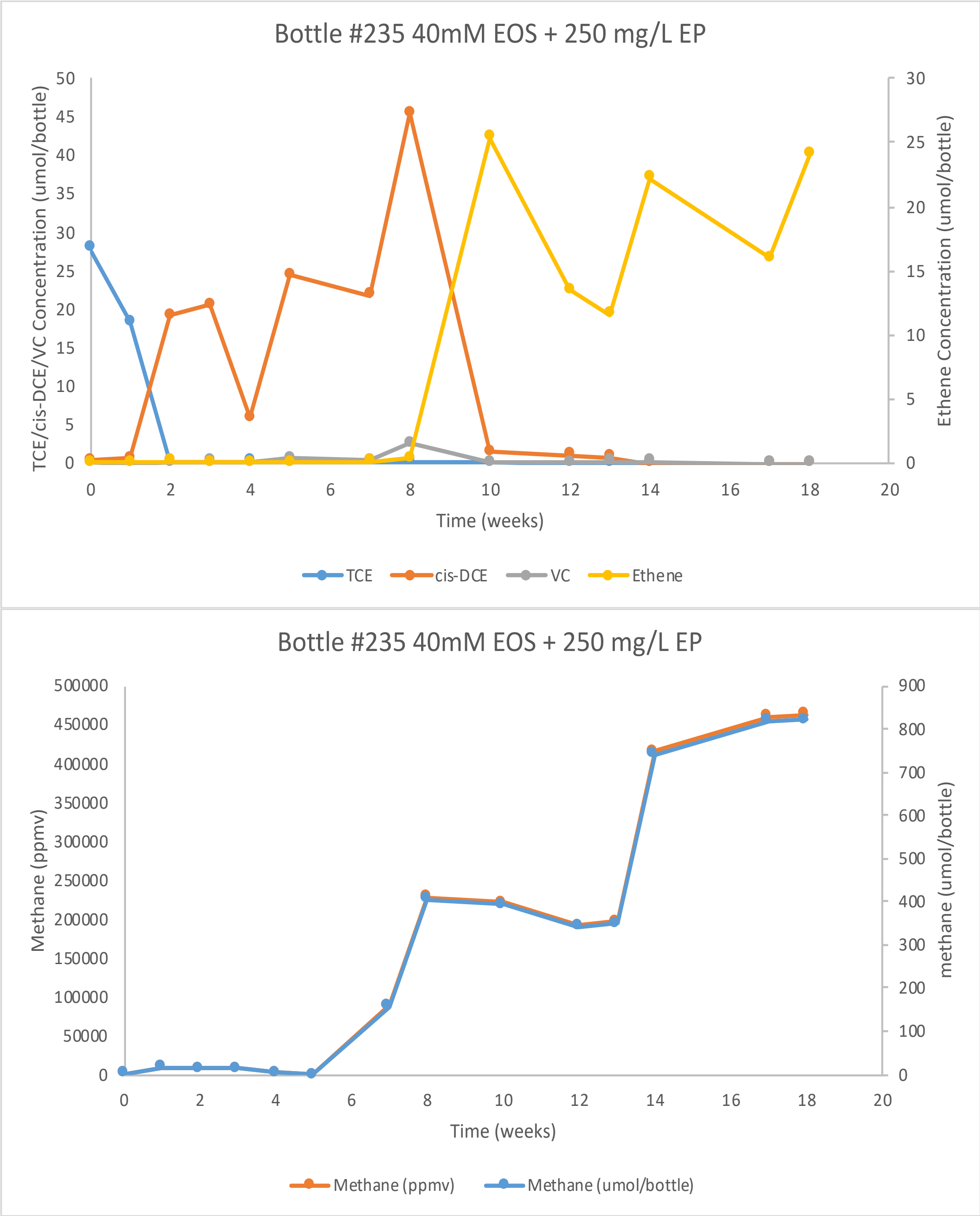


Figure B.30: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

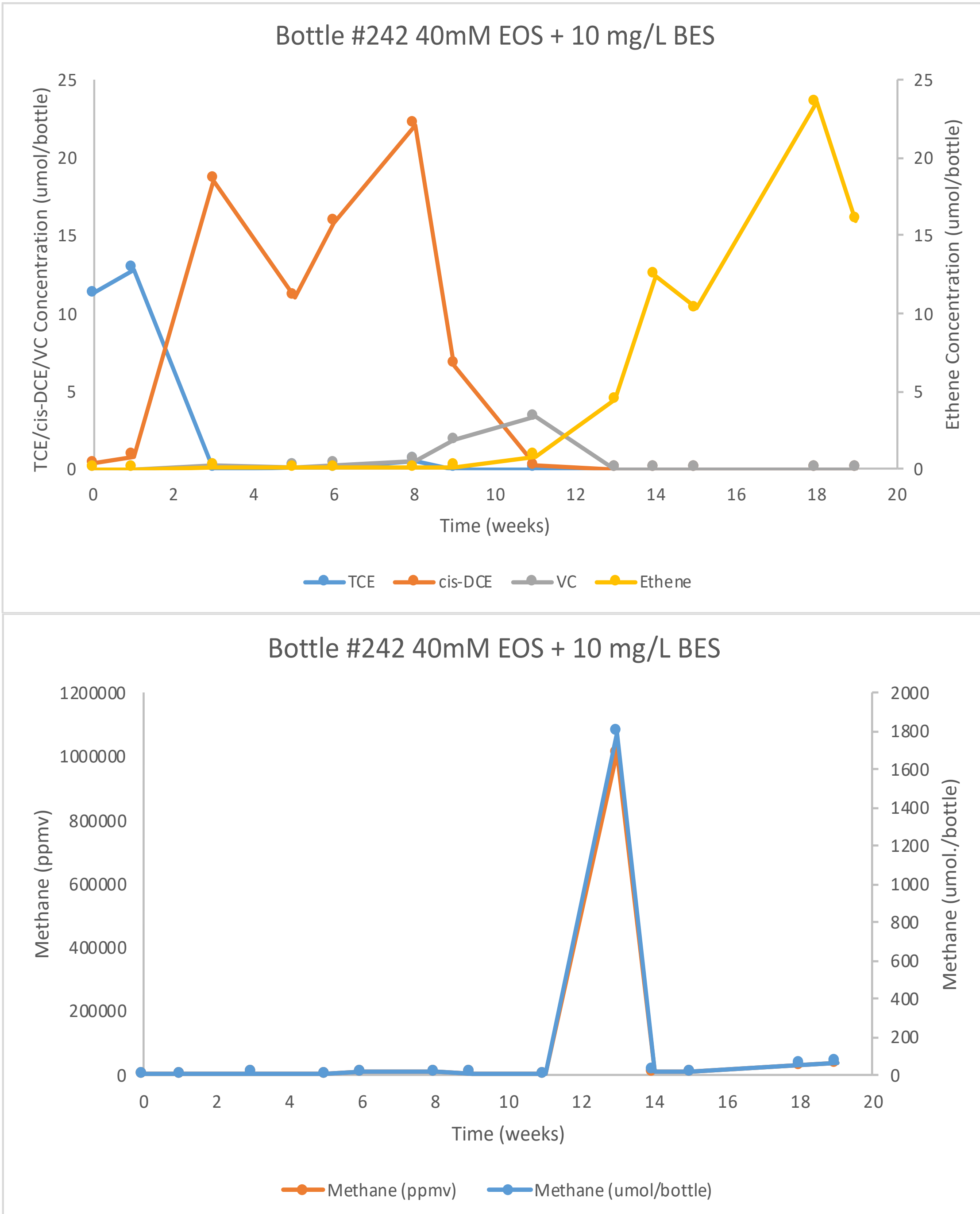


Figure B.31: TCE reduction and methane reduction for aquifer material with 40mM EOS and 10 mg/L BES. Plot indicates replicate one of two.

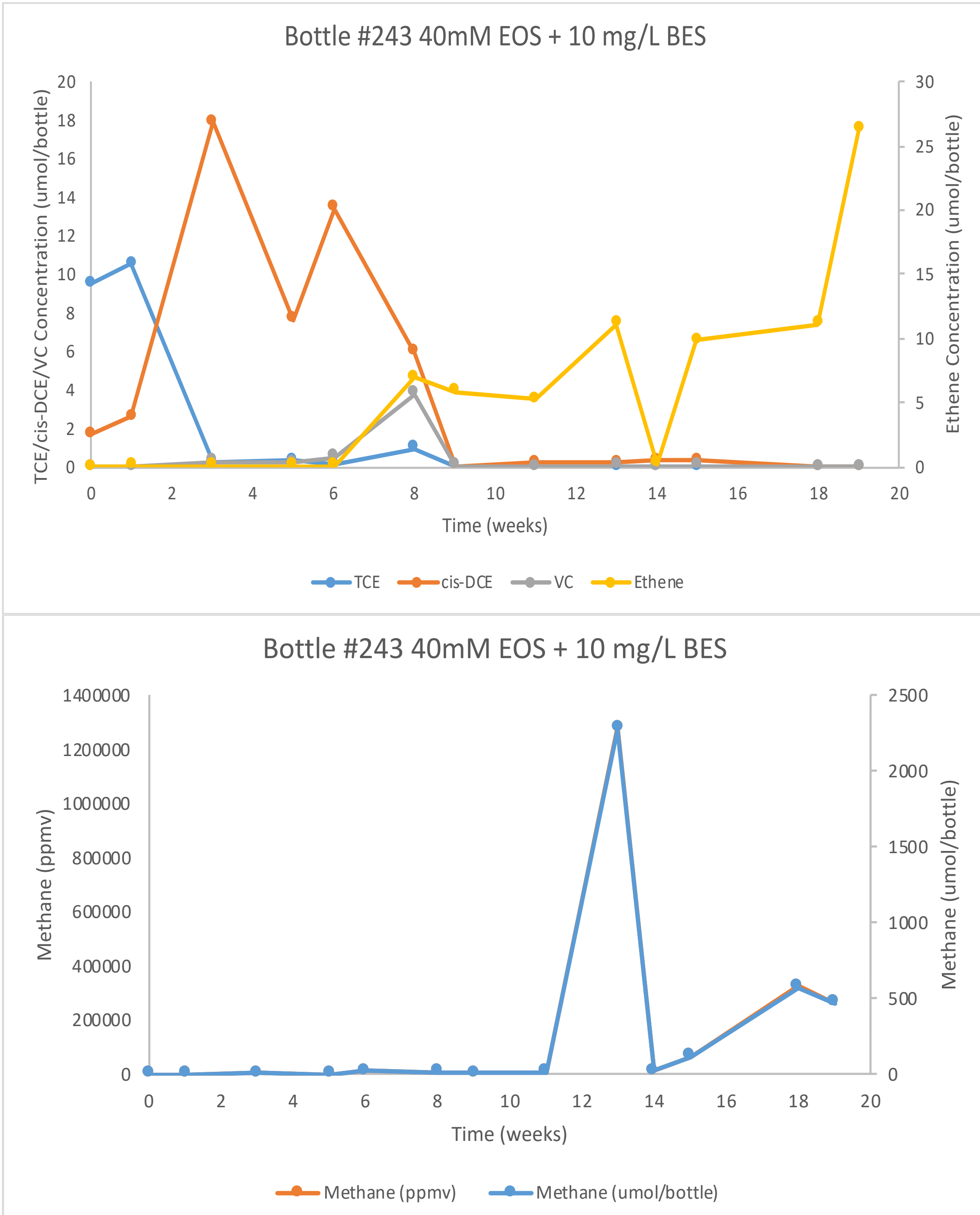


Figure B.32: TCE reduction and methane reduction for aquifer material with 40mM EOS and 10 mg/L BES. Plot indicates replicate two of two.

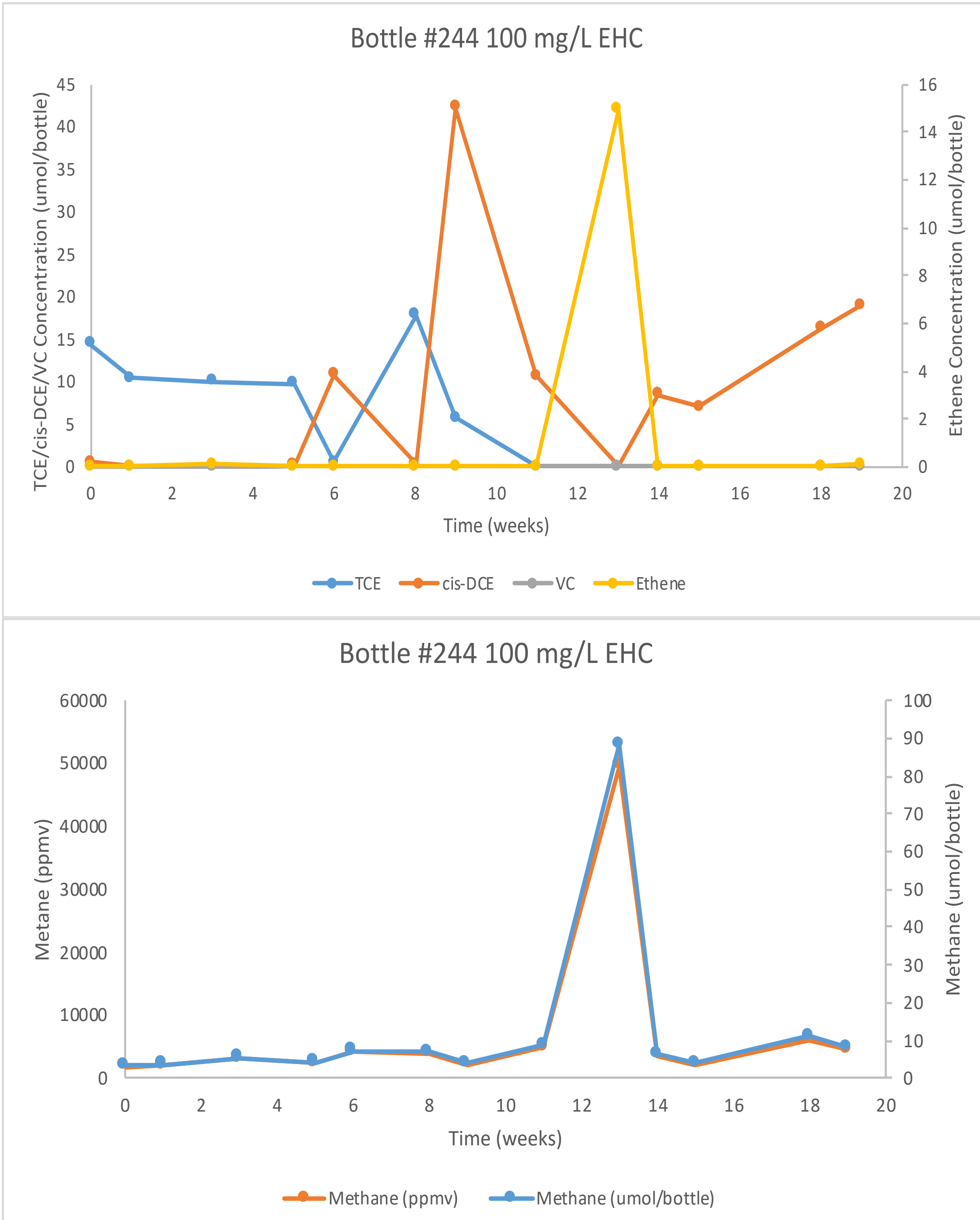
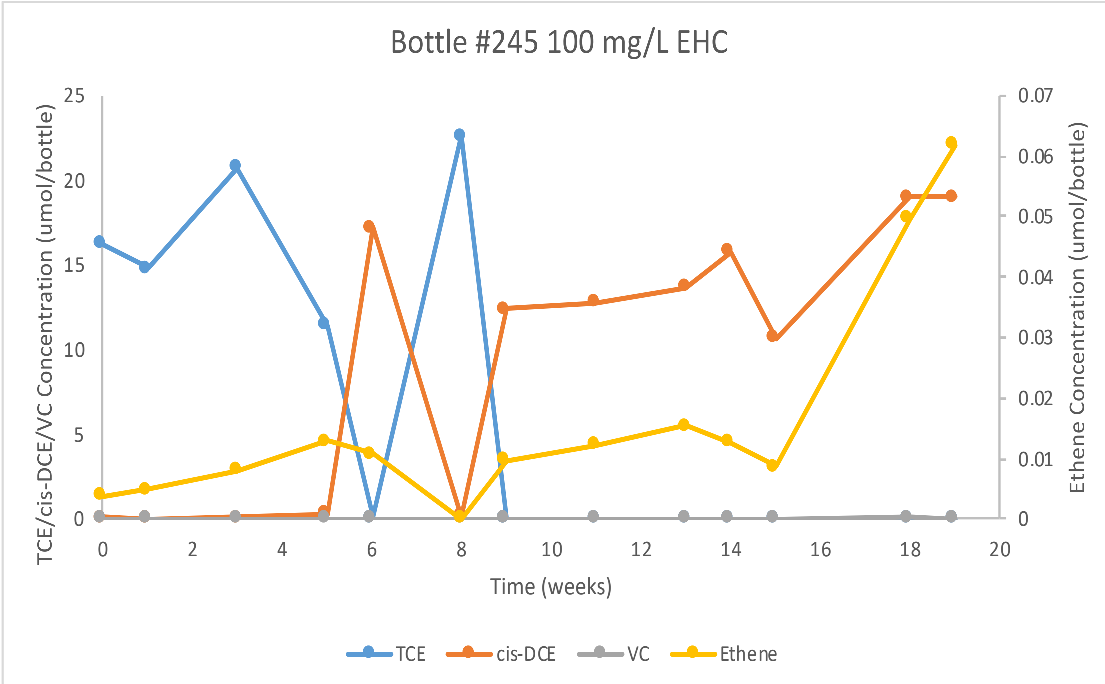


Figure B.33: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC. Plot indicates replicate one of two.



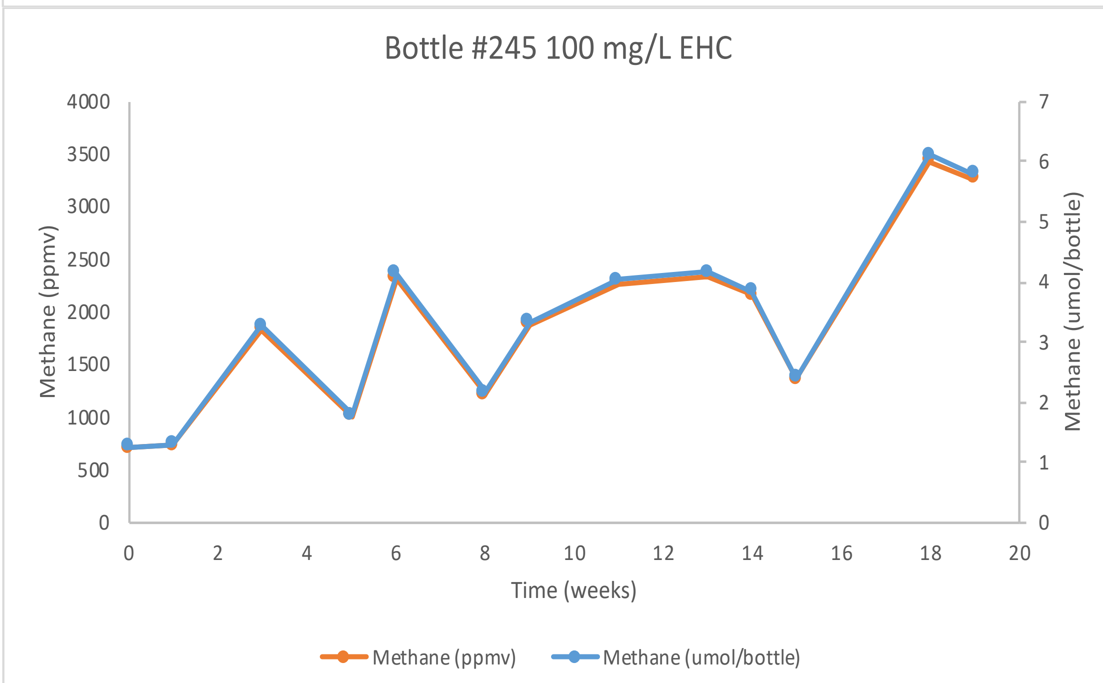
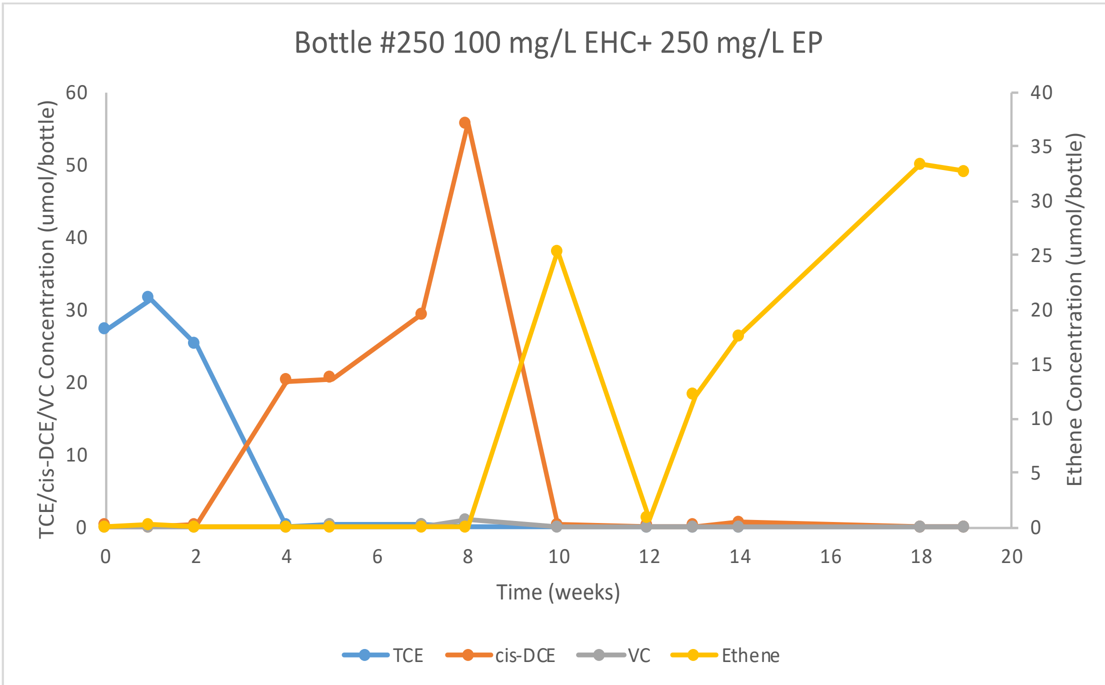


Figure B.34: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC. Plot indicates replicate two of two.



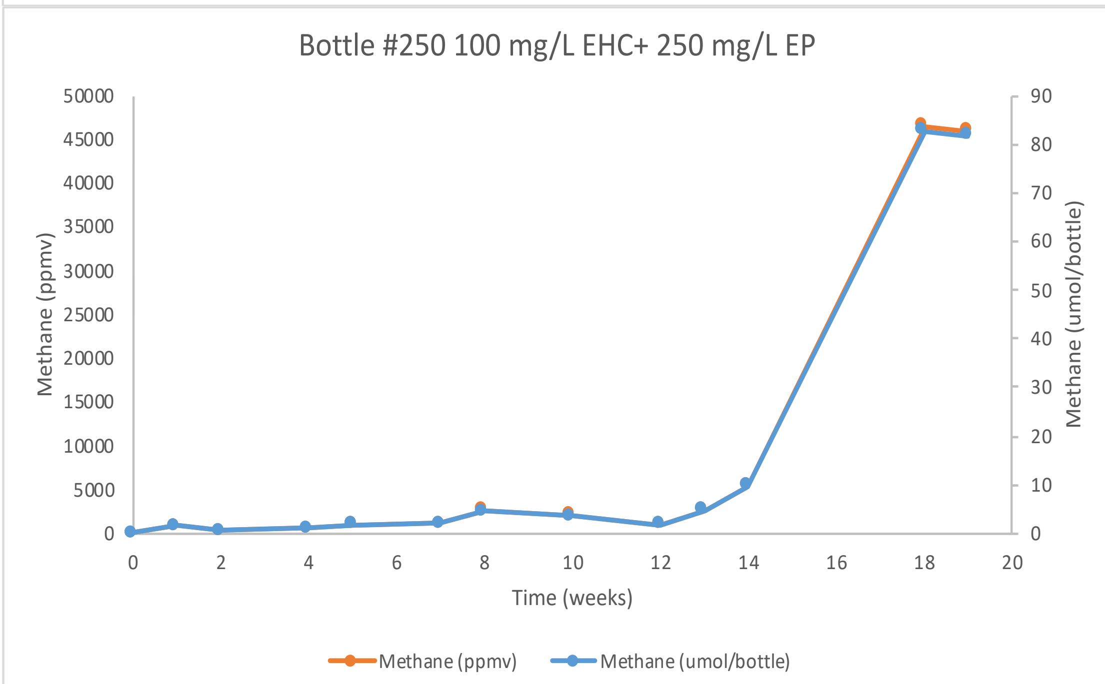


Figure B.35: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

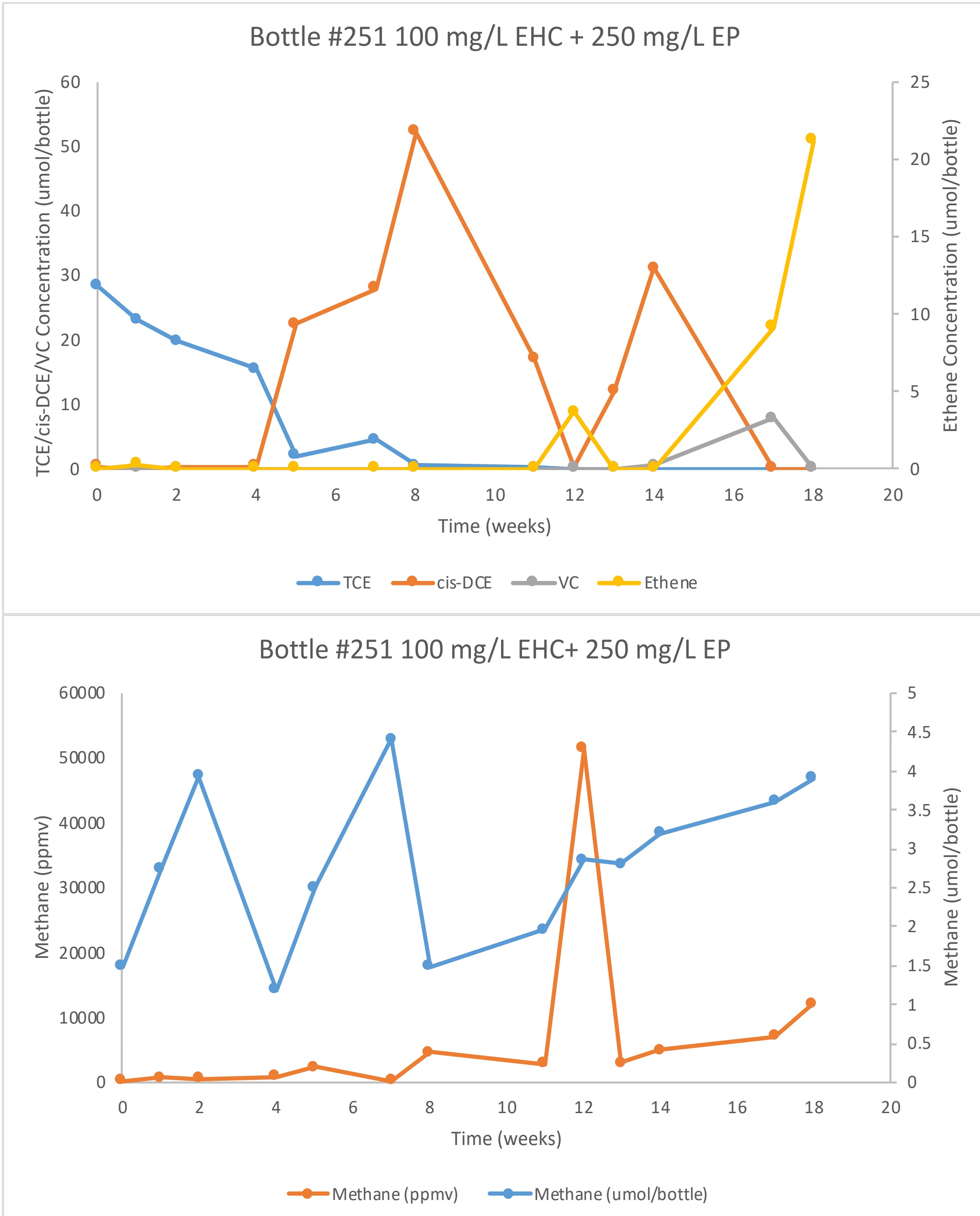
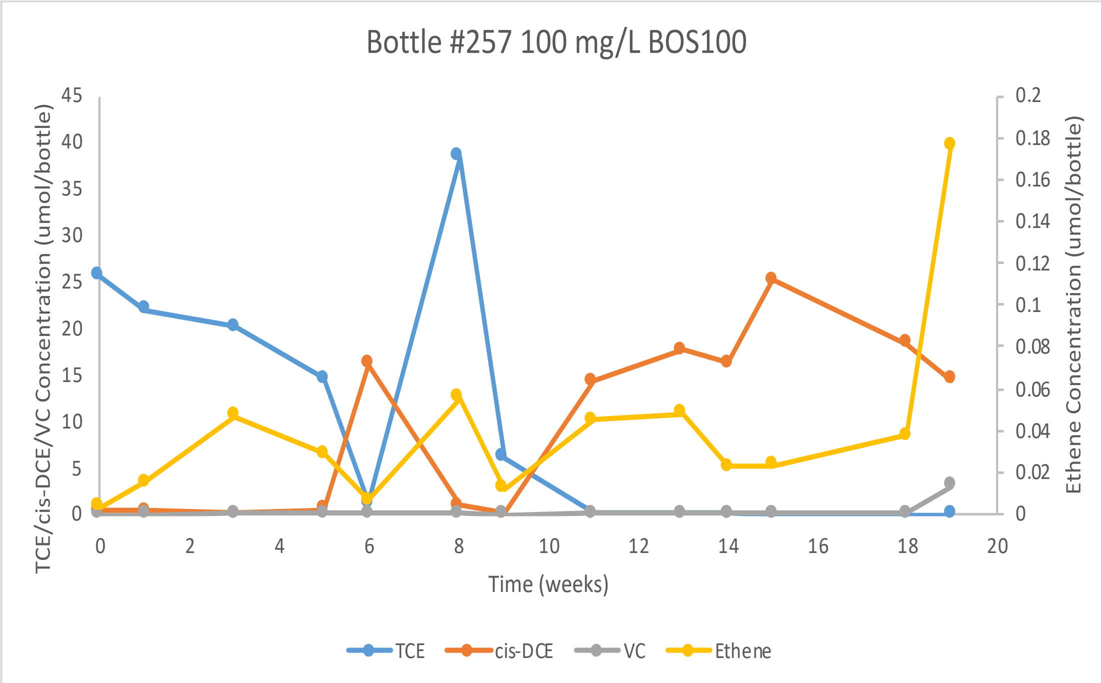


Figure B.36: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.

Figure B.37: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100. Plot indicates replicate one of two.



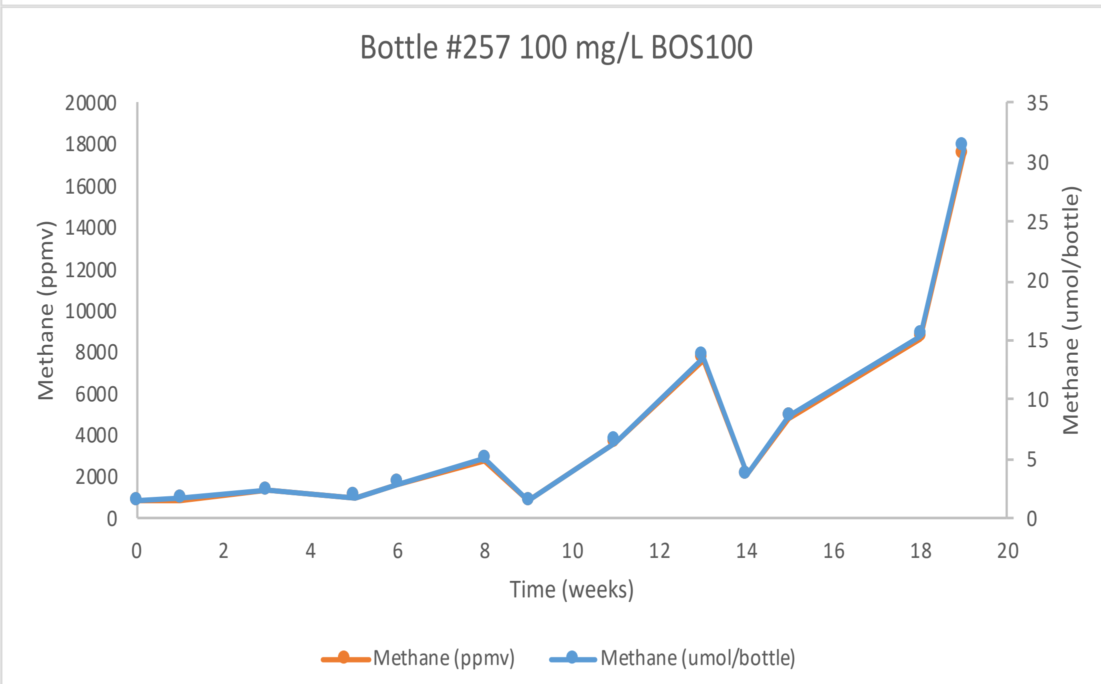
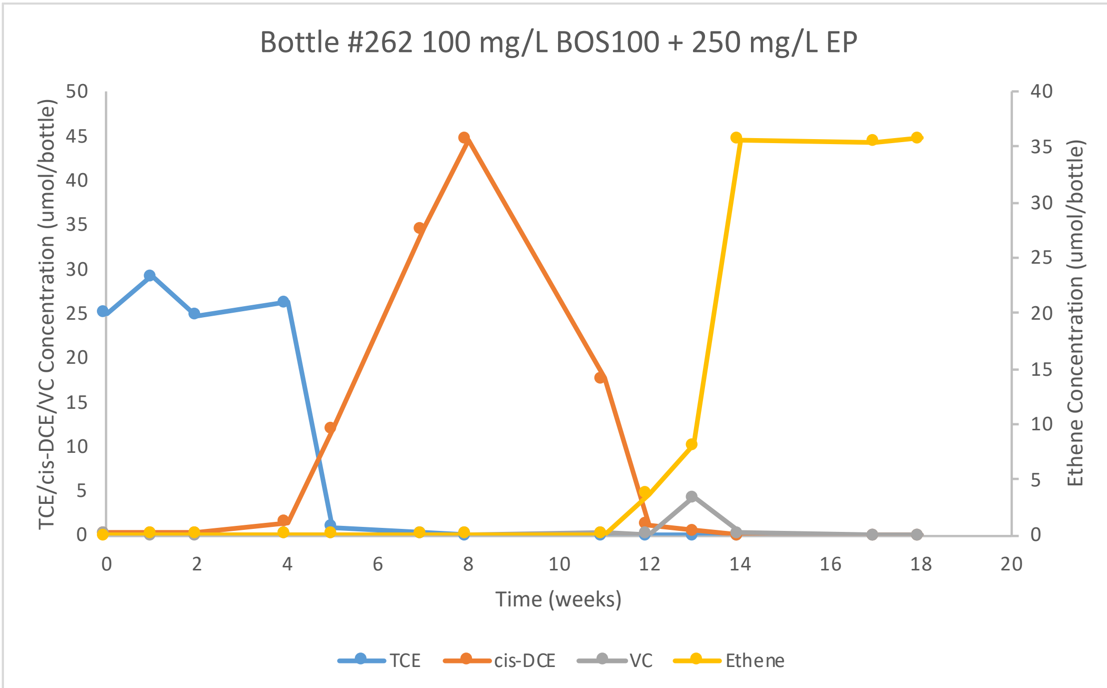


Figure B.38: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100. Plot indicates replicate two of two.



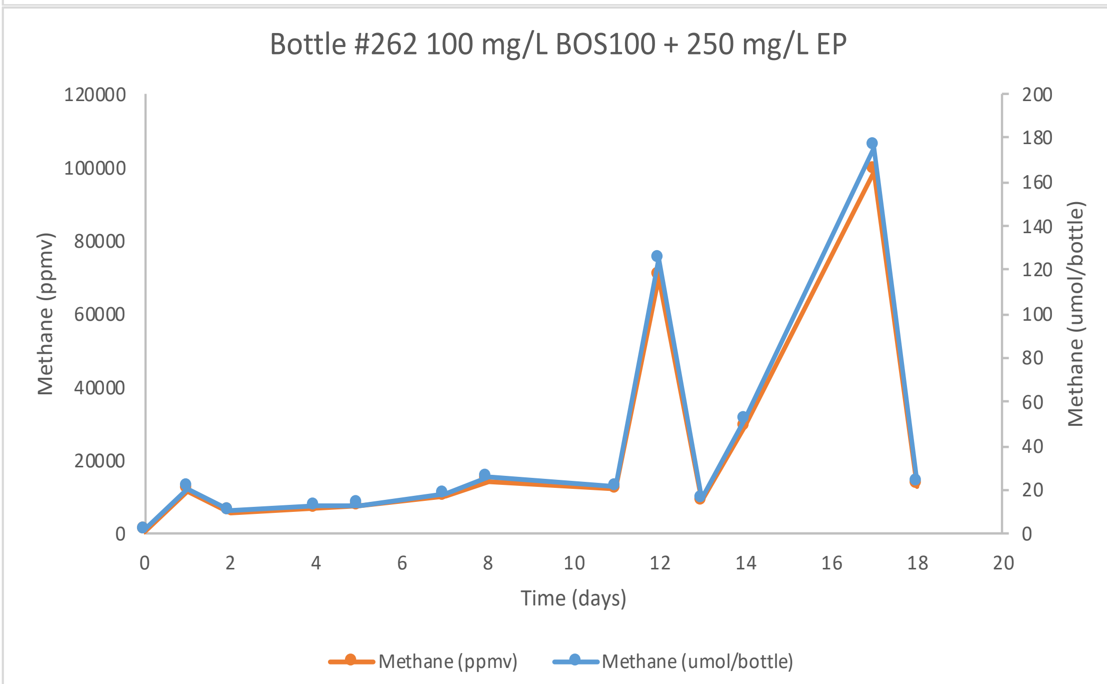


Figure B.39: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Ethyl Propionate. Plot indicates replicate one of two.

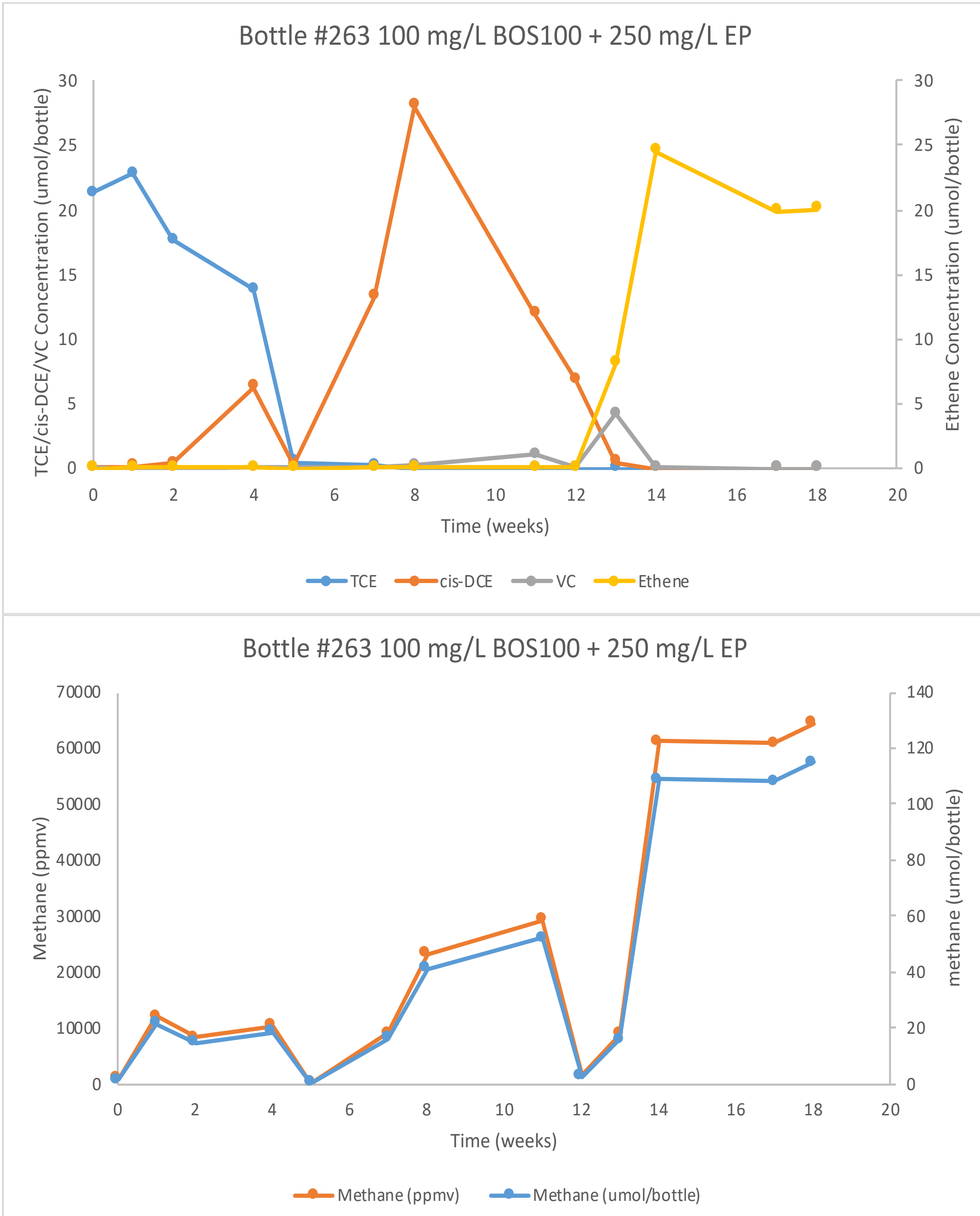
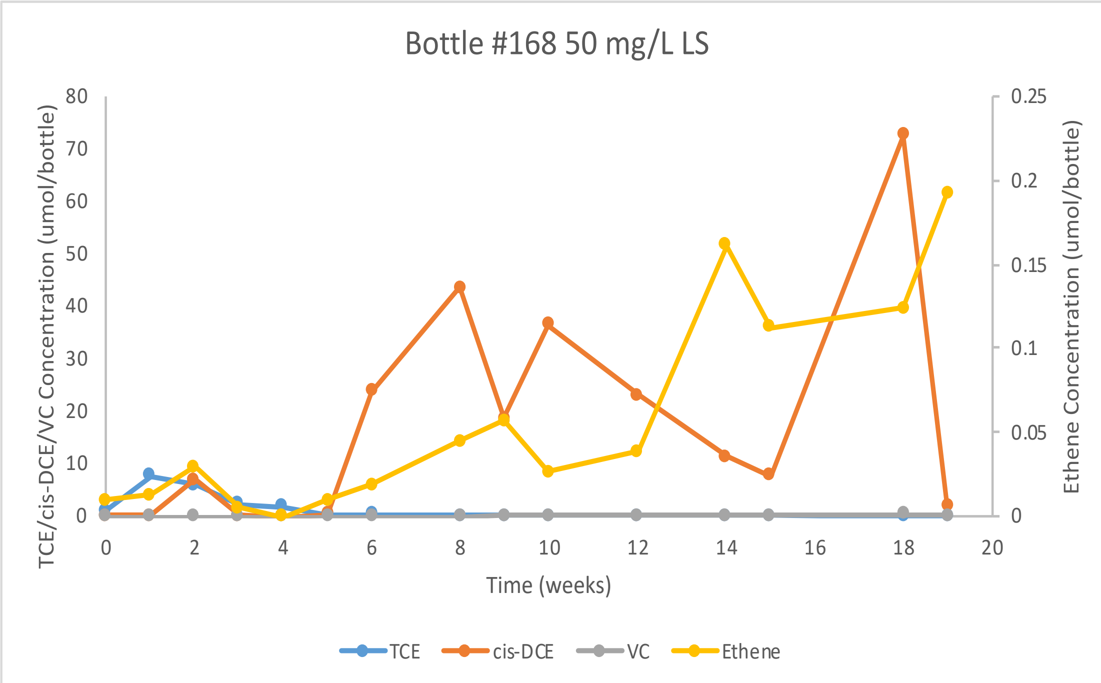


Figure B.40: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Ethyl Propionate. Plot indicates replicate two of two.



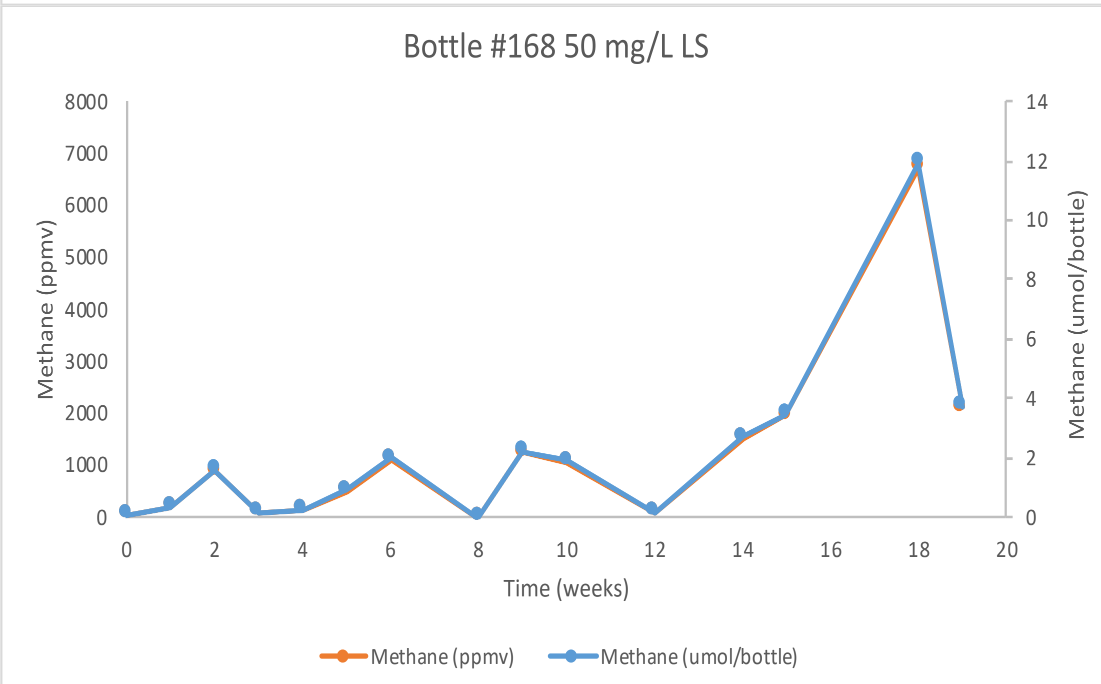
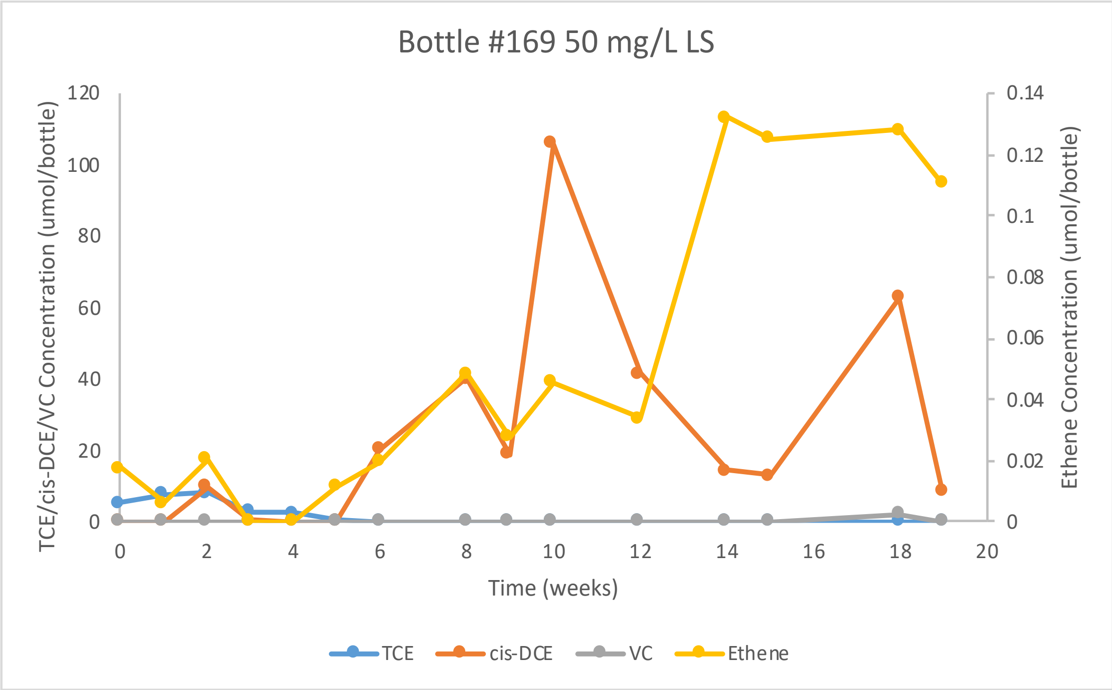


Figure B.41: TCE reduction and methane reduction for aquifer material with 50 mg/L Lovastatin. Plot indicates replicate one of two.



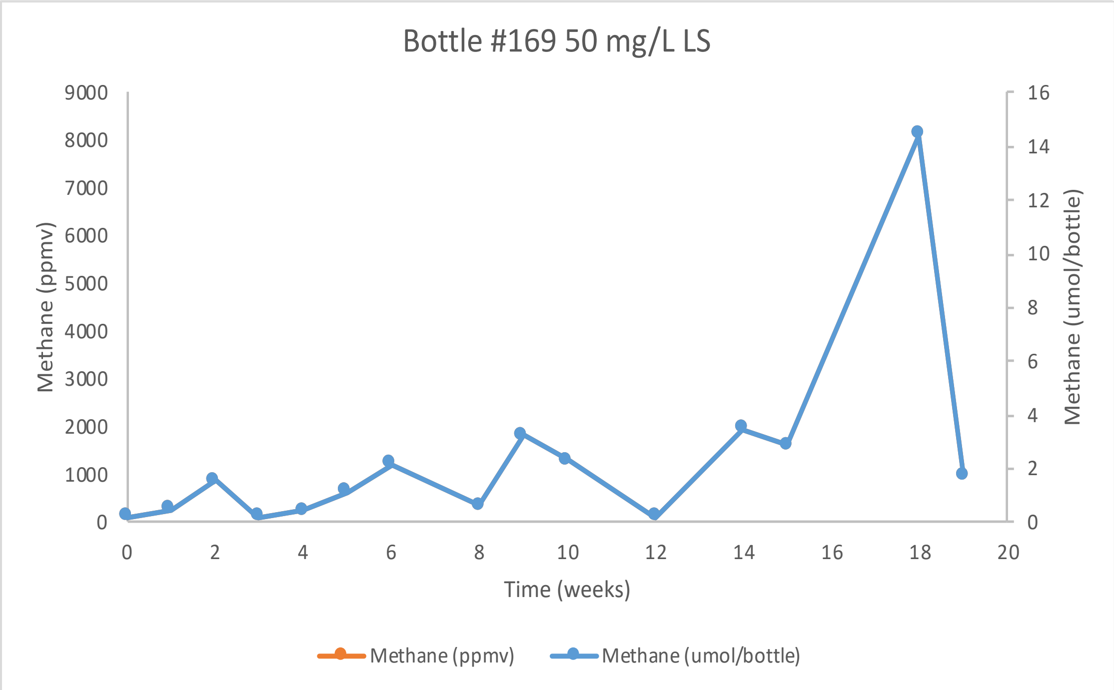
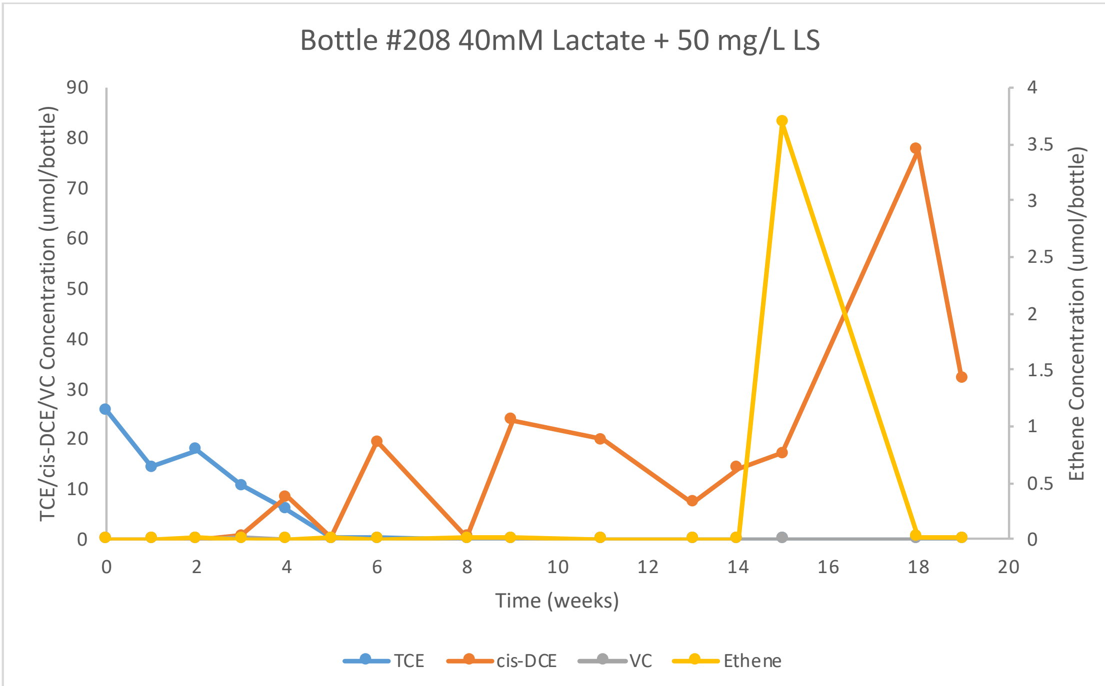


Figure B.42: TCE reduction and methane reduction for aquifer material with 50 mg/L Lovastatin. Plot indicates replicate two of two.



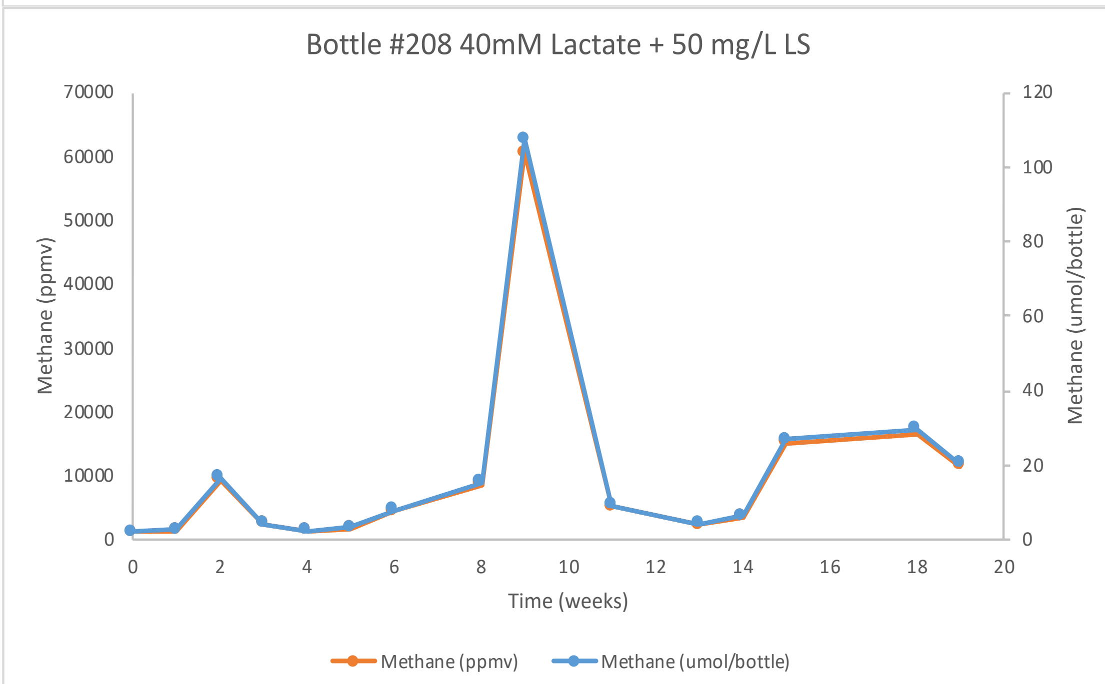
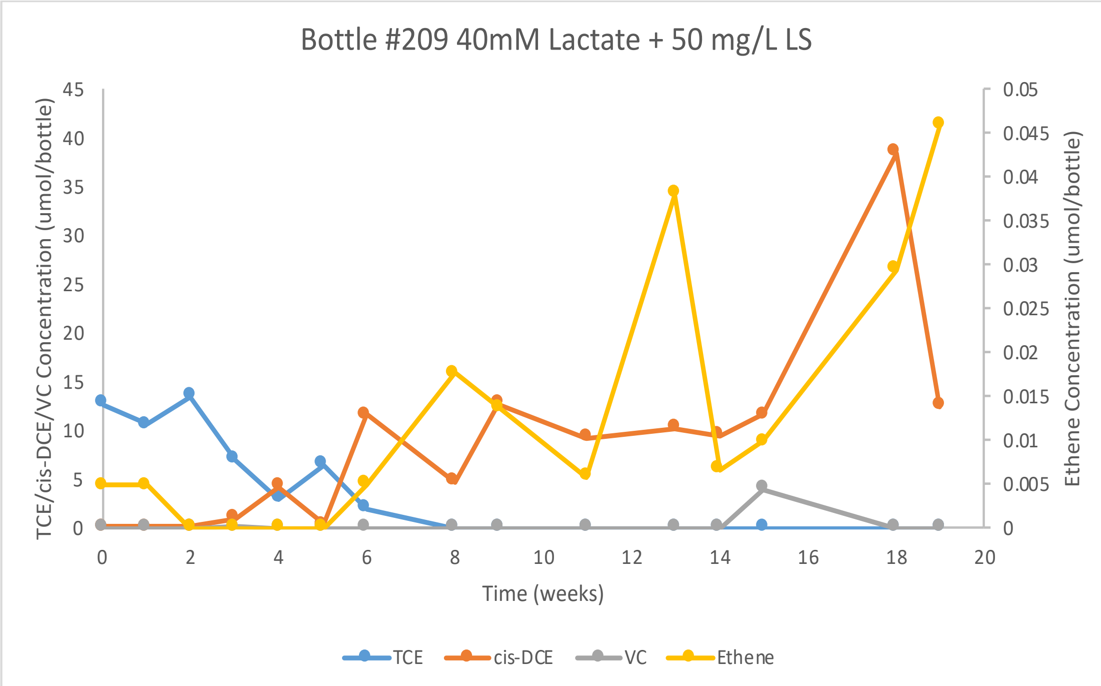


Figure B.43: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 50 mg/L Lovastatin. Plot indicates replicate one of two.



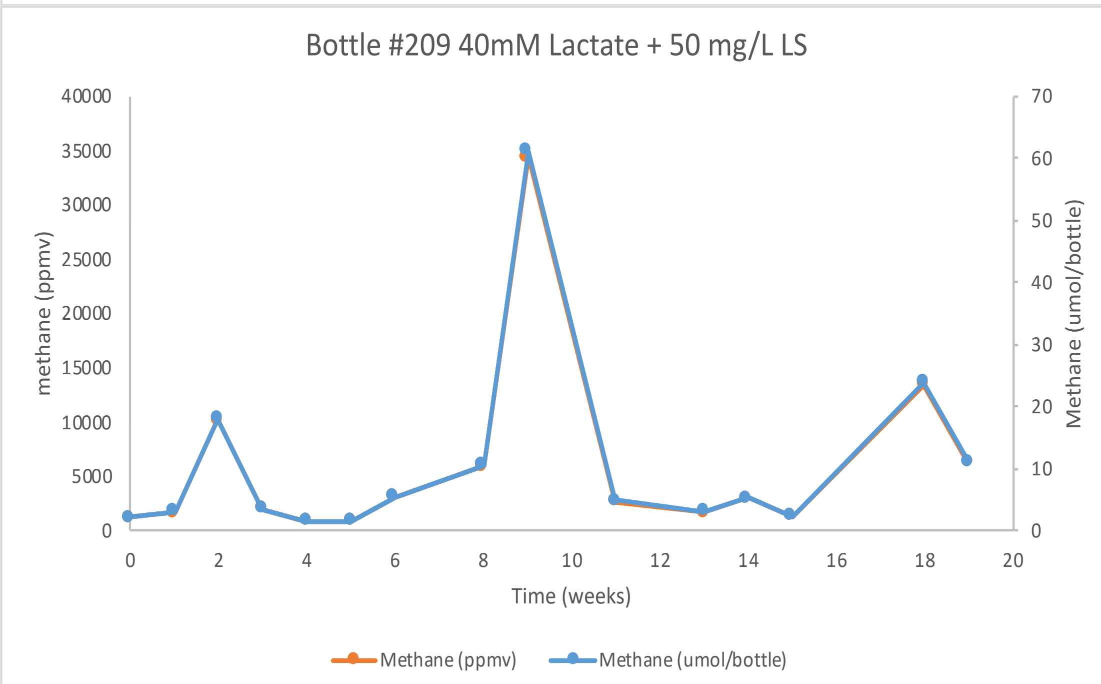


Figure B.44: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 50 mg/L Lovastatin. Plot indicates replicate two of two.

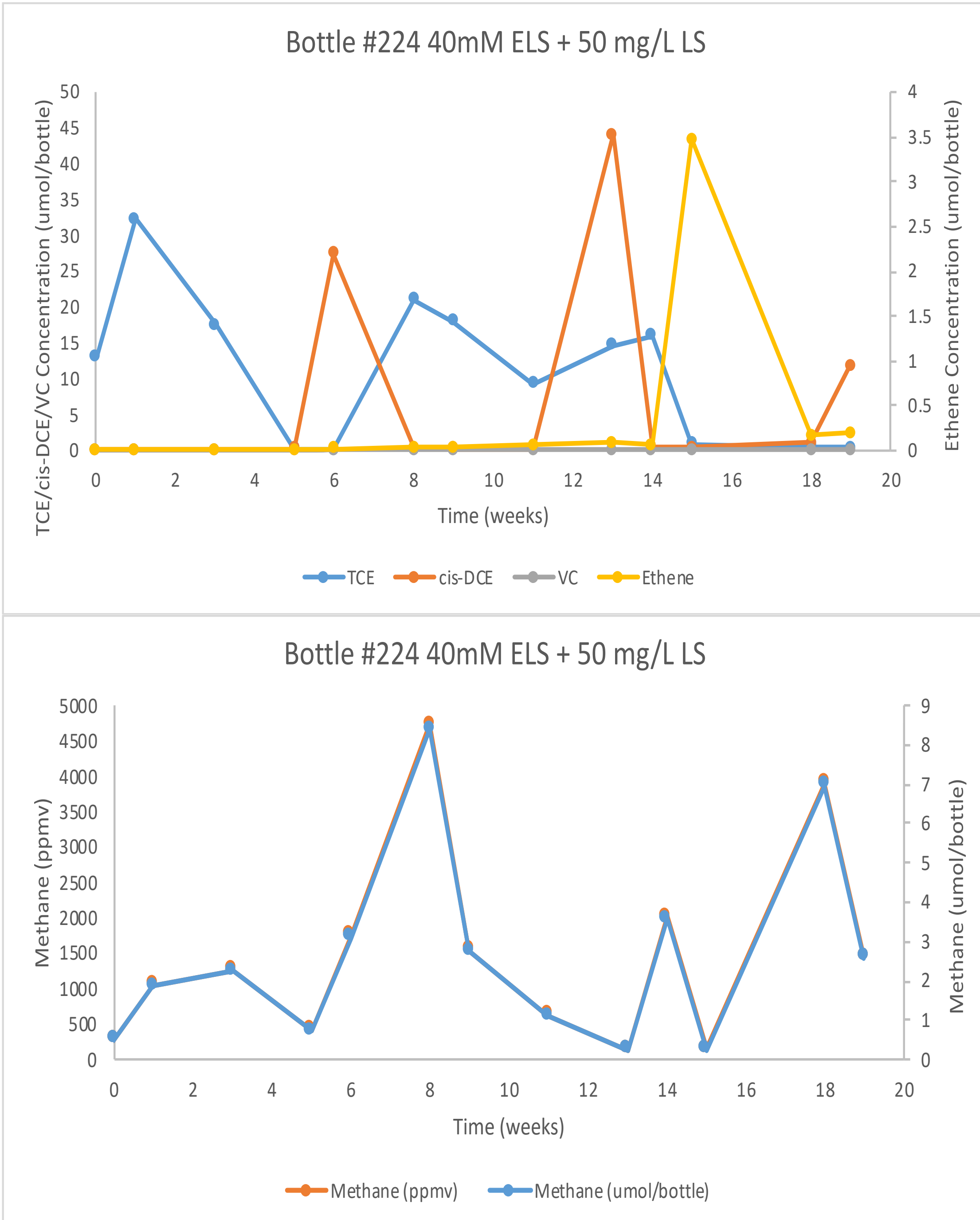
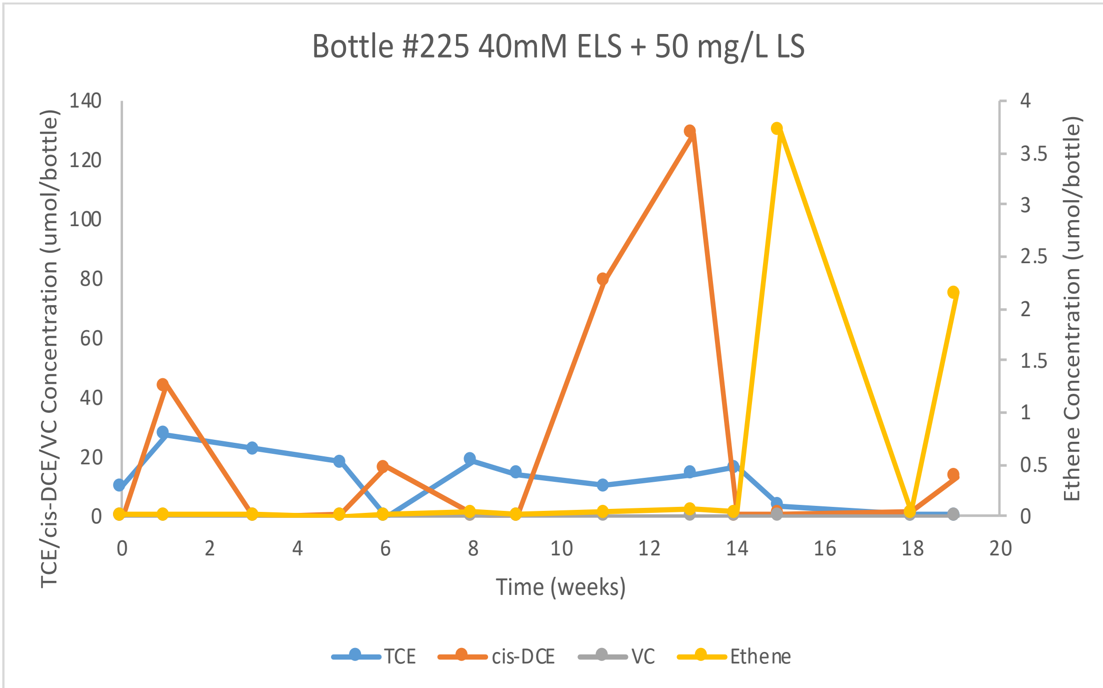


Figure B.45: TCE reduction and methane reduction for aquifer material with 40mM ELS and 50 mg/L Lovastatin. Plot indicates replicate one of two.



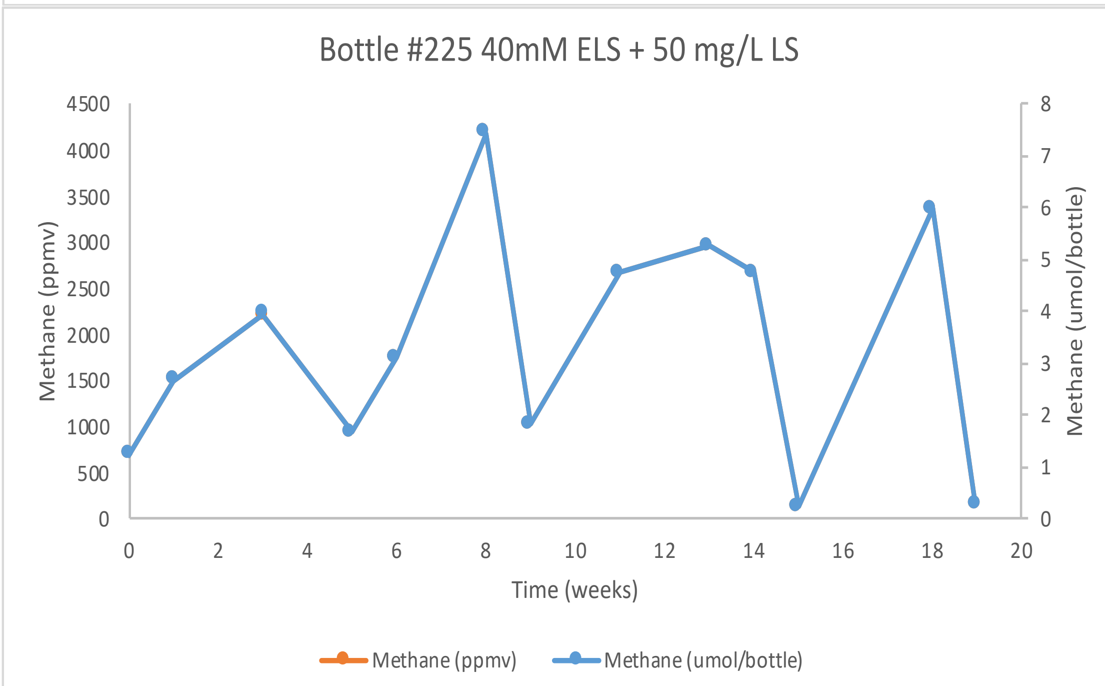


Figure B.46: TCE reduction and methane reduction for aquifer material with 40mM ELS and 50 mg/L Lovastatin. Plot indicates replicate two of two.

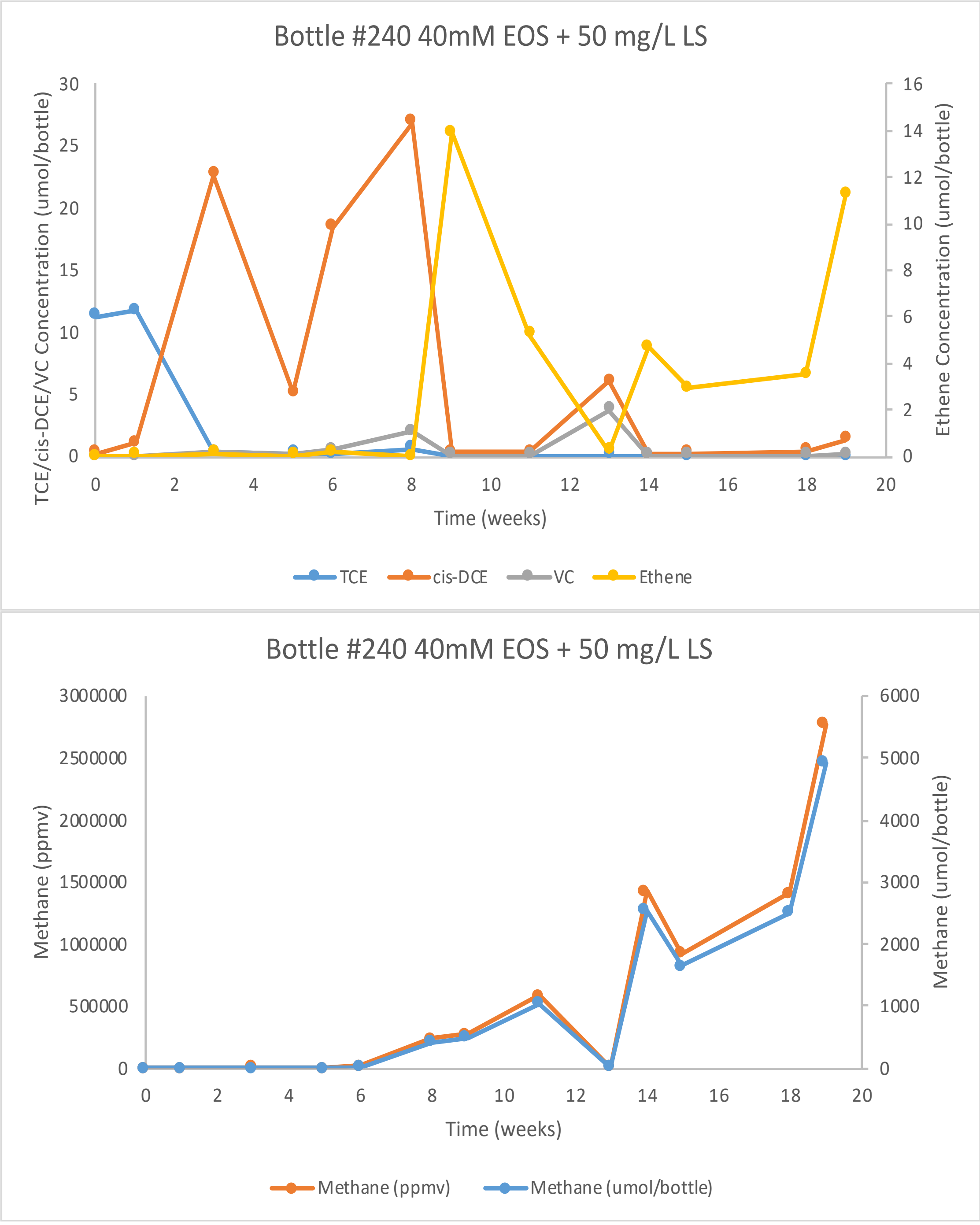
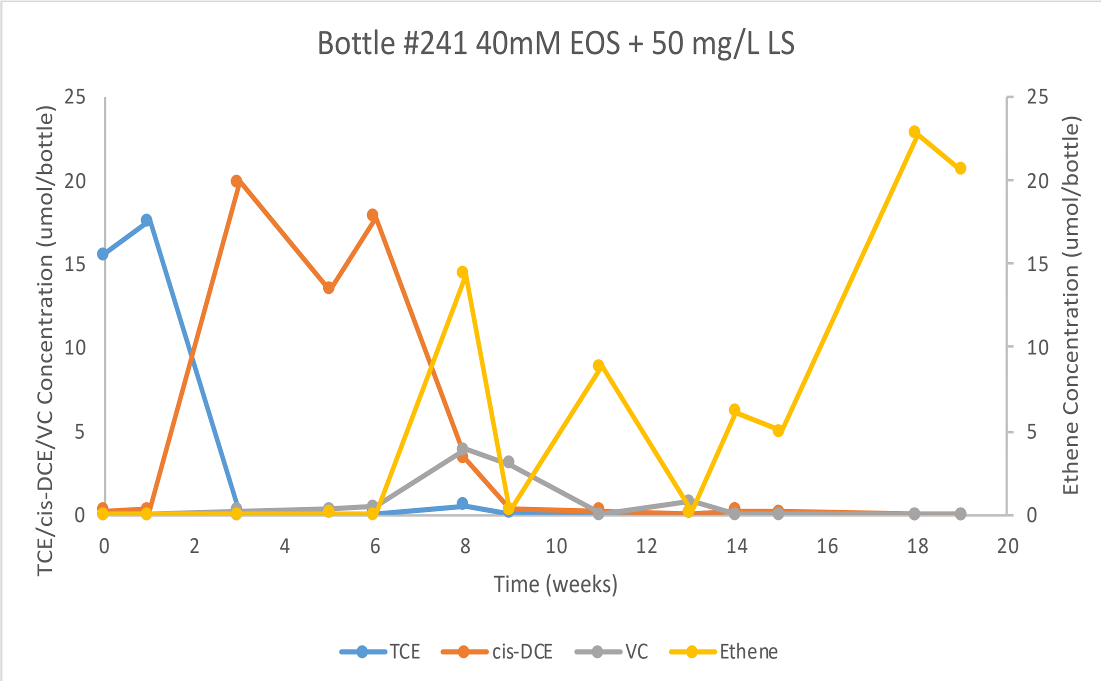


Figure B.47: TCE reduction and methane reduction for aquifer material with 40mM EOS and 50 mg/L Lovastatin. Plot indicates replicate one of two.



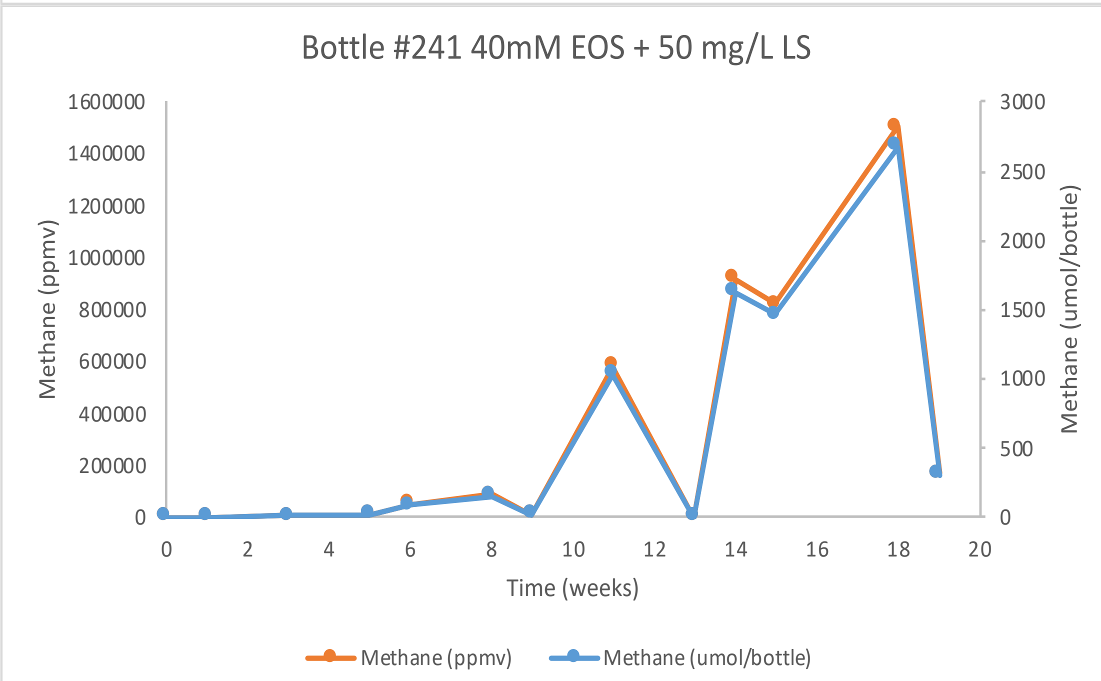


Figure B.48: TCE reduction and methane reduction for aquifer material with 40mM EOS and 50 mg/L Lovastatin. Plot indicates replicate two of two.

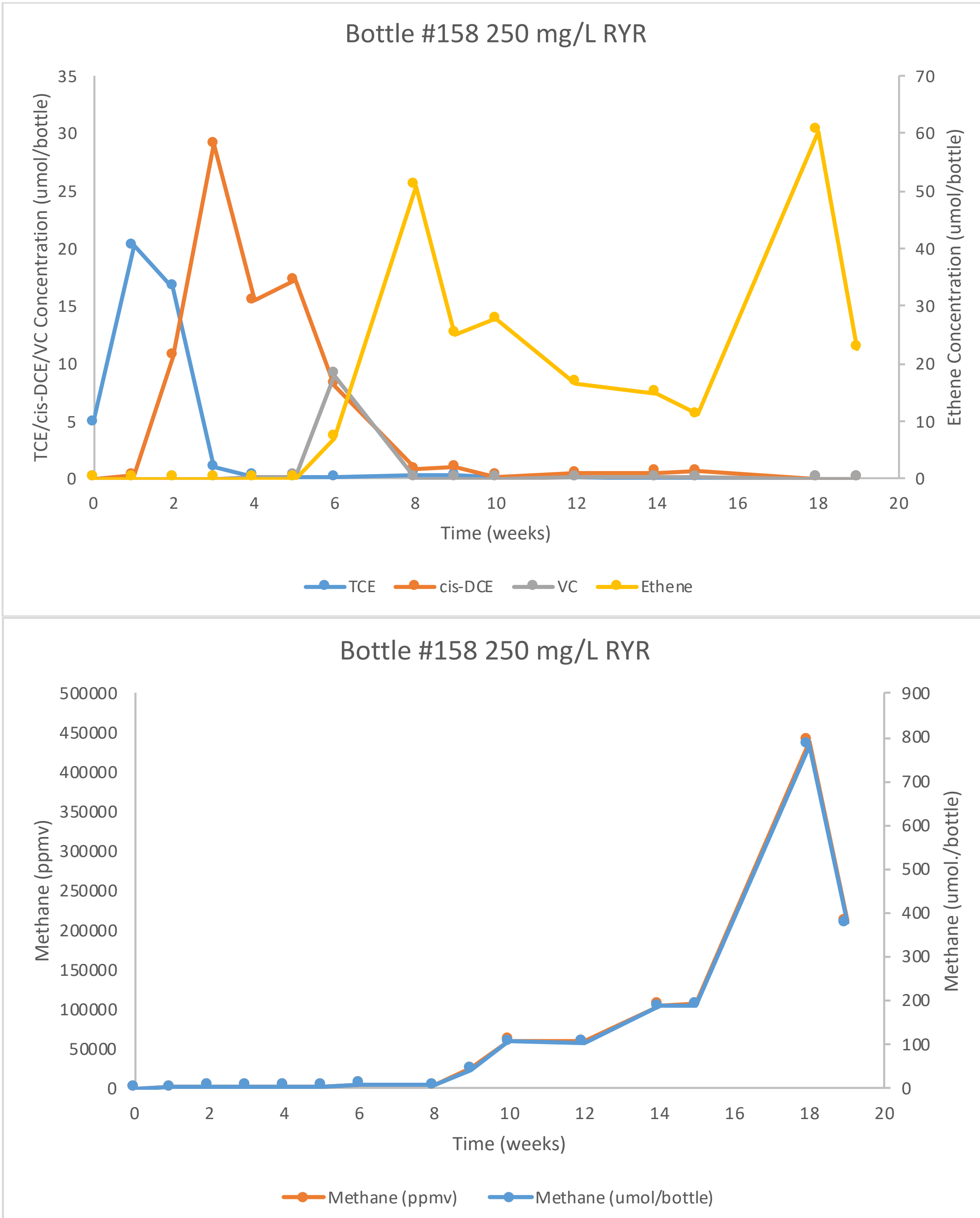
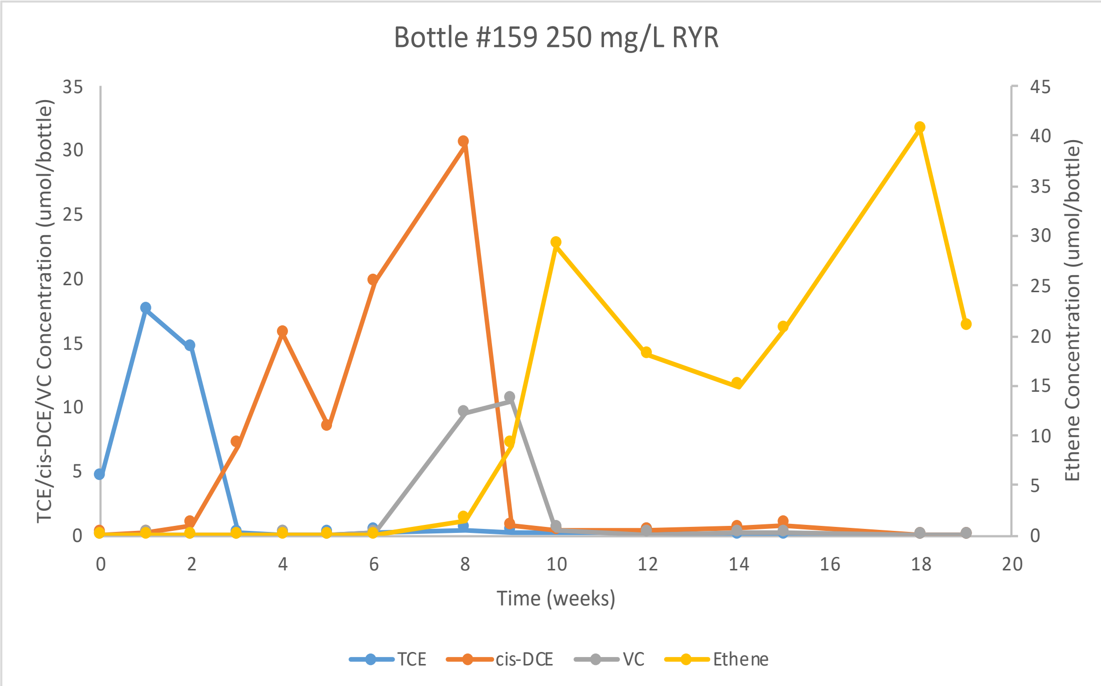


Figure B.49: TCE reduction and methane reduction for aquifer material with 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



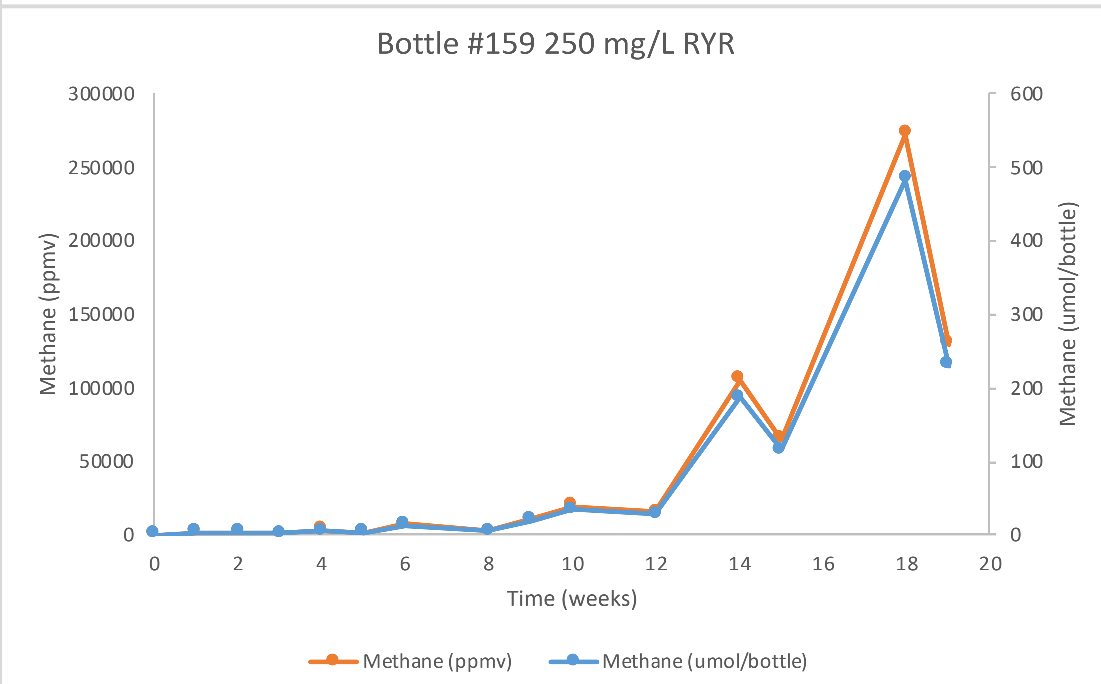


Figure B.50: TCE reduction and methane reduction for aquifer material with 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.

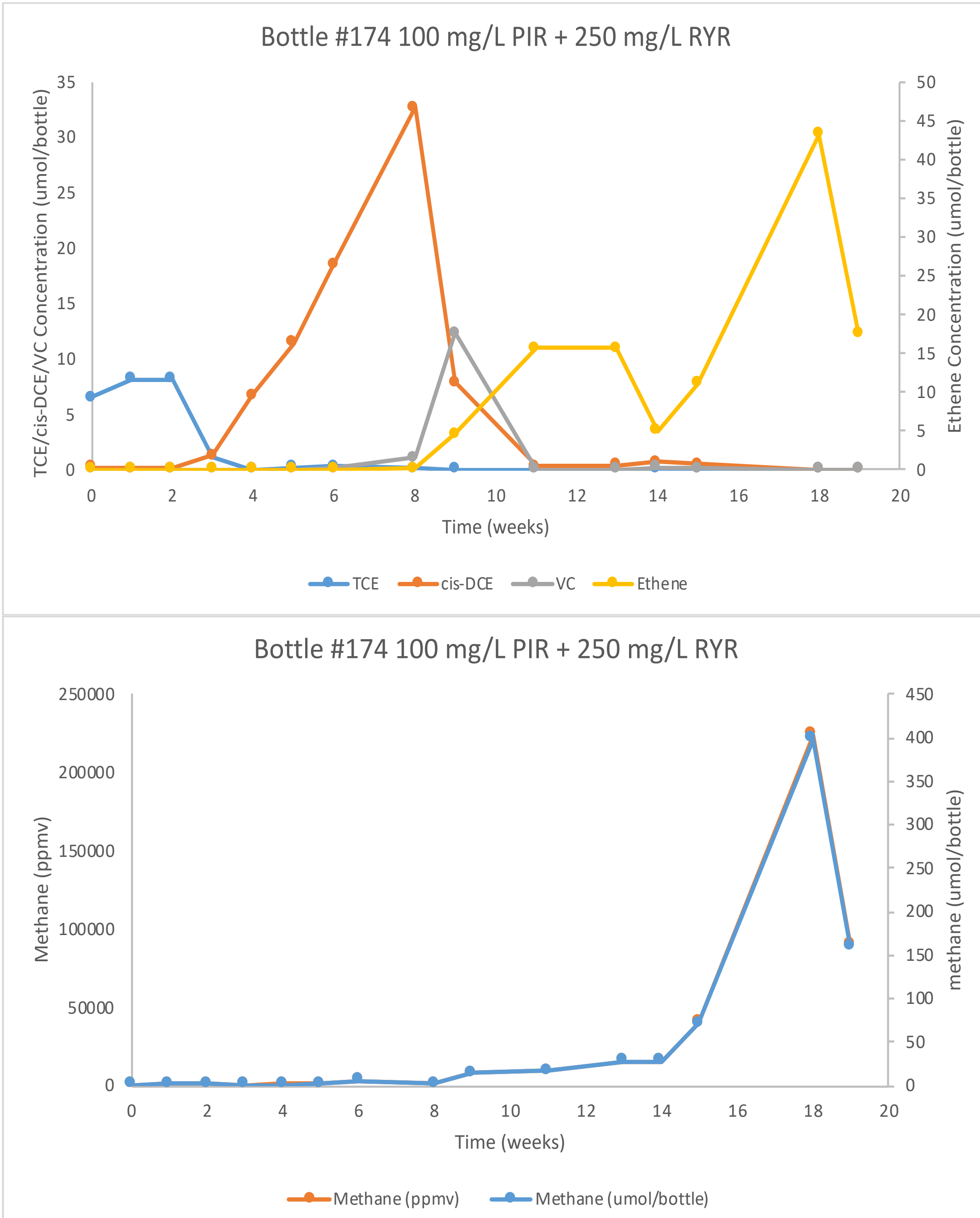
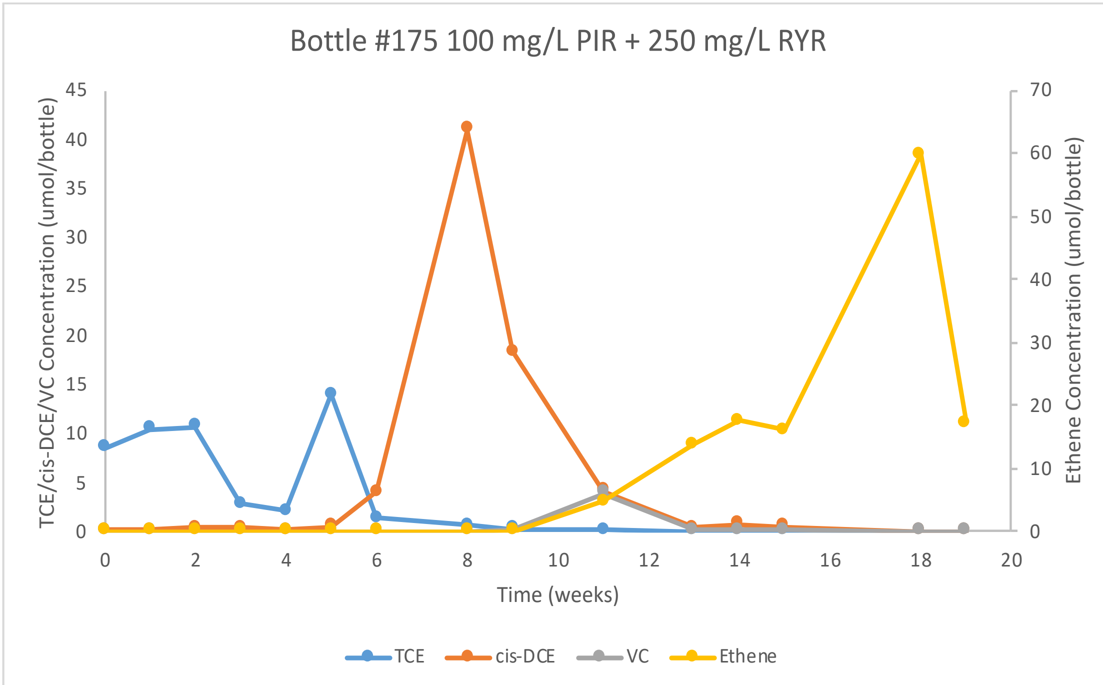


Figure B.51: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



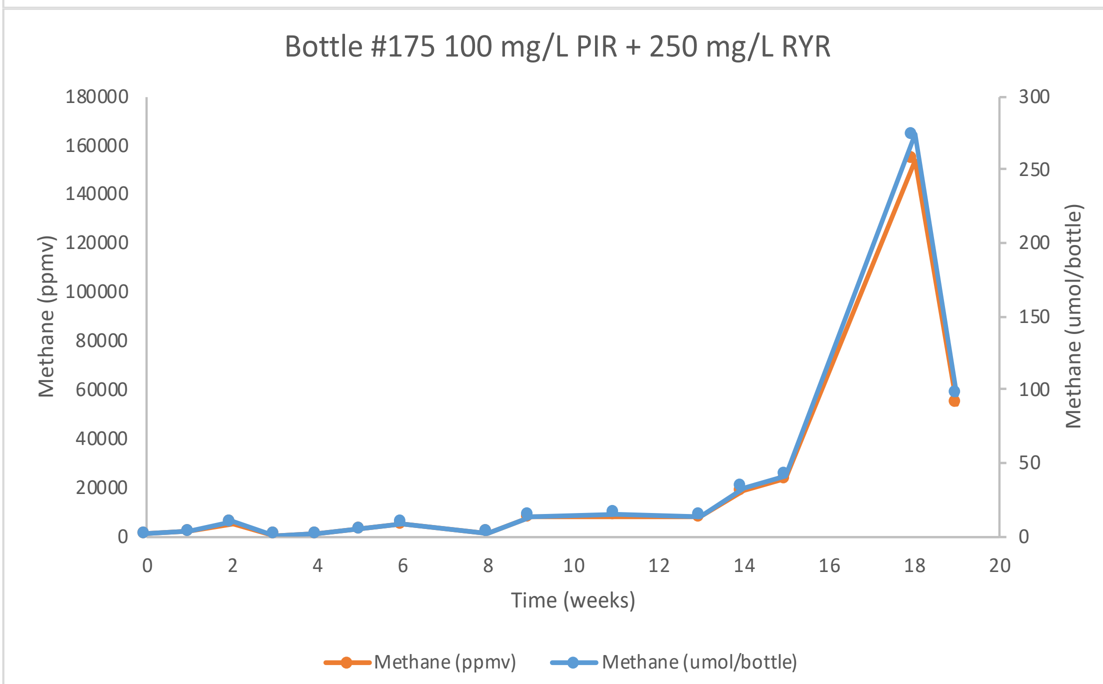


Figure B.52: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.

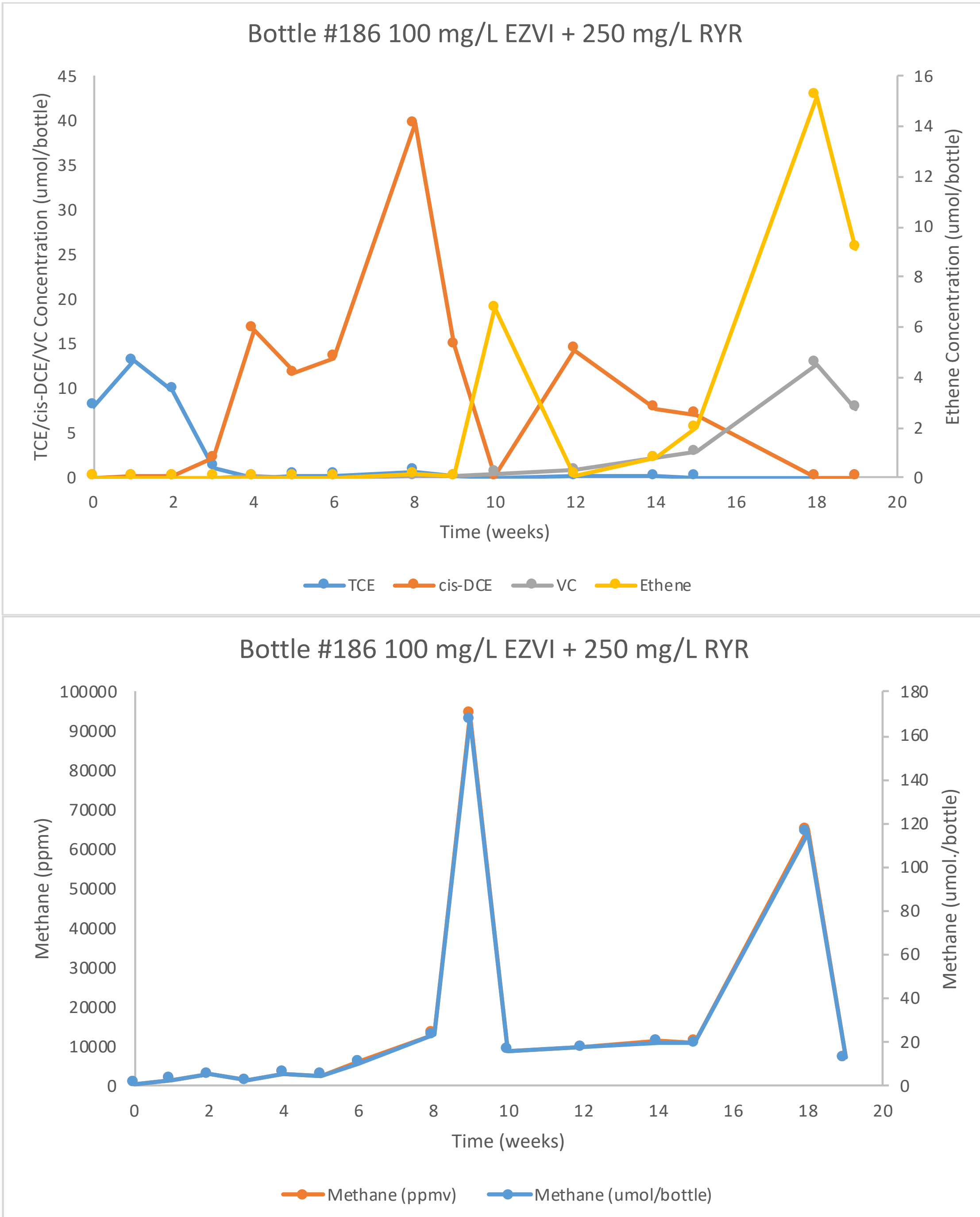


Figure B.53: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.

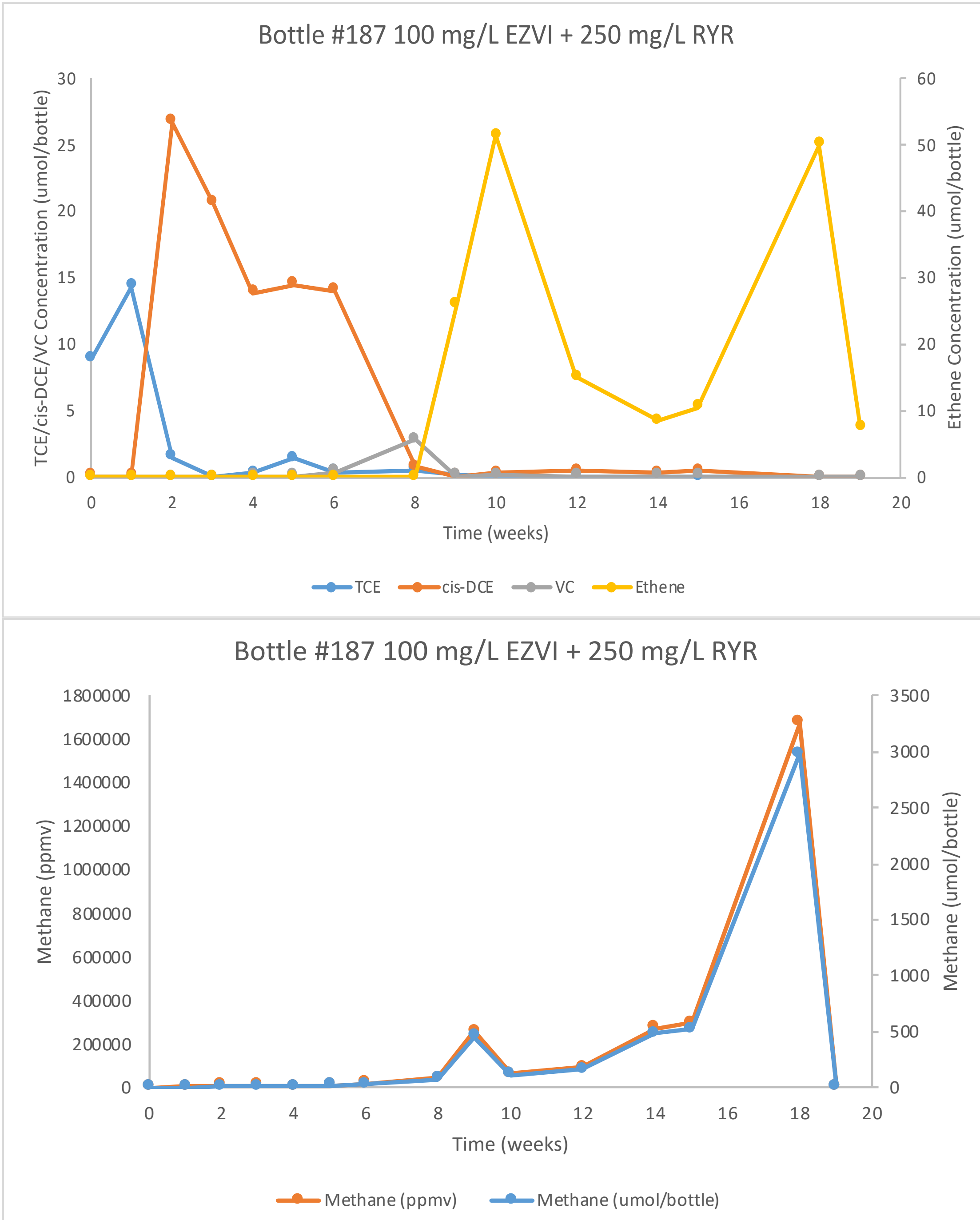


Figure B.54: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.

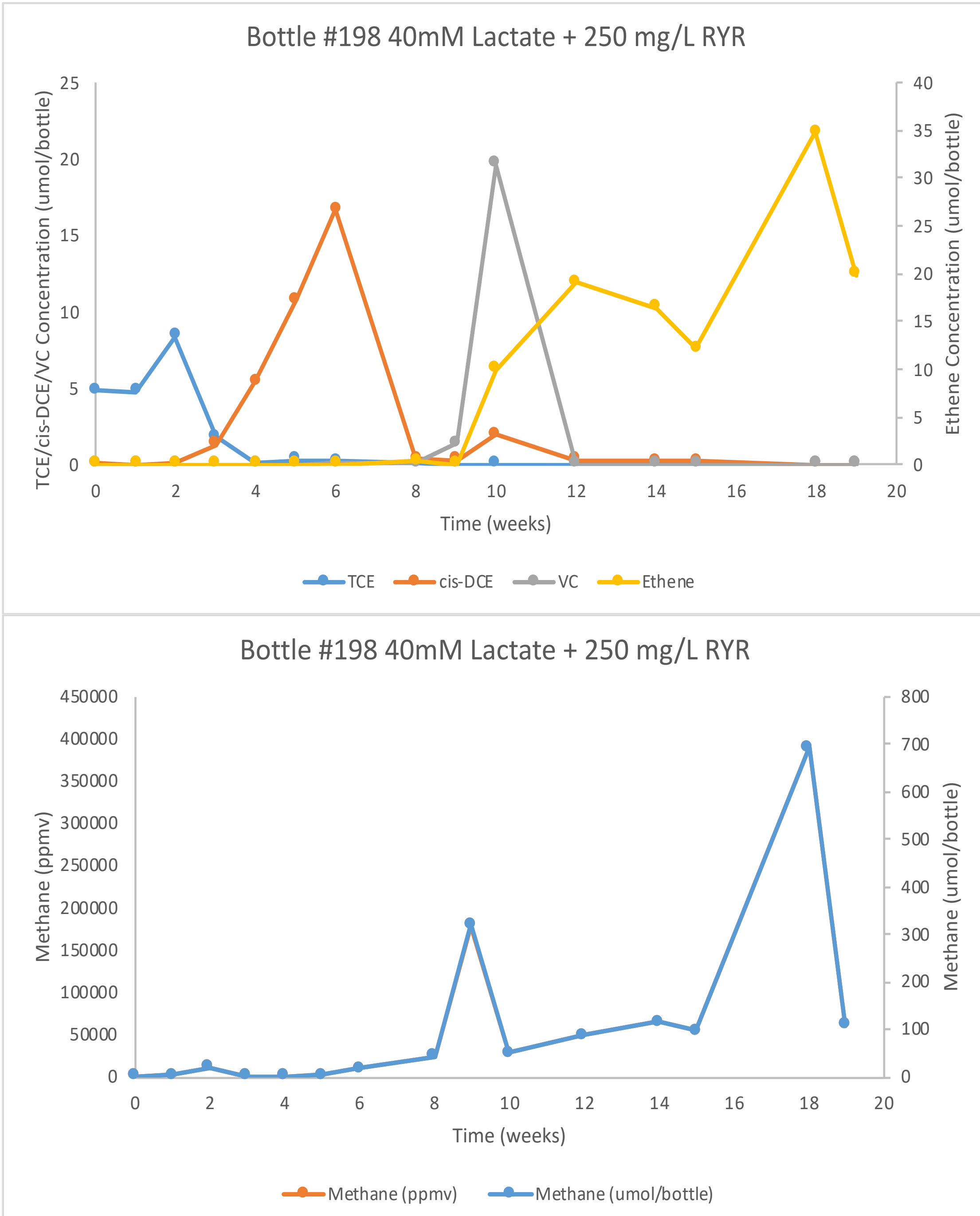
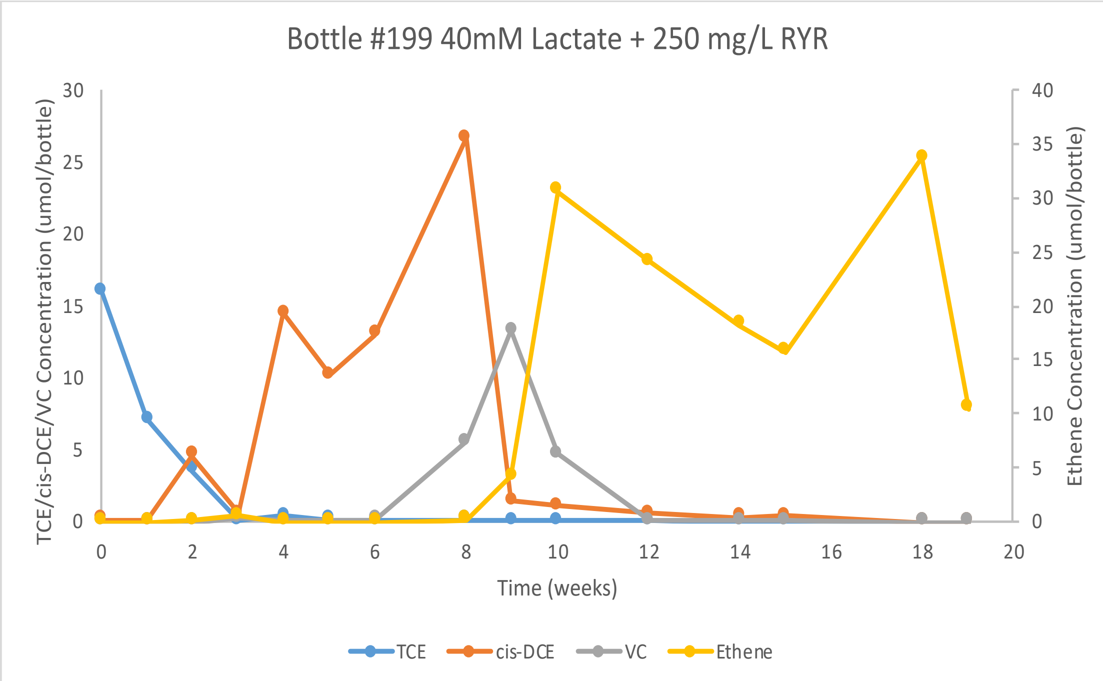


Figure B.55: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



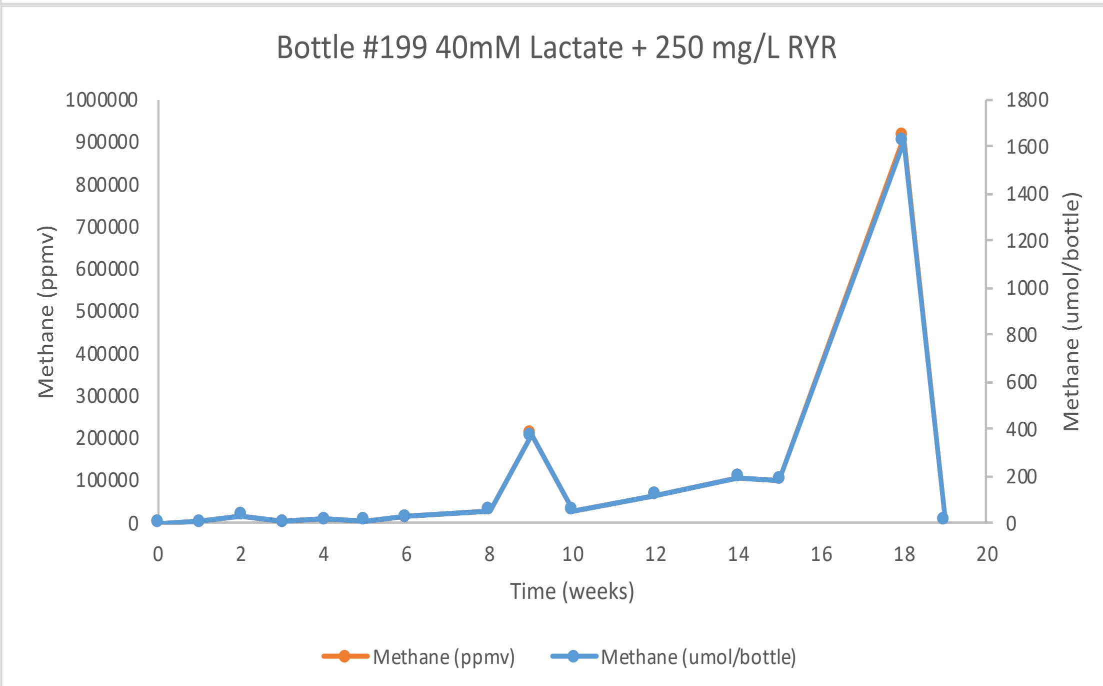


Figure B.56: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.

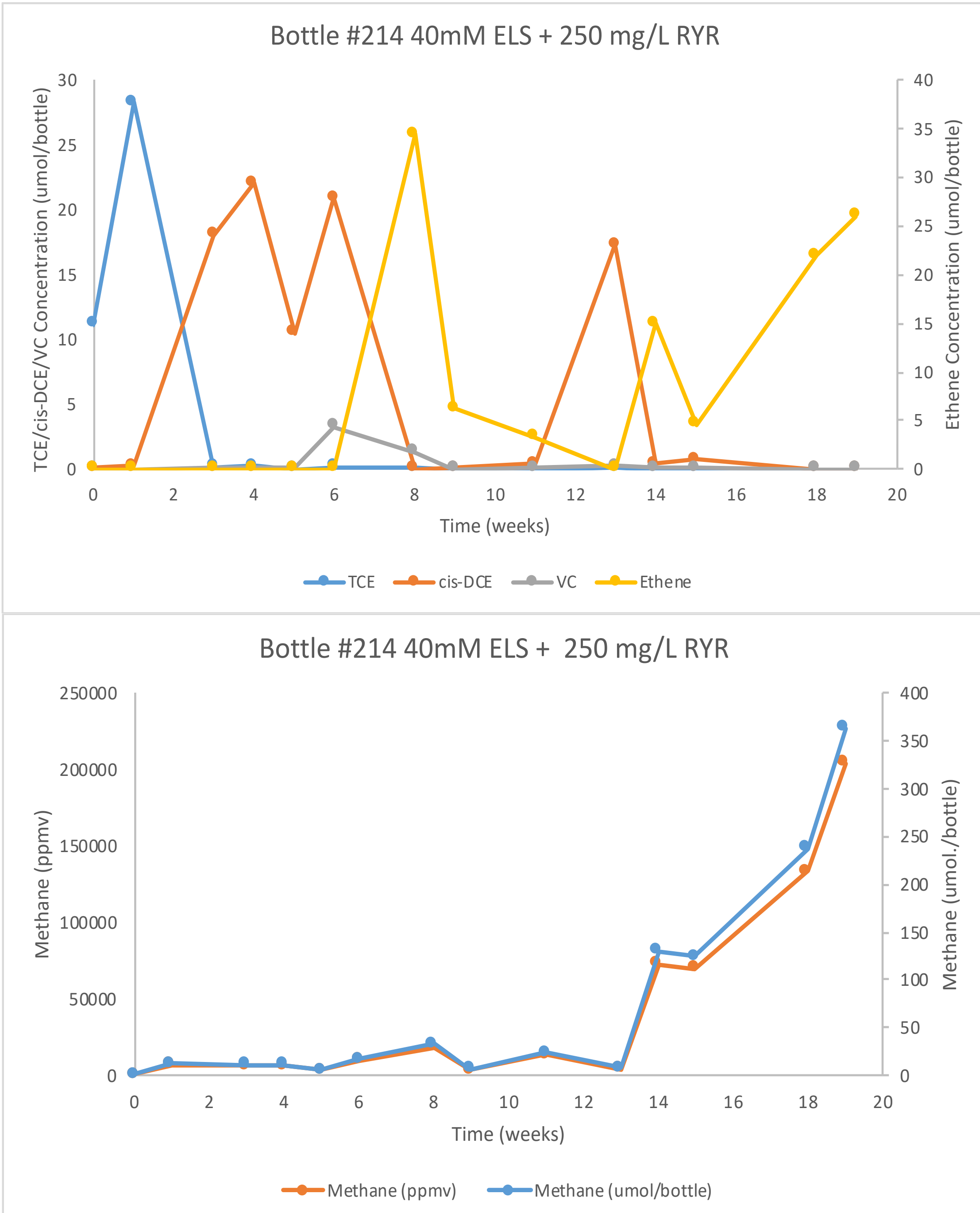
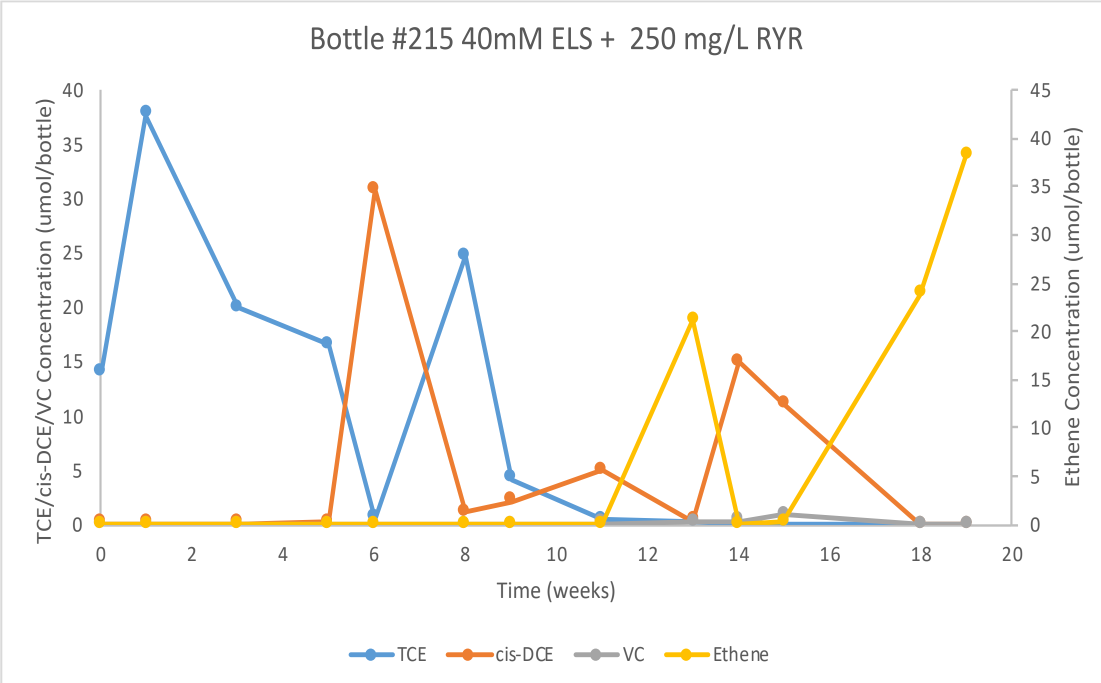


Figure B.57: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



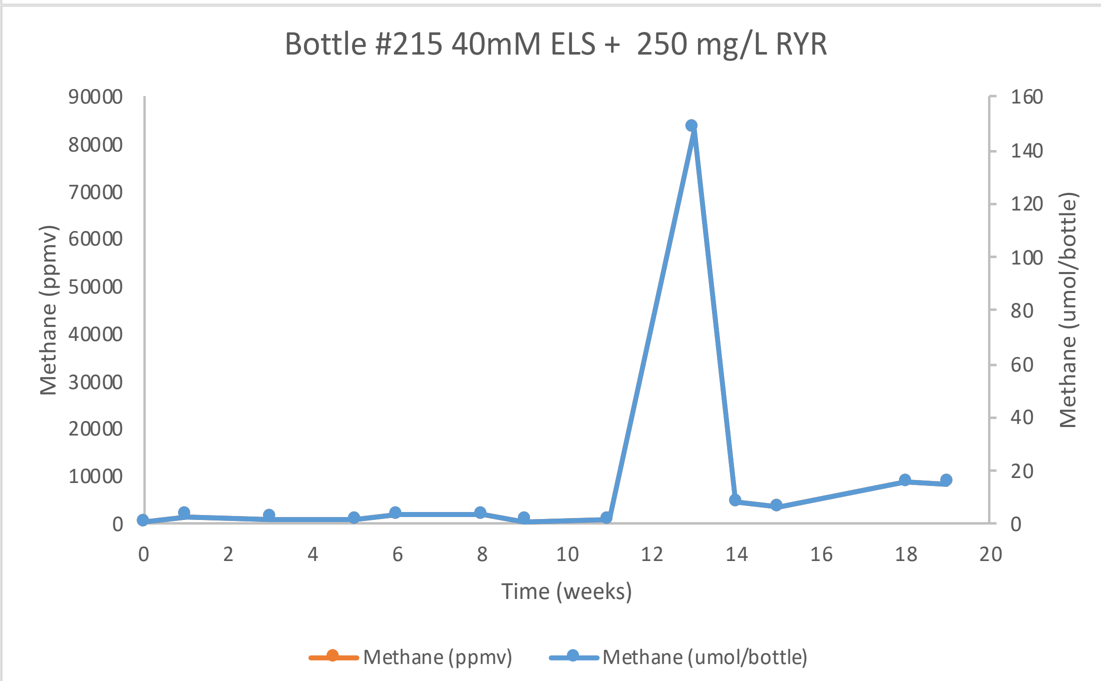


Figure B.58: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.

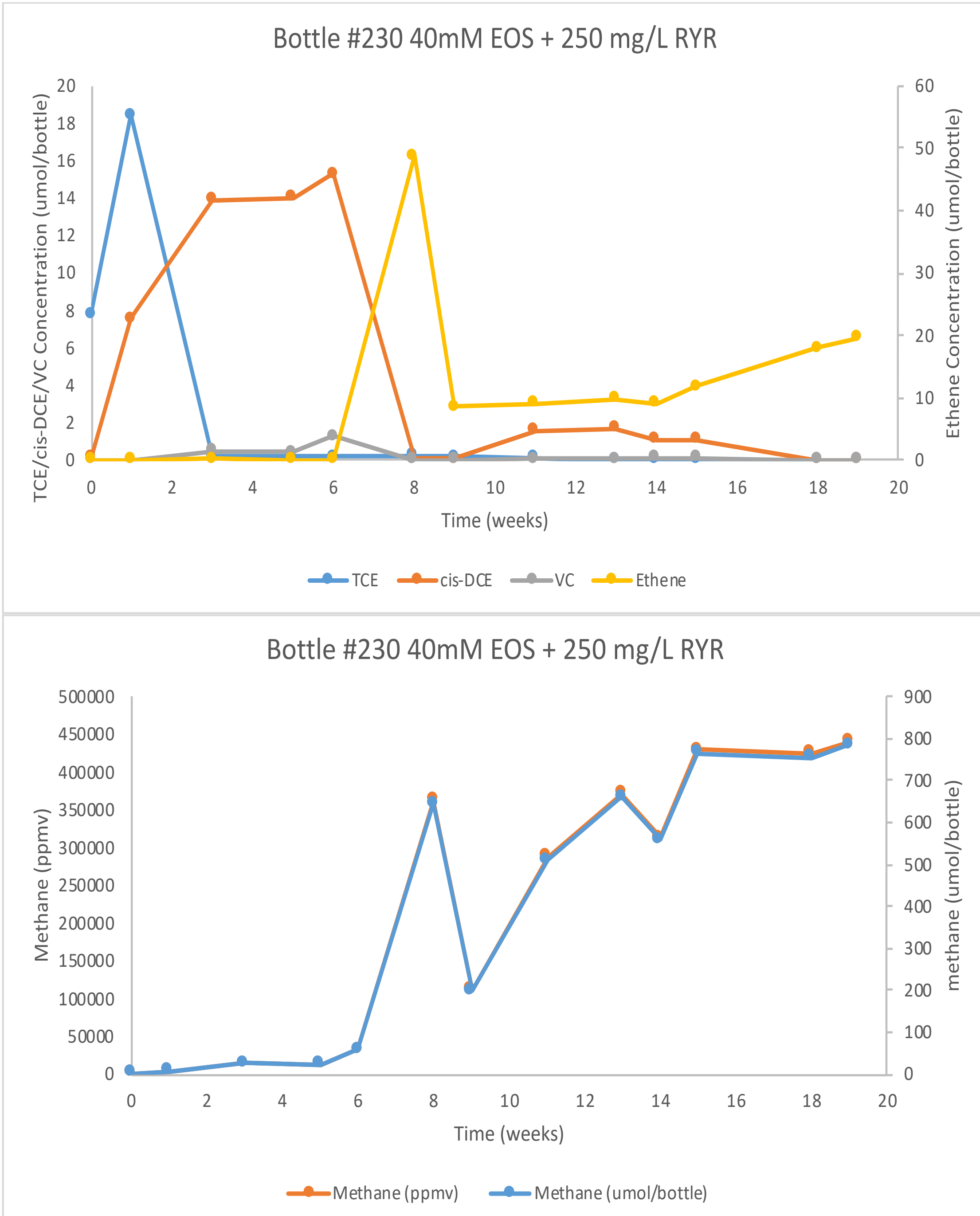
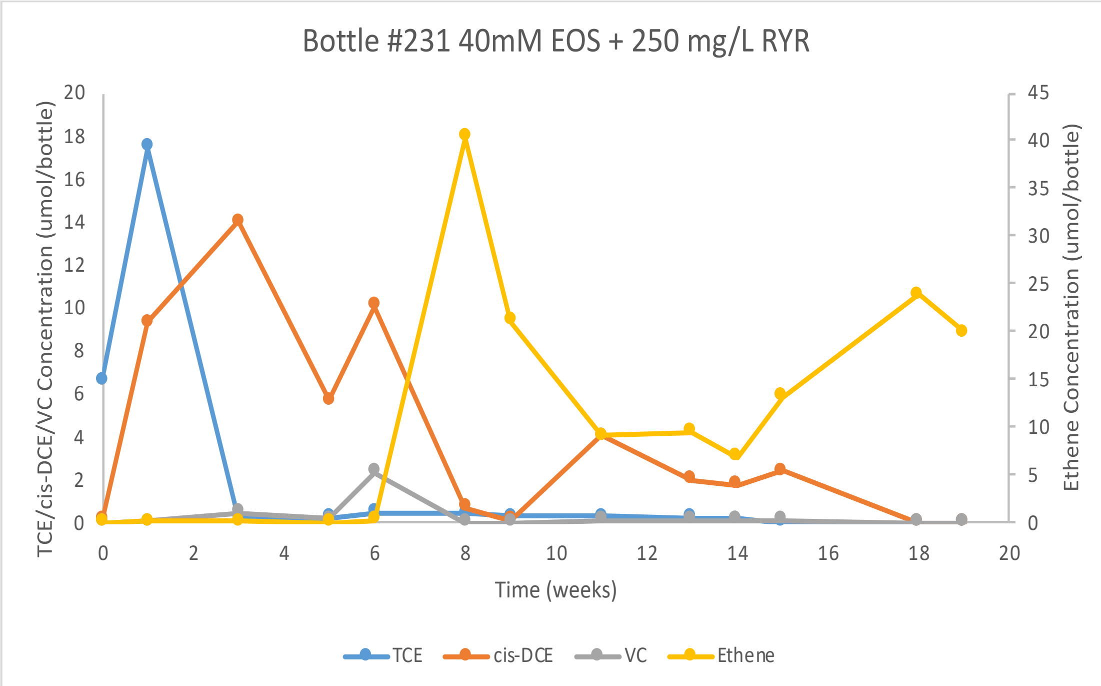


Figure B.59: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



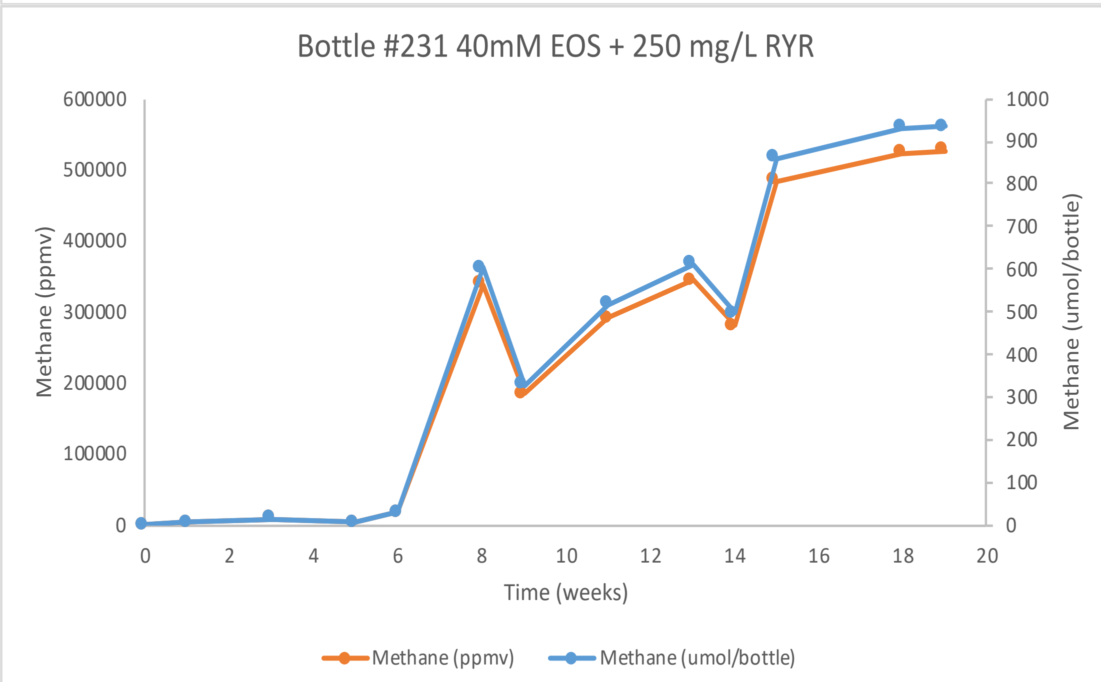
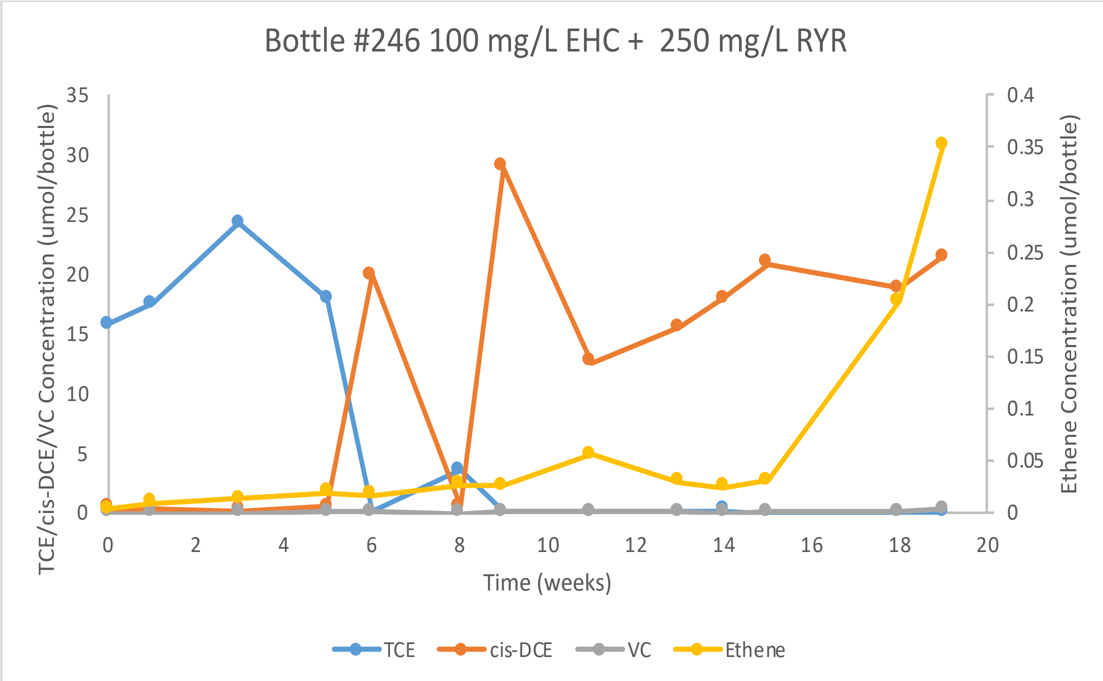


Figure B.60: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.



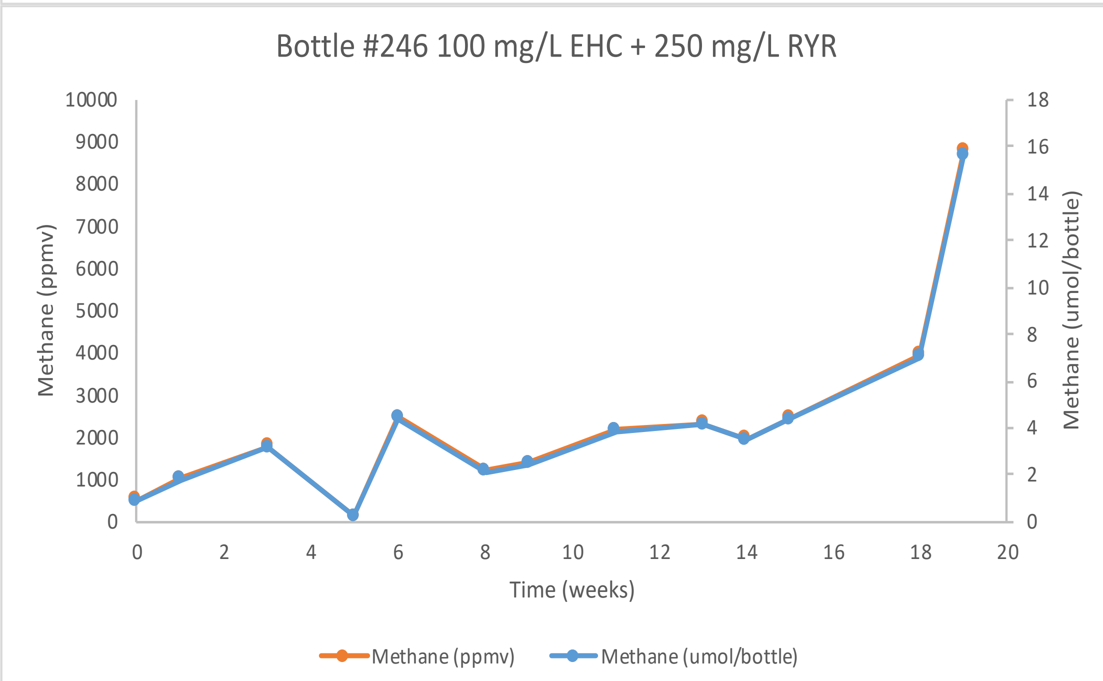


Figure B.61: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.

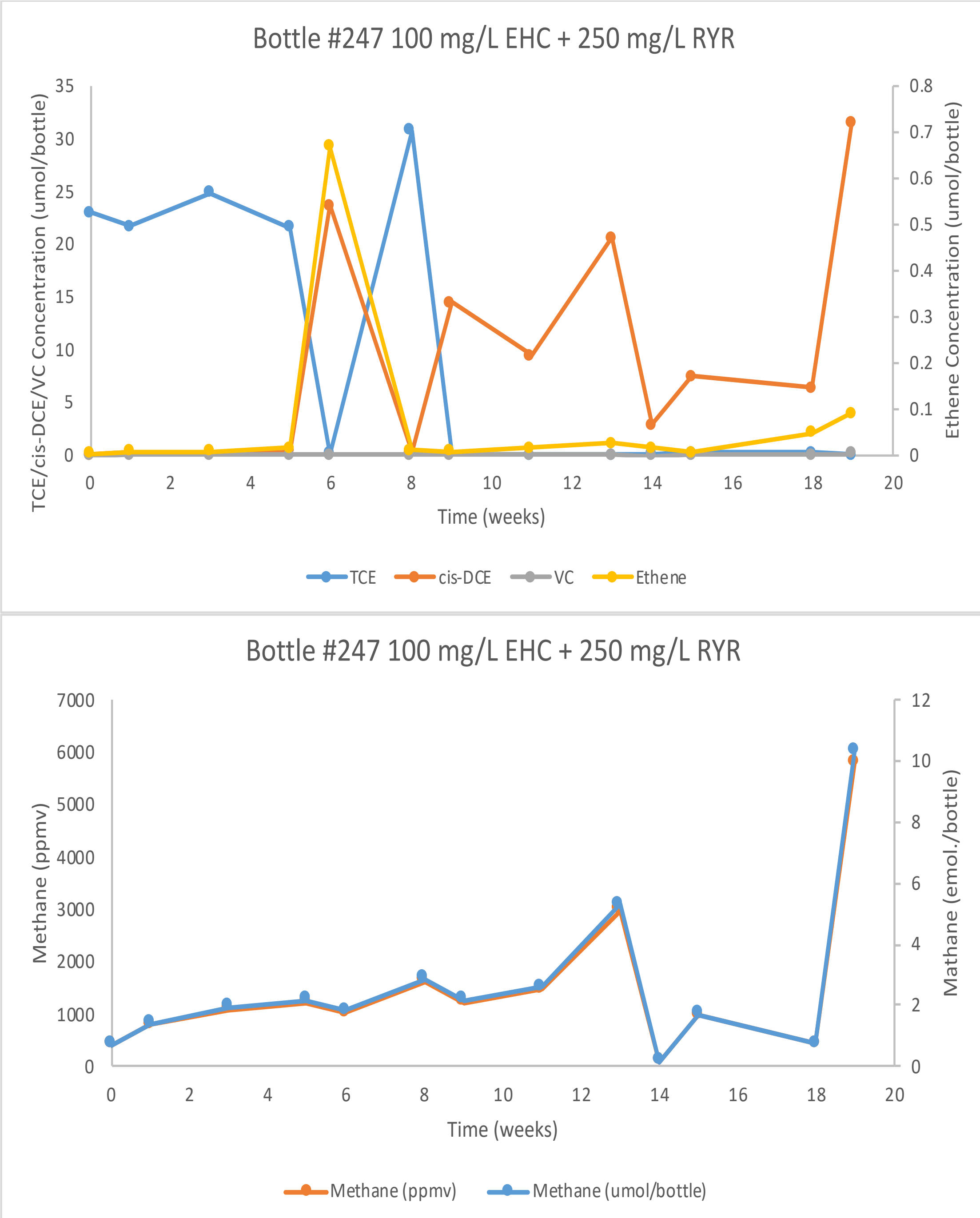
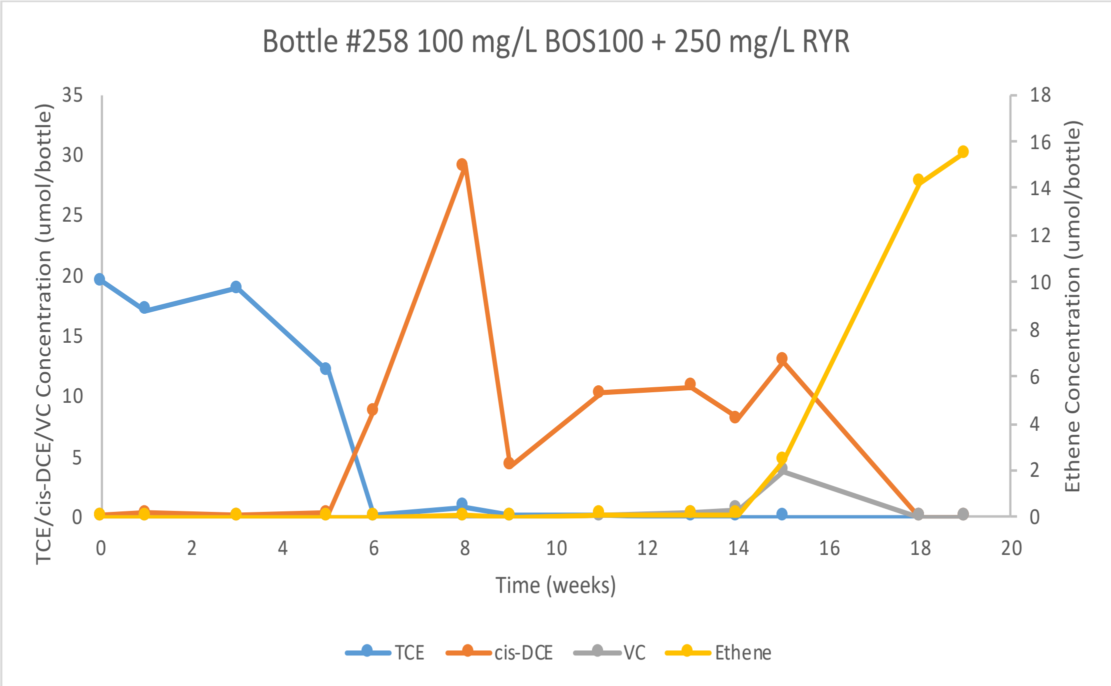


Figure B.62: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.



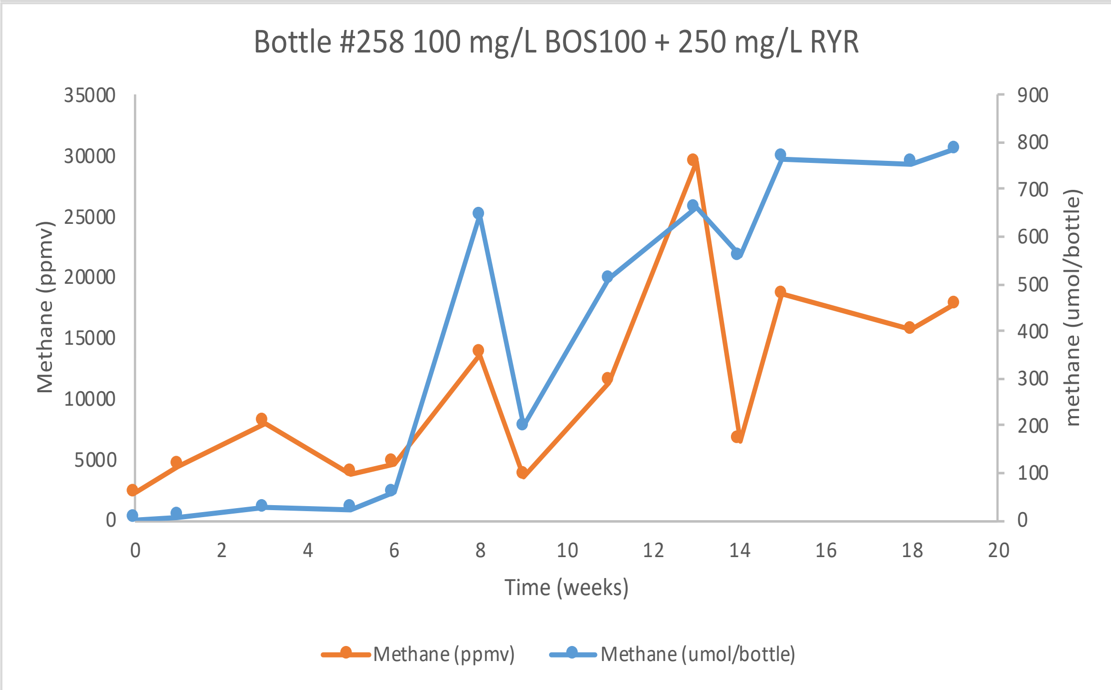
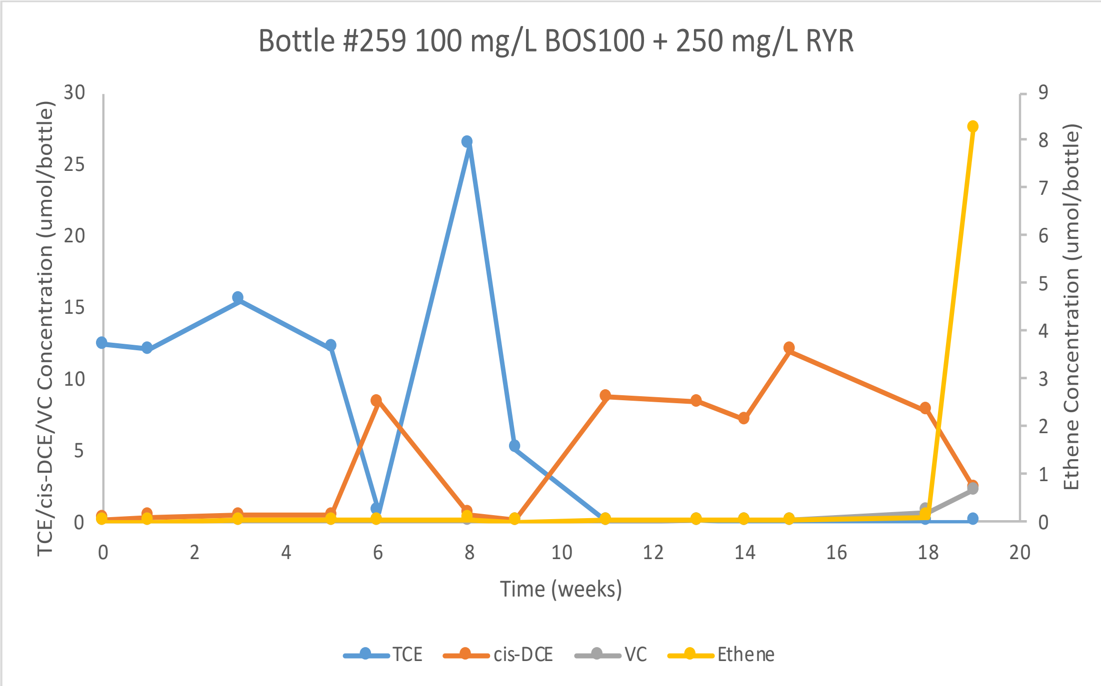


Figure B.63: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Red Yeast Rice. Plot indicates replicate one of two.



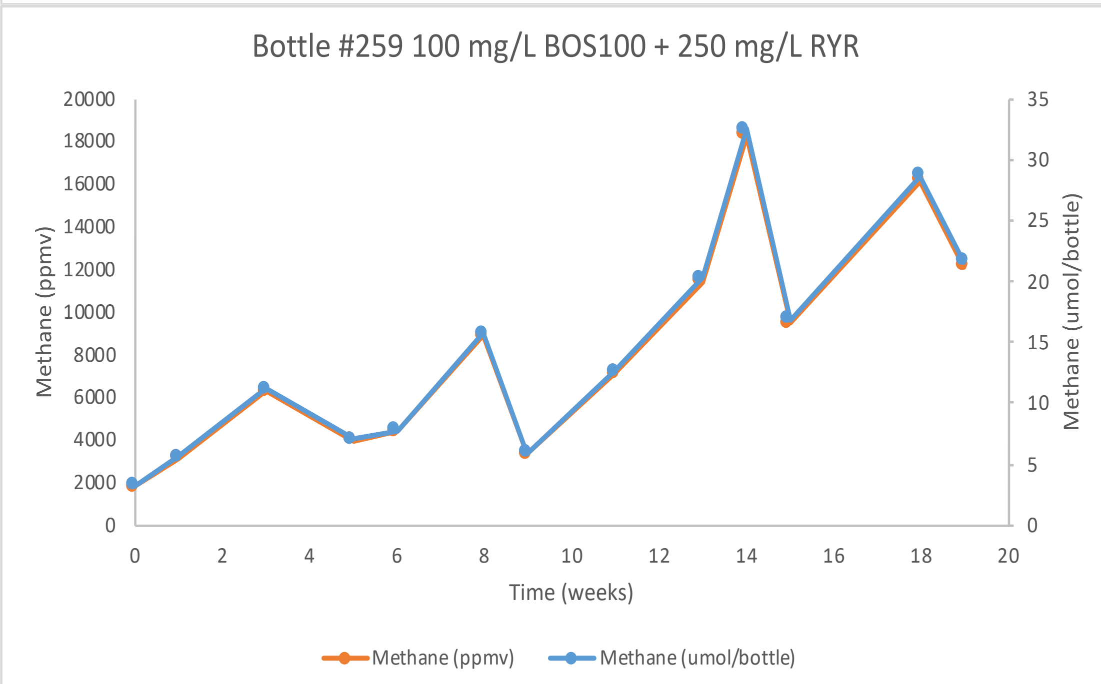
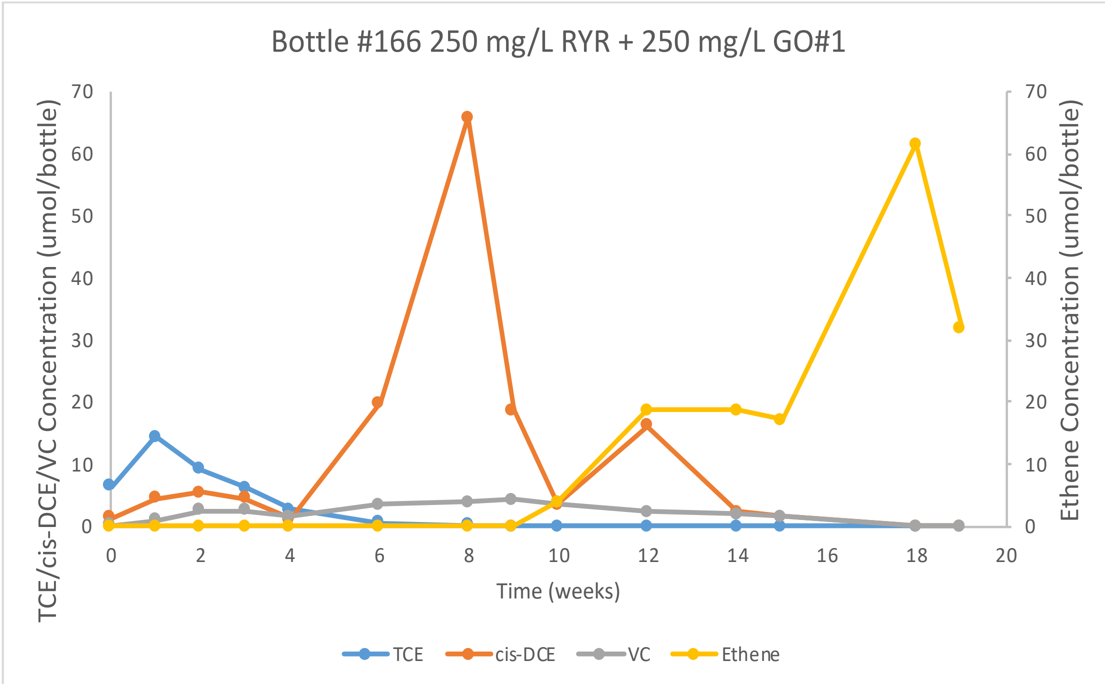


Figure B.64: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Red Yeast Rice. Plot indicates replicate two of two.



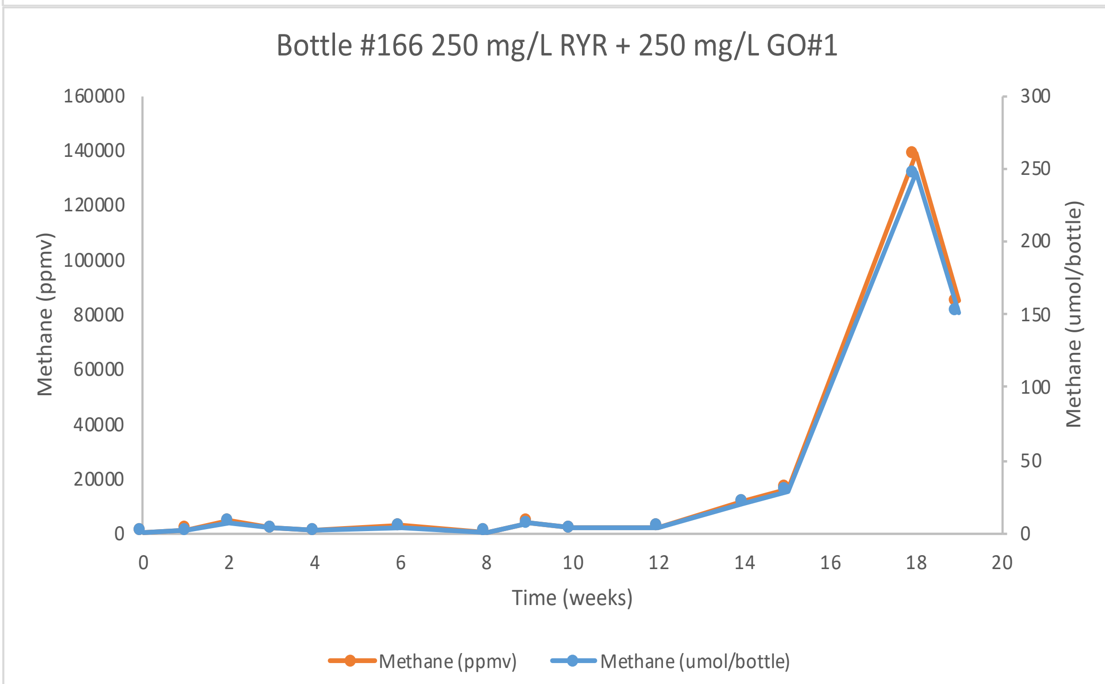


Figure B.65: TCE reduction and methane reduction for aquifer material with 250 mg/L Red Yeast Rice and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

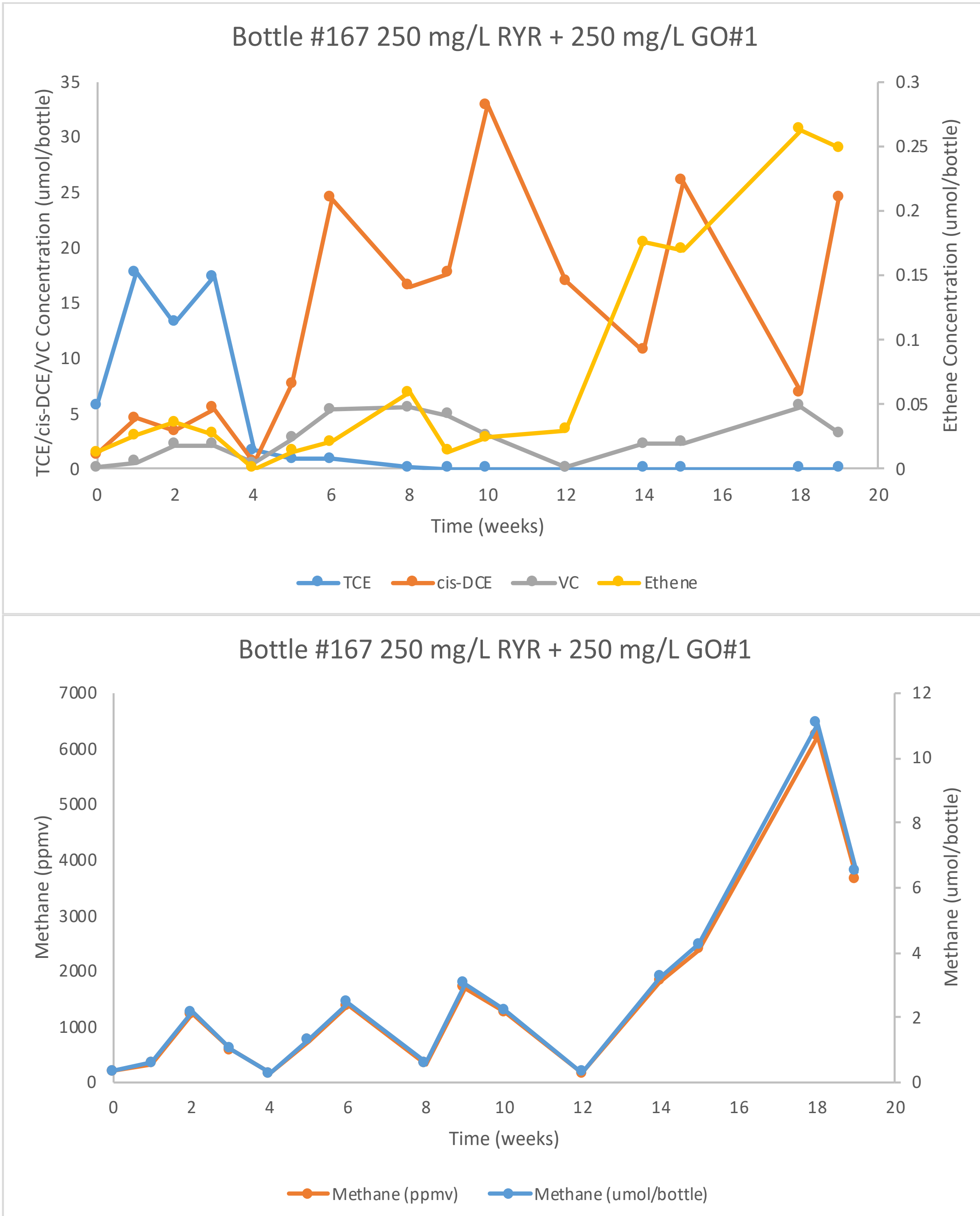
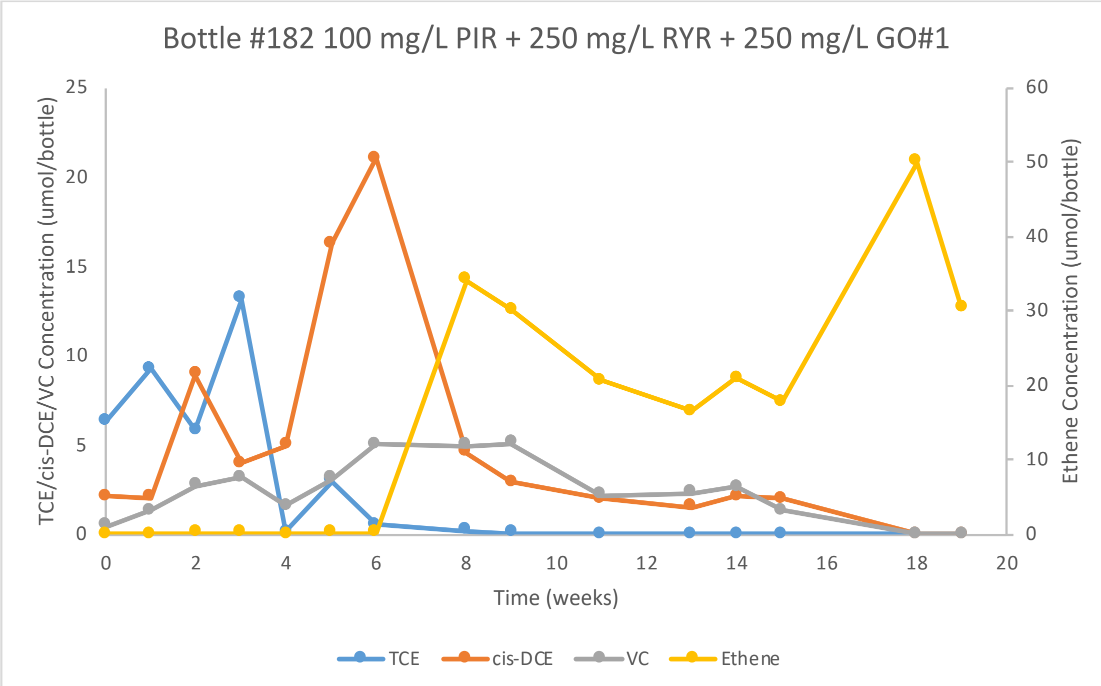


Figure B.66: TCE reduction and methane reduction for aquifer material with 250 mg/L Red Yeast Rice and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



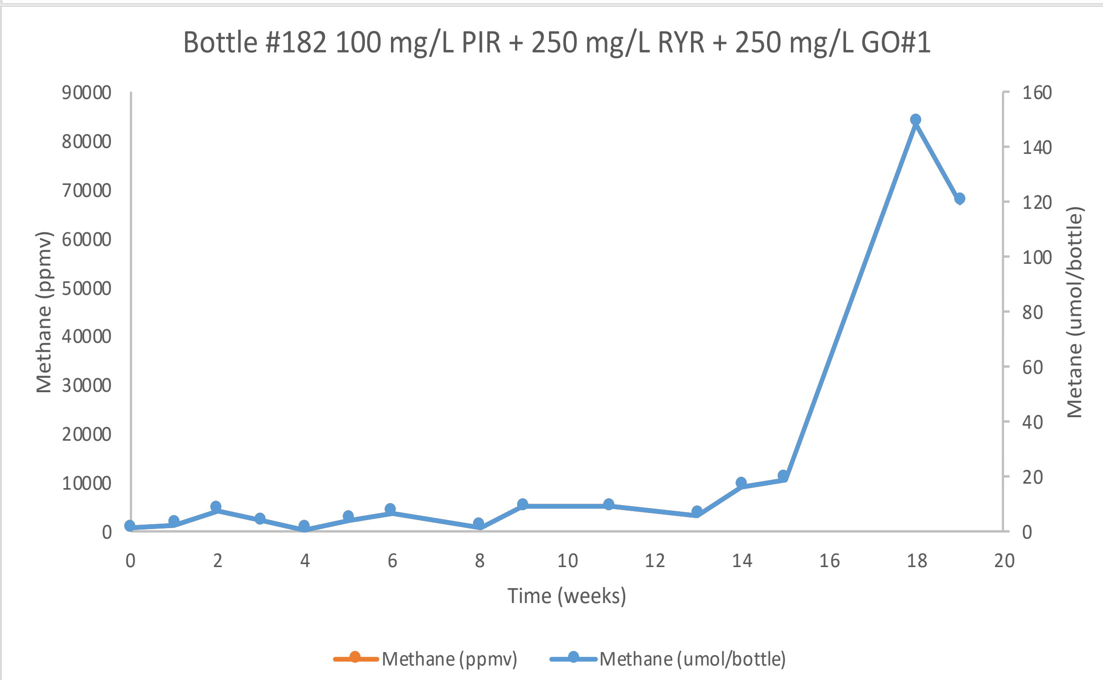
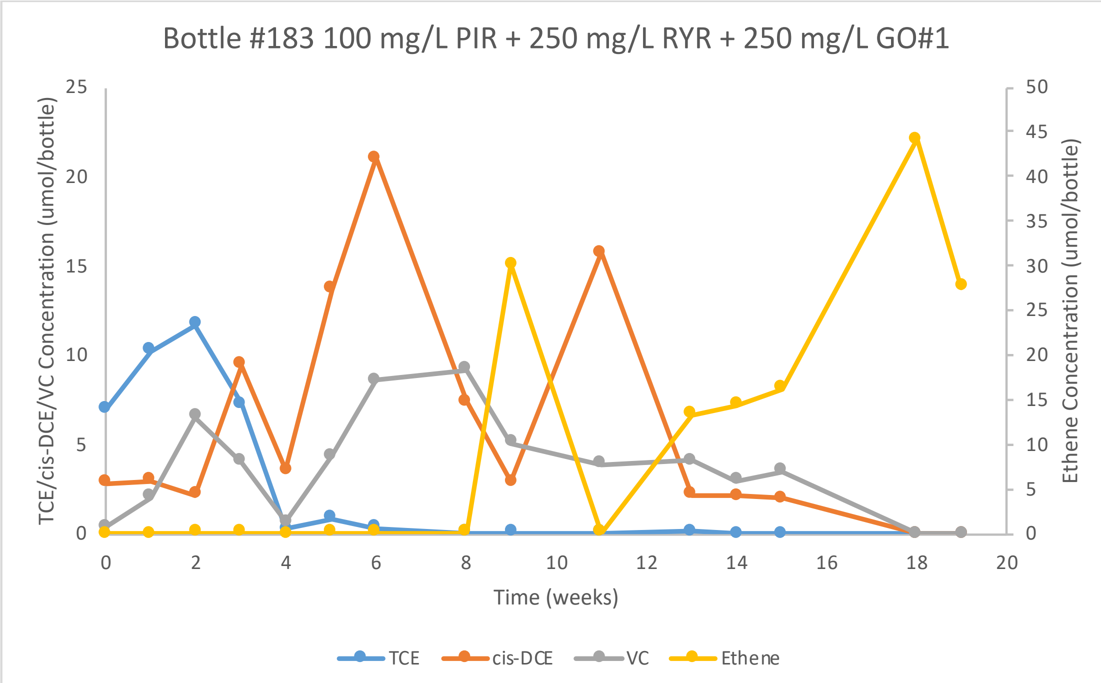


Figure B.67: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



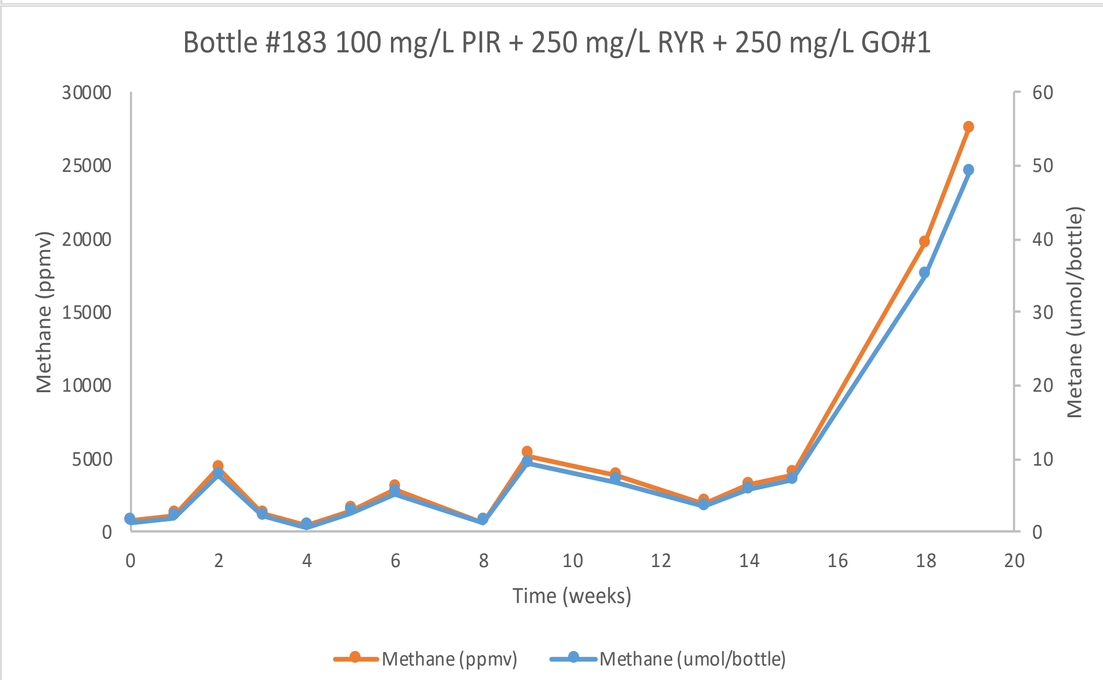
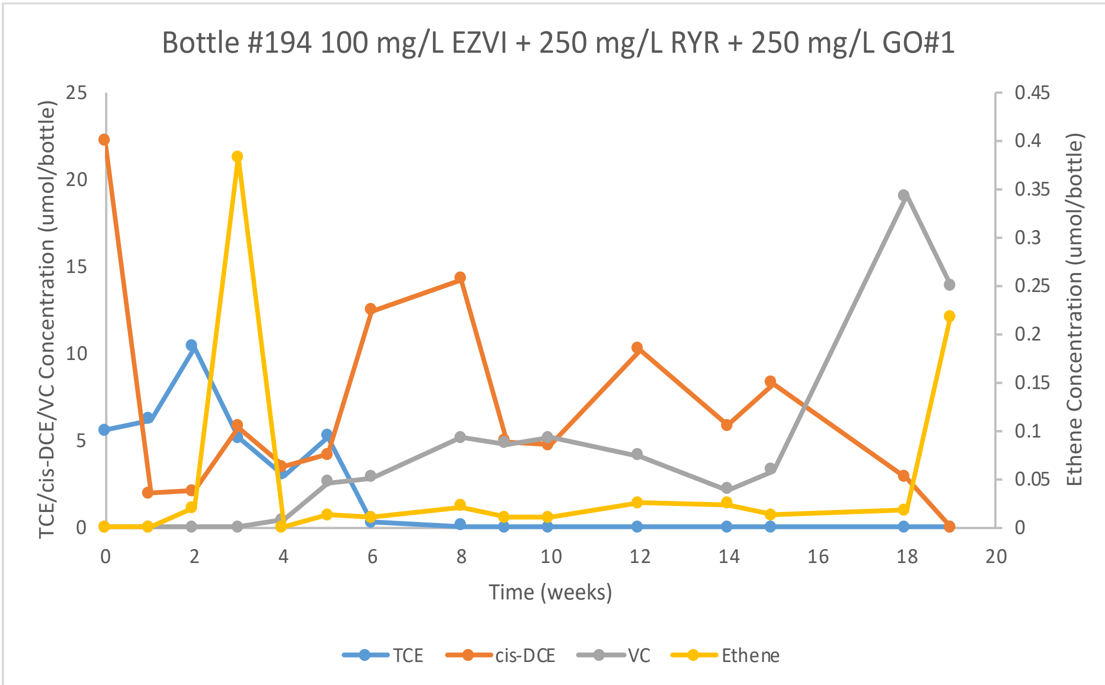


Figure B.68: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



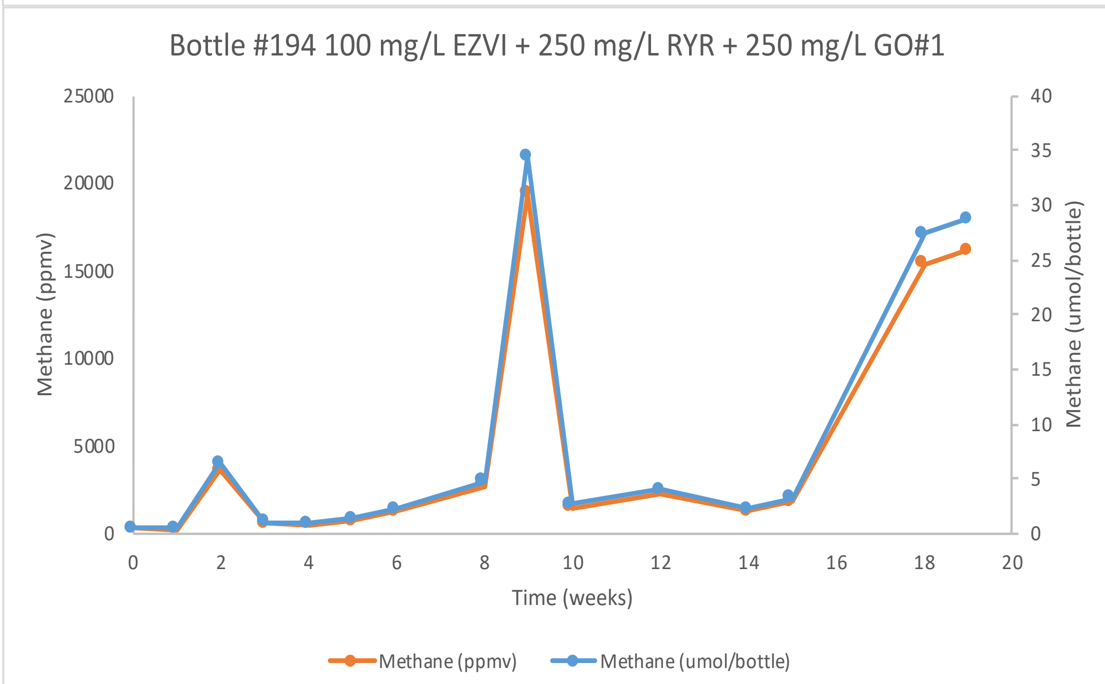
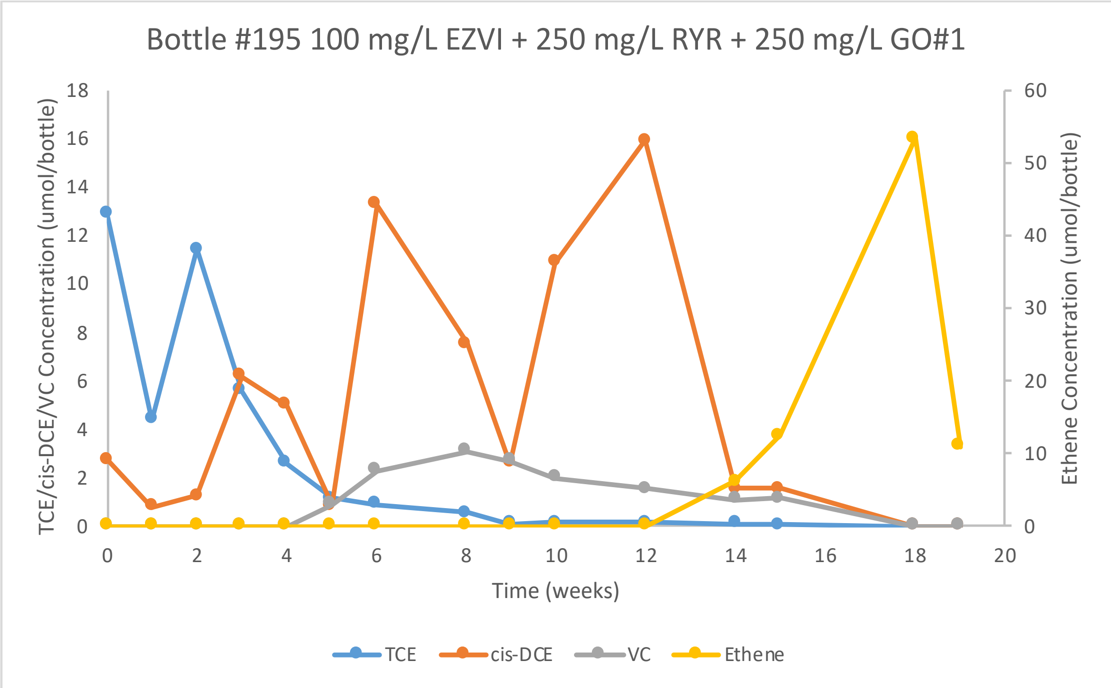


Figure B.69: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



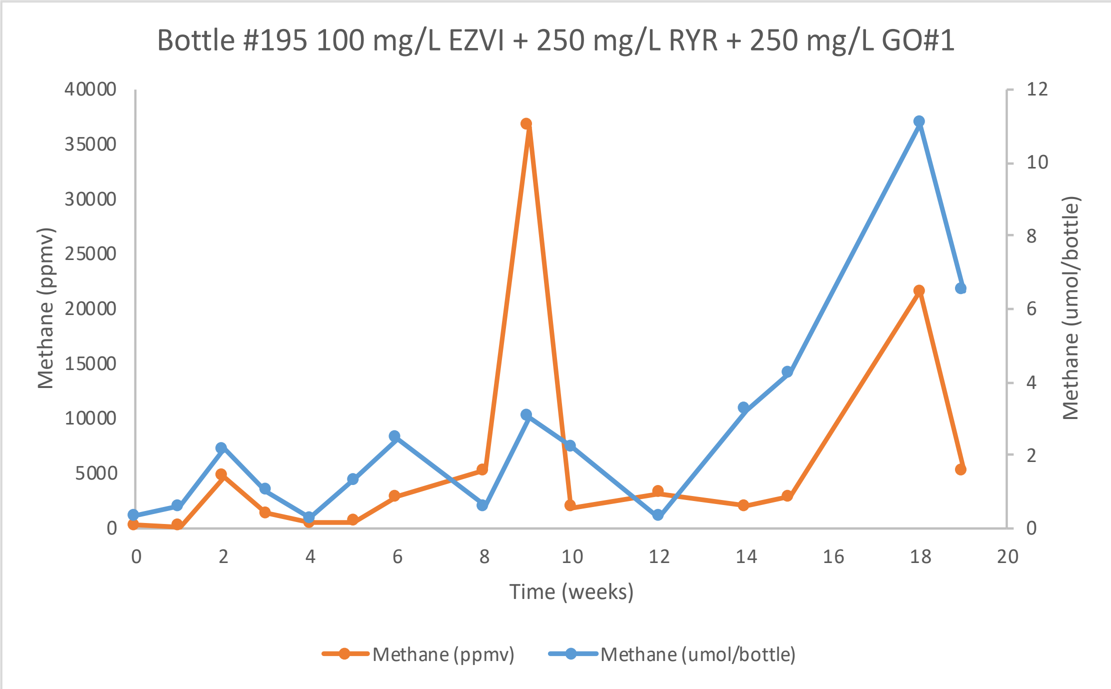


Figure B.70: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

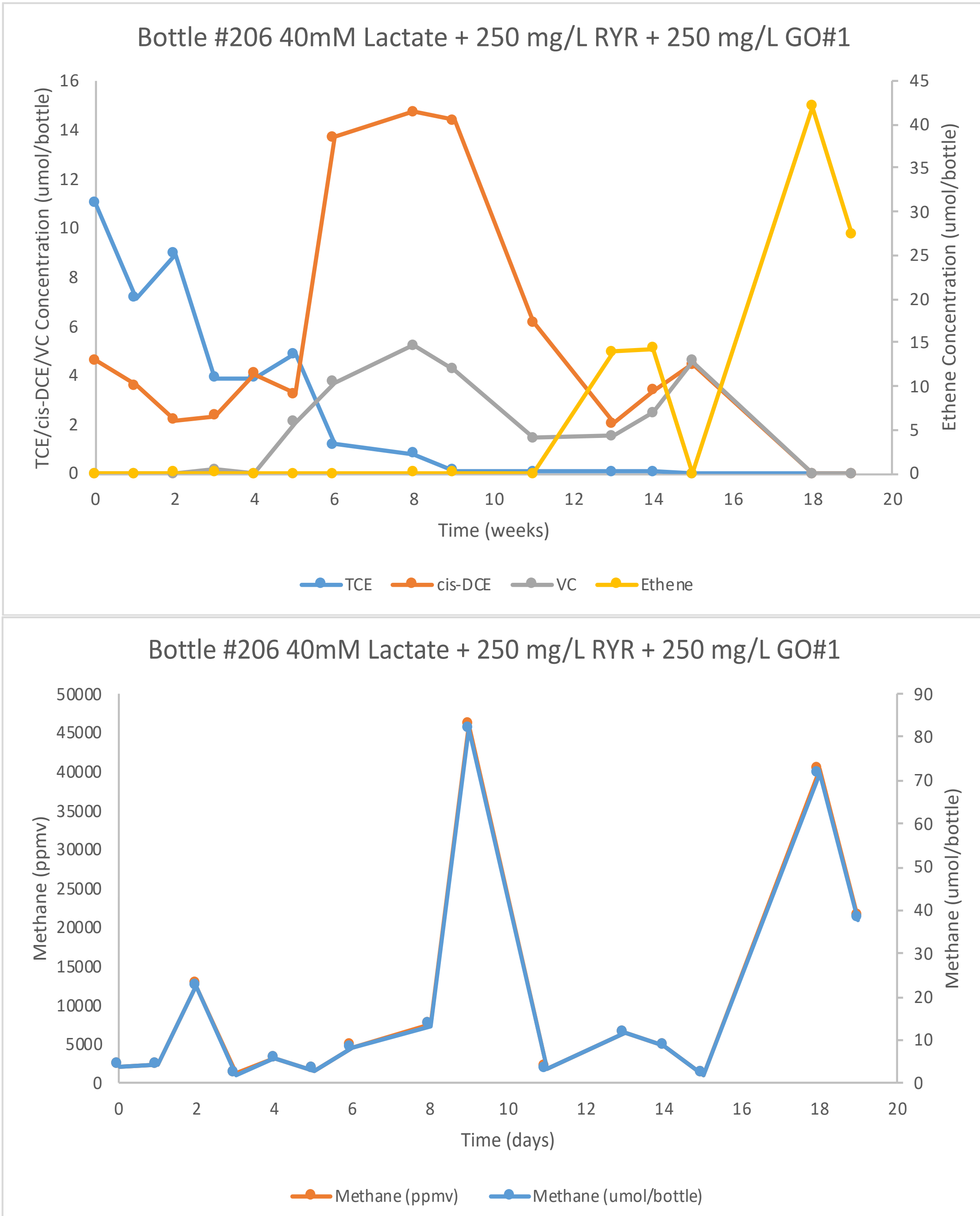


Figure B.71: TCE reduction and methane reduction for aquifer material with 40mM Lactate, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

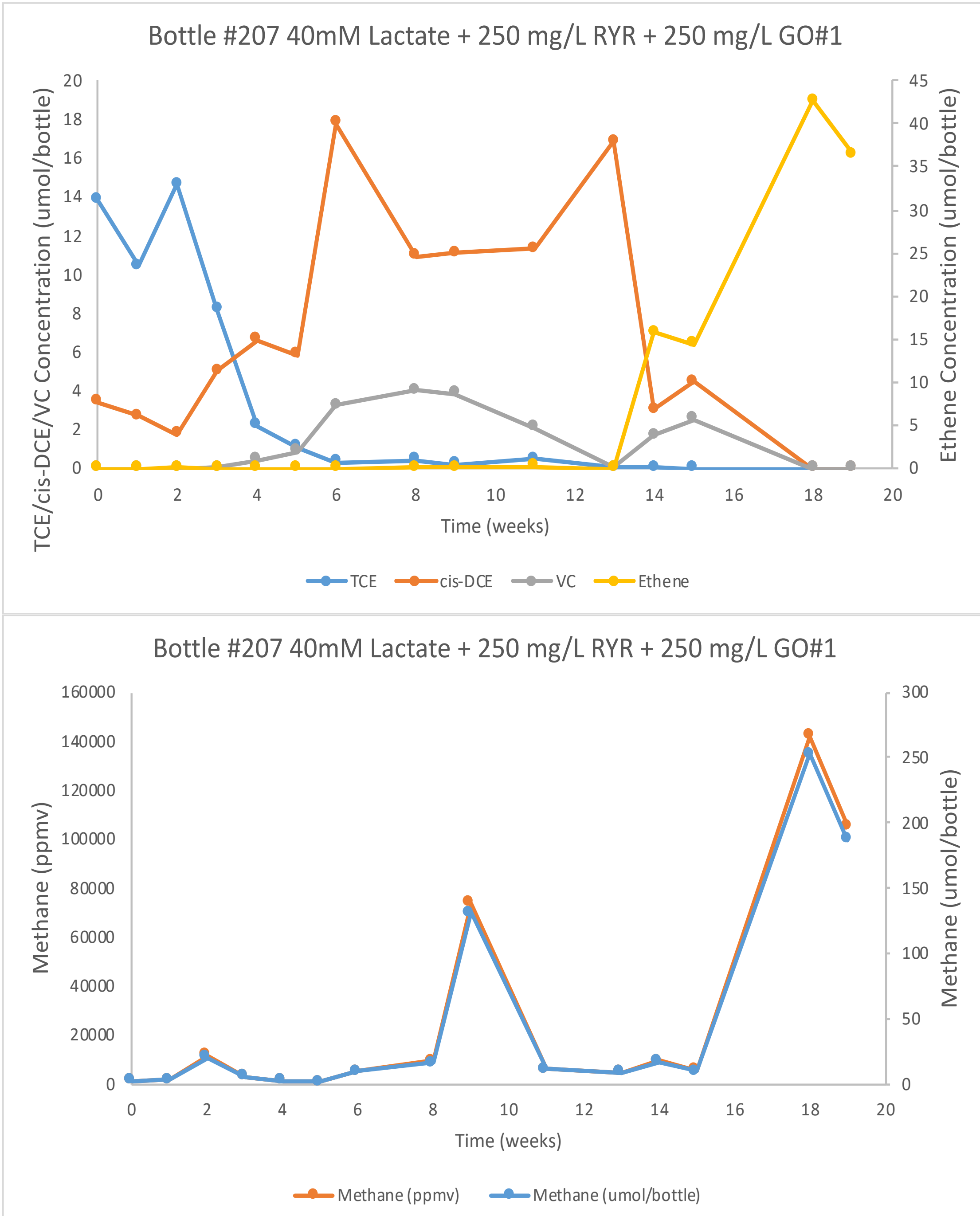
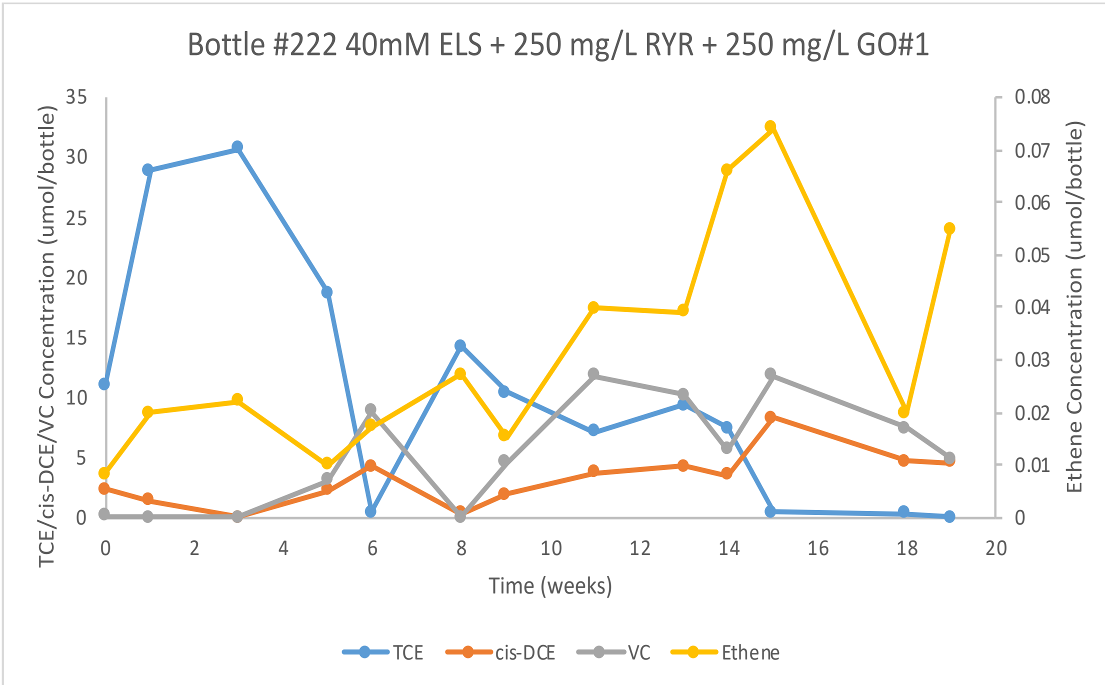


Figure B.72: TCE reduction and methane reduction for aquifer material with 40mM Lactate, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



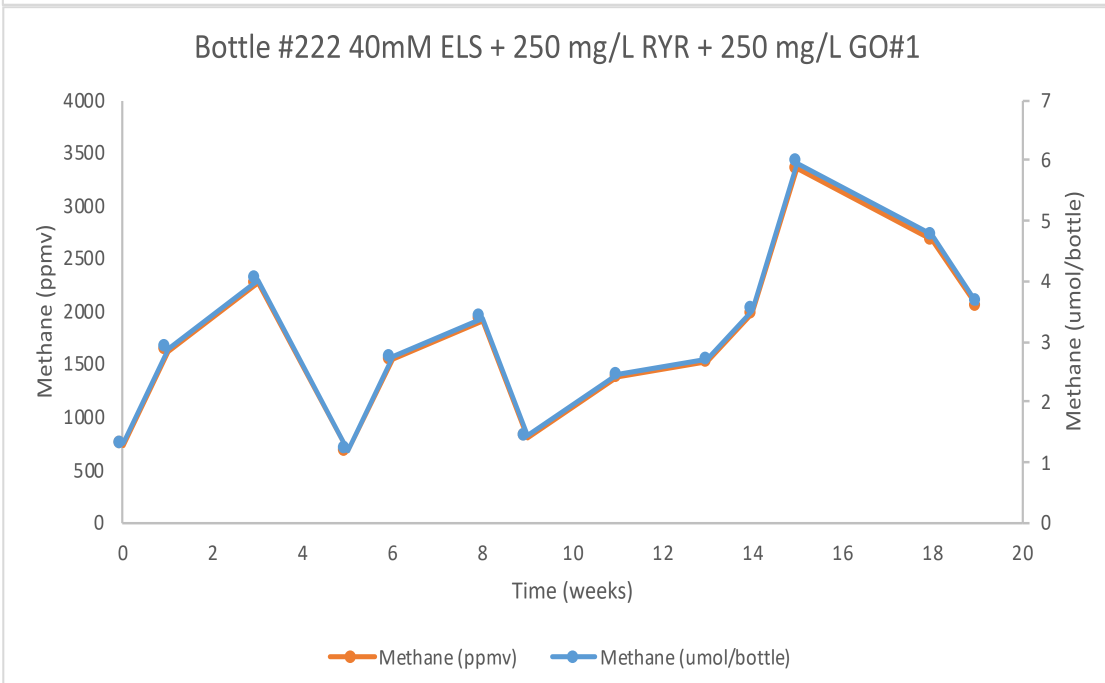


Figure B.73: TCE reduction and methane reduction for aquifer material with 40mM ELS, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

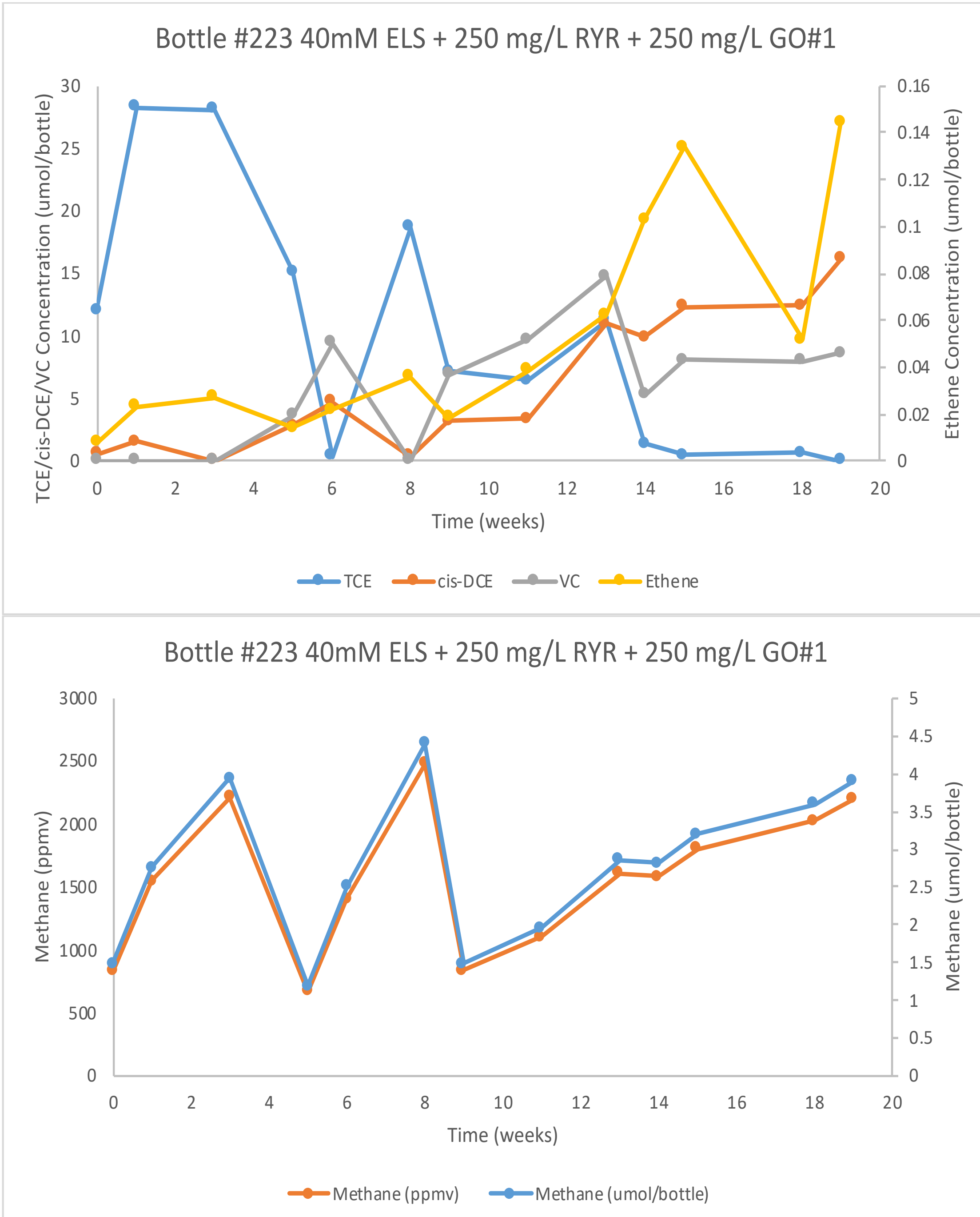
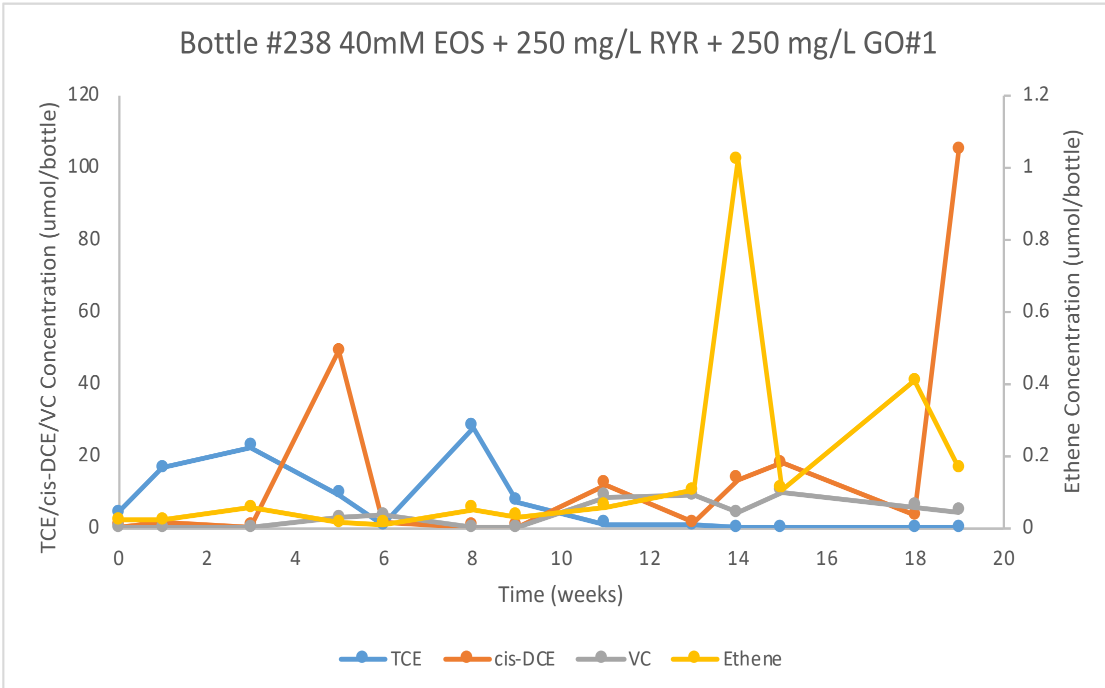


Figure B.74: TCE reduction and methane reduction for aquifer material with 40mM ELS, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



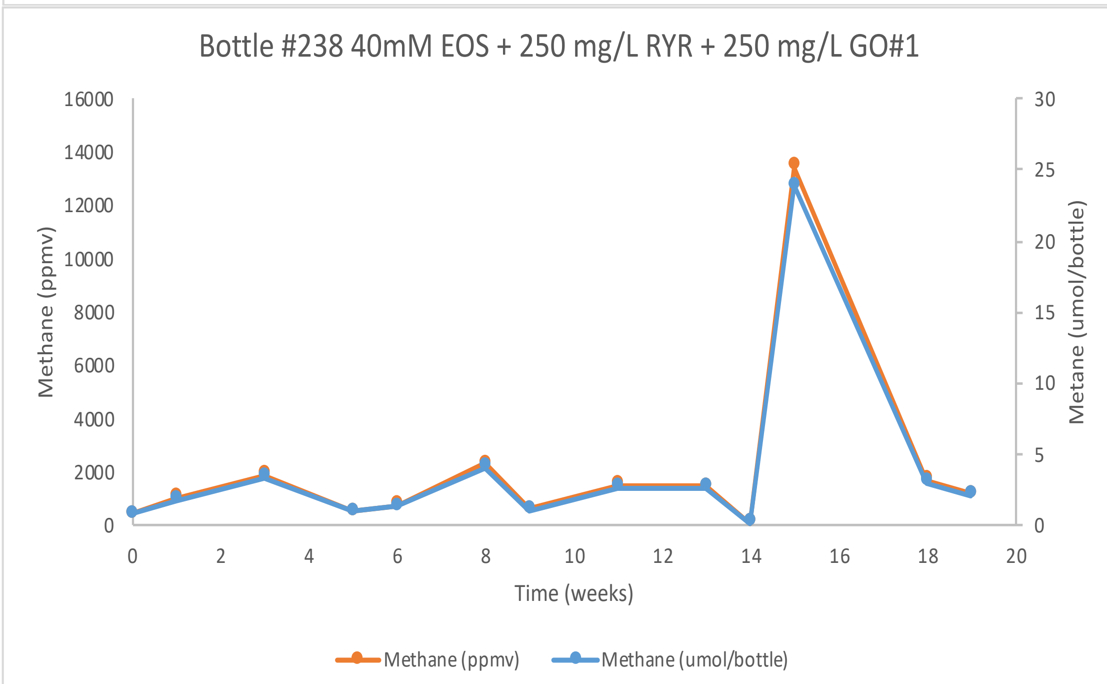
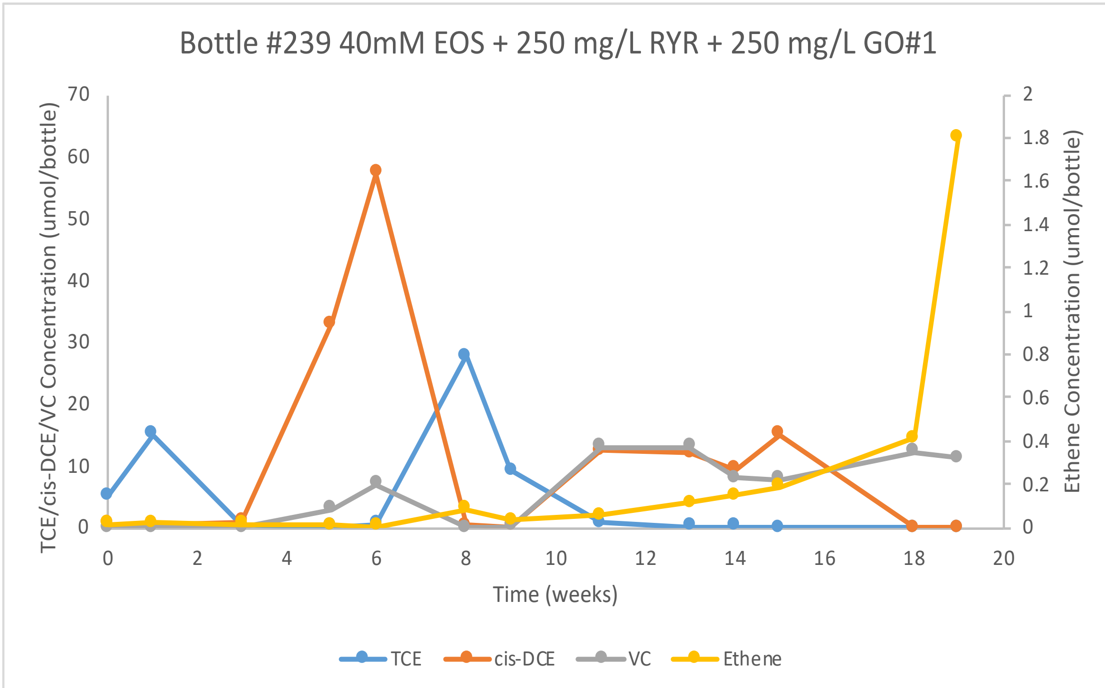


Figure B.75: TCE reduction and methane reduction for aquifer material with 40mM EOS, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



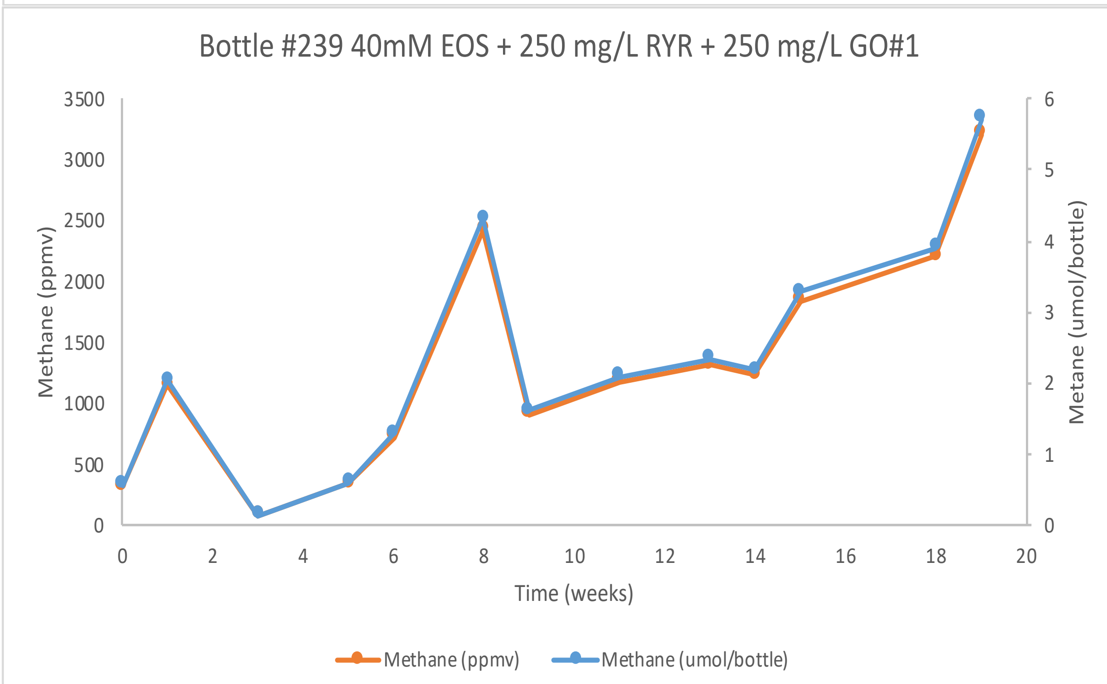


Figure B.76: TCE reduction and methane reduction for aquifer material with 40mM EOS, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

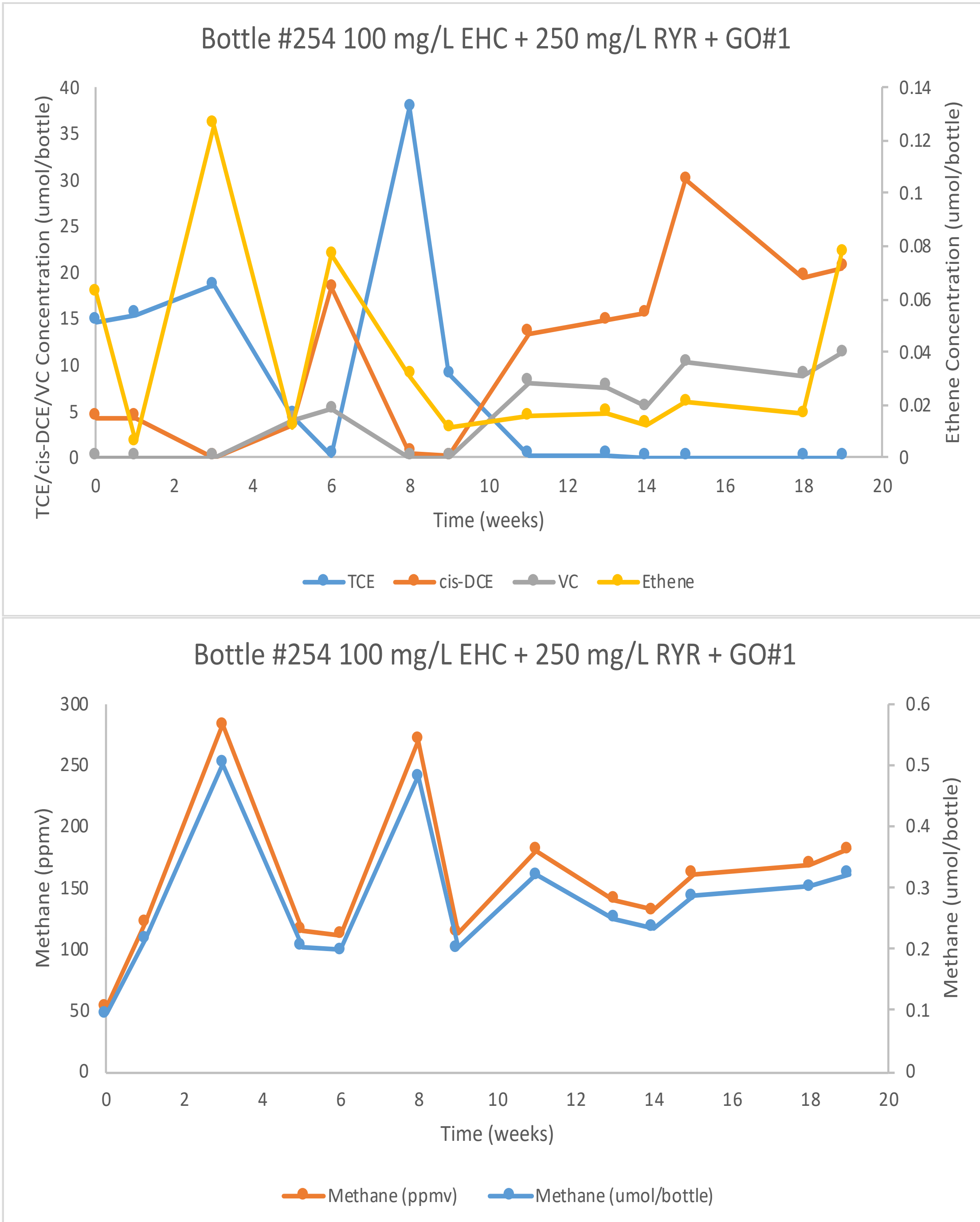
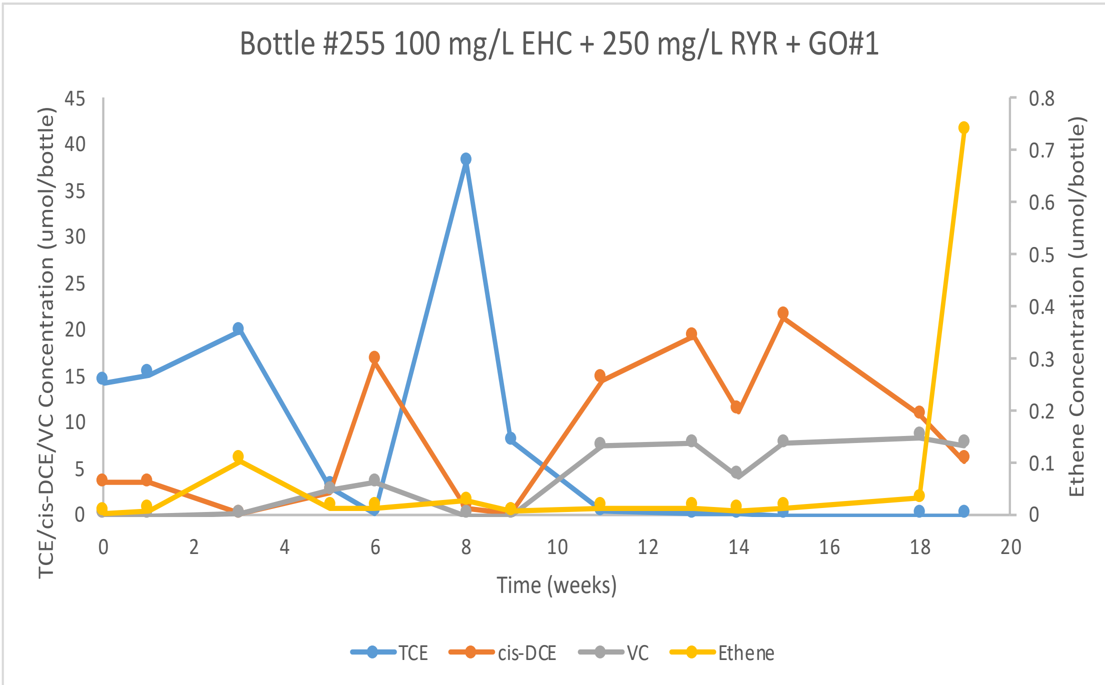


Figure B.77: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



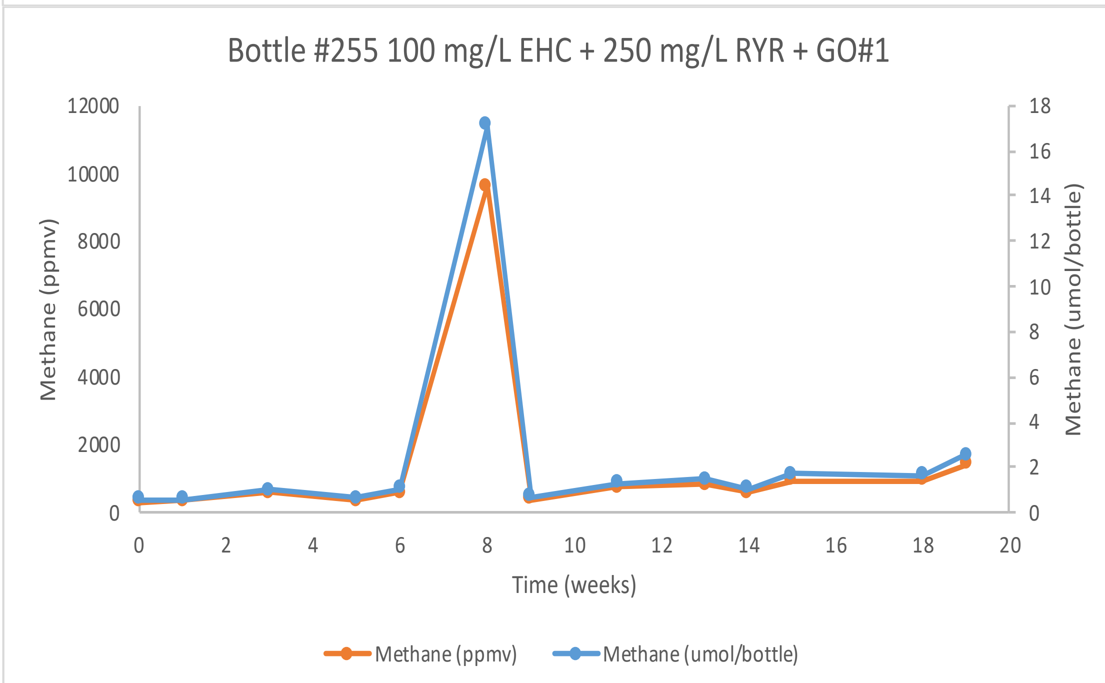
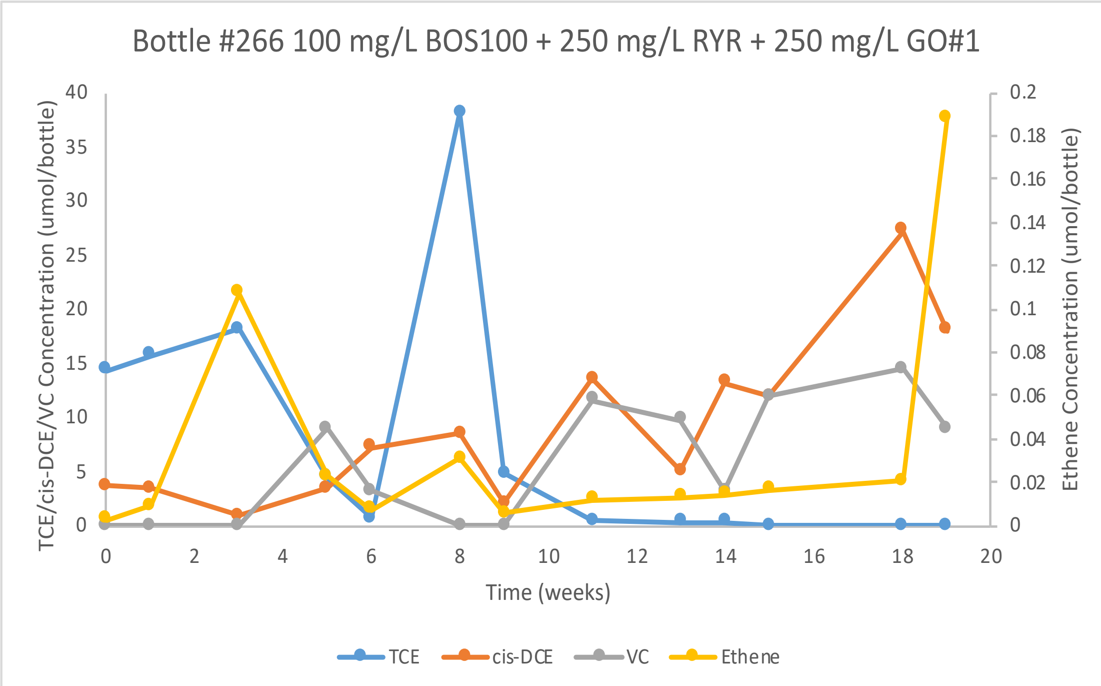


Figure B.78: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



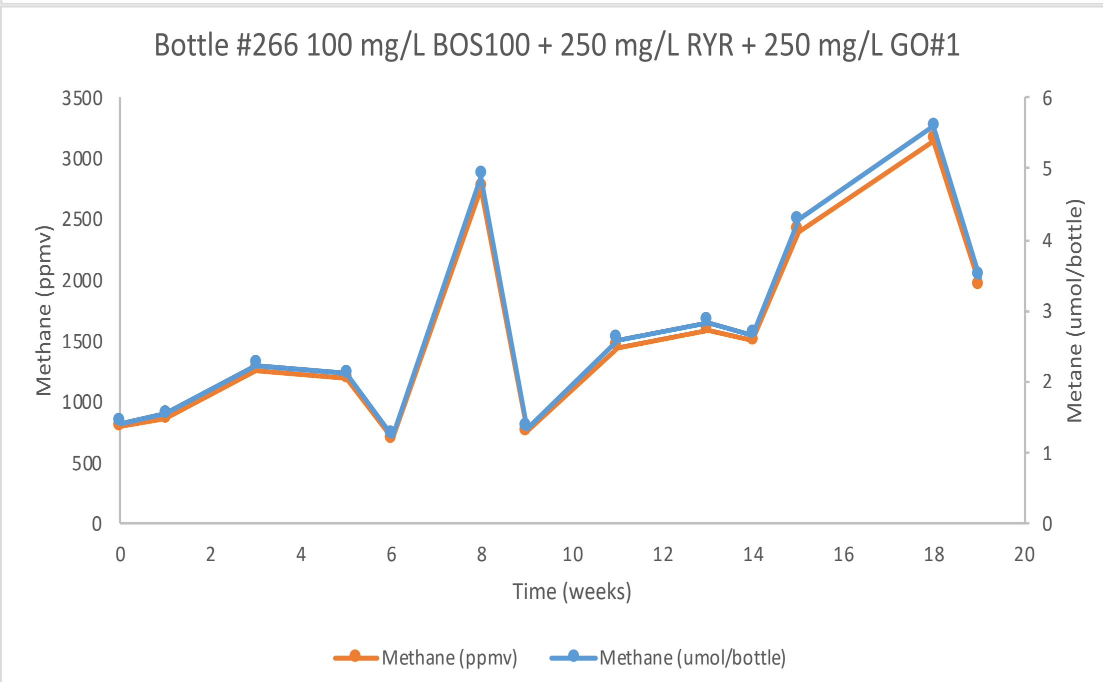
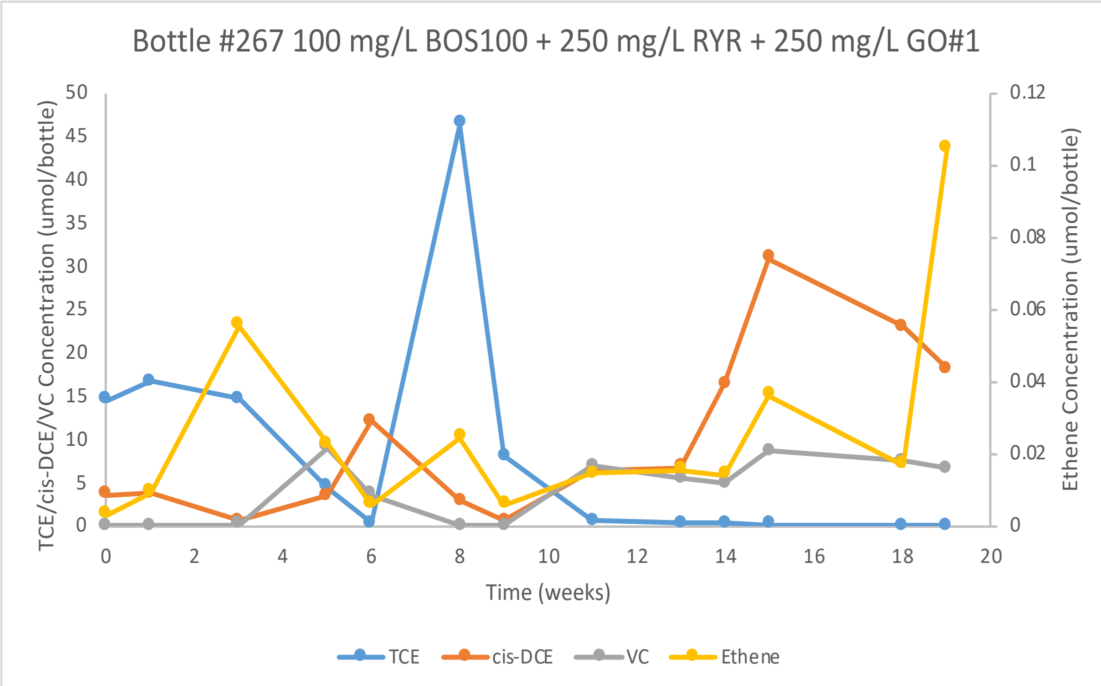


Figure B.79: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



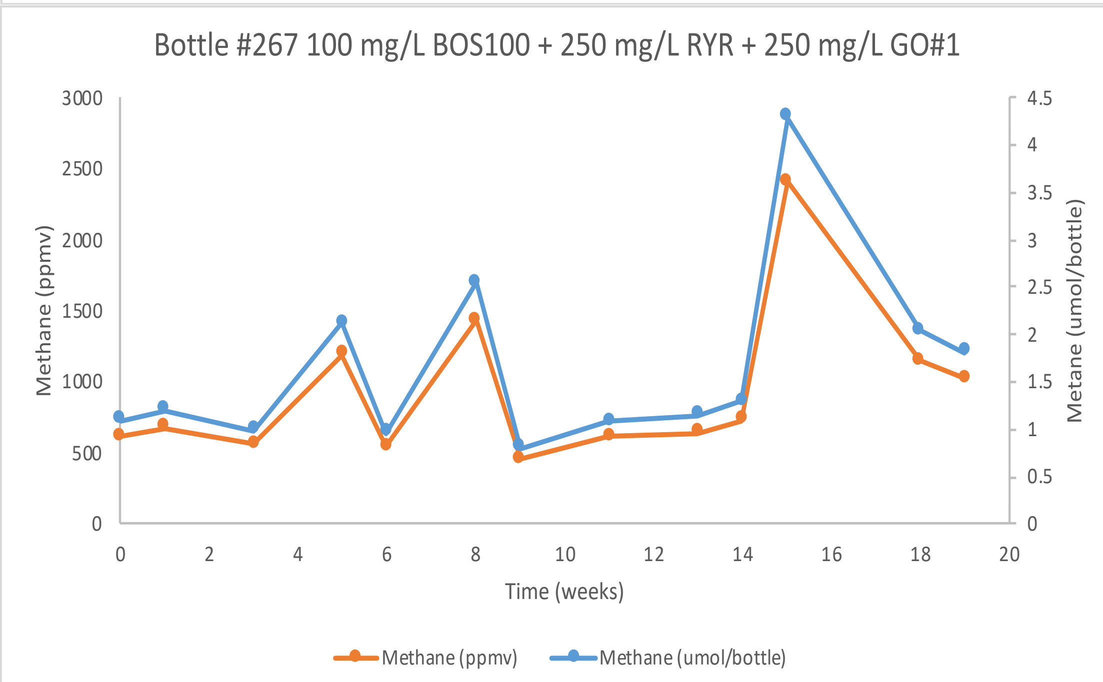


Figure B.80: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100, 250 mg/L Red Yeast Rice, and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

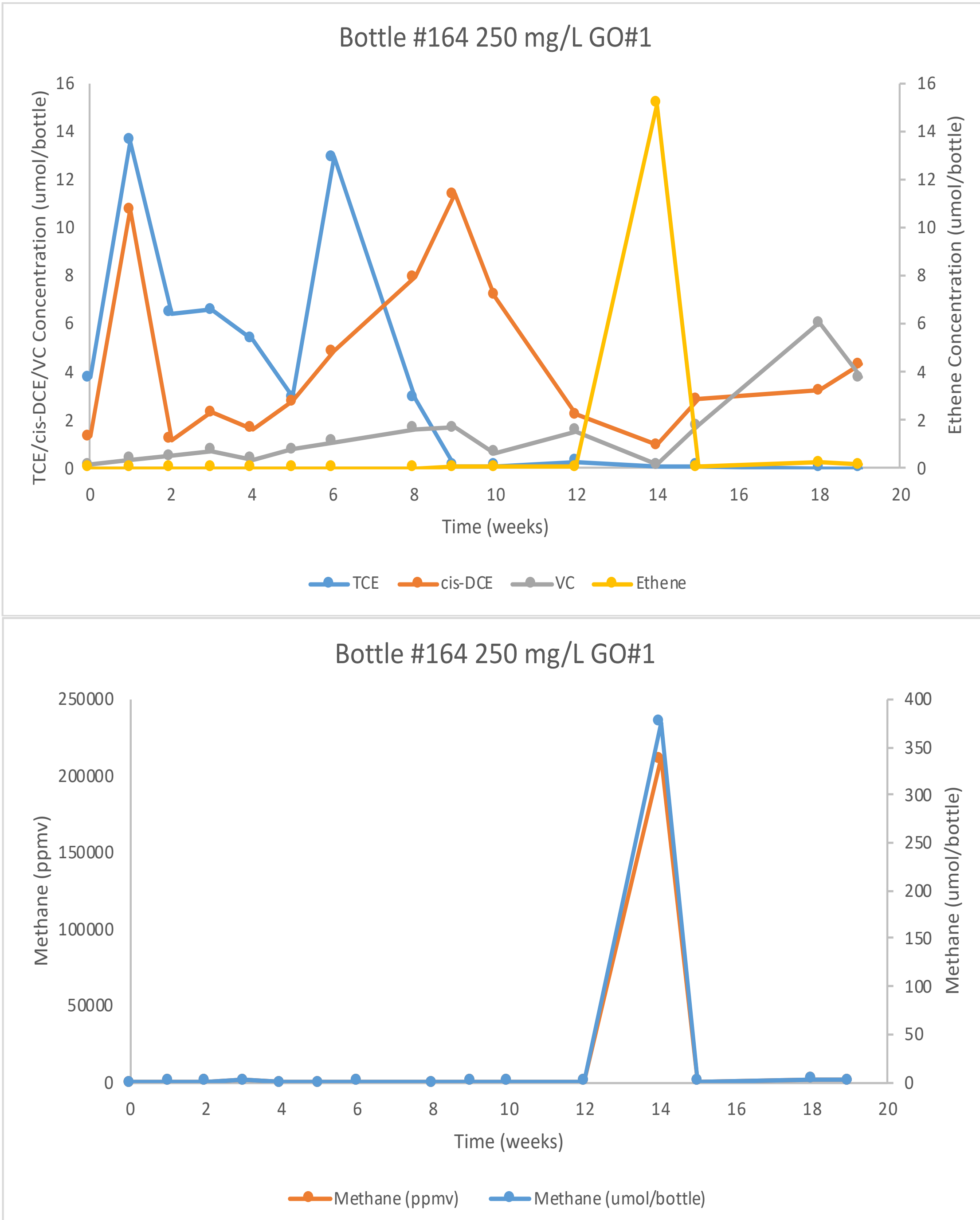


Figure B.81: TCE reduction and methane reduction for aquifer material with 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

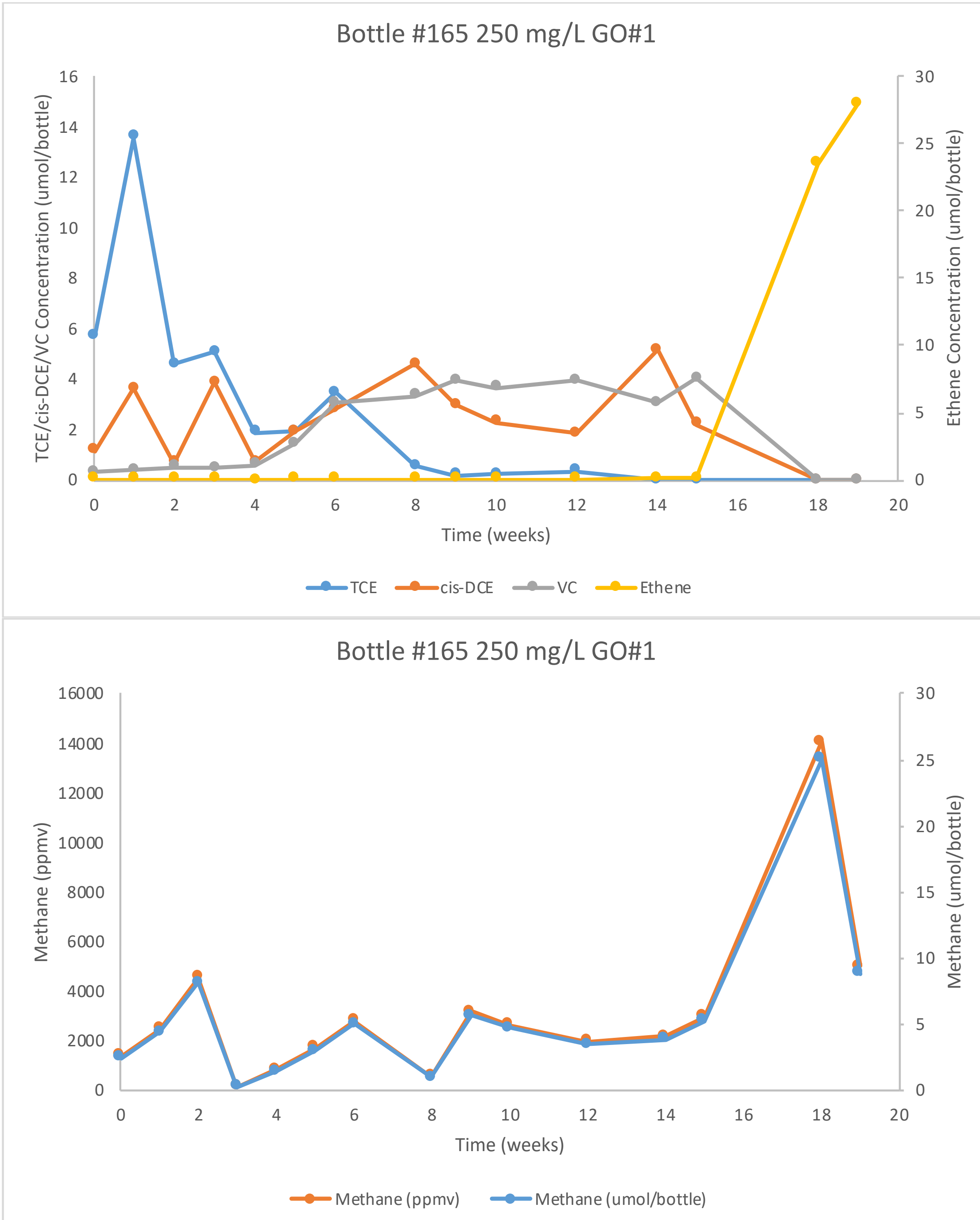
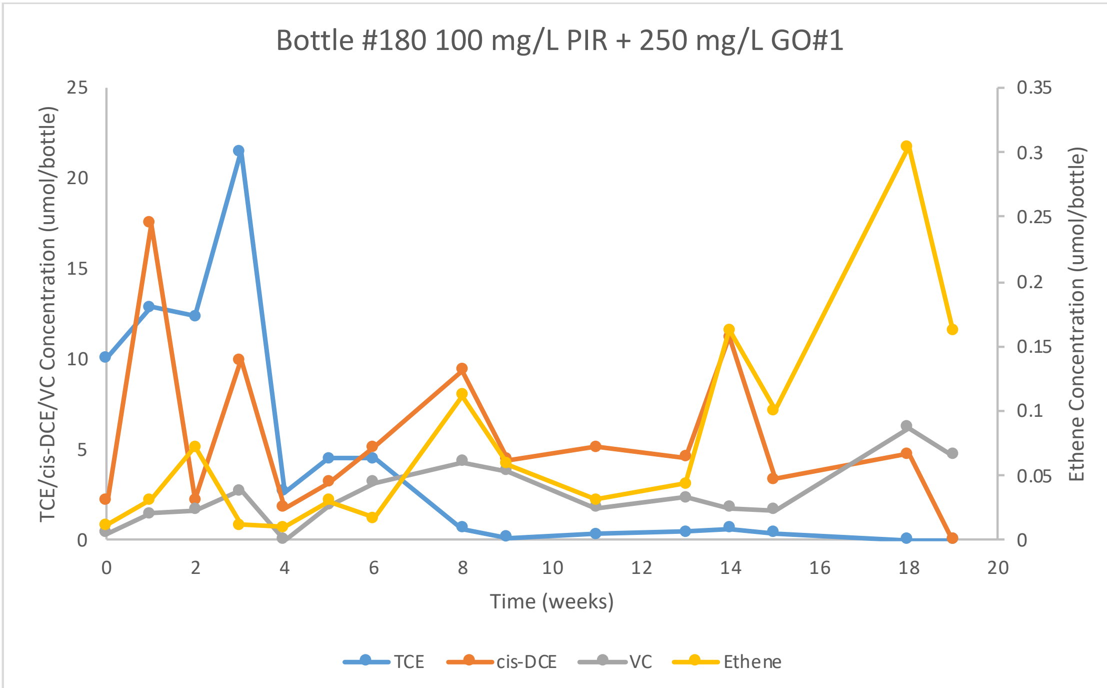


Figure B.82: TCE reduction and methane reduction for aquifer material with 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



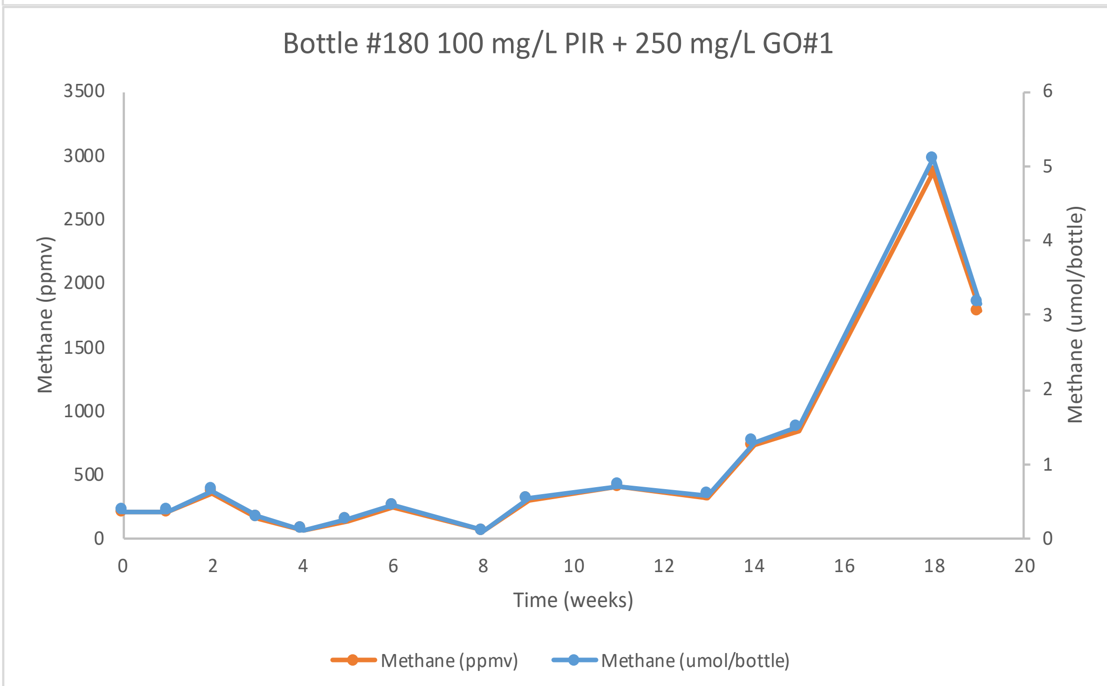
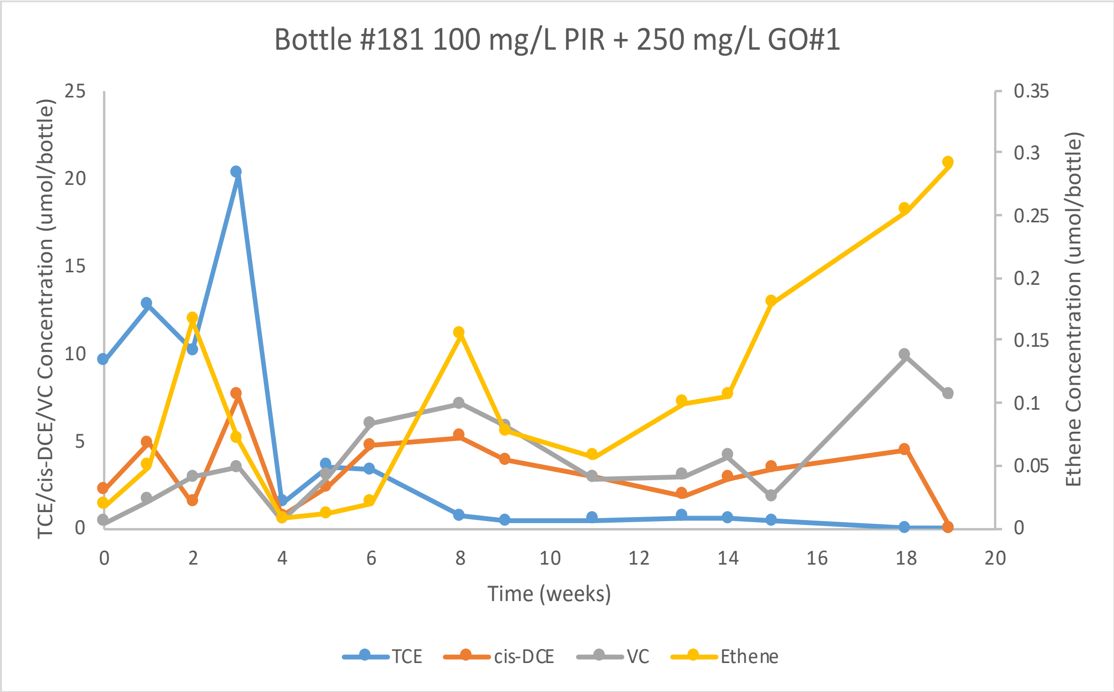


Figure B.83: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



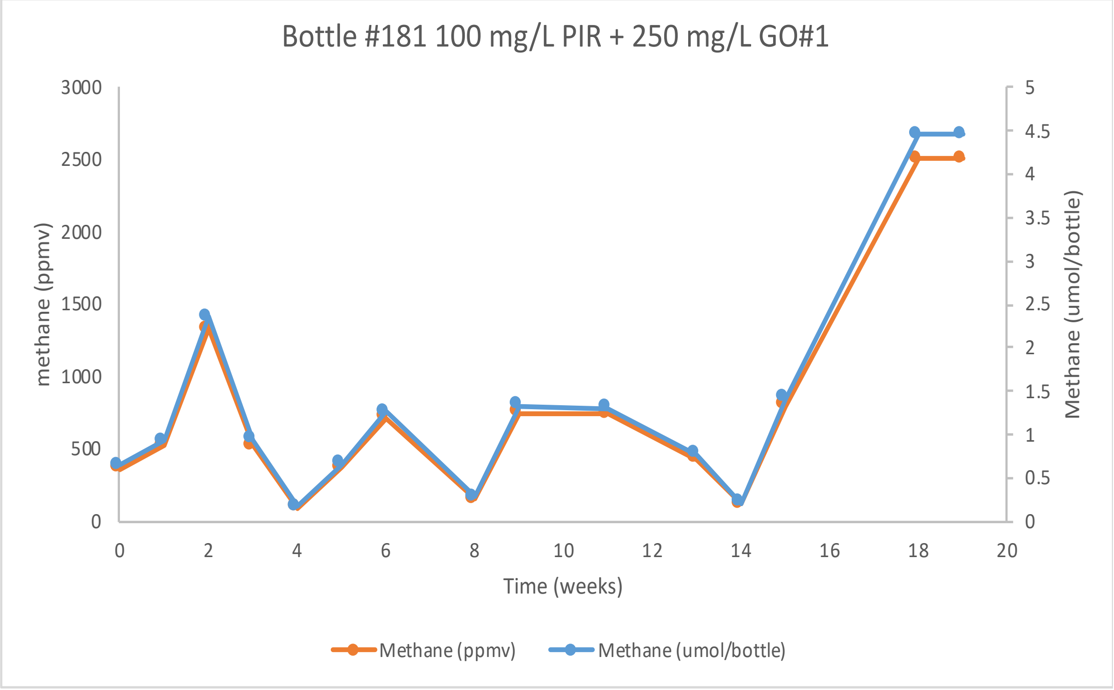
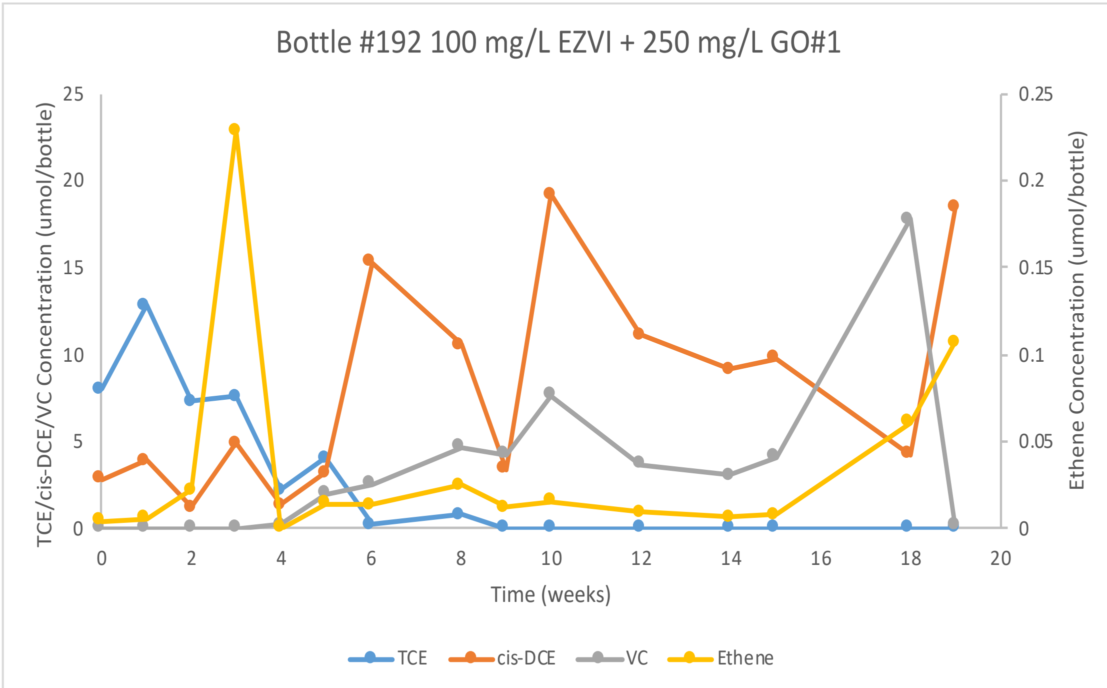


Figure B.84: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



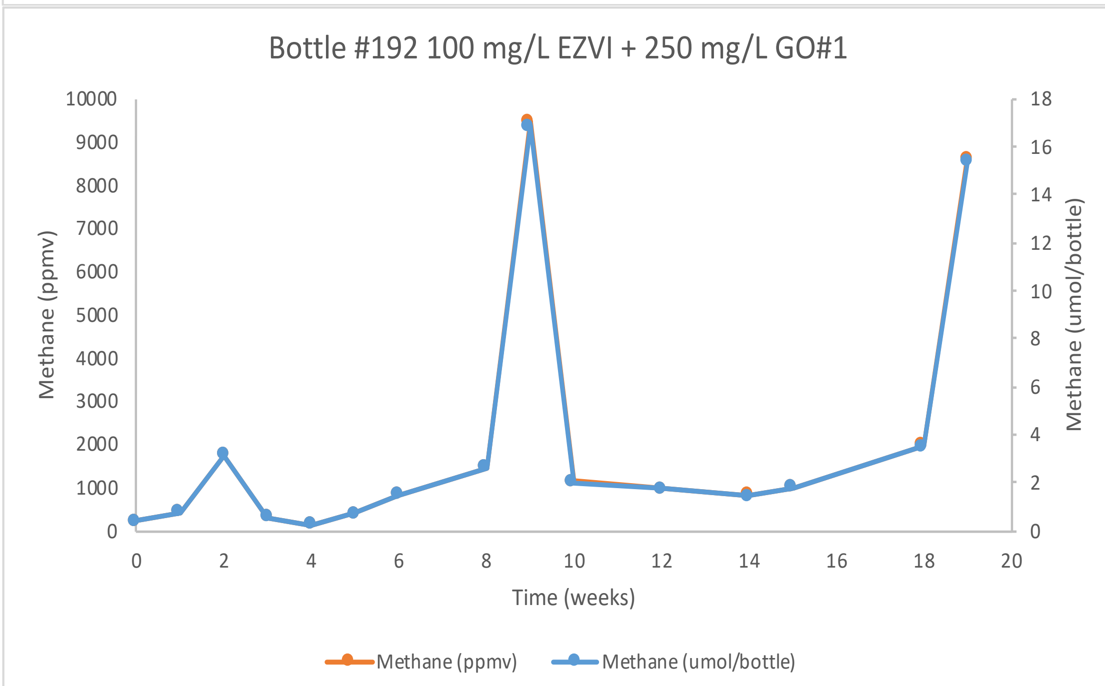


Figure B.85: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

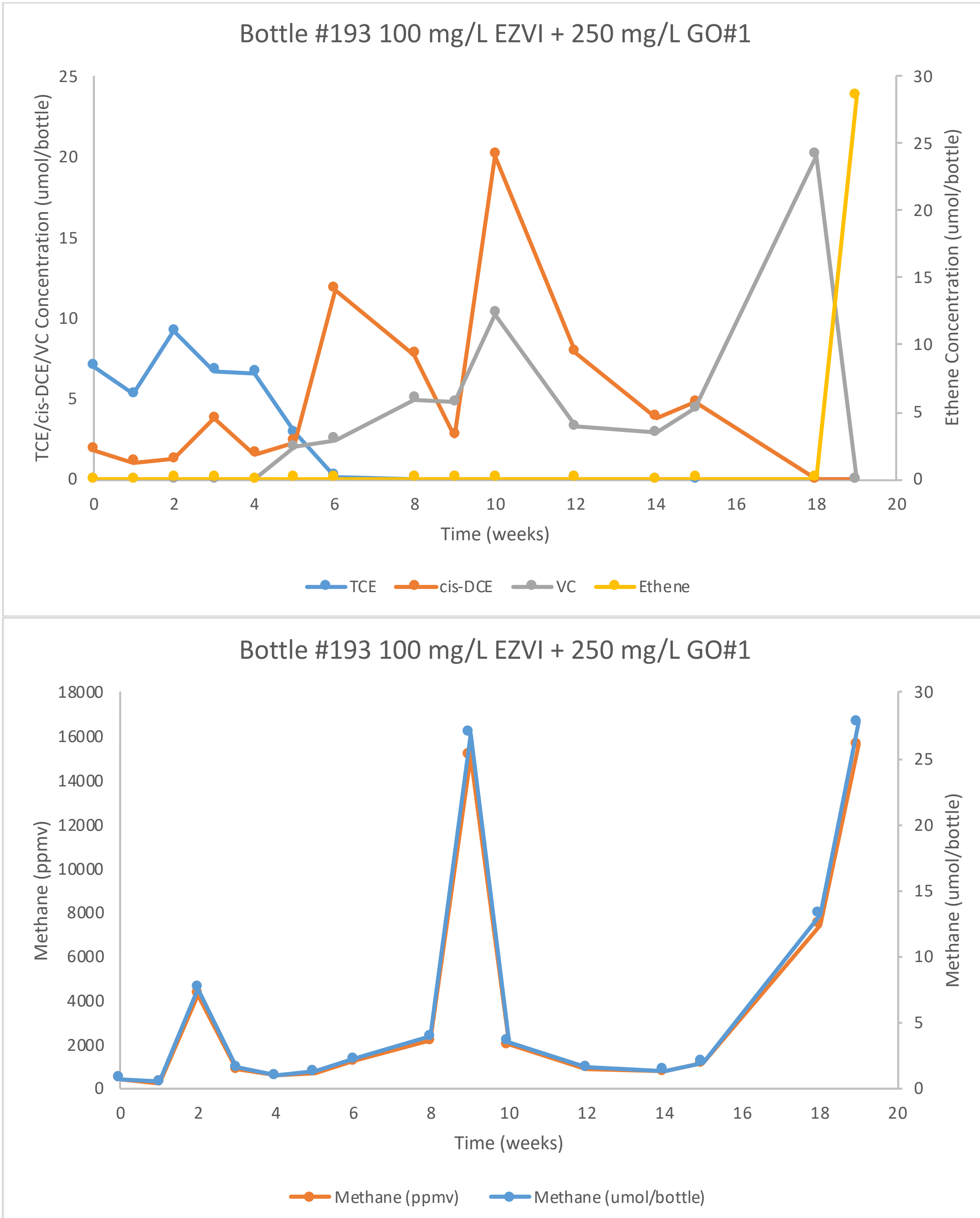


Figure B.86: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

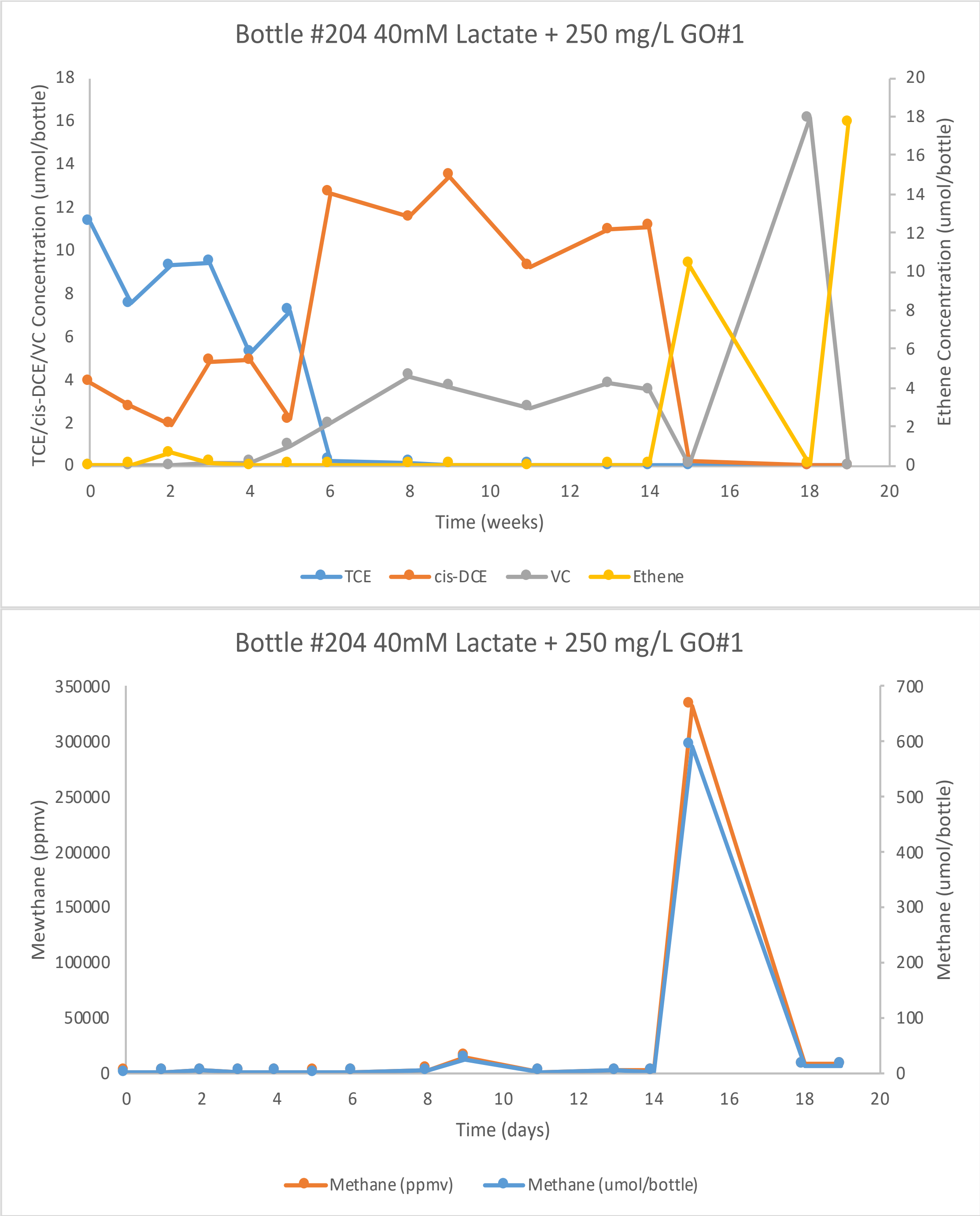


Figure B.87: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

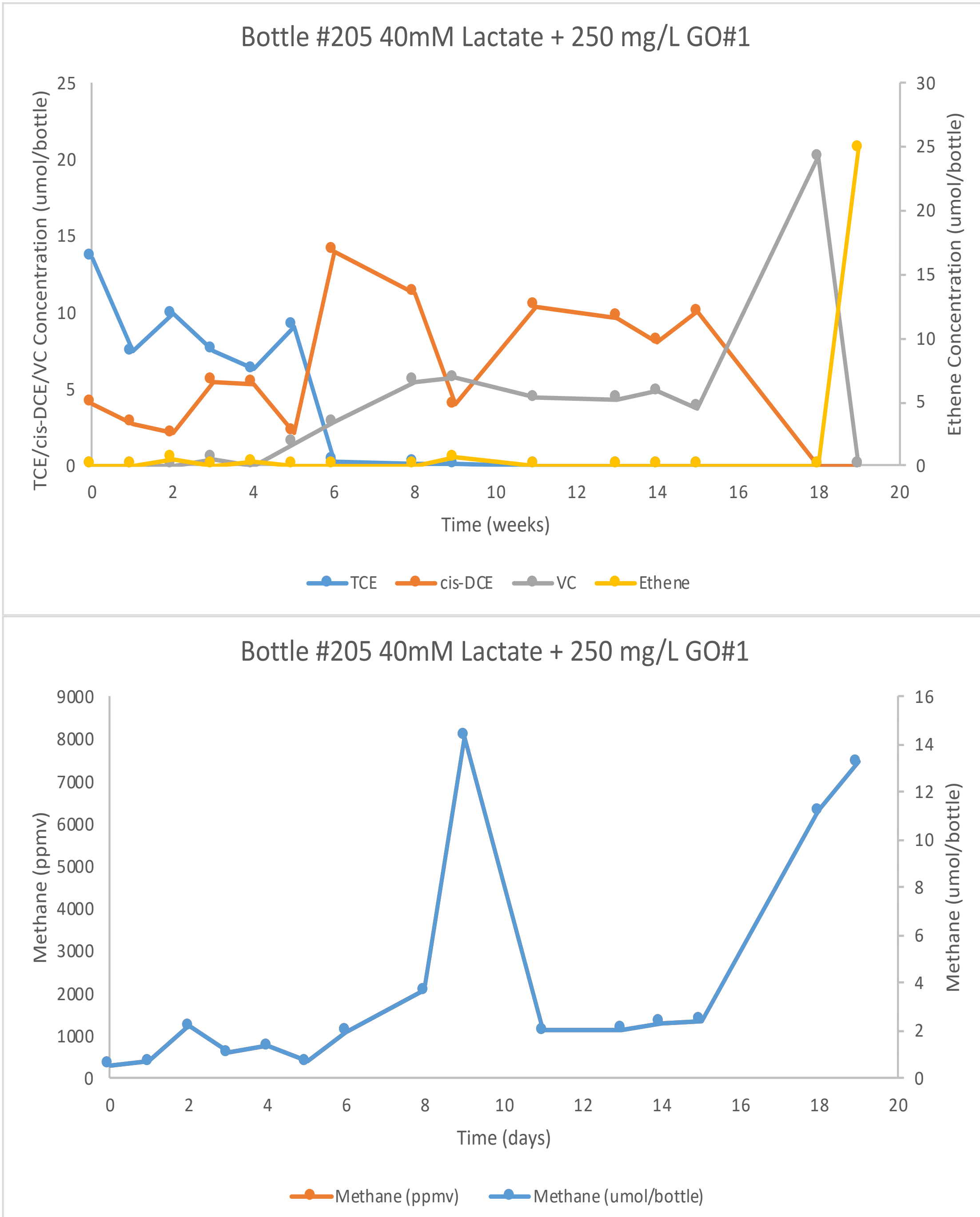
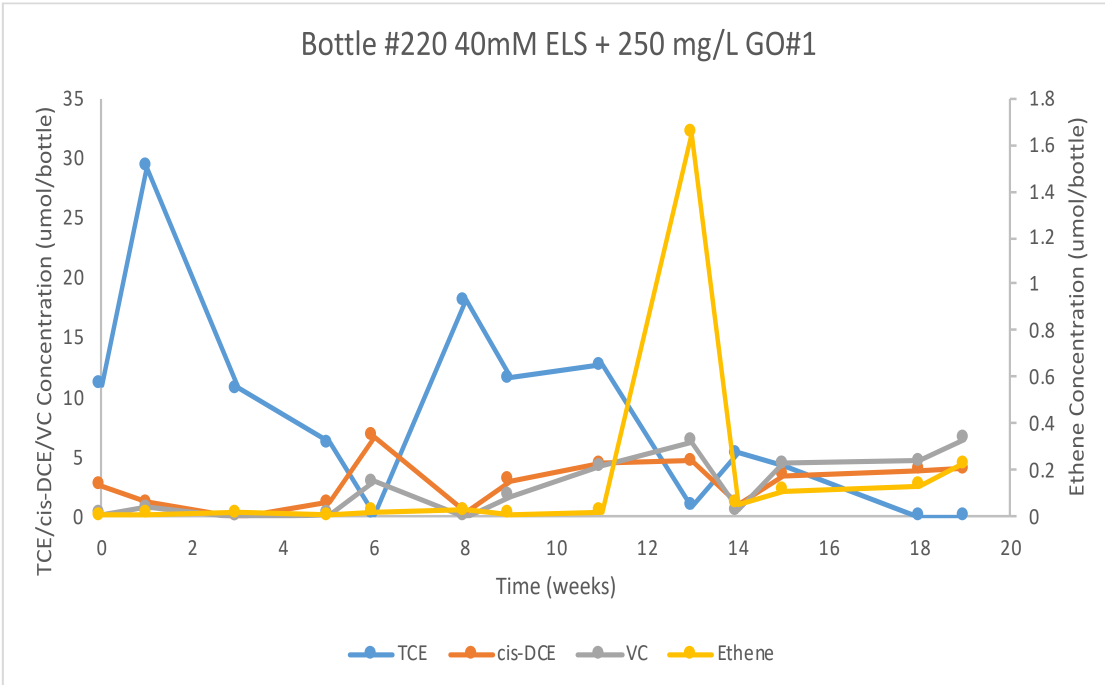


Figure B.88: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



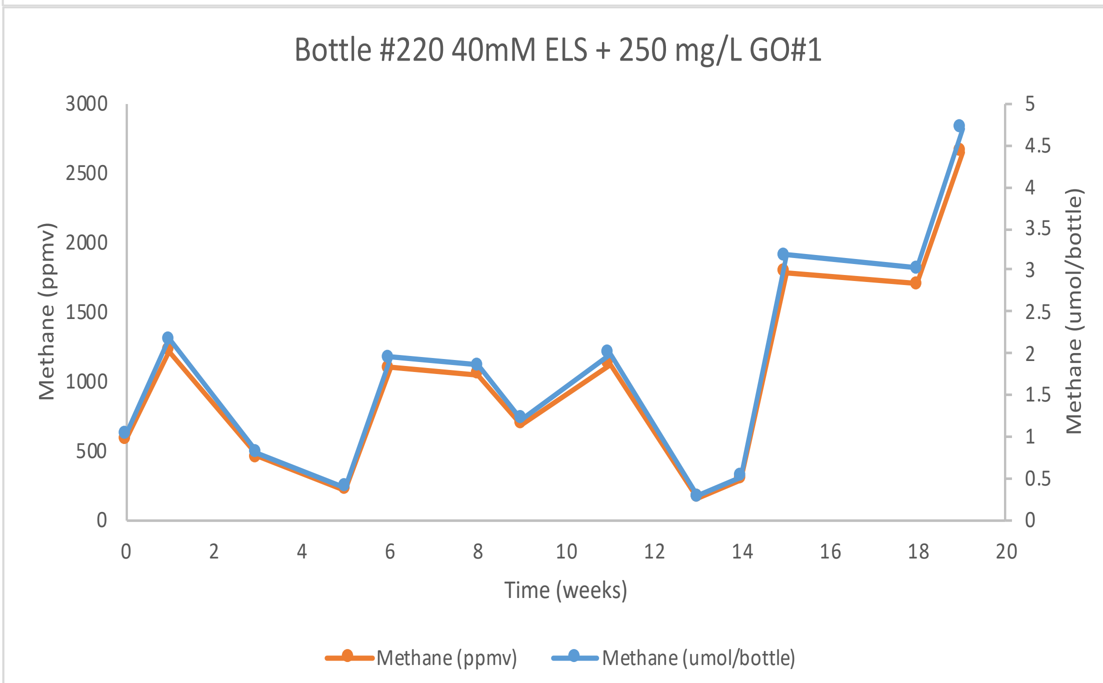
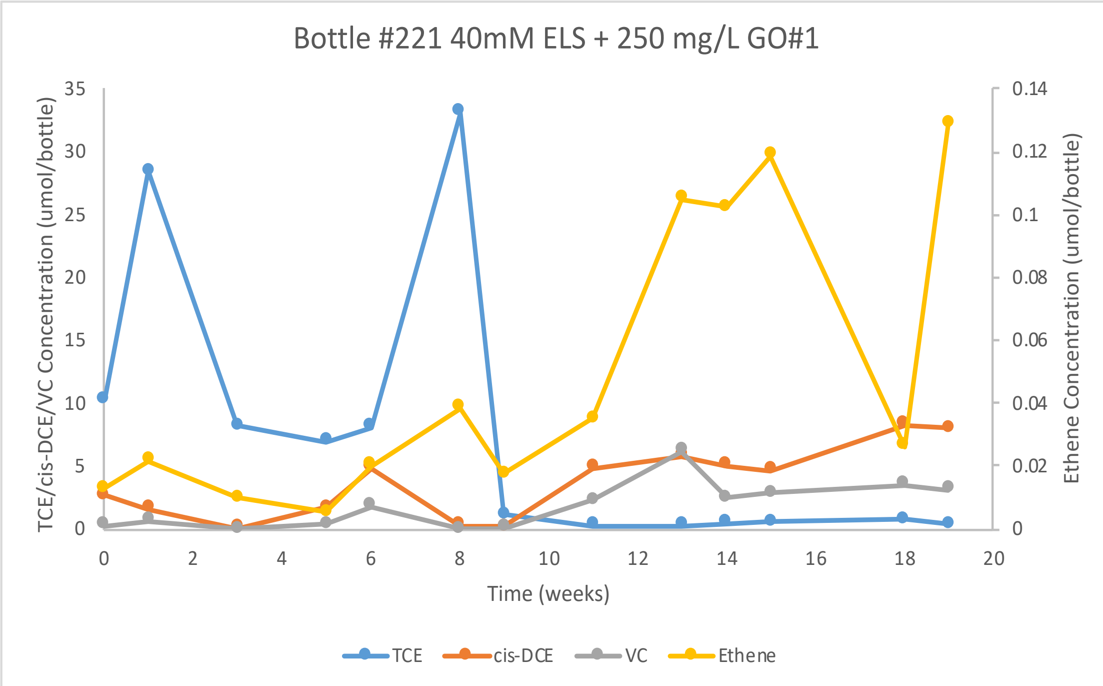


Figure B.89: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.



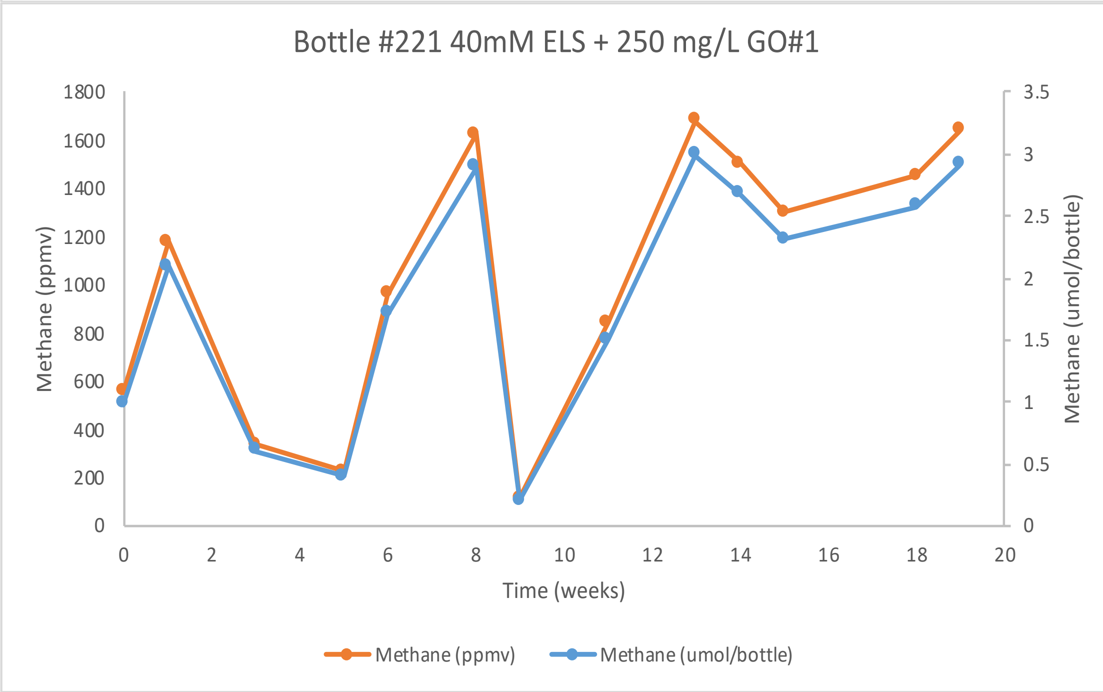
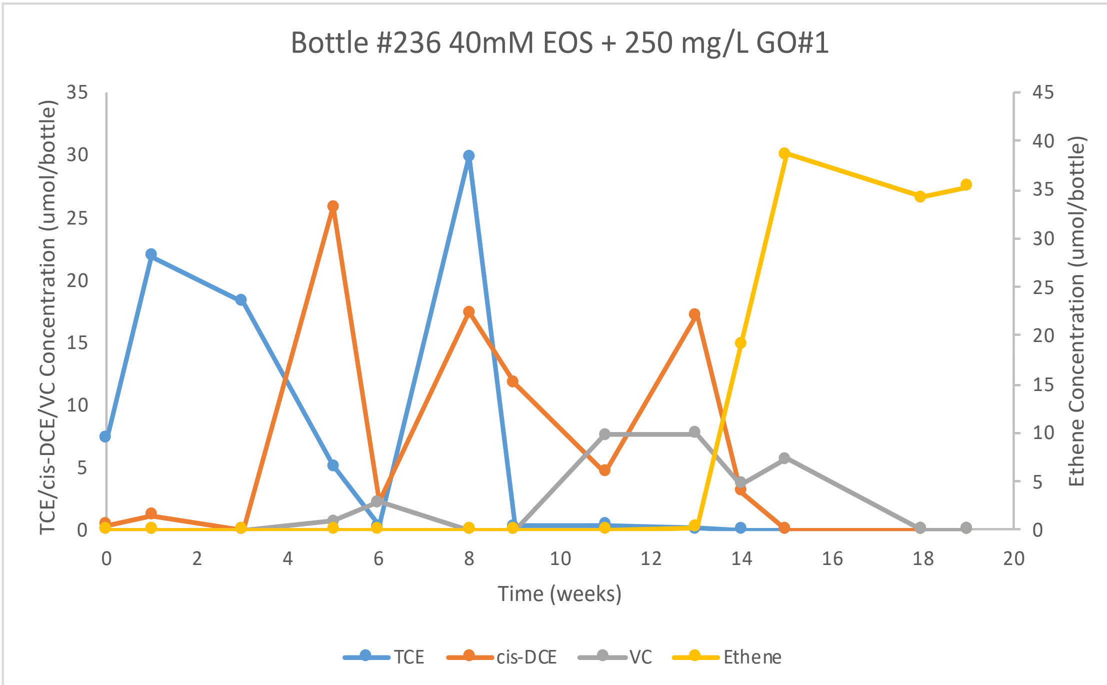


Figure B.90: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.



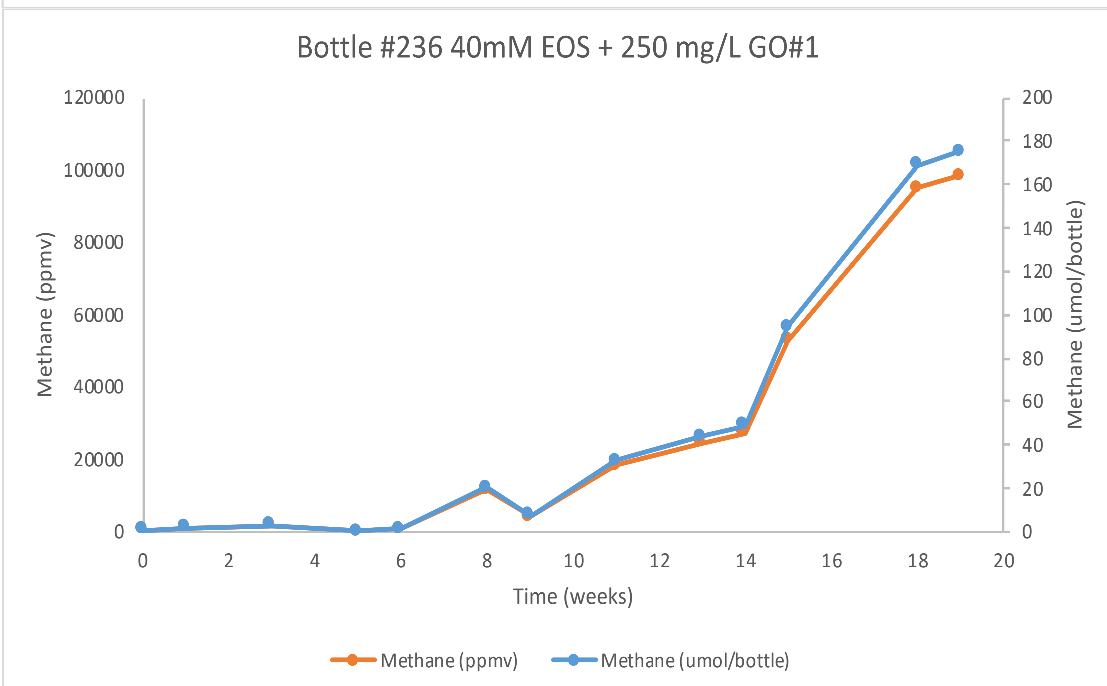


Figure B.91: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

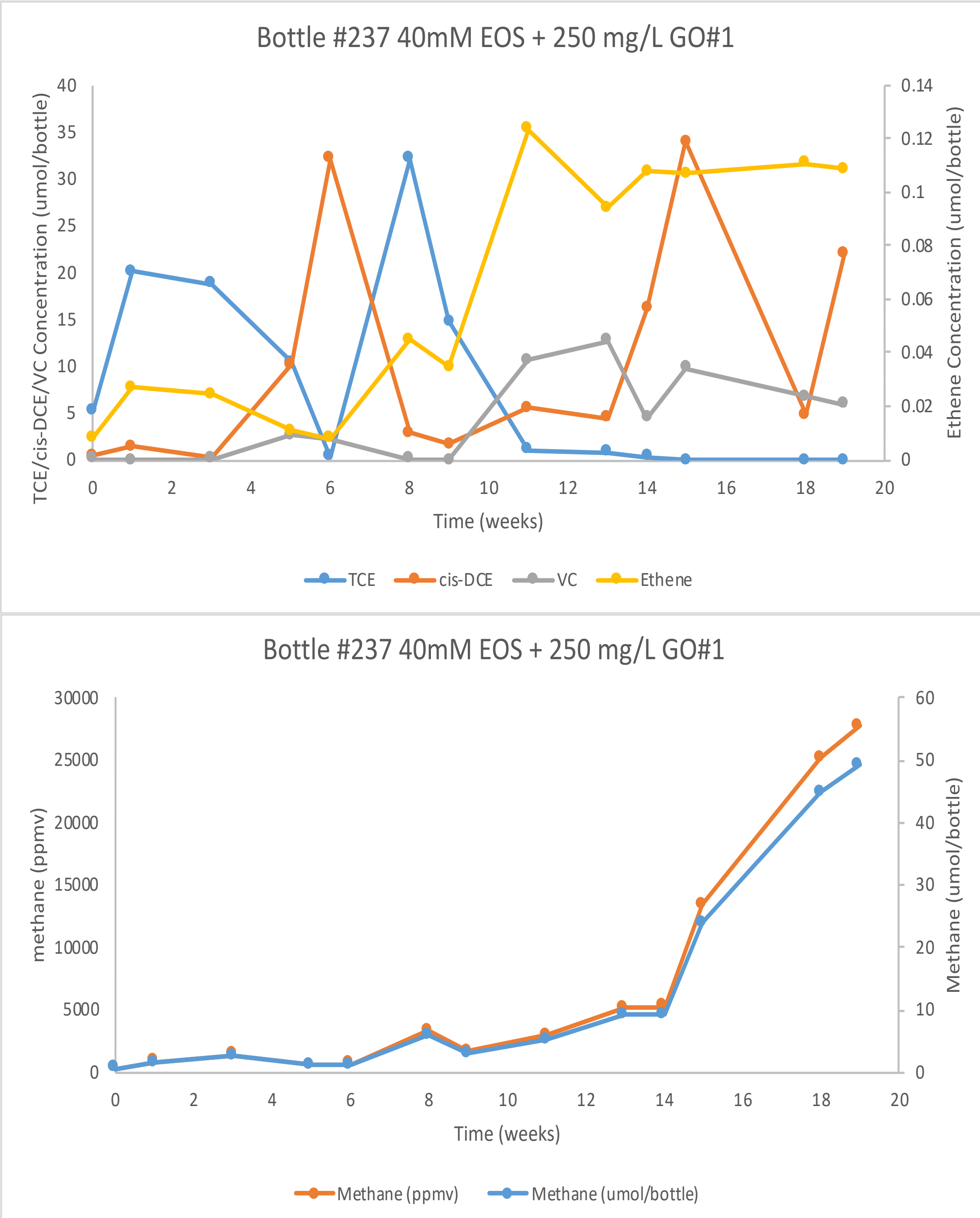


Figure B.92: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

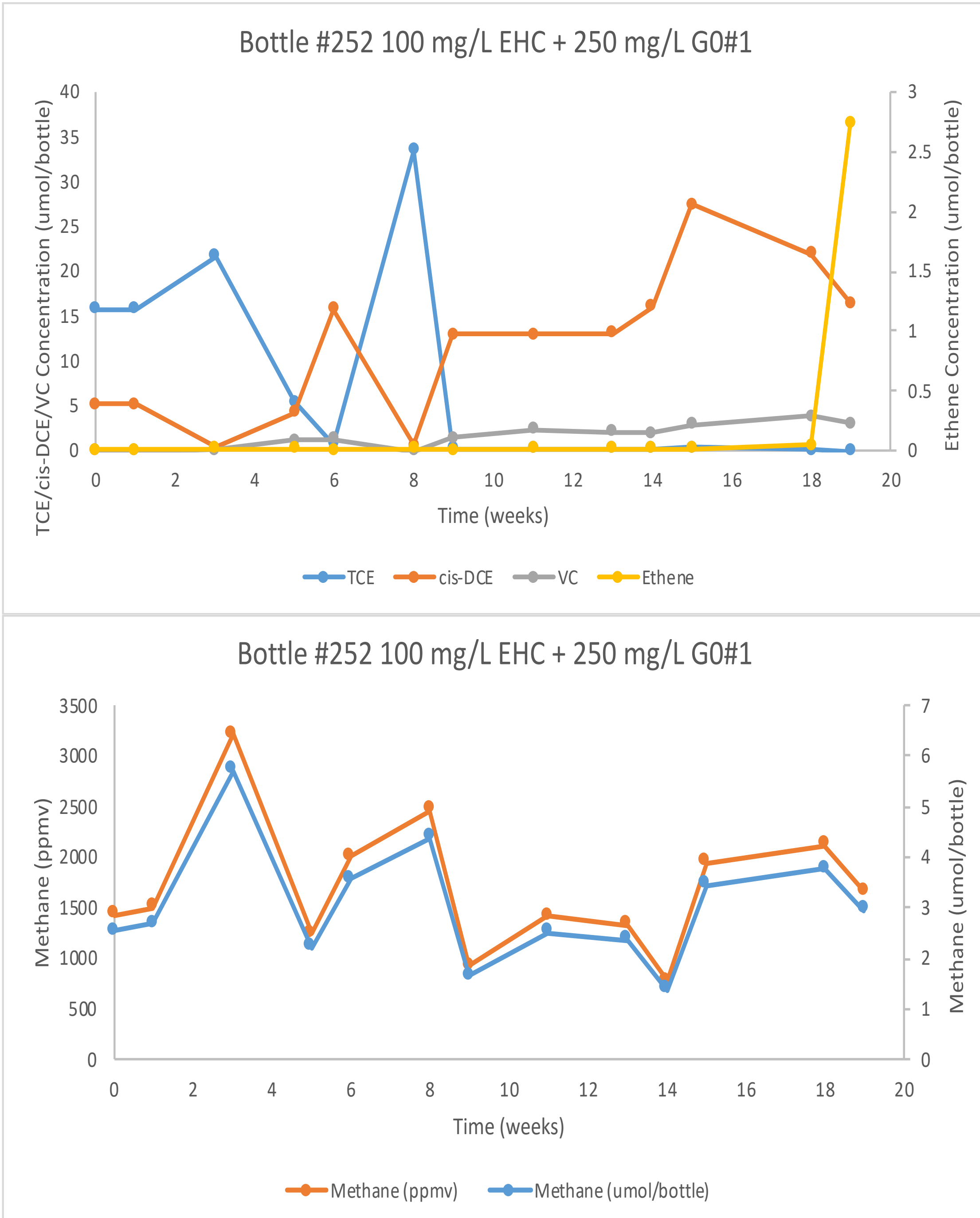


Figure B.93: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

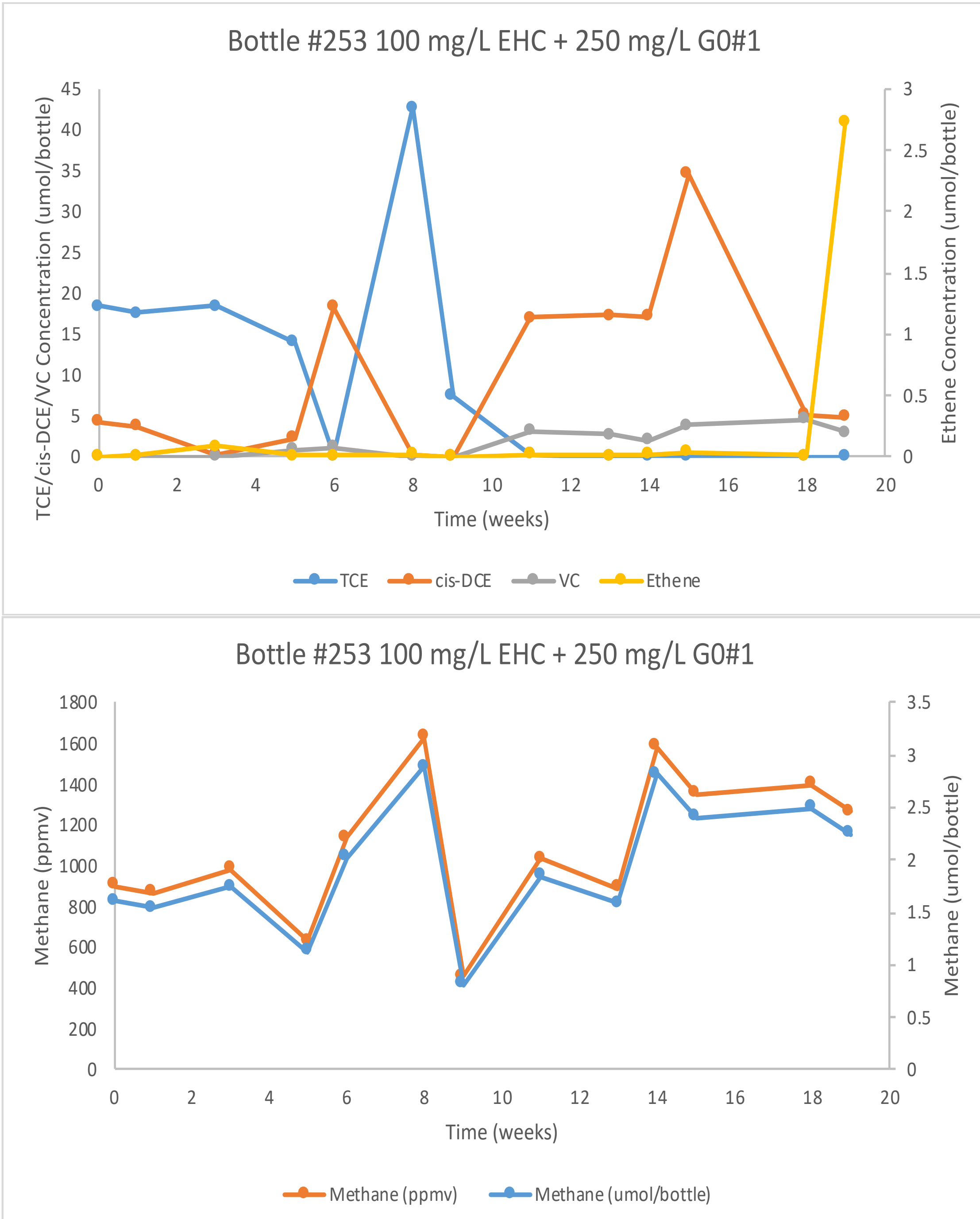


Figure B.94: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

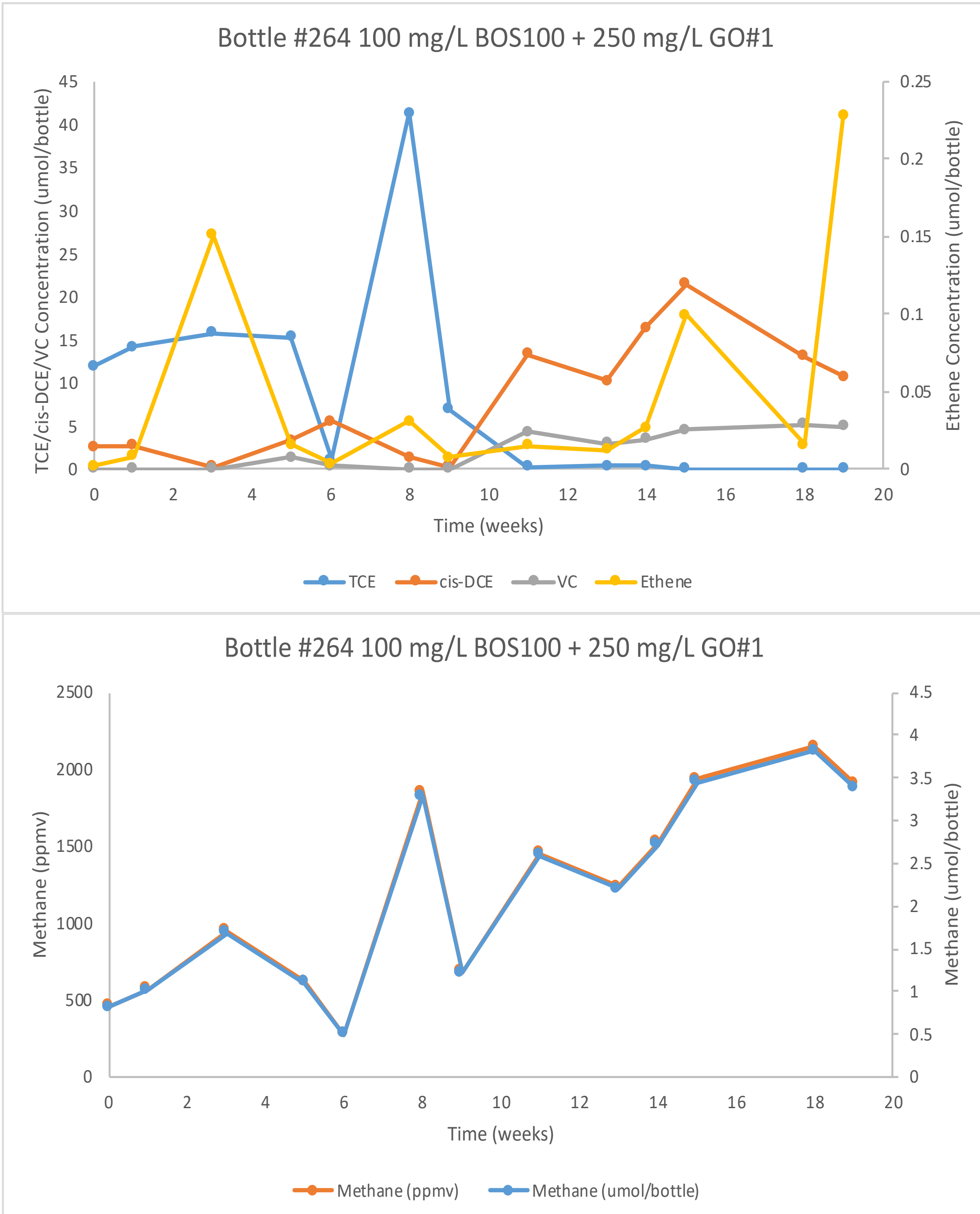


Figure B.95: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Garlic Oil #1. Plot indicates replicate one of two.

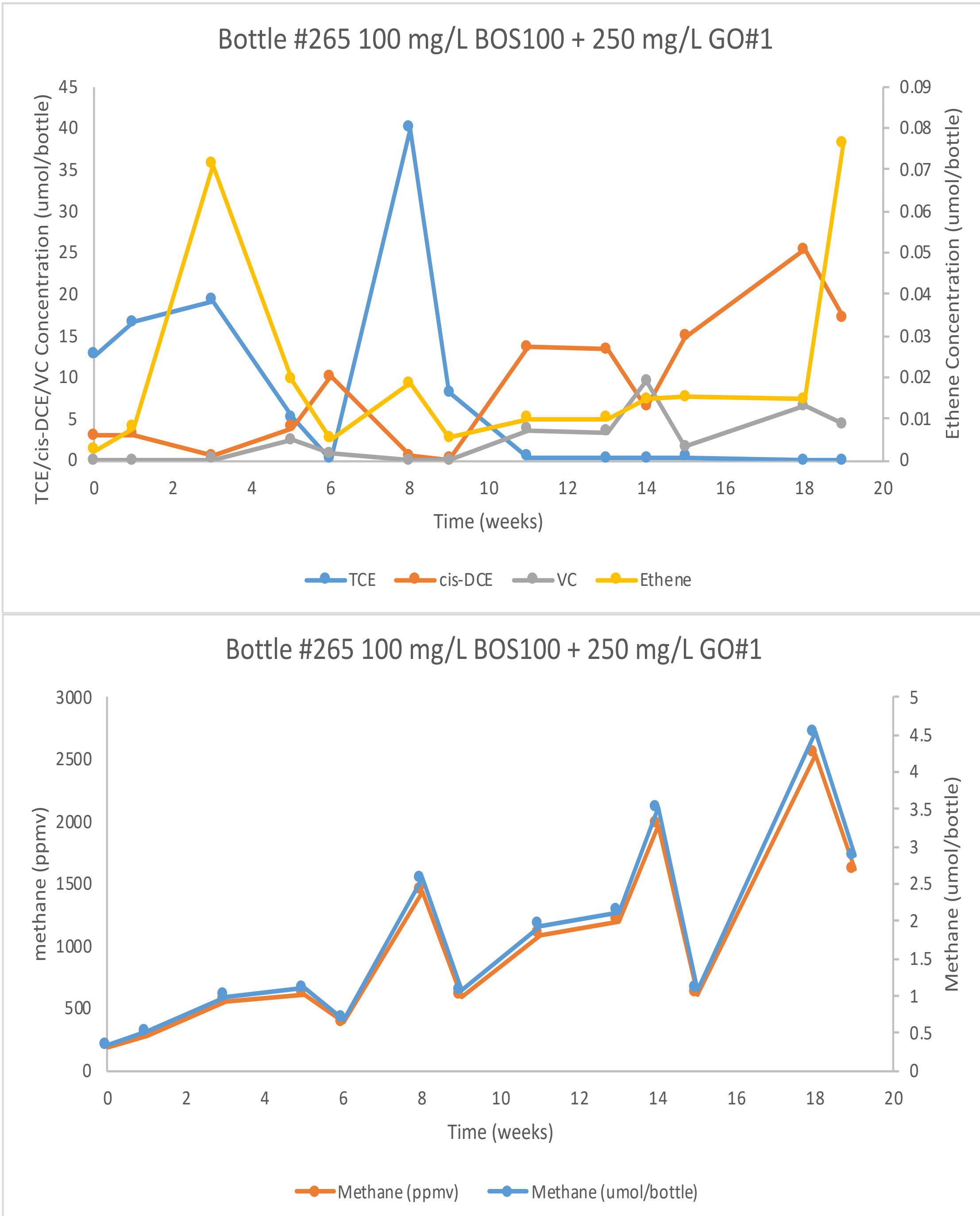


Figure B.96: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Garlic Oil #1. Plot indicates replicate two of two.

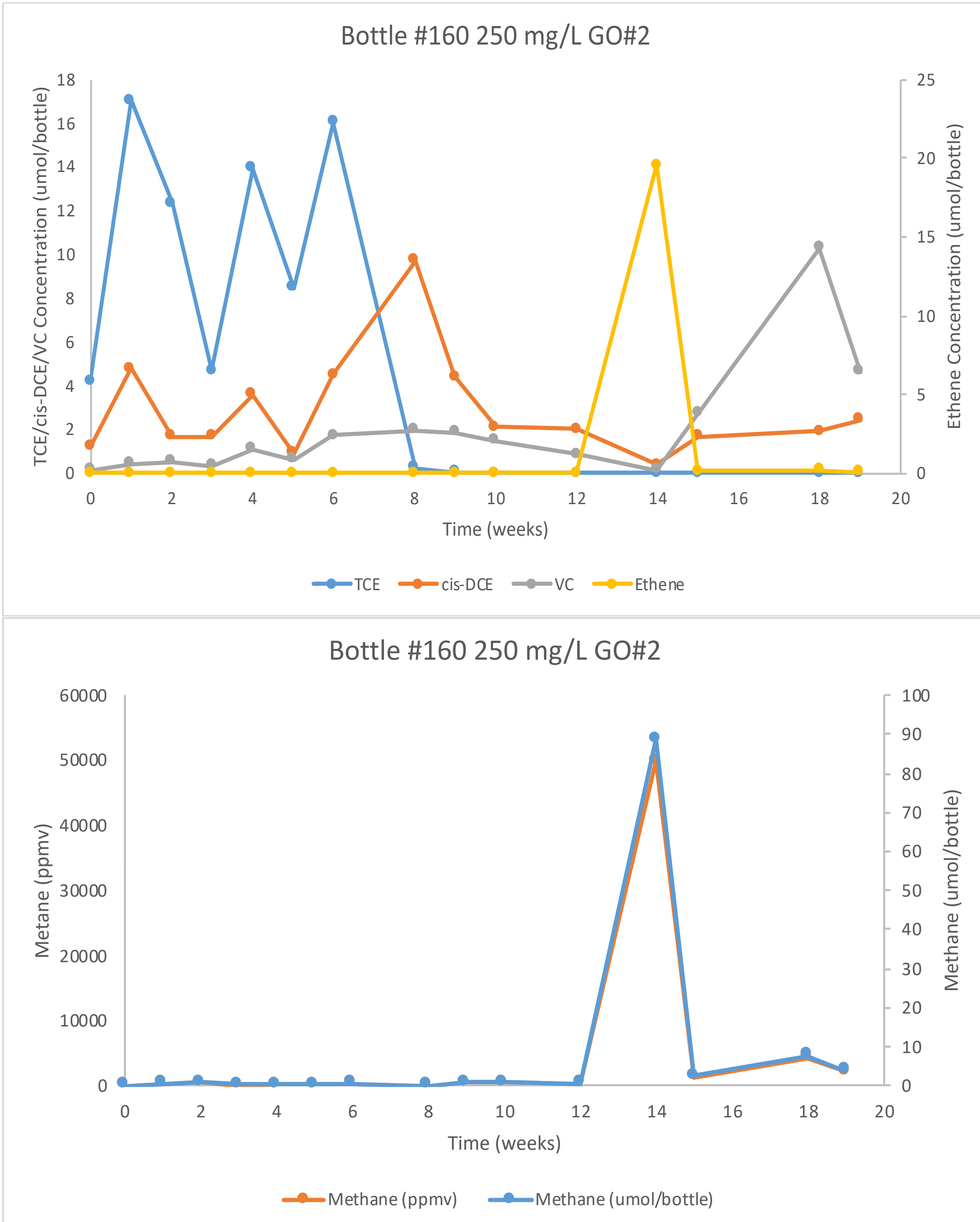


Figure B.97: TCE reduction and methane reduction for aquifer material with 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

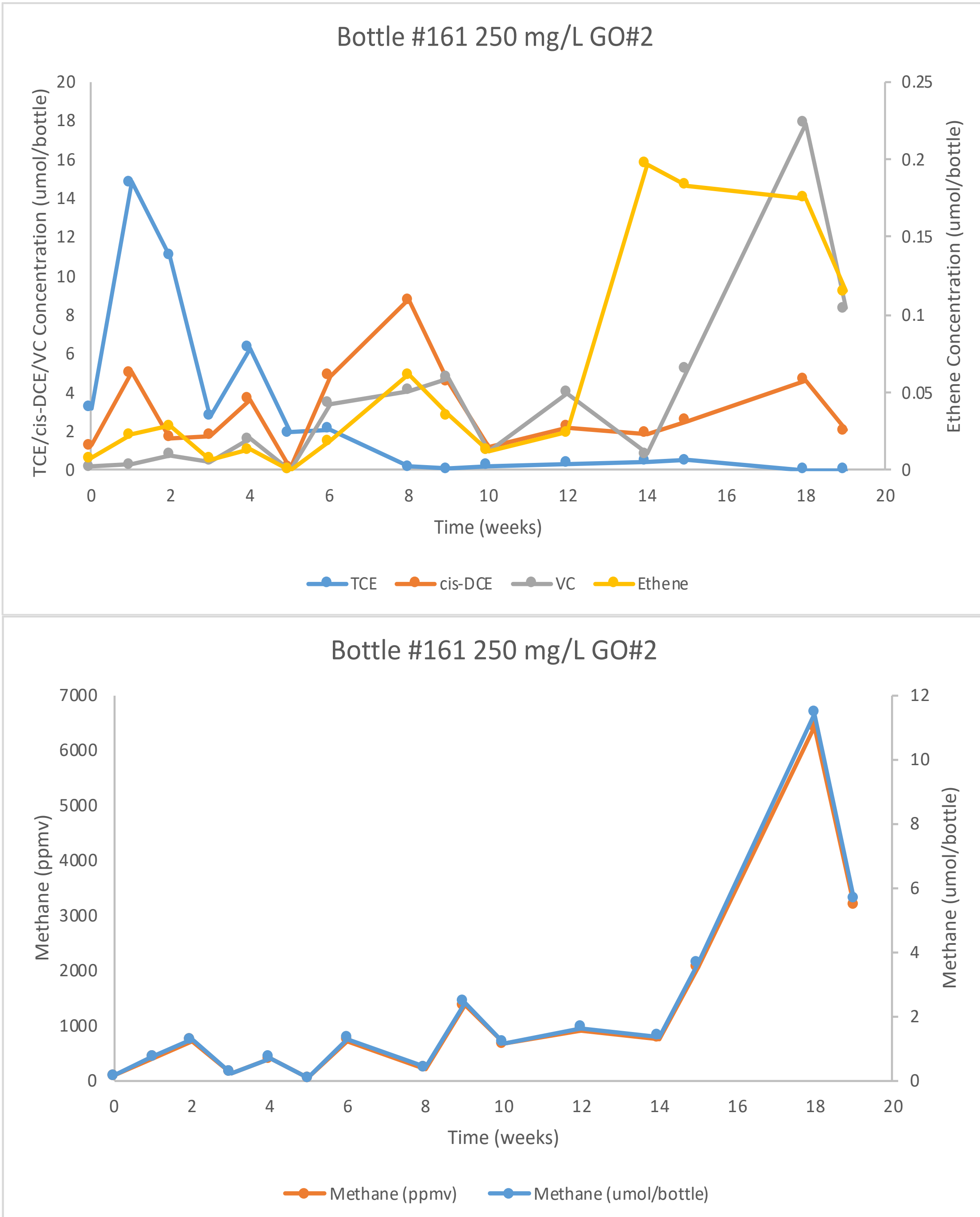


Figure B.98: TCE reduction and methane reduction for aquifer material with 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.

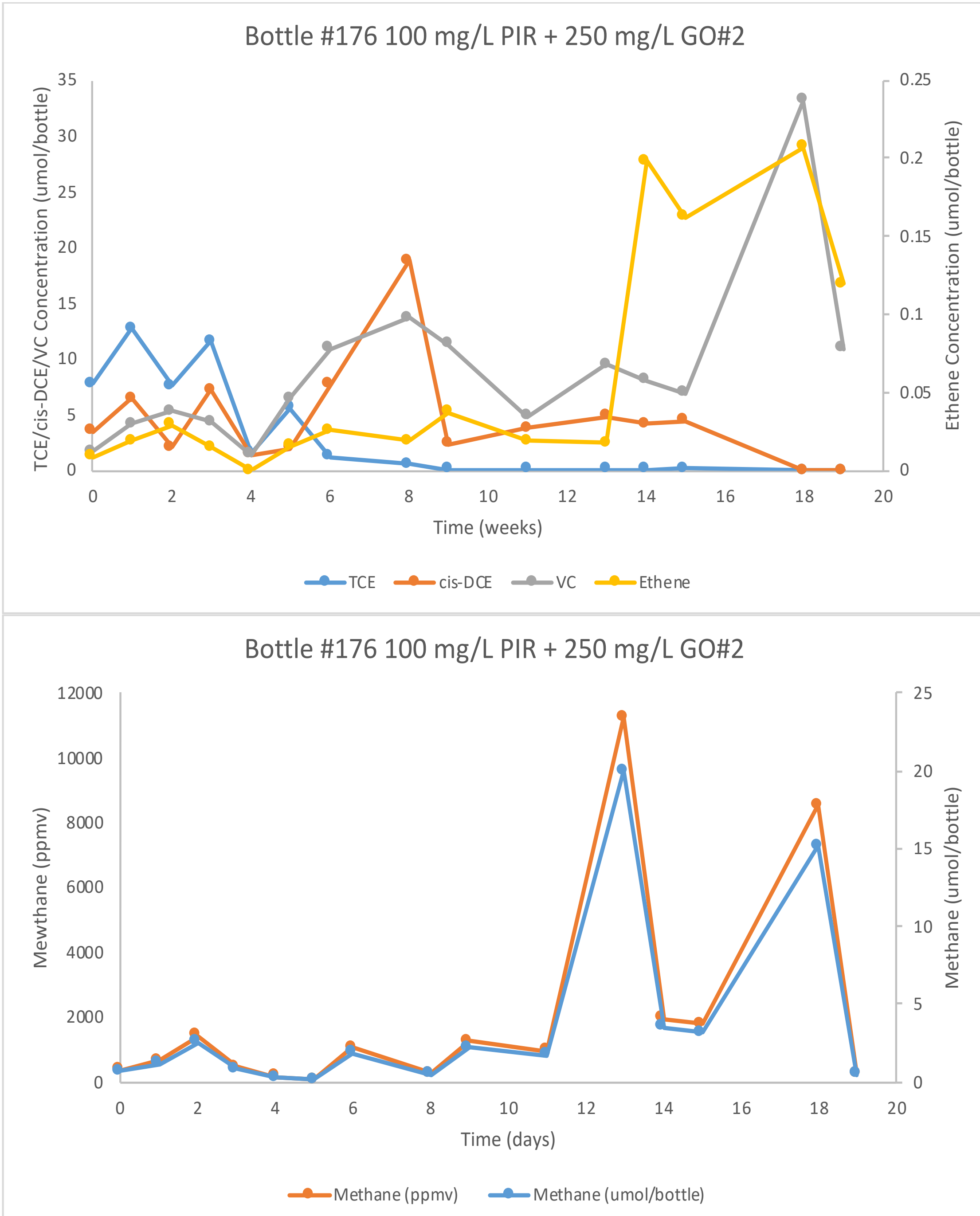
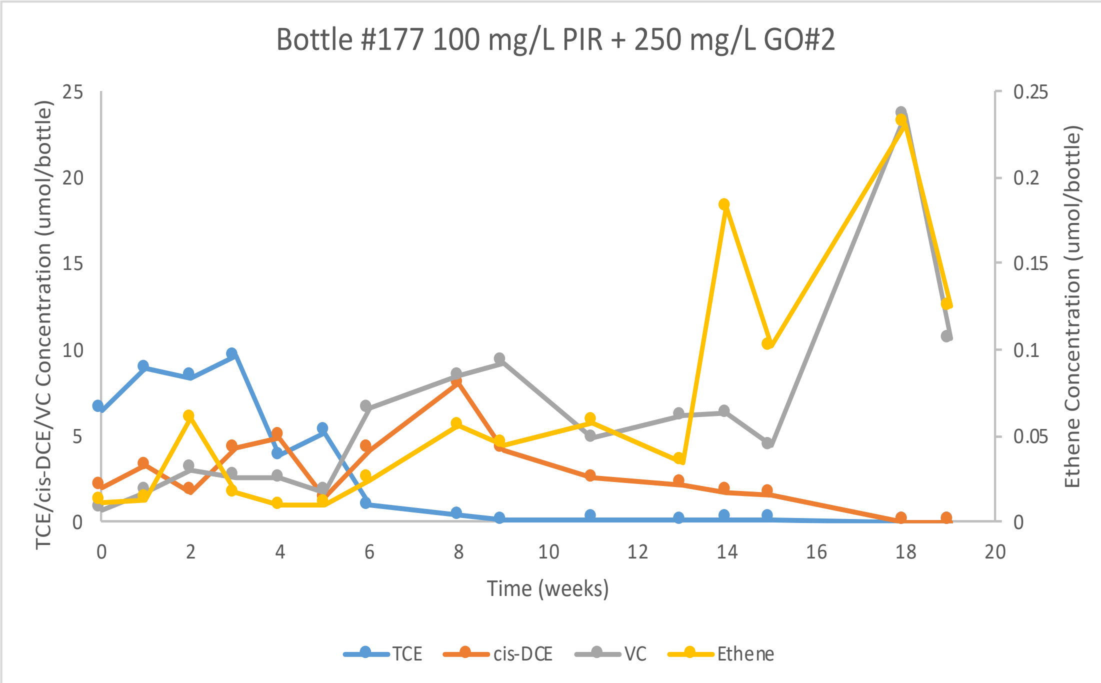


Figure B.99: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.



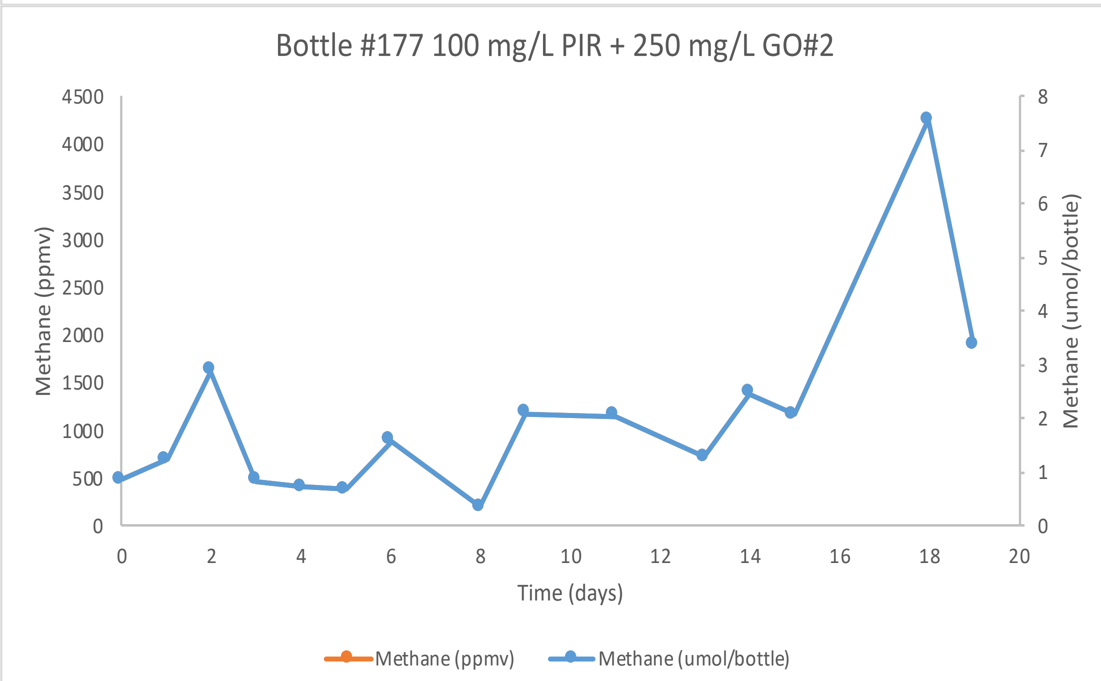
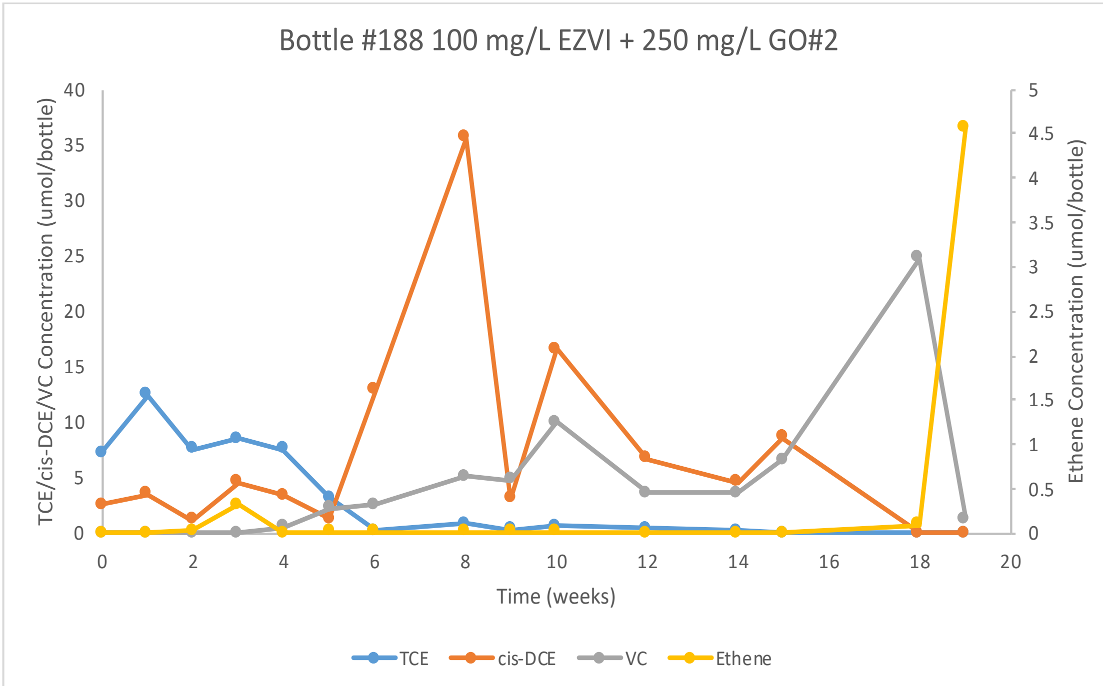


Figure B.100: TCE reduction and methane reduction for aquifer material with 100 mg/L Provect-IR and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.



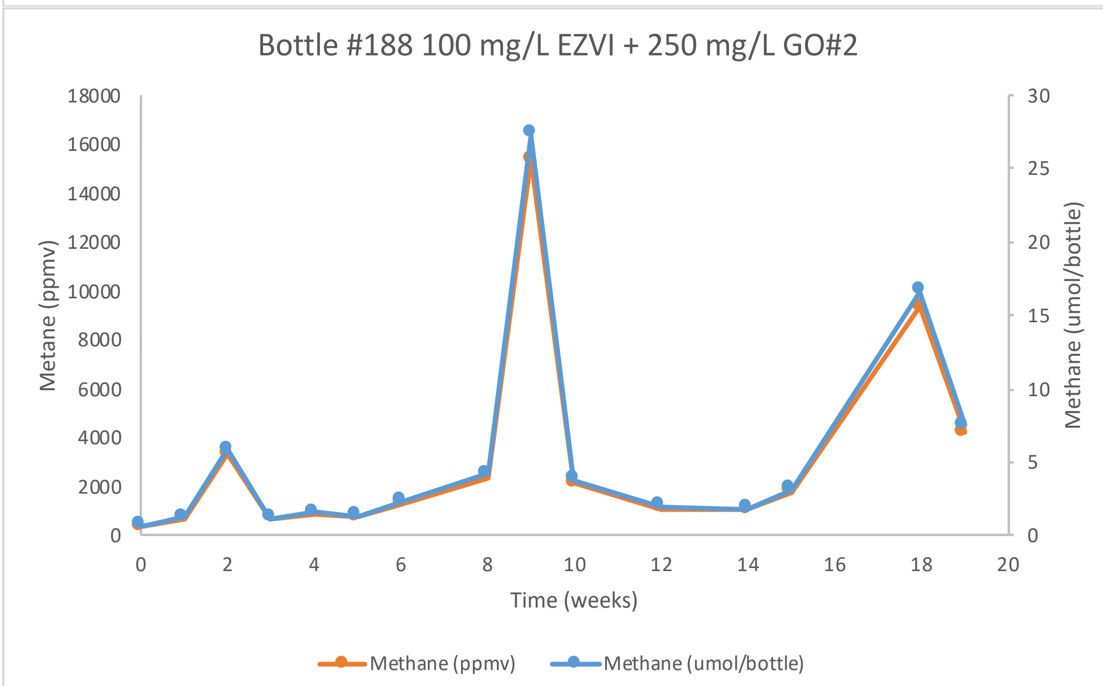


Figure B.101: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

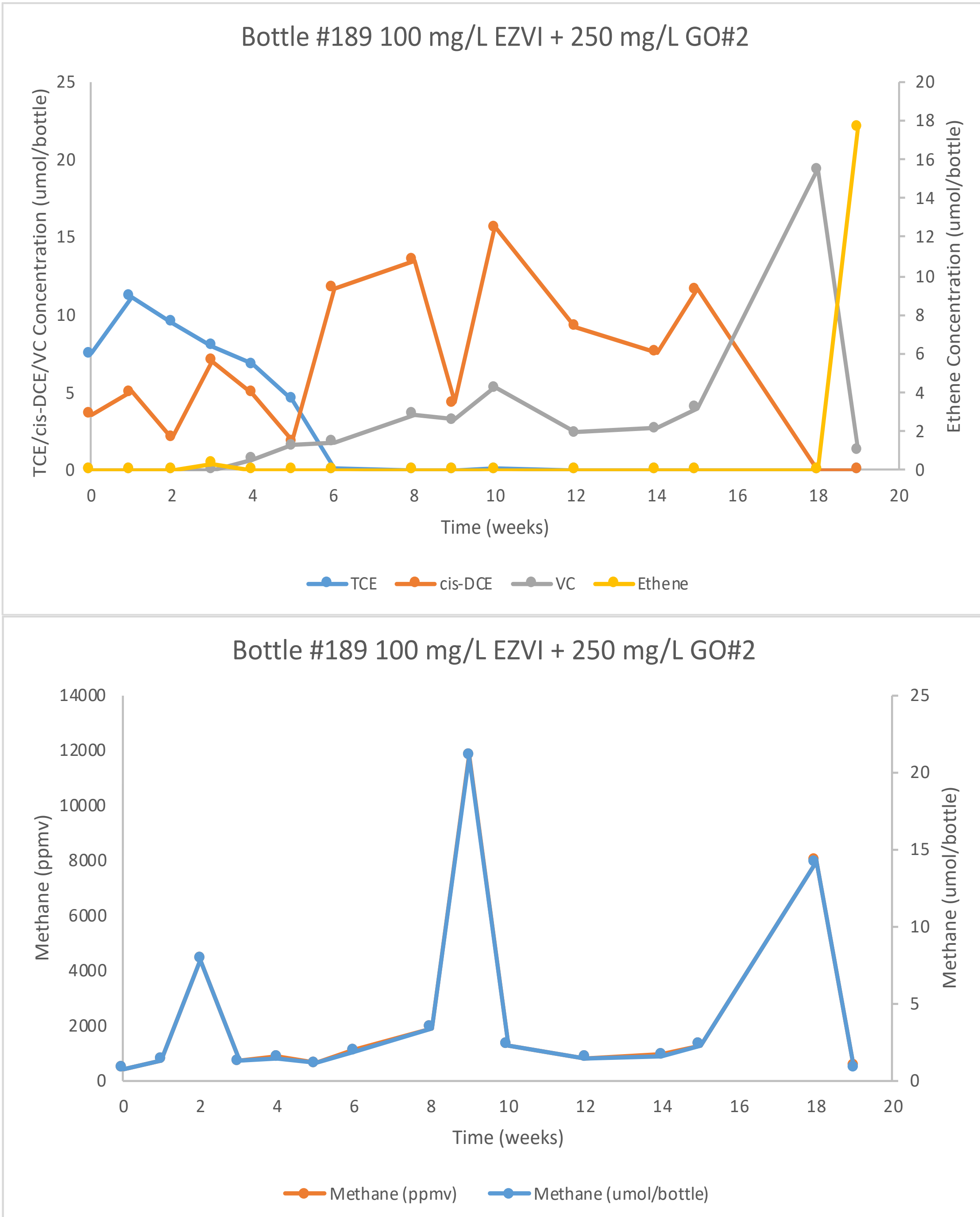


Figure B.102: TCE reduction and methane reduction for aquifer material with 100 mg/L EZVI and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.

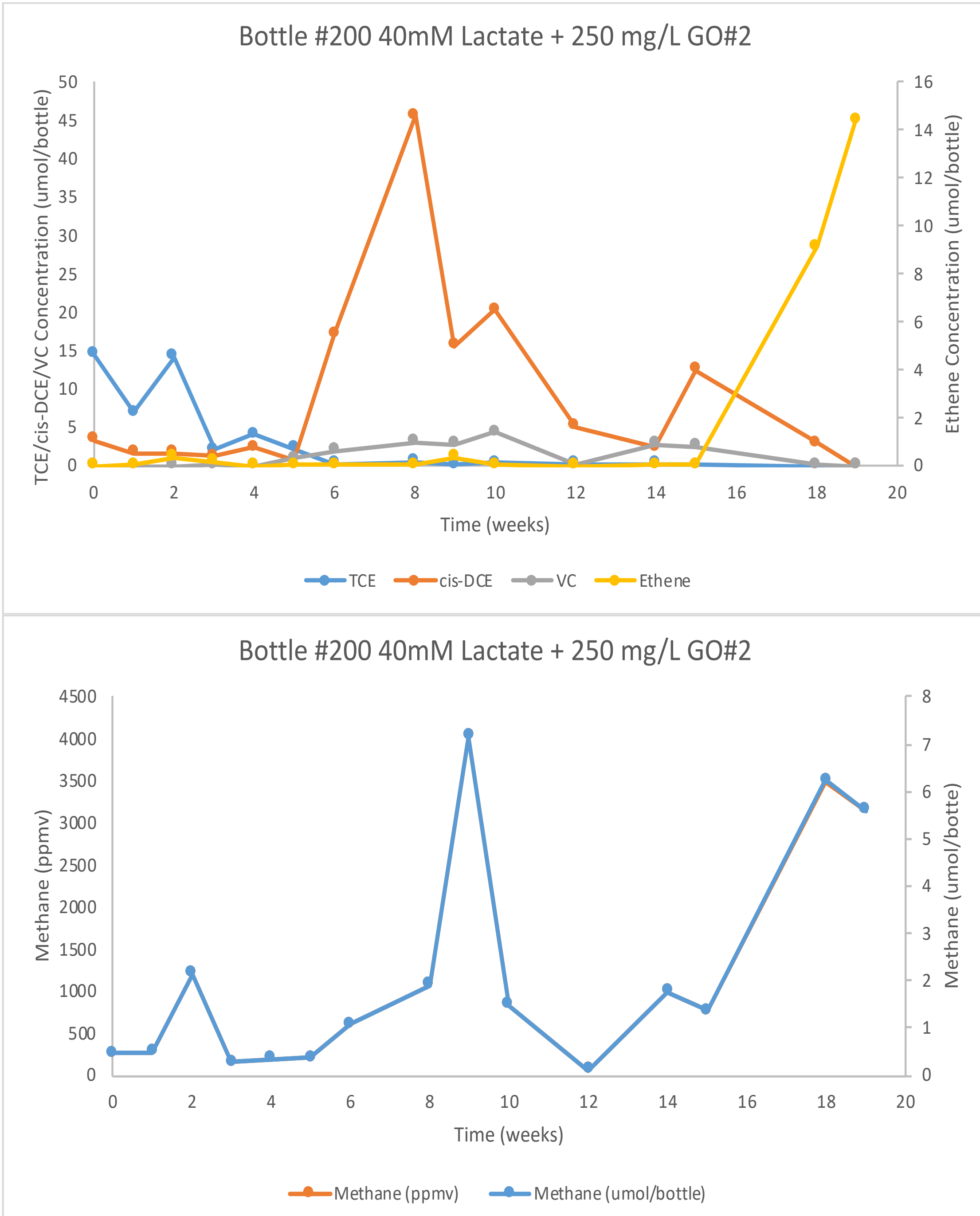
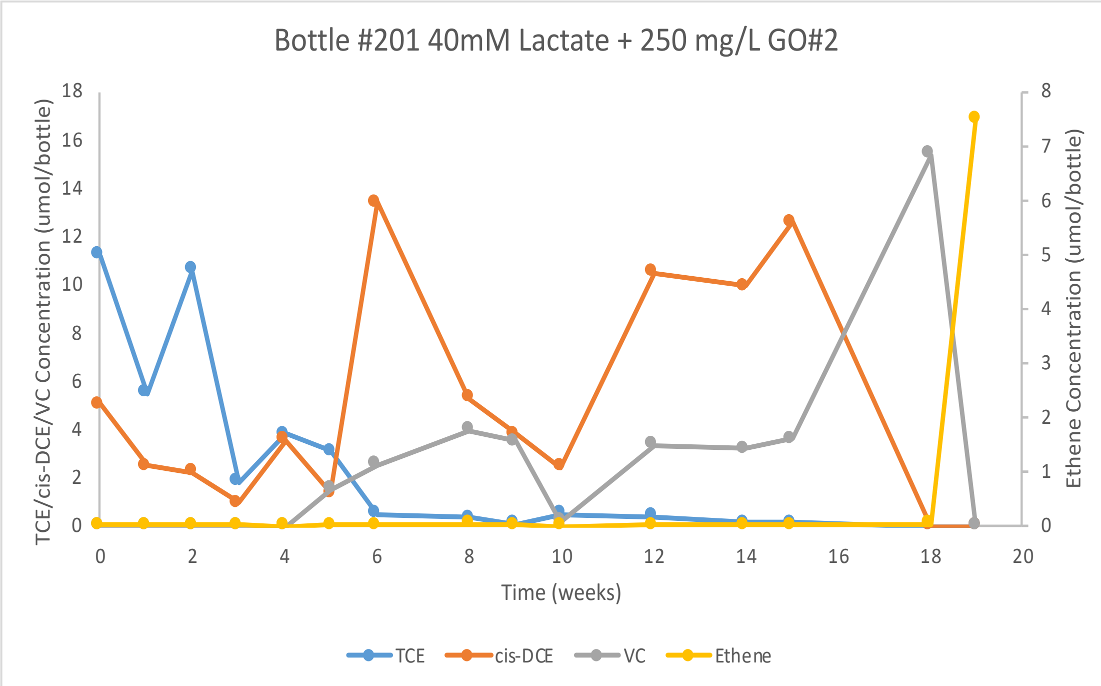


Figure B.103: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.



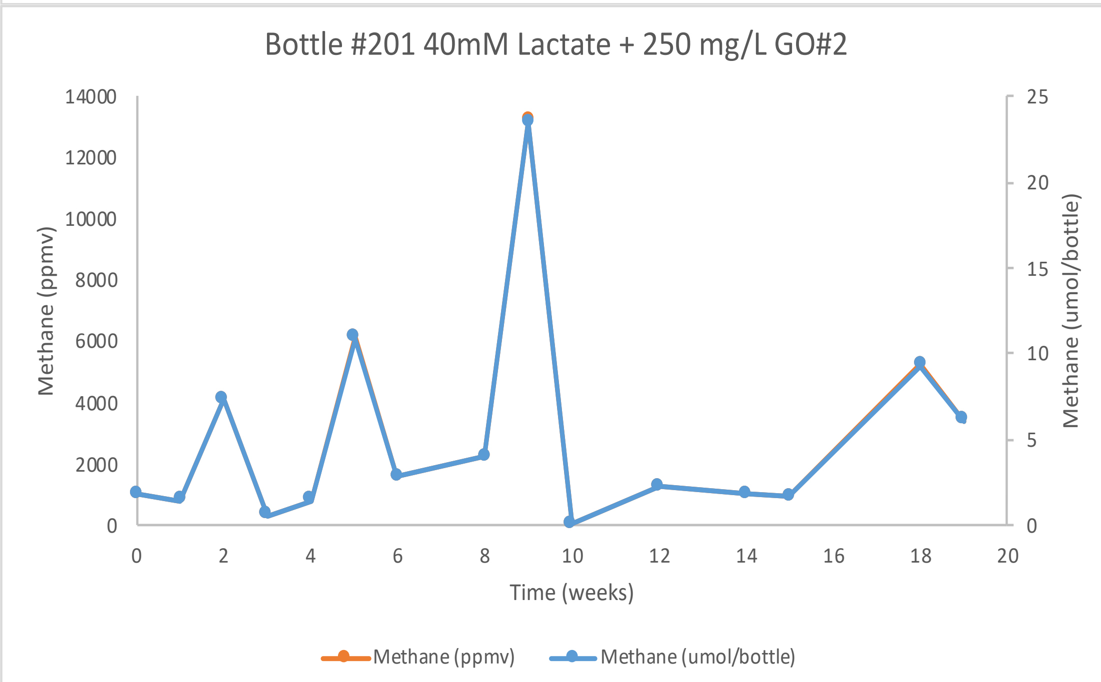
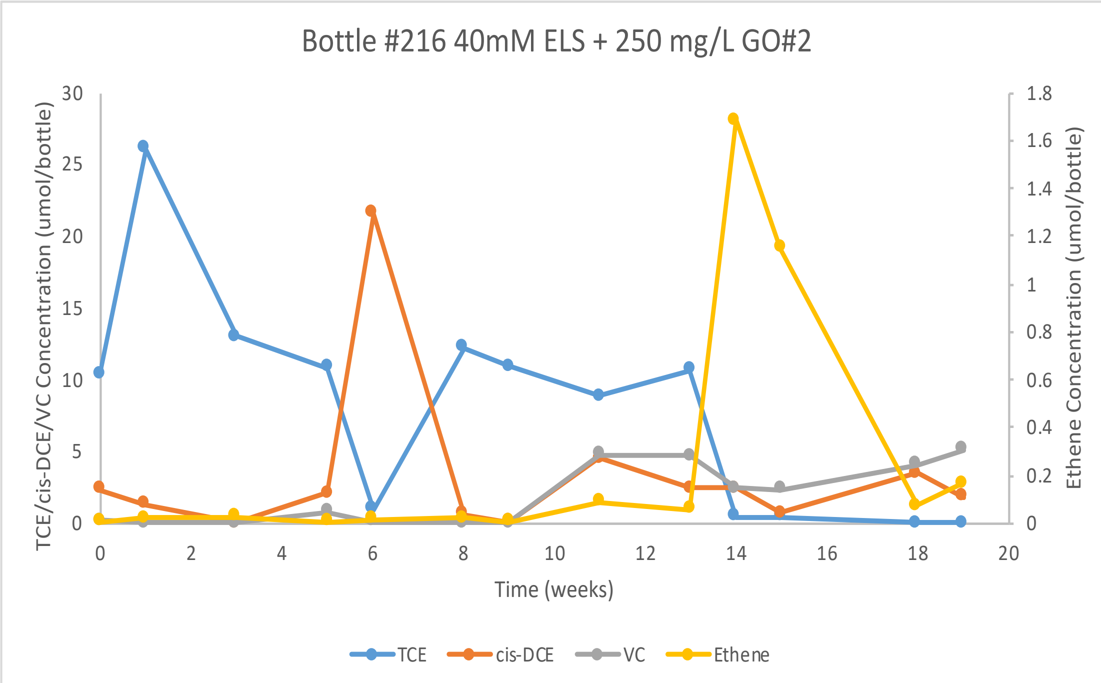


Figure B.104: TCE reduction and methane reduction for aquifer material with 40mM Lactate and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.



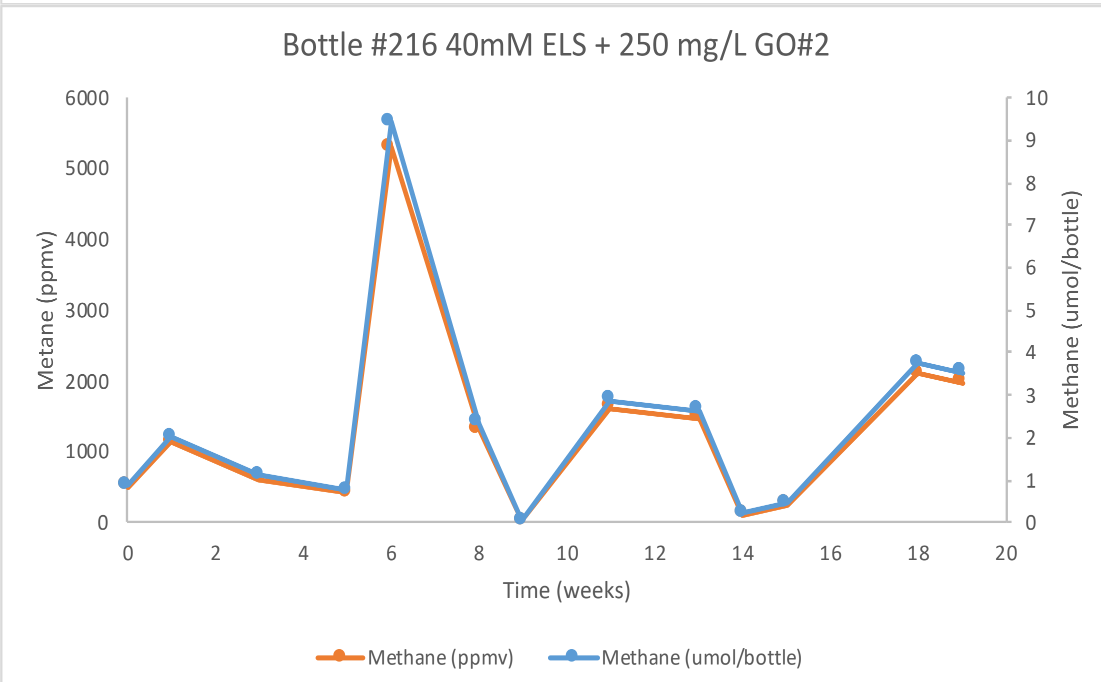


Figure B.105: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

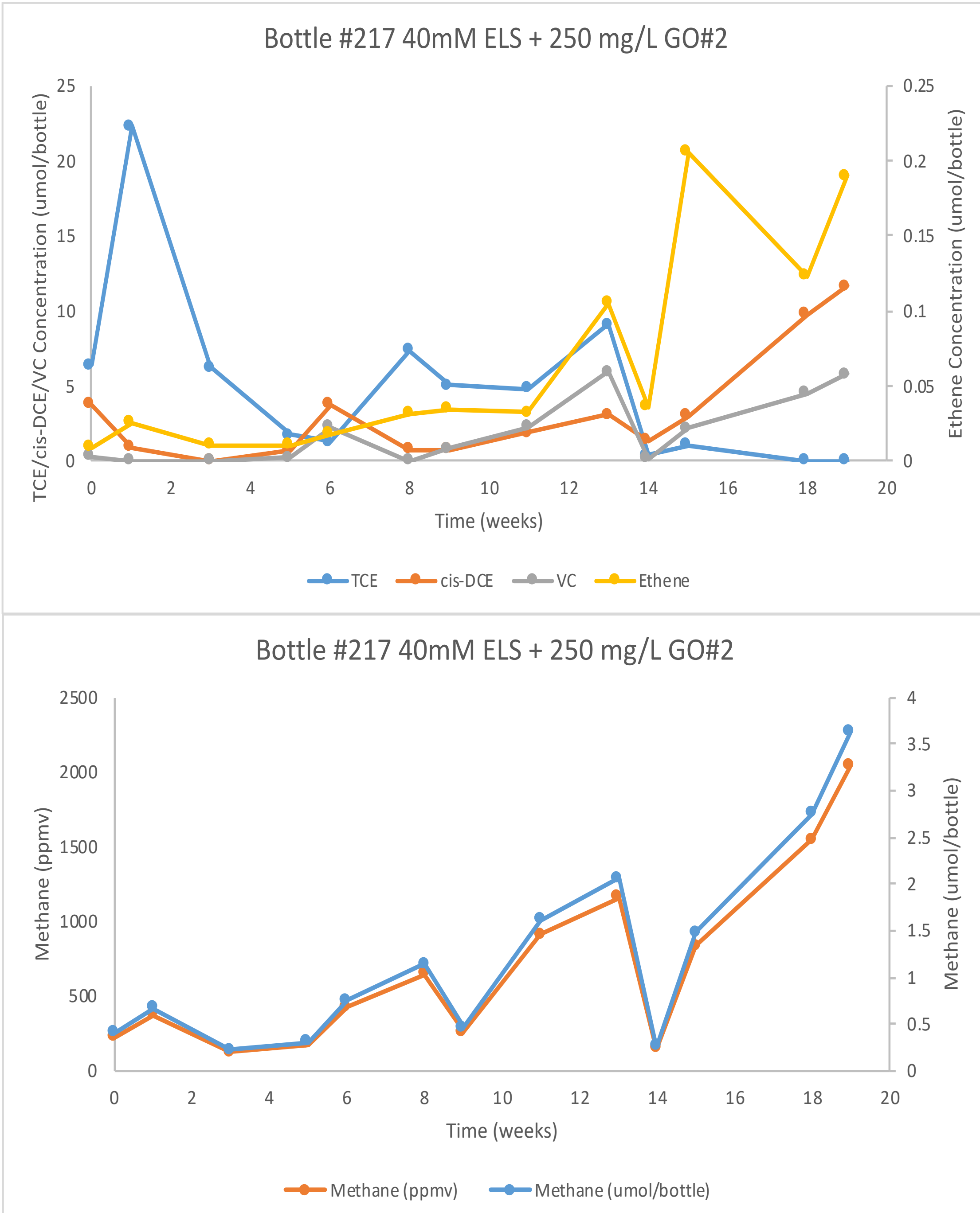


Figure B.106: TCE reduction and methane reduction for aquifer material with 40mM ELS and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.

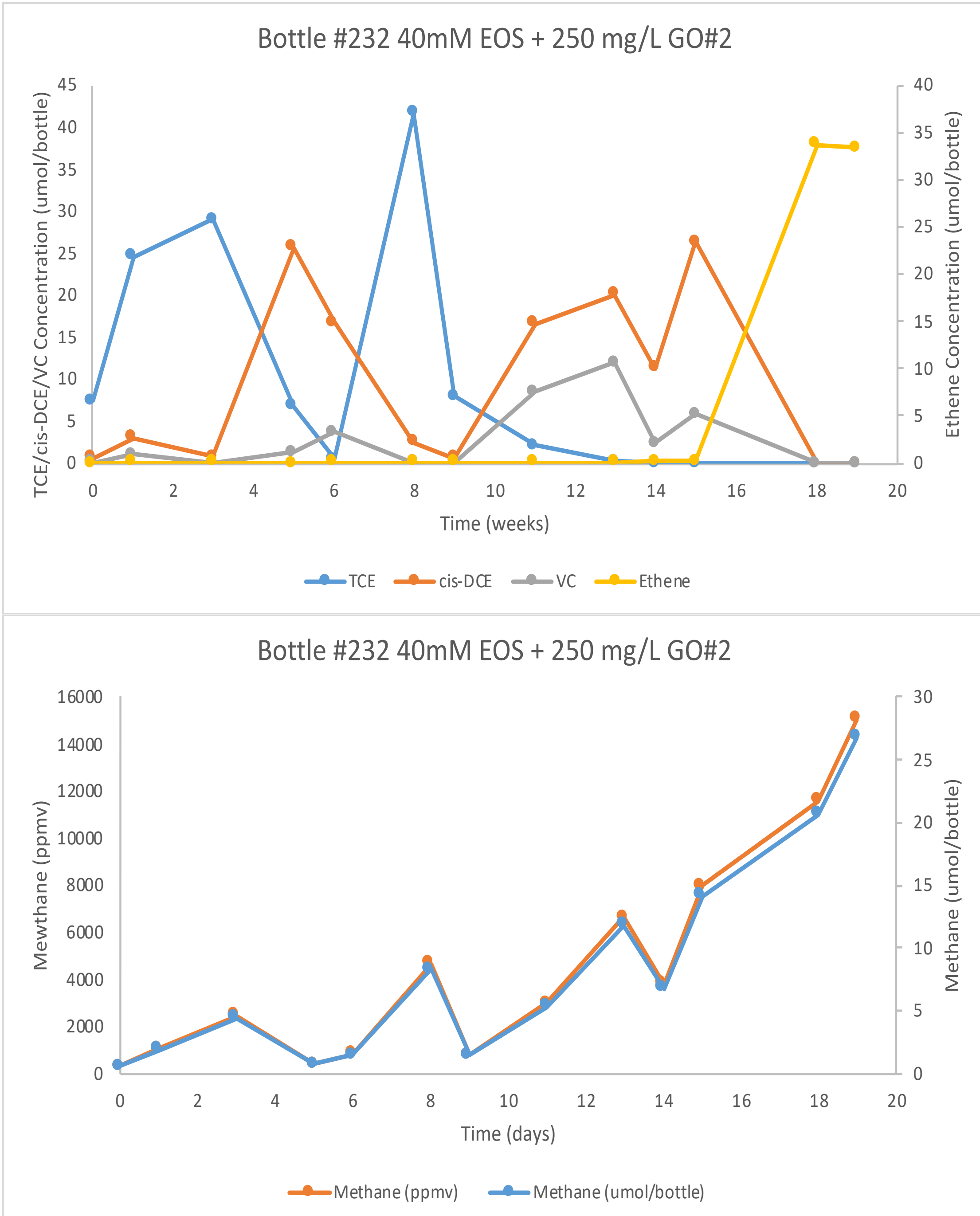


Figure B.107: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

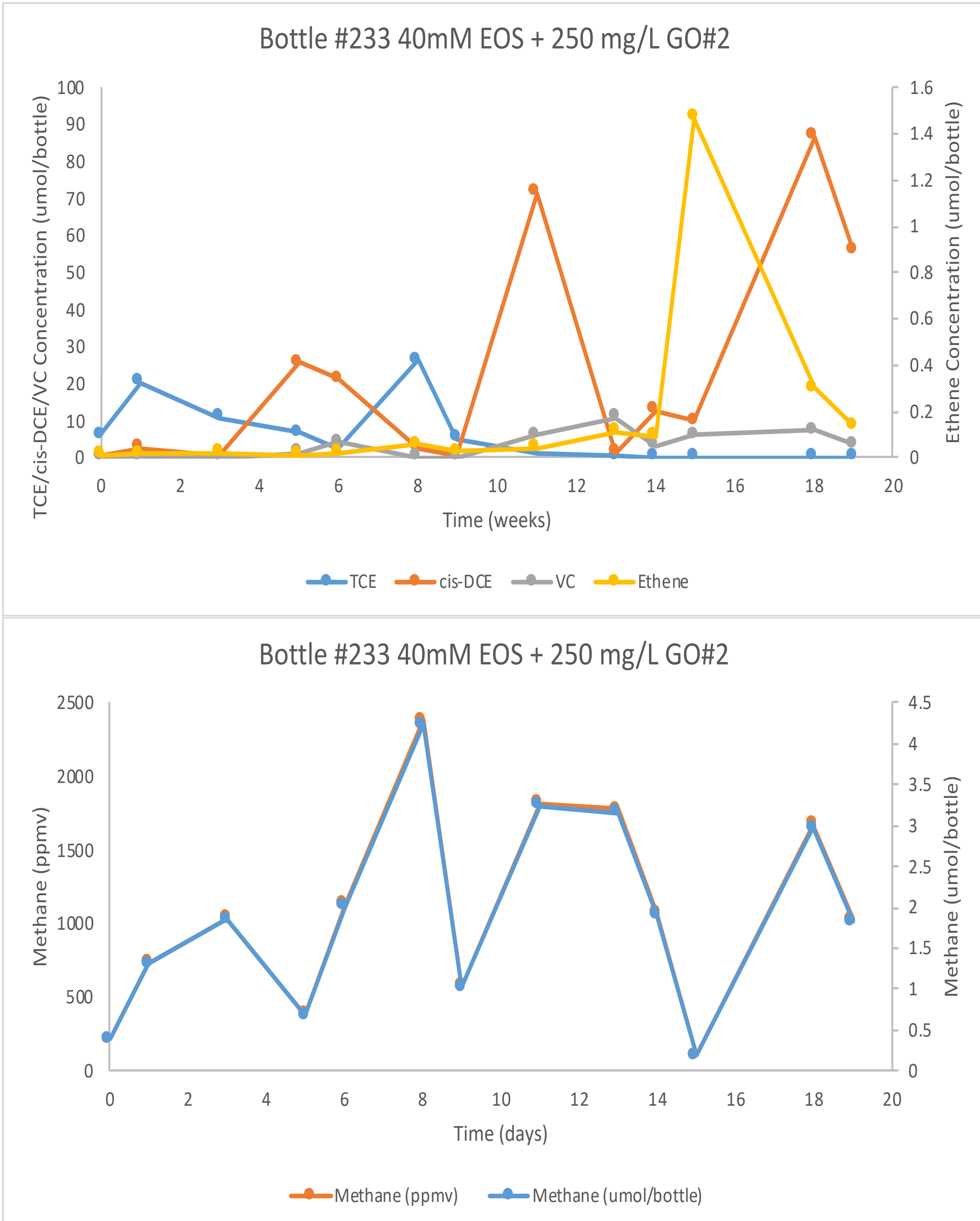
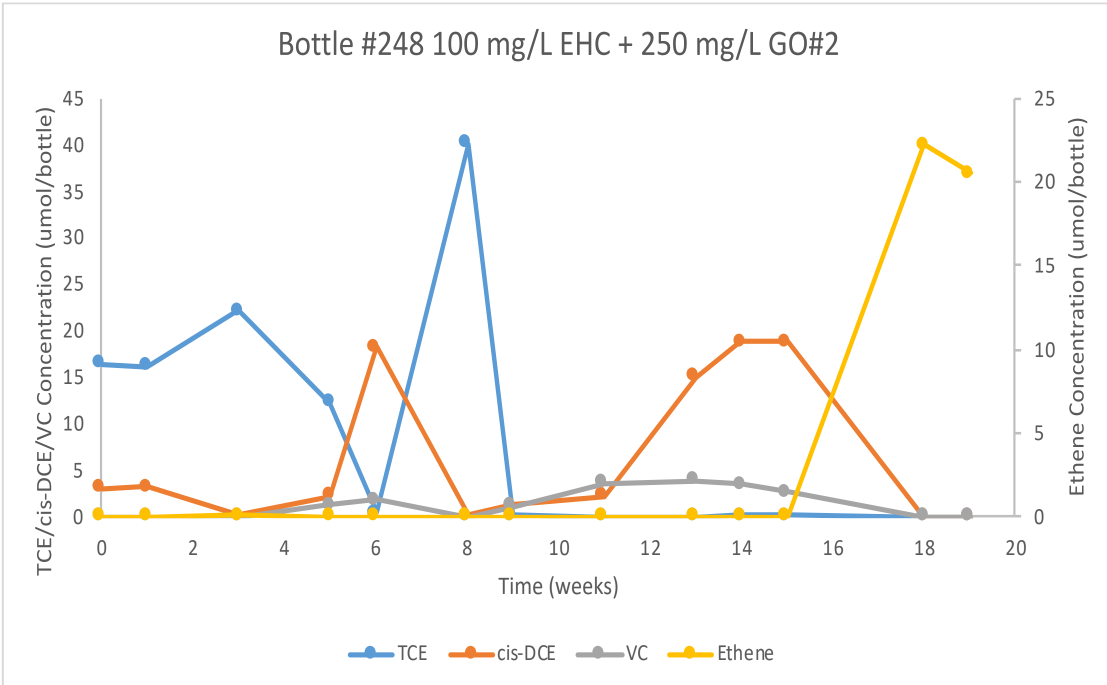


Figure B.108: TCE reduction and methane reduction for aquifer material with 40mM EOS and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.



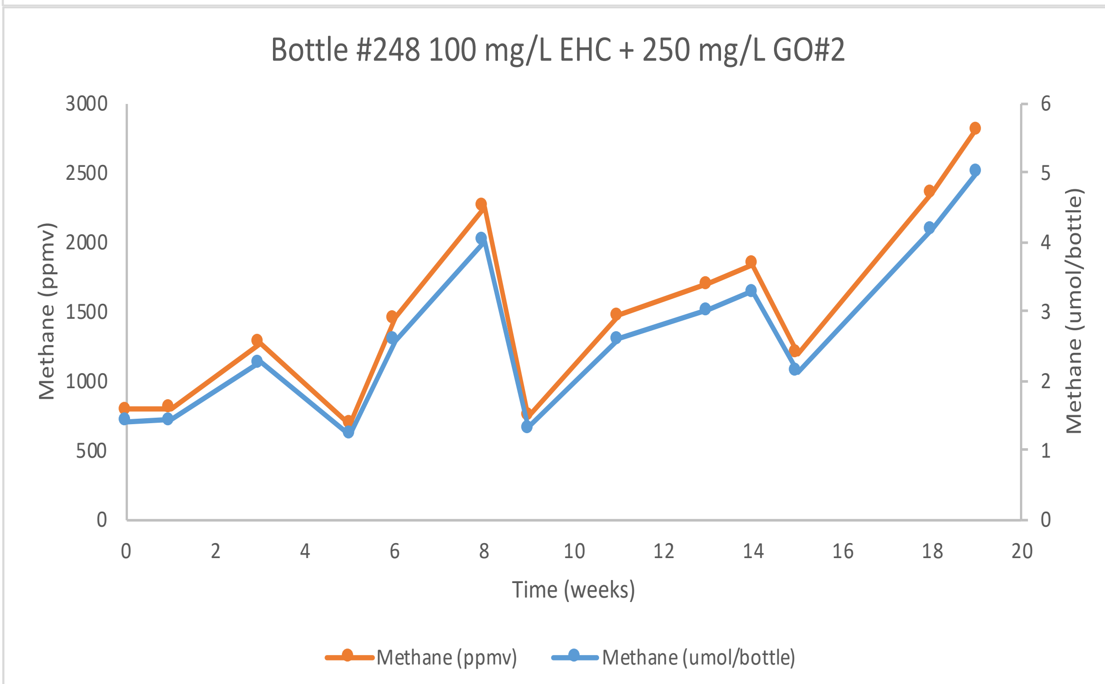


Figure B.109: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

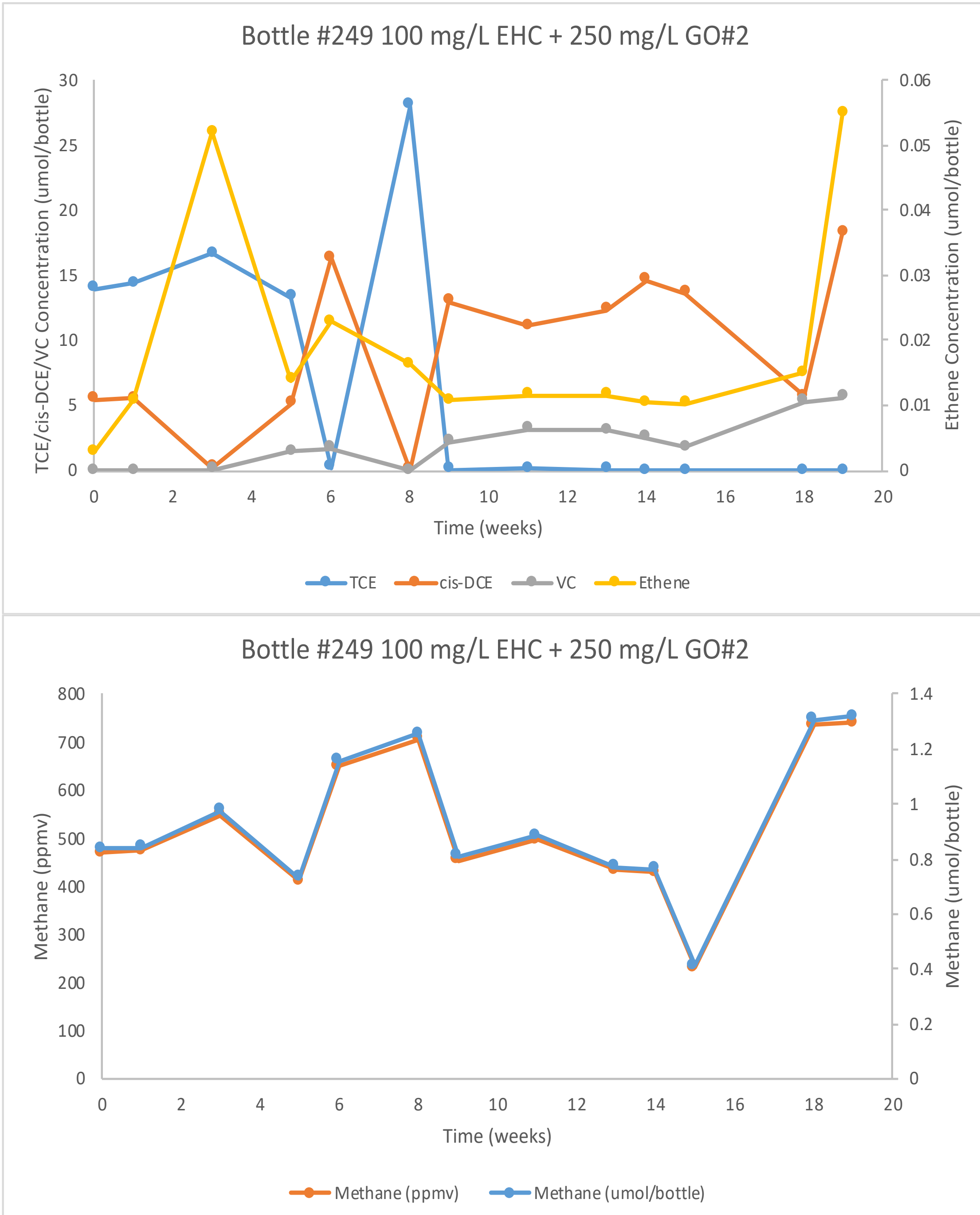


Figure B.110: TCE reduction and methane reduction for aquifer material with 100 mg/L EHC and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.

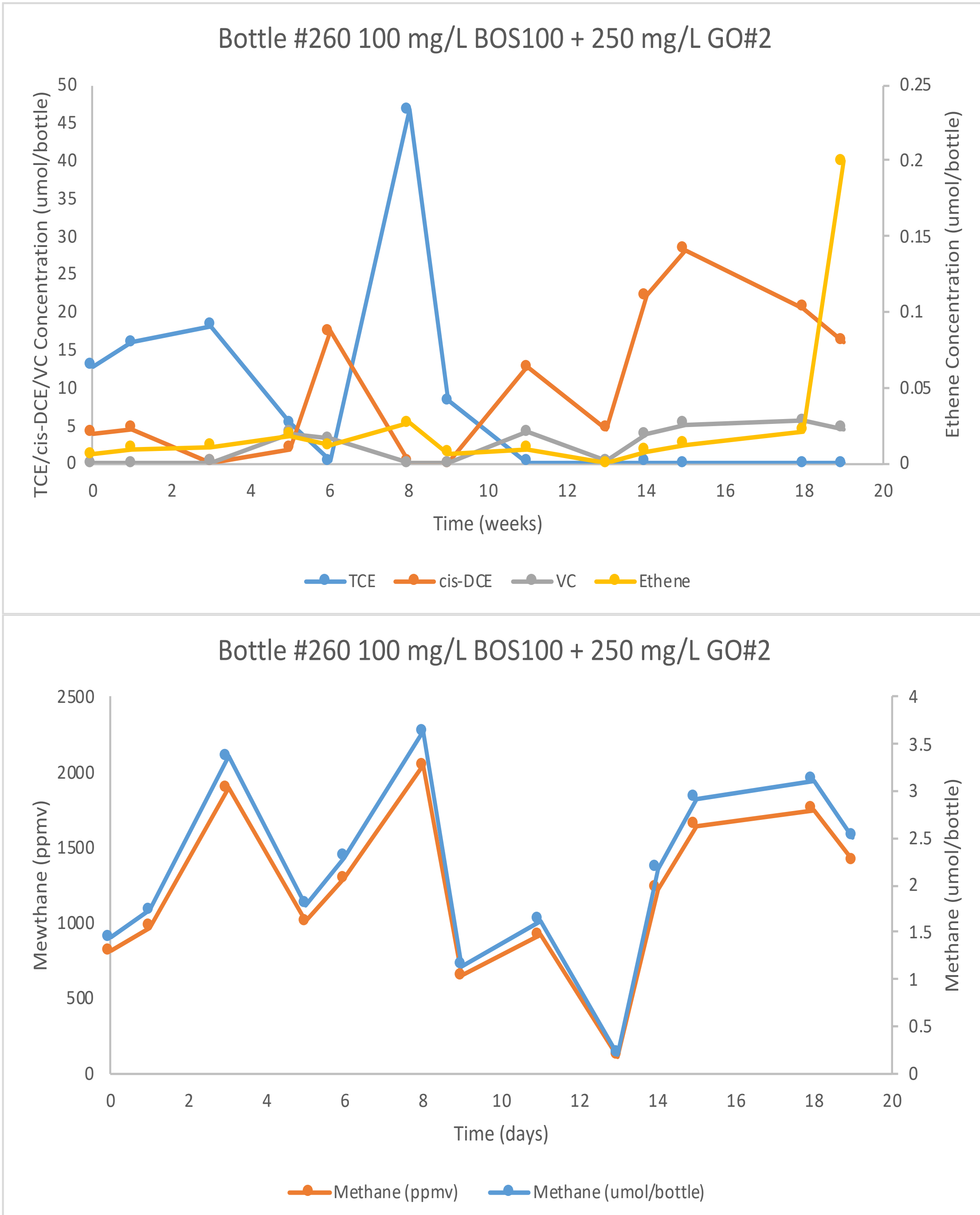


Figure B.111: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Garlic Oil #2. Plot indicates replicate one of two.

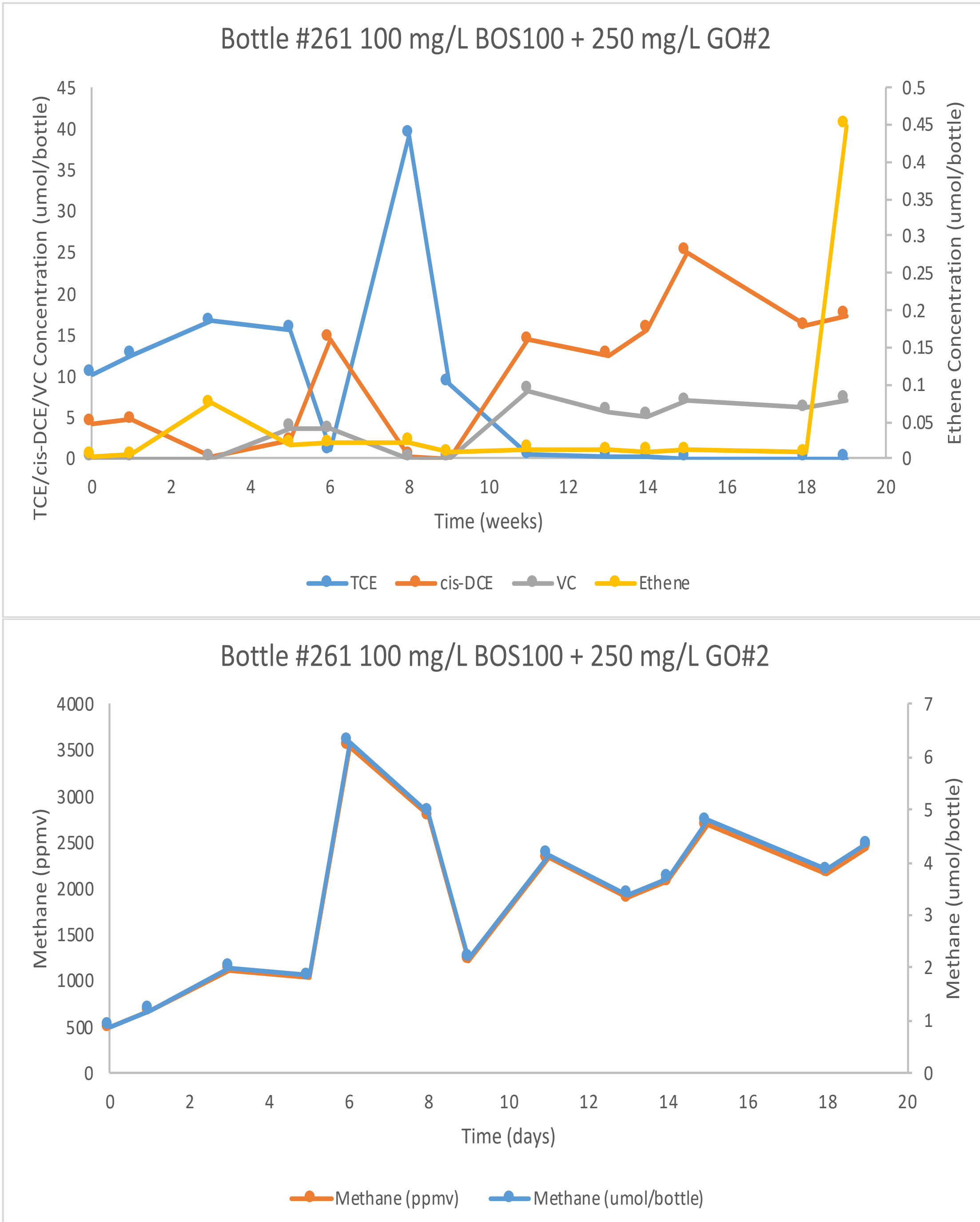


Figure B.112: TCE reduction and methane reduction for aquifer material with 100 mg/L BOS100 and 250 mg/L Garlic Oil #2. Plot indicates replicate two of two.

Appendix C

Percent Statin Analysis Experimental Data

February 21, 2018: 100 mg/L RYR#1 with 10% SLS	
Peak Area	0.0212
Concentration (mg/L)	0.001

Table C.1: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#1 and 10% SLS.

February 27, 2018: 100 mg/L RYR#1 with 0.1% SLS	
Peak Area	Concentration
0.02	0.0065
0.0166	0.0055
0.0125	0.0041
Average Concentration (mg/L)	0.0054

Table C.2: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#1 and 0.1% SLS.

March 6, 2018: 100 mg/L RYR#2 with 1% SLS at pH 4	
Peak Area	Concentration
0.017	0.0051
0.021	0.0063
0.024	0.0072
Average Concentration (mg/L)	0.0062

Table C.3: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#2 and 1% SLS at pH4.

March 14, 2018			
100 mg/L RYR#1 with 0.1% SLS at pH2		**100 mg/L RYR#2 with 0.1% SLS at pH2**	
Peak Area	Concentration	Peak Area	Concentration
0.1026	0.0341	0.0839	0.0278
0.1031	0.0342	0.0776	0.0257
0.1053	0.0349	0.0723	0.0240
Average Concentration (mg/L)	0.0344	Average Concentration (mg/L)	0.0259

Table C.4: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#1 and 0.1% SLS at pH2 and 100 mg/L RYR #2 with 0.1% SLS at pH 2.

May 24, 2018			
100 mg/L RYR#1 with 10% SLS (Sonicated)		**100 mg/L RYR#2 with 10% SLS (Sonicated)**	
Peak Area	Concentration	Peak Area	Concentration
0.001	0.0003	0	0
0.000	0.000	0	0
0.14	0.04647	0	0
Average Concentration (mg/L)	0.0155	Average Concentration (mg/L)	0

Table C.5: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#1 and 10% SLS and 100 mg/L RYR #2 with 10% SLS. Both samples were sonicated for 10 minutes prior to filtration and analysis.

May 24, 2018			
100 mg/L RYR#1 with 10% SLS at pH 2		**100 mg/L RYR#2 with 10% SLS at pH 2 (Sonicated)**	
Peak Area	Concentration	Peak Area	Concentration
0.022	0.0073	0	0
0.098	0.0325	0	0
0.261	0.0866	0.099	0.0328
Average Concentration (mg/L)	0.4321	Average Concentration (mg/L)	0.0109

Table C.6: Red Yeast Rice Statin Content analysis with 100 mg/L RYR#1 and 10% SLS at pH 2 and 100 mg/L RYR #2 with 10% SLS at pH 2. The samples on the right were sonicated for 10 minutes prior to filtration and analysis.

Appendix D

pH Adjustments Experimental Data

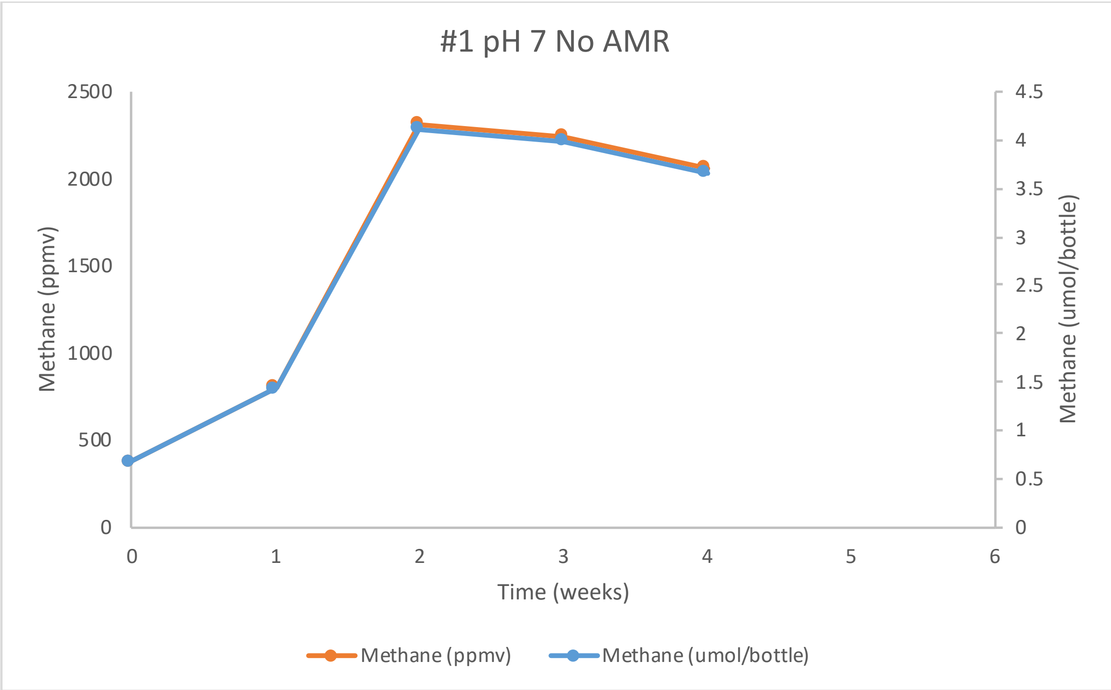


Figure D.1: Methane reduction for methane culture from aquifer material with no amendments at pH 7. Plot indicates replicate one of three.

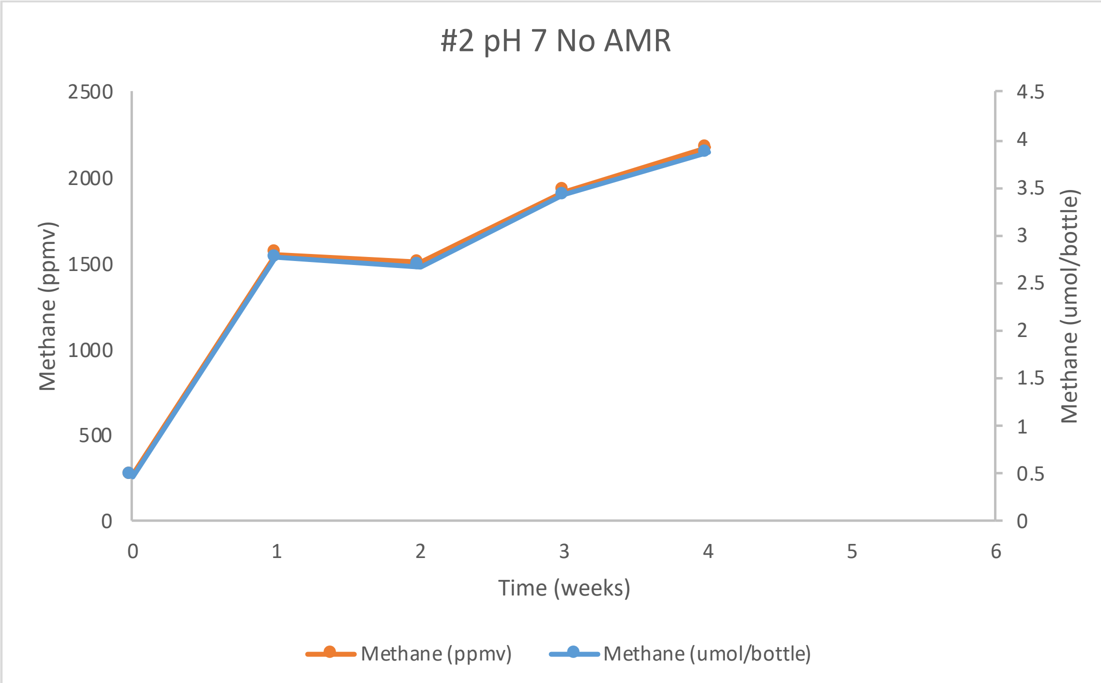


Figure D.2: Methane reduction for methane culture from aquifer material with no amendments at pH 7. Plot indicates replicate two of three.

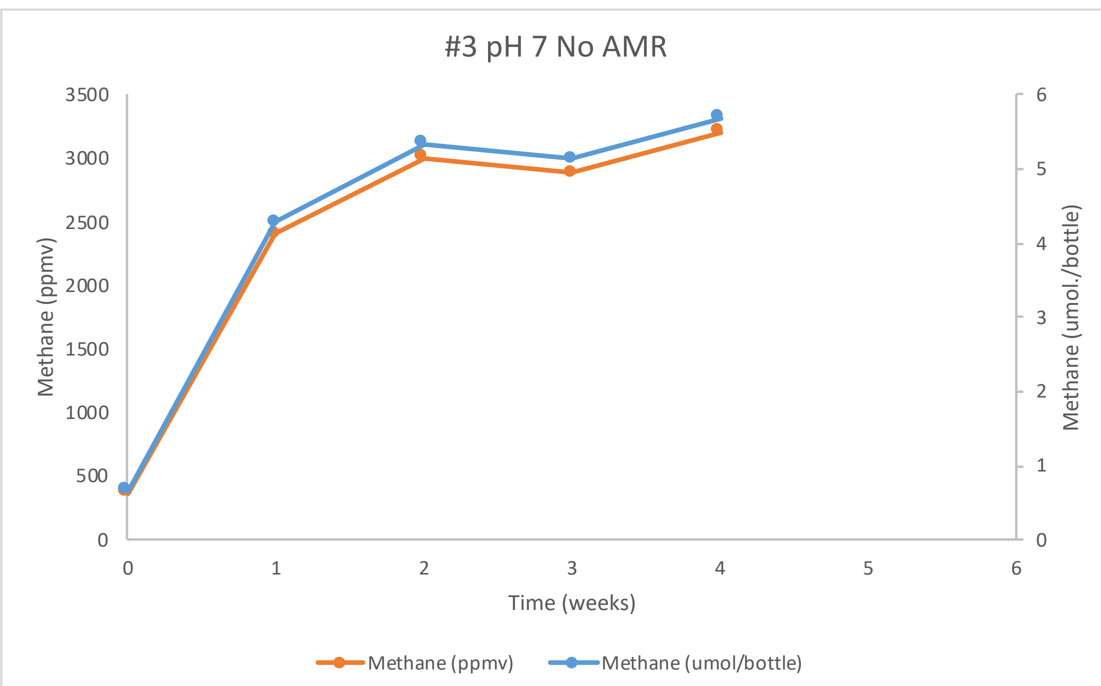


Figure D.3: Methane reduction for methane culture from aquifer material with no amendments at pH 7. Plot indicates replicate three of three.

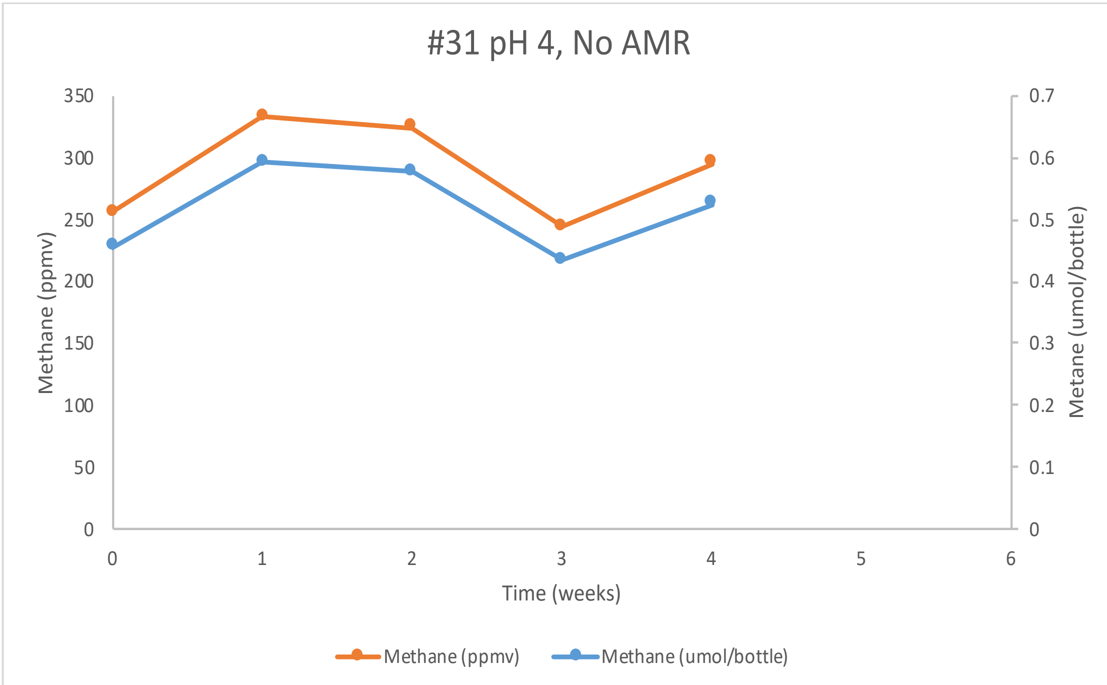


Figure D.4: Methane reduction for methane culture from aquifer material with no amendments at pH 4. Plot indicates replicate one of three.

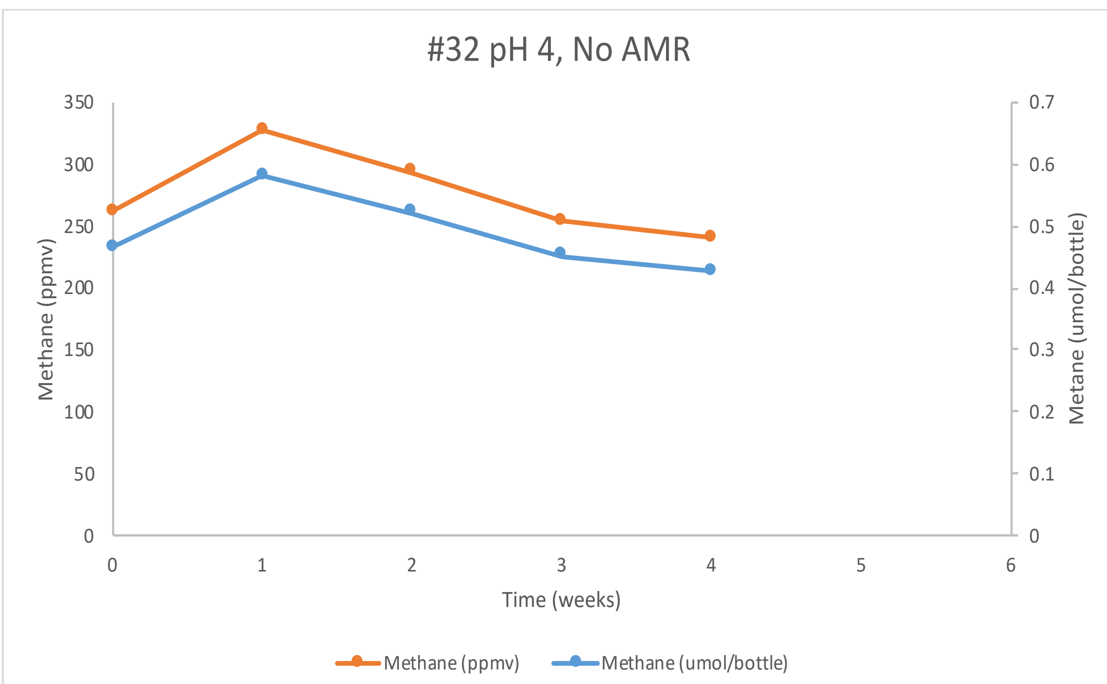


Figure D.5: Methane reduction for methane culture from aquifer material with no amendments at pH 4. Plot indicates replicate two of three.

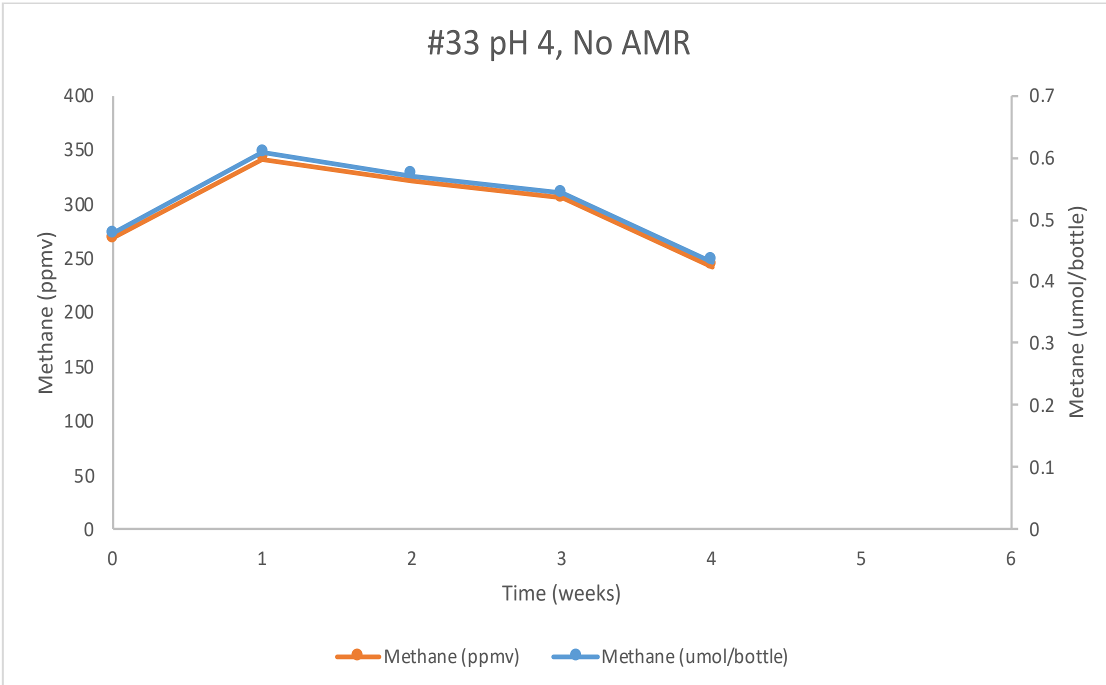


Figure D.6: Methane reduction for methane culture from aquifer material with no amendments at pH 4. Plot indicates replicate three of three.

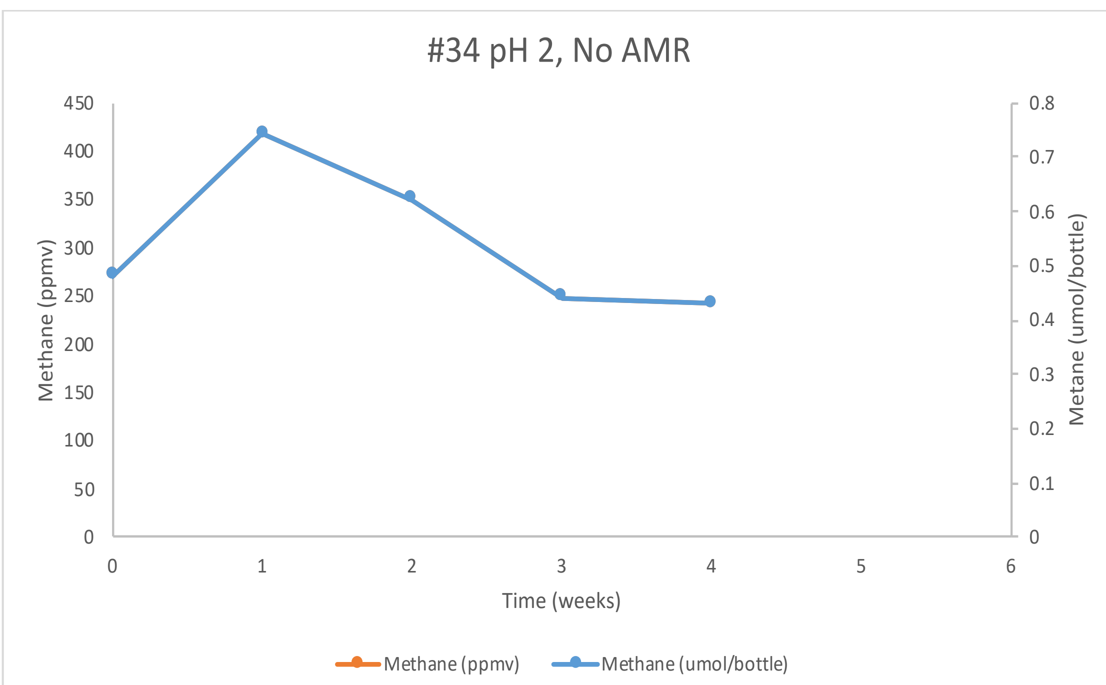


Figure D.7: Methane reduction for methane culture from aquifer material with no amendments at pH 2. Plot indicates replicate one of three.

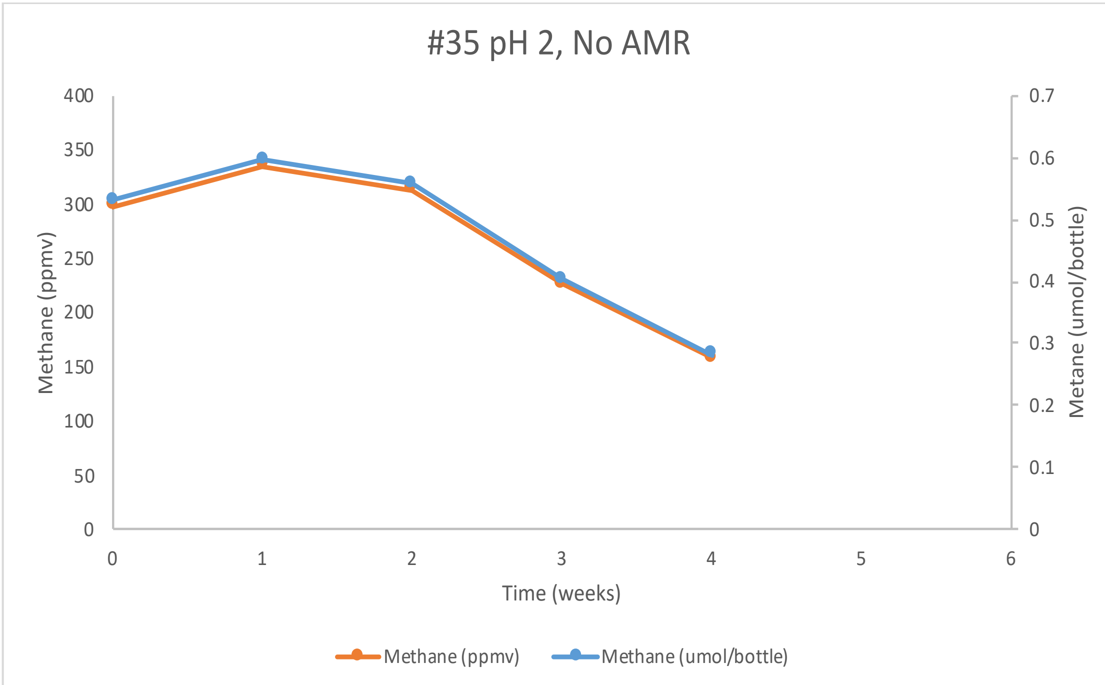


Figure D.8: Methane reduction for methane culture from aquifer material with no amendments at pH 2. Plot indicates replicate two of three.

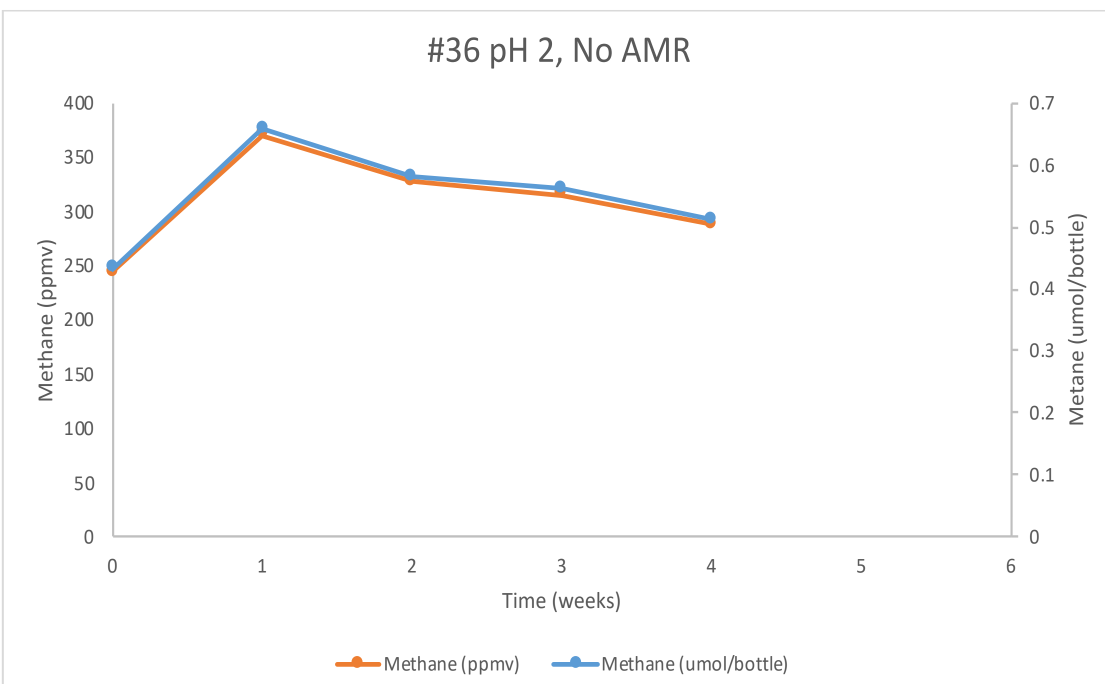


Figure D.9: Methane reduction for methane culture from aquifer material with no amendments at pH 2. Plot indicates replicate three of three.

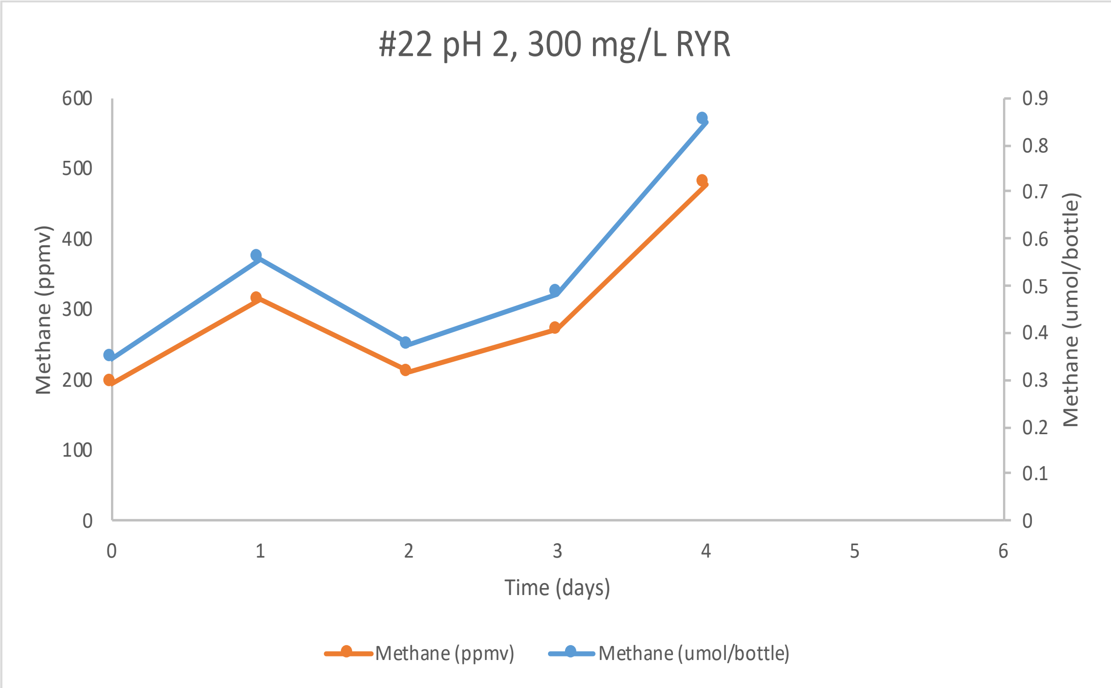


Figure D.10: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 2. Plot indicates replicate one of three.

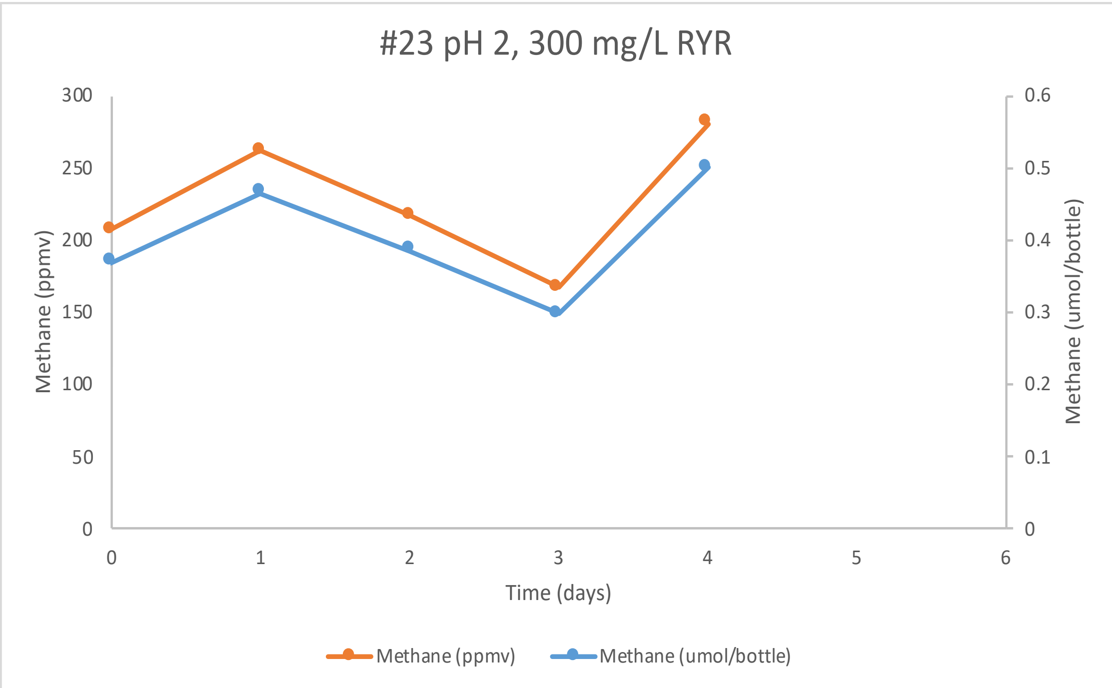


Figure D.11: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 2. Plot indicates replicate two of three.

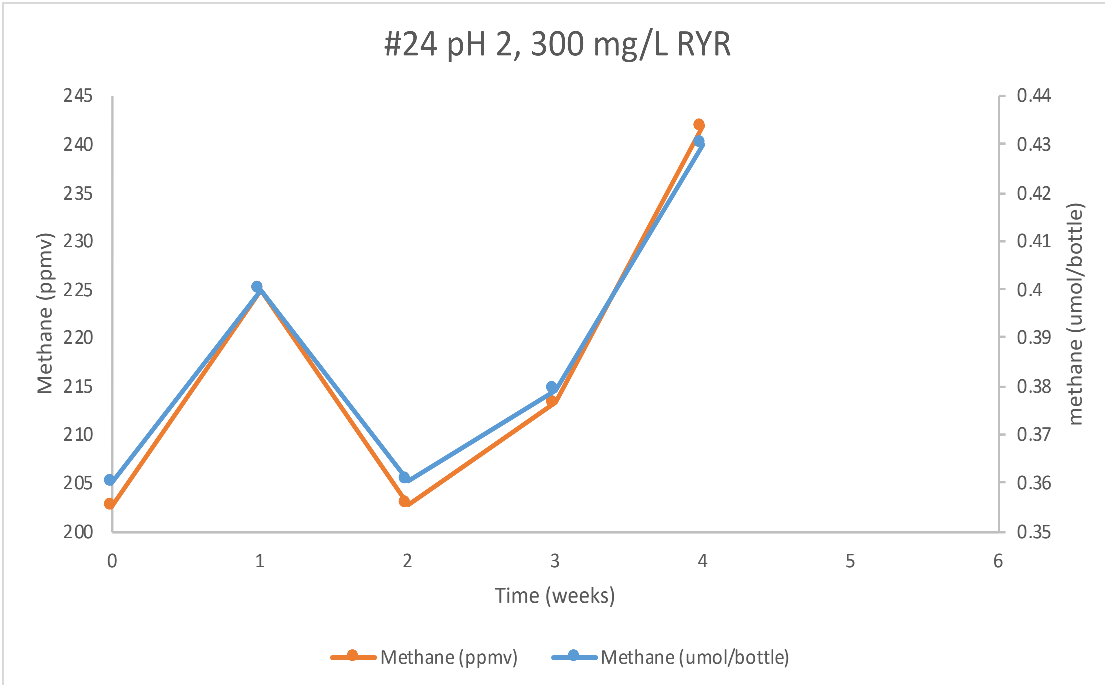


Figure D.12: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 2. Plot indicates replicate three of three.

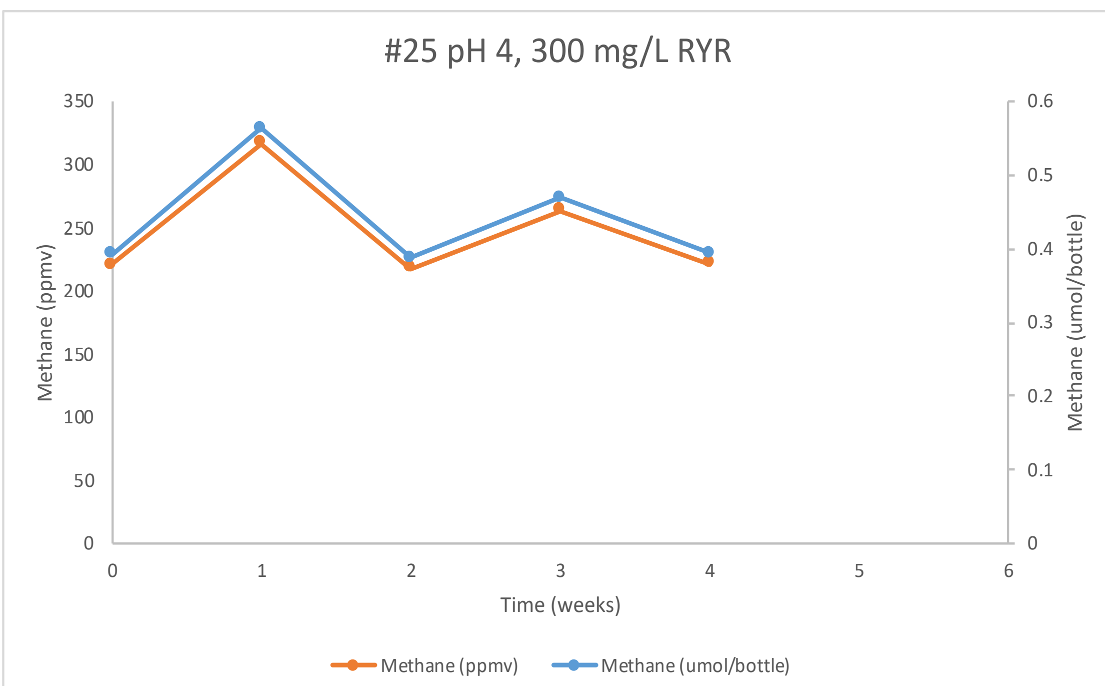


Figure D.13: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 4. Plot indicates replicate one of three.

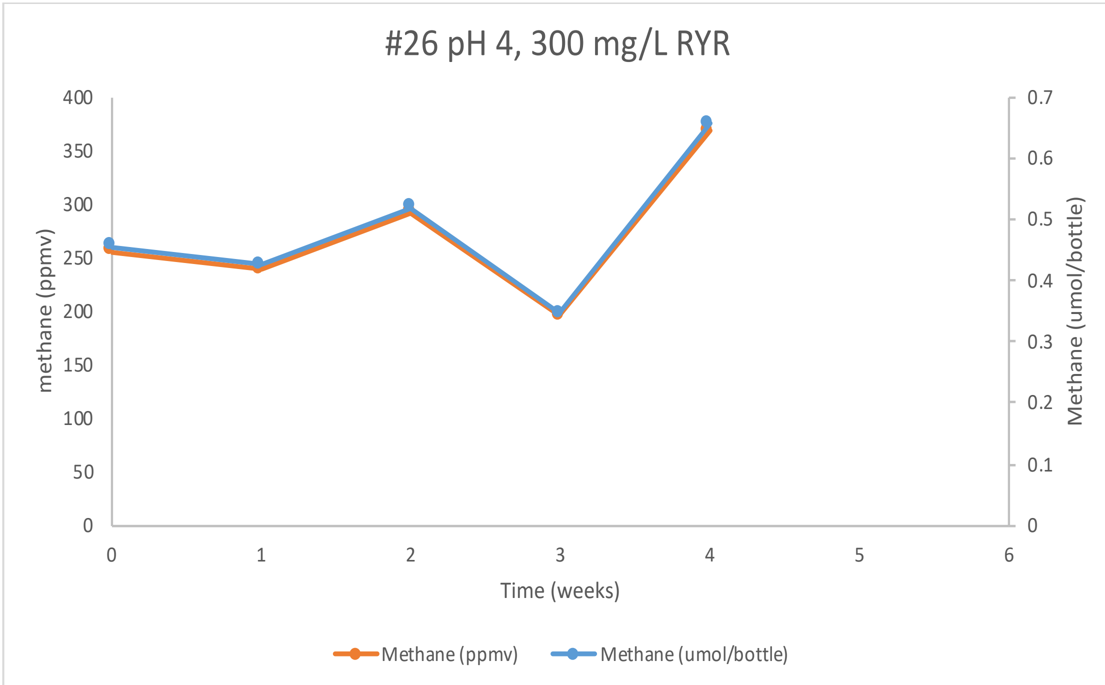


Figure D.14: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 4. Plot indicates replicate two of three.

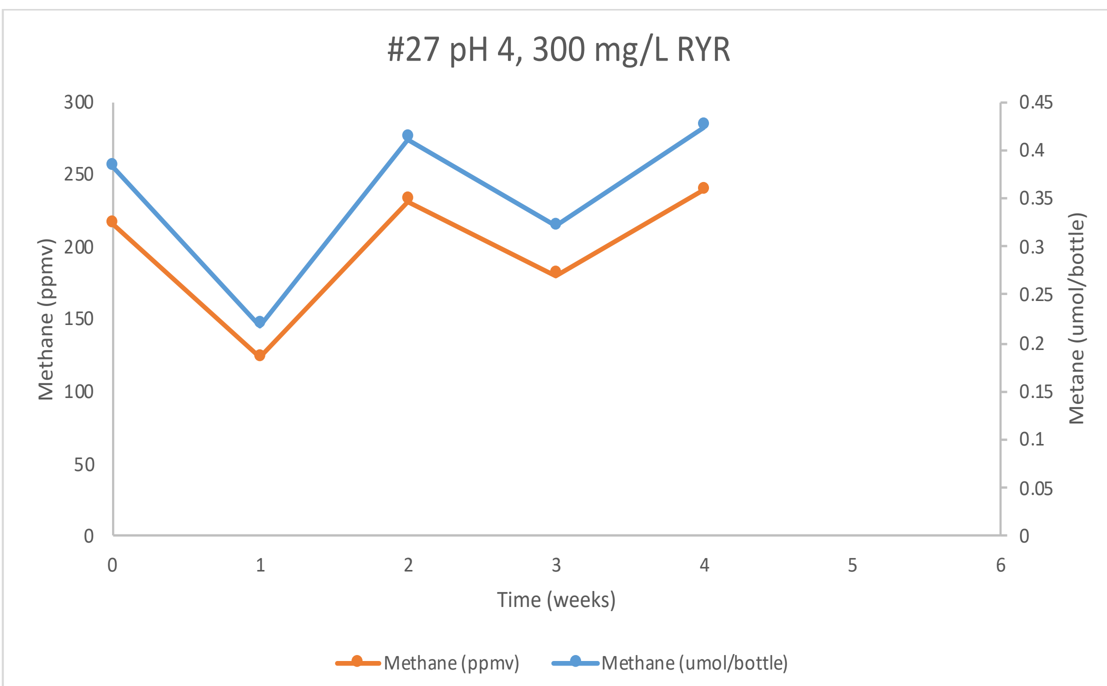


Figure D.15: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 4. Plot indicates replicate three of three.

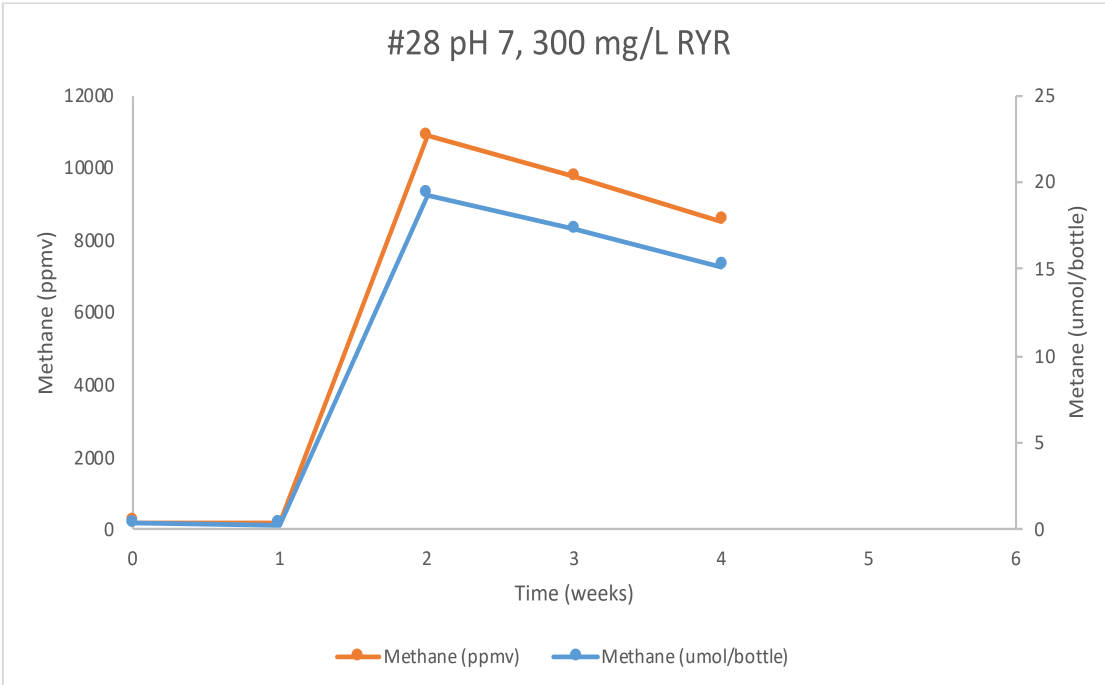


Figure D.16: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 7. Plot indicates replicate one of three.

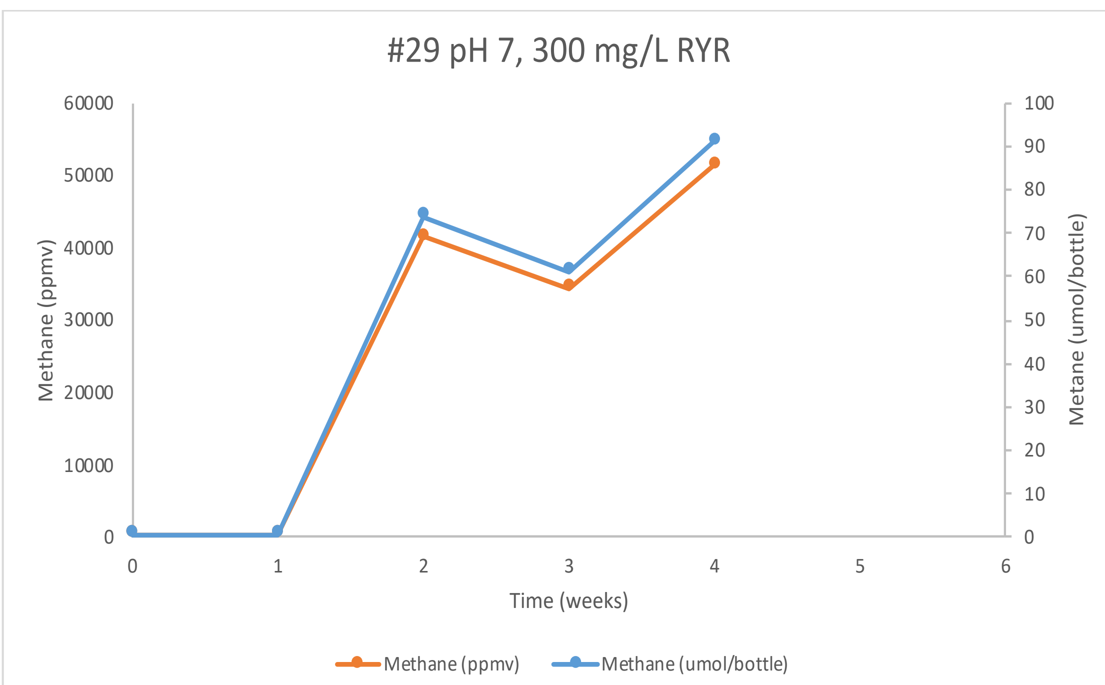


Figure D.17: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 7. Plot indicates replicate two of three.

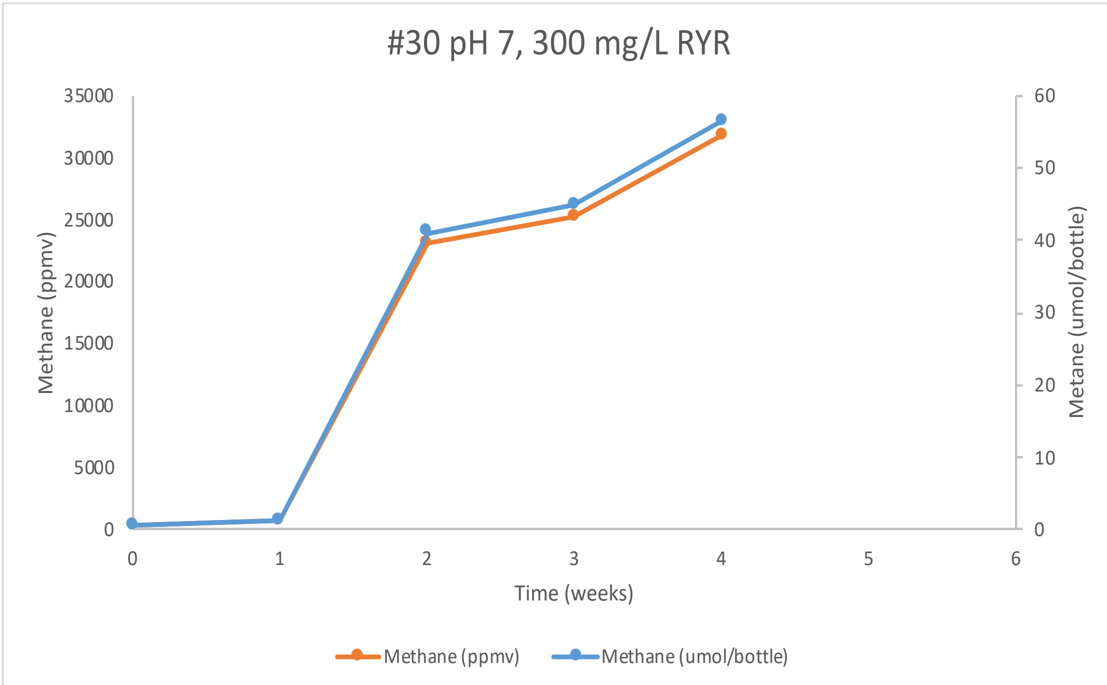


Figure D.18: Methane reduction for methane culture from aquifer material with 300 mg/L Red Yeast Rice at pH 7. Plot indicates replicate three of three.

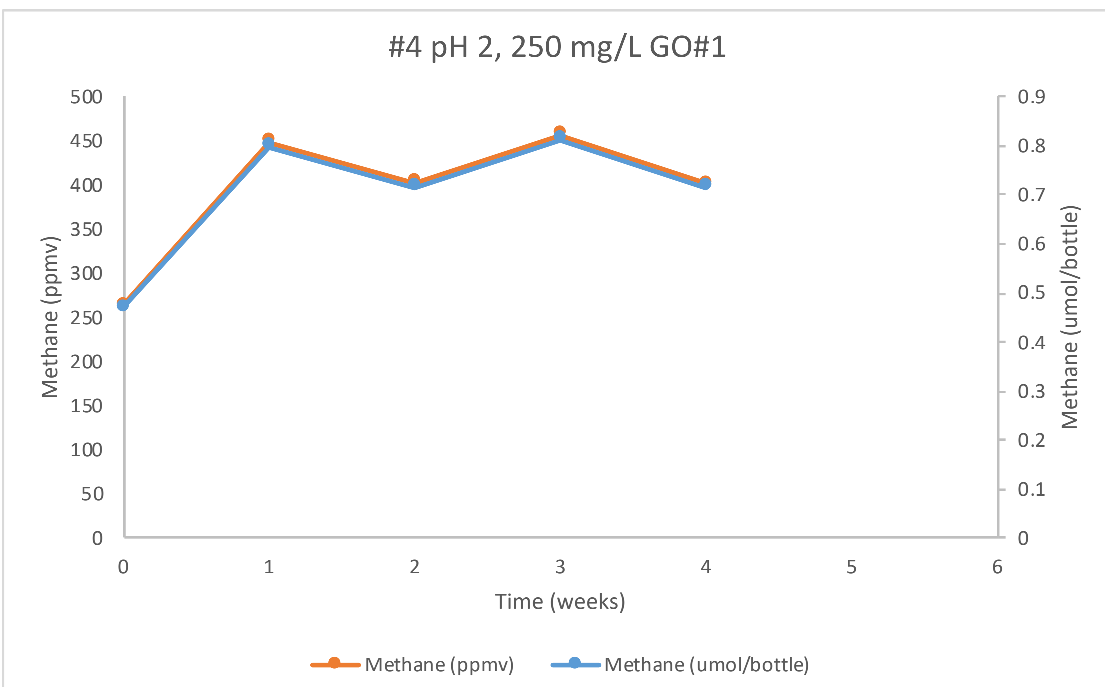


Figure D.19: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 2. Plot indicates replicate one of three.

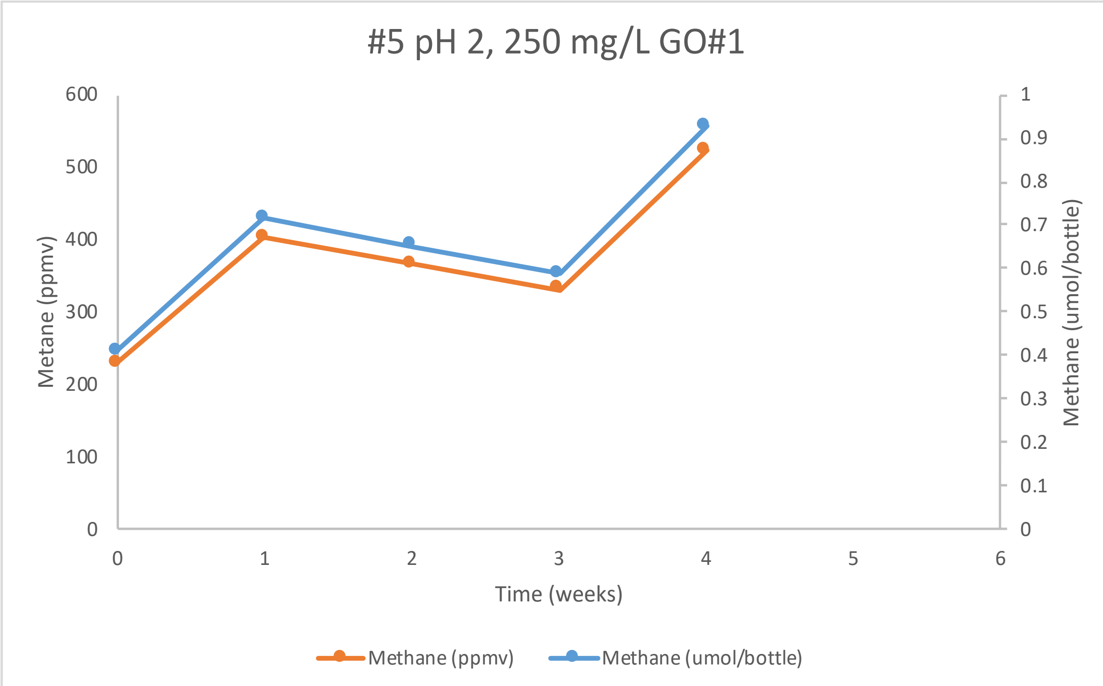


Figure D.20: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 2. Plot indicates replicate two of three.

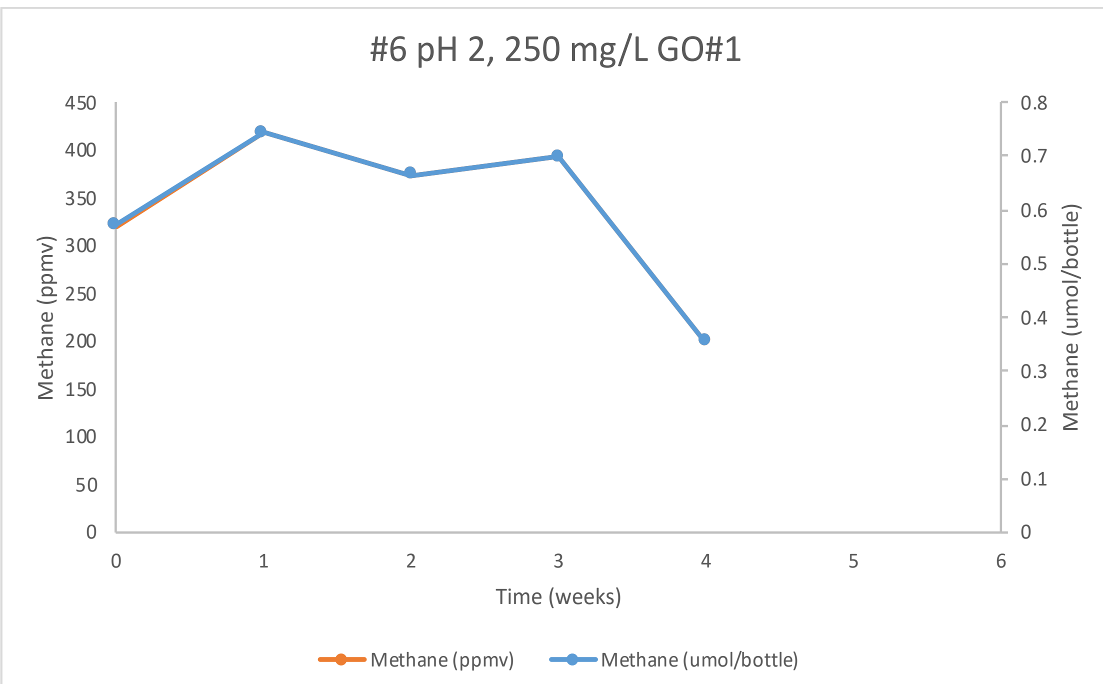


Figure D.21: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 2. Plot indicates replicate three of three.

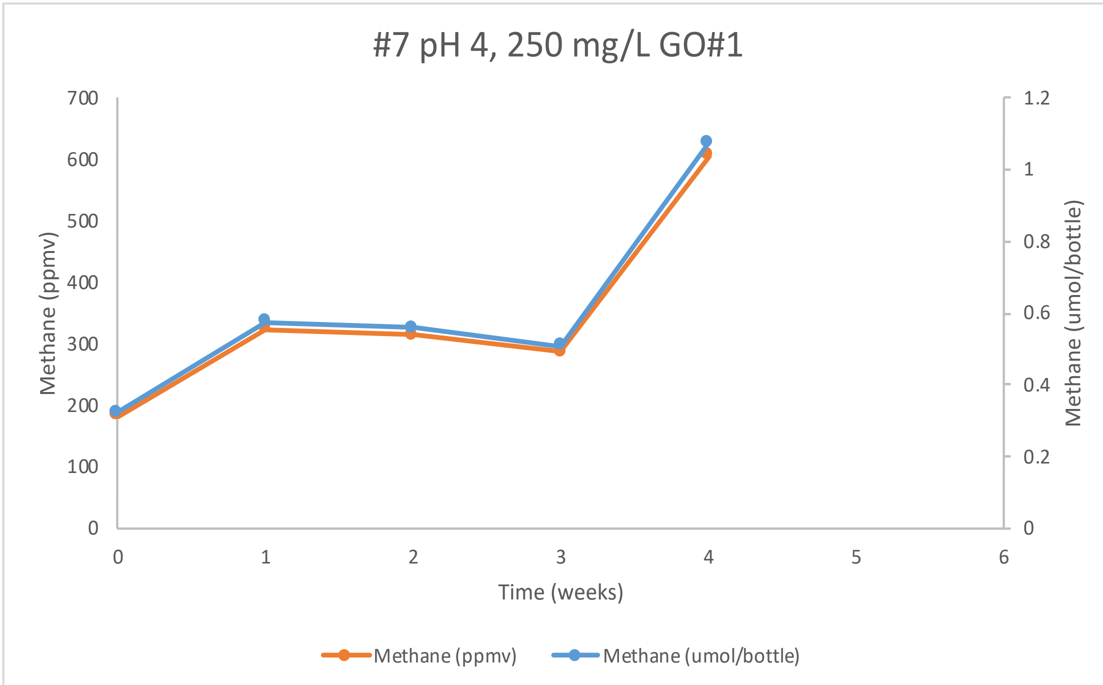


Figure D.22: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 4. Plot indicates replicate one of three.

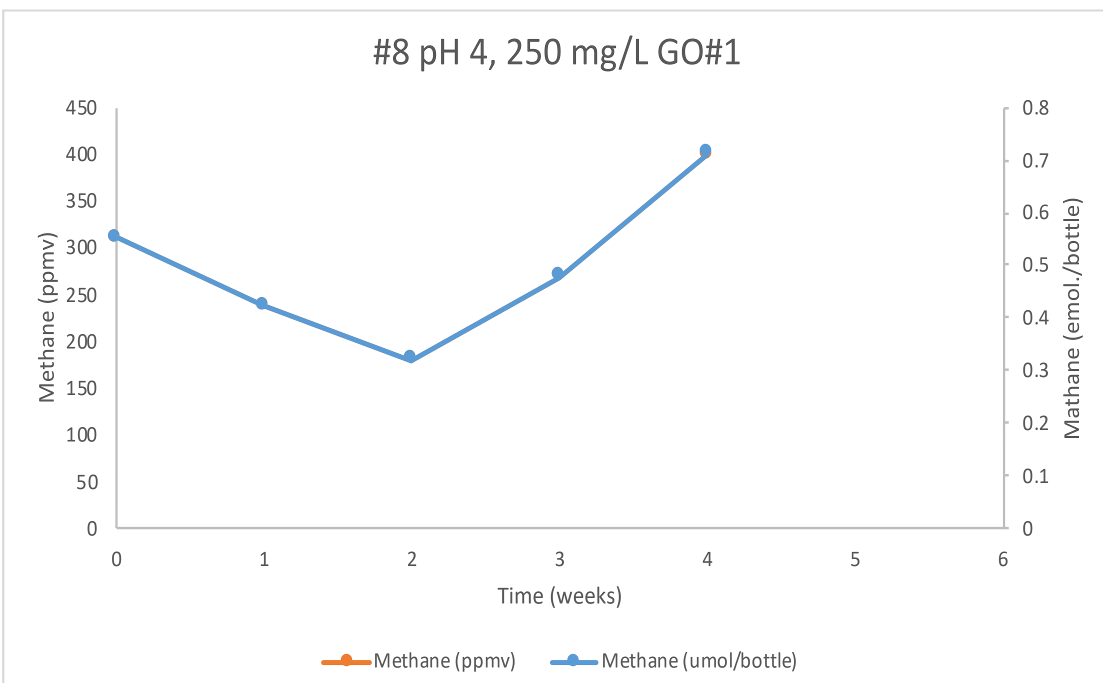


Figure D.23: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 4. Plot indicates replicate two of three.

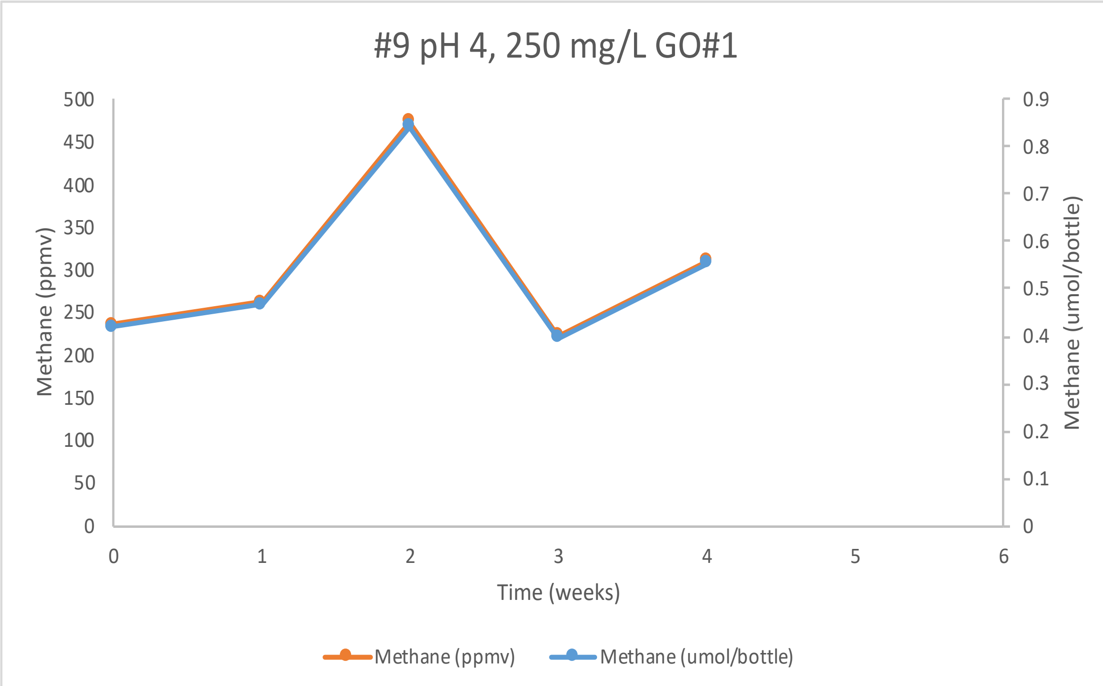


Figure D.24: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 4. Plot indicates replicate three of three.

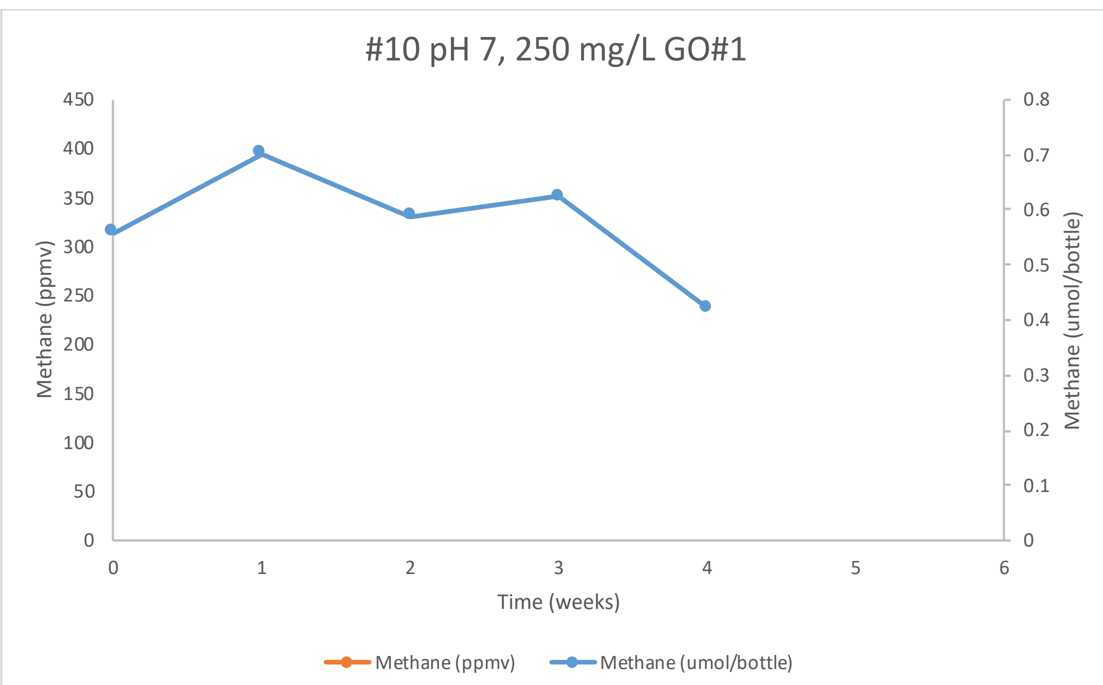


Figure D.25: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 7. Plot indicates replicate one of three.

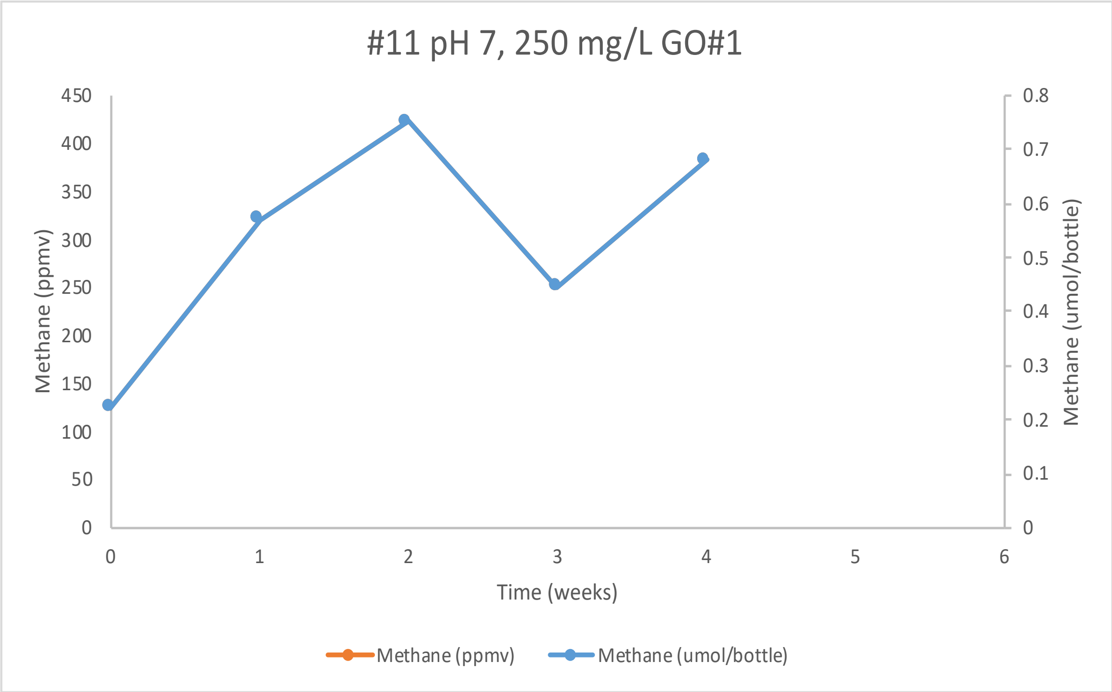


Figure D.26: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 7. Plot indicates replicate two of three.

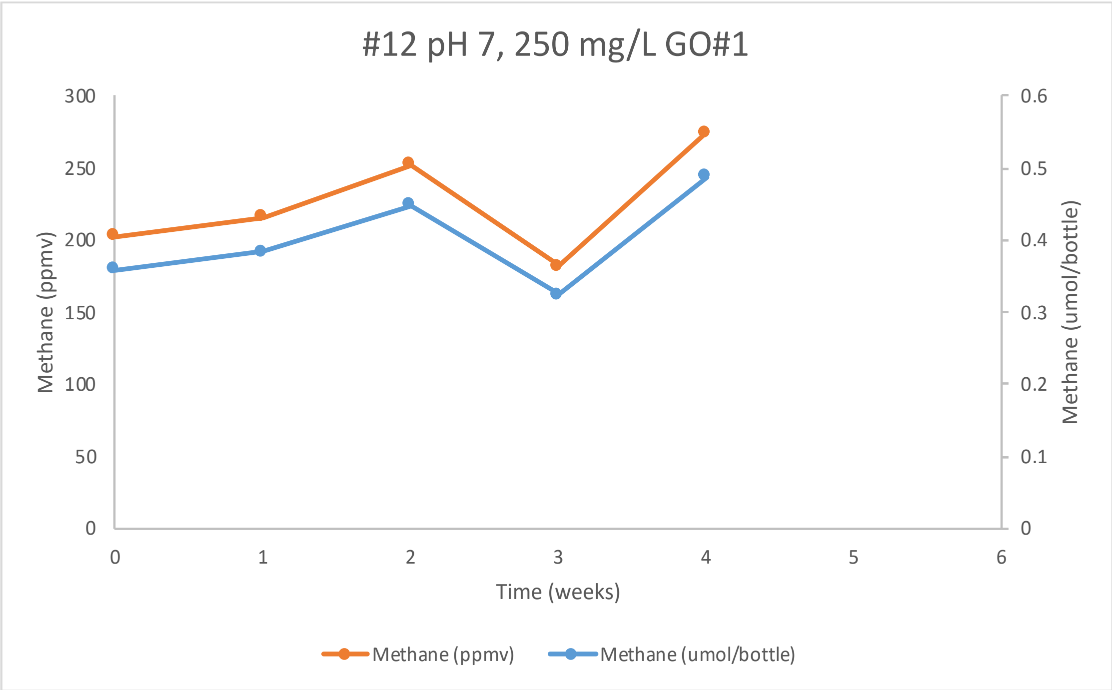


Figure D.27: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #1 at pH 7. Plot indicates replicate three of three.

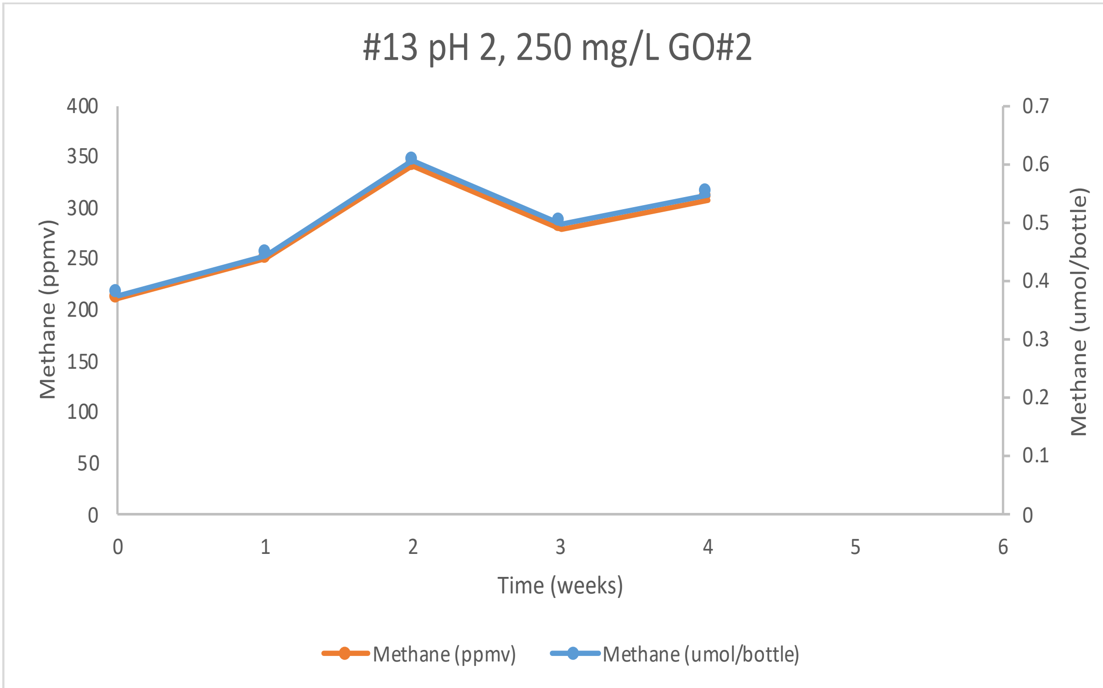


Figure D.28: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 2. Plot indicates replicate one of three.

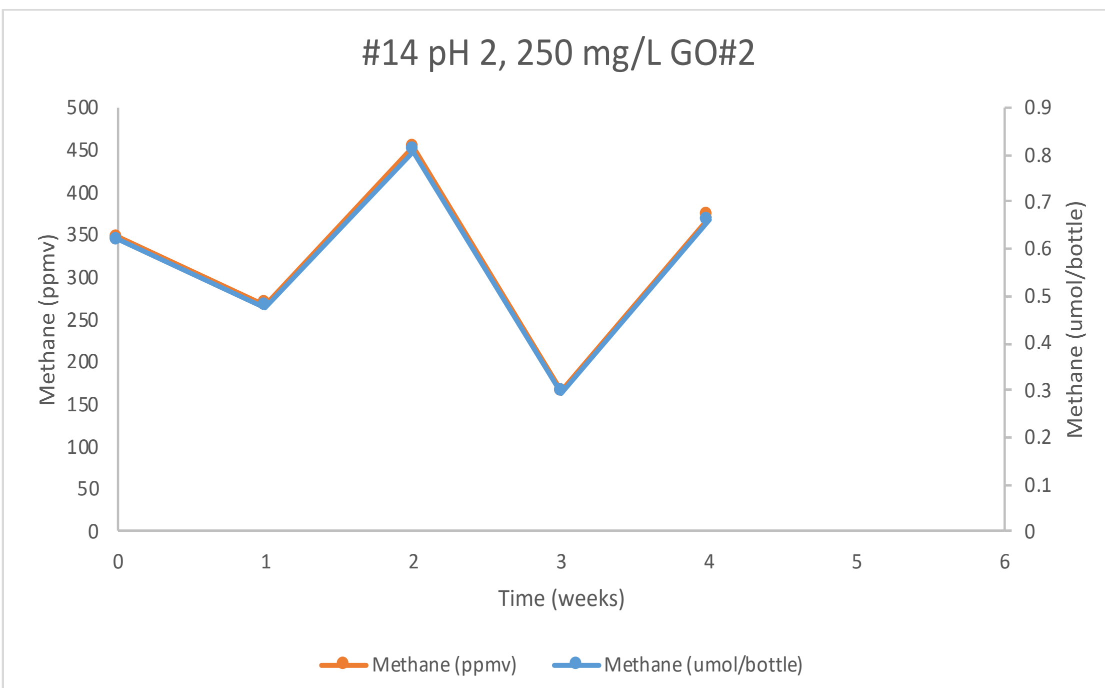


Figure D.29: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 2. Plot indicates replicate two of three.

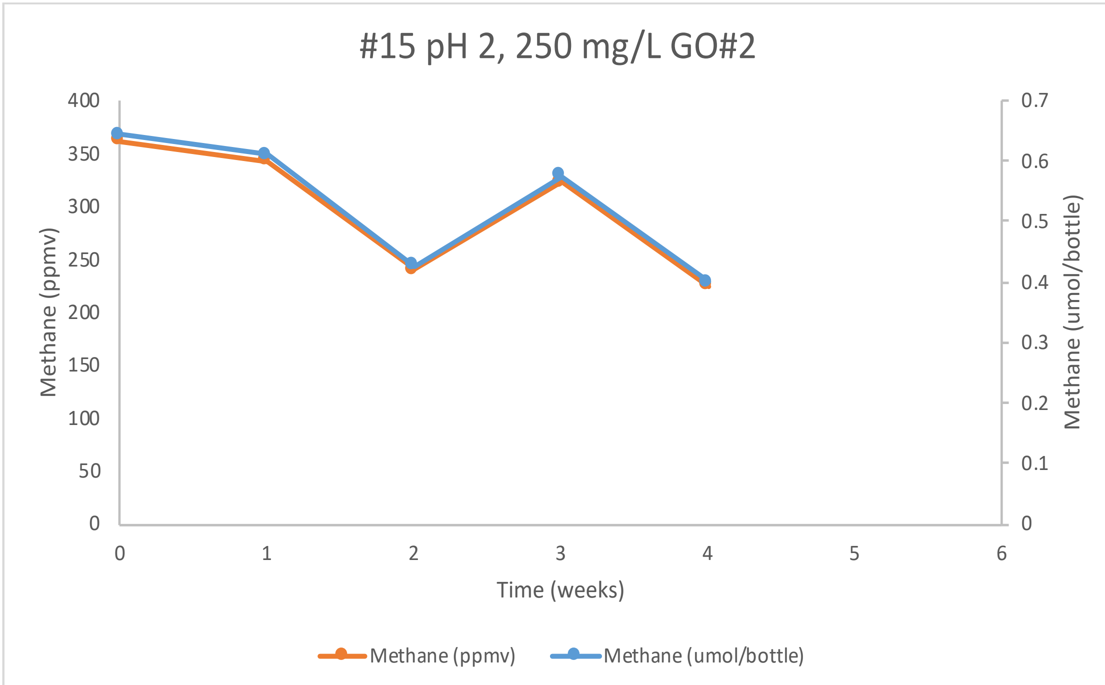


Figure D.30: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 2. Plot indicates replicate three of three.

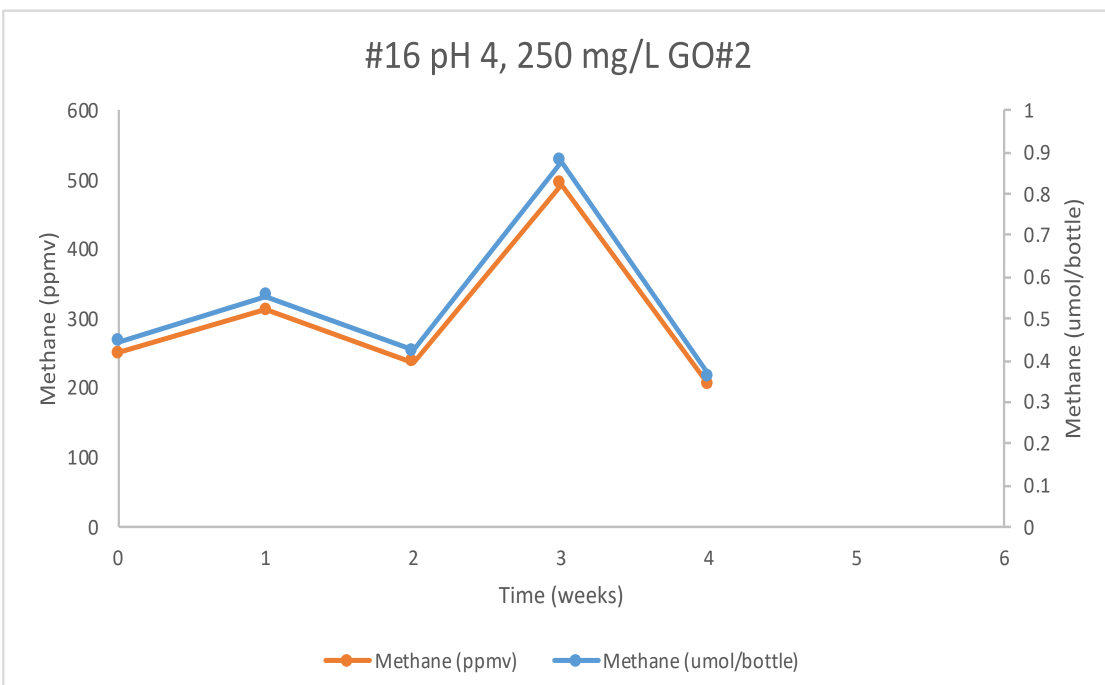


Figure D.31: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 4. Plot indicates replicate one of three.

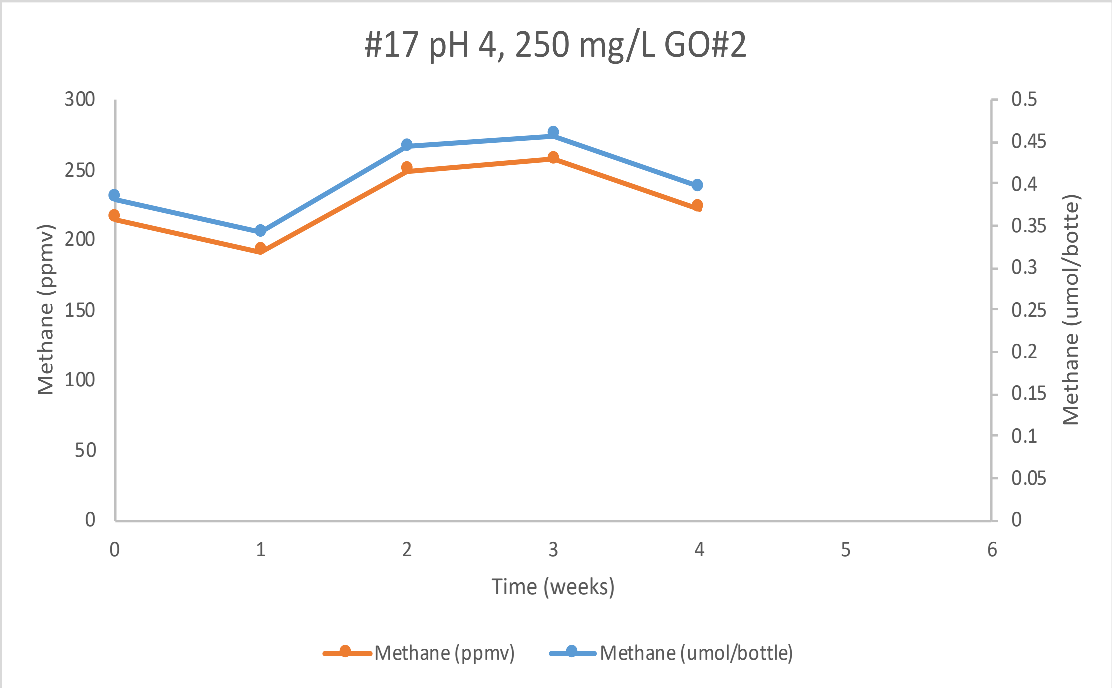


Figure D.32: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 4. Plot indicates replicate two of three.

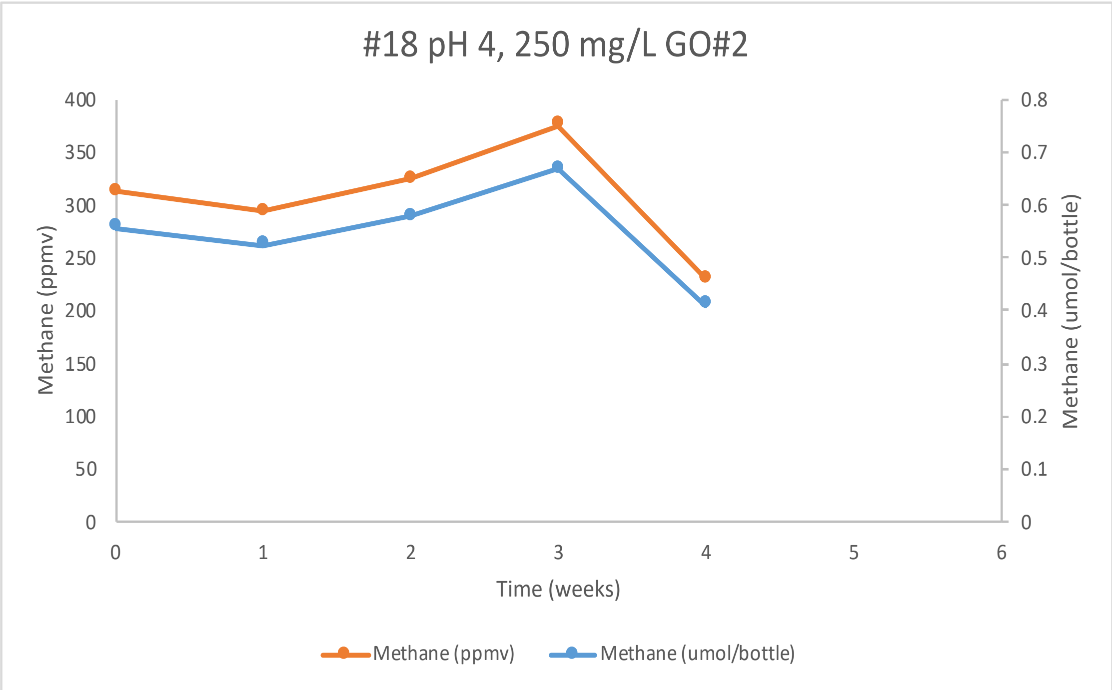


Figure D.33: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 4. Plot indicates replicate three of three.

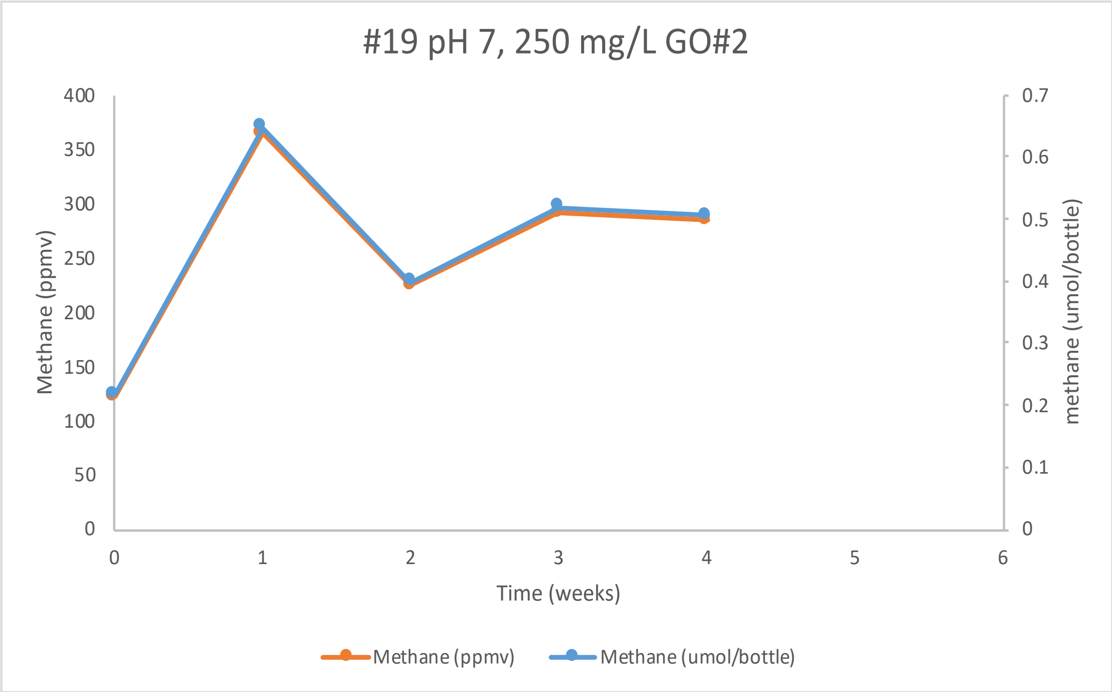


Figure D.34: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 7. Plot indicates replicate one of three.

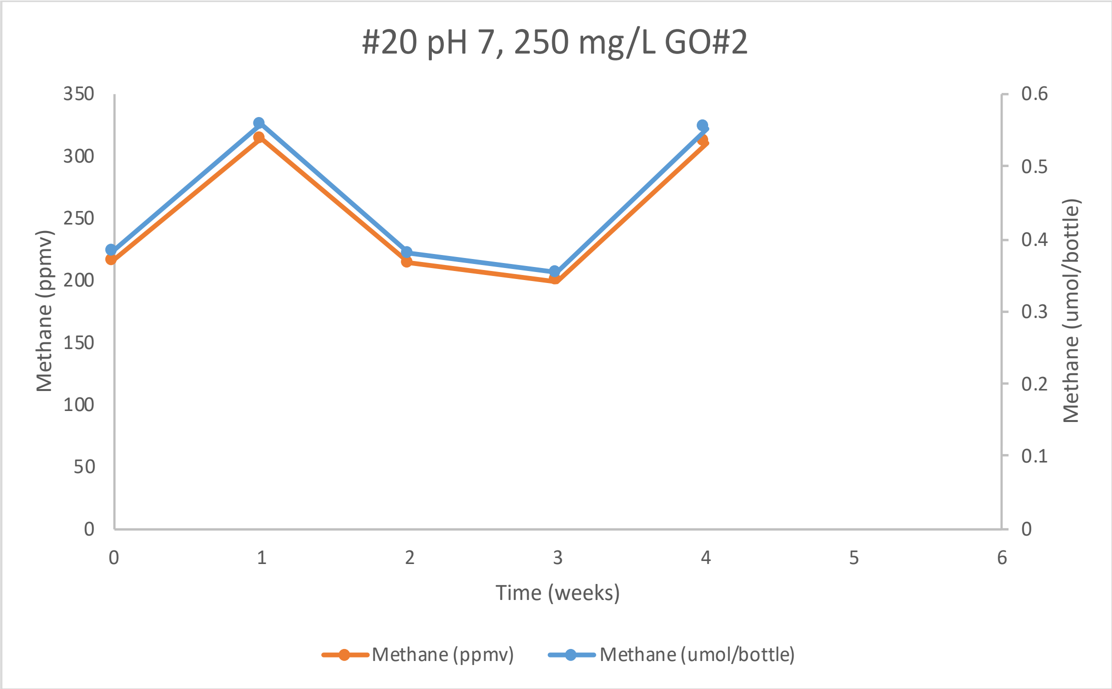


Figure D.35: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 7. Plot indicates replicate two of three.

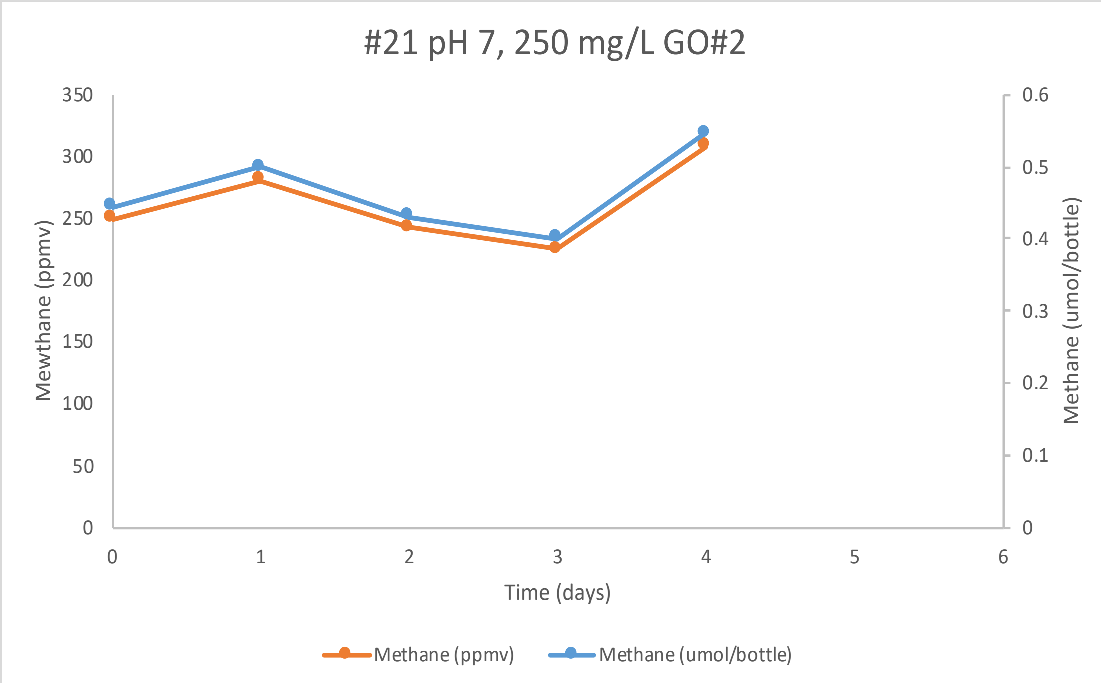


Figure D.36: Methane reduction for methane culture from aquifer material with 250 mg/L Garlic Oil #2 at pH 7. Plot indicates replicate three of three.

Appendix E

Preliminary Batch Study Experiment List

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
1	Unamended			
2	Unamended			
3	Unamended			
94	Unamended			
4	Lactate	5mM		
5	Lactate	5mM		
6	Lactate	5mM		
7	Lactate	10mM		
8	Lactate	10mM		
9	Lactate	10mM		
10	Lactate	40mM		
11	Lactate	40mM		
12	Lactate	40mM		
13	Acetate	5mM		
14	Acetate	5mM		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
15	Acetate	5mM		
16	Acetate	40mM		
17	Acetate	40mM		
18	Acetate	40mM		
73	ELS	5mM		
114	ELS	40mM		
115	ELS	40mM		
116	ELS	40mM		
76	ELS	50mM		
92	BOS100	100mg/L		
82	BOS100, Lactate	1mg/L, 40mM		
85	BOS100, Lactate	50mg/L, 40mM		
89	BOS100, Lactate	100mg/L, 40mM		
96	Bone Meal #1	1g		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
98	Bone Meal #2	1g		
100	Chicken Fat	5mL		
101	Chicken Fat	5mL		
126	EHS	100mg/L		
127	EHS	100mg/L		
128	EHS	100mg/L		
117	EHS, Lactate	1mg/L, 40mM		
118	EHS, Lactate	1mg/L, 40mM		
119	EHS, Lactate	1mg/L, 40mM		
120	EHS, Lactate	50mg/L, 40mM		
121	EHS, Lactate	50mg/L, 40mM		
122	EHS, Lactate	50mg/L, 40mM		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
123	EHS, Lactate	100mg/L, 40mM		
124	EHS, Lactate	100mg/L, 40mM		
125	EHS, Lactate	100mg/L, 40mM		
19	Lactate	40mM	RYR	1 mg/L
20	Lactate	40mM	RYR	1 mg/L
21	Lactate	40mM	RYR	1 mg/L
22	Lactate	40mM	RYR	5 mg/L
23	Lactate	40mM	RYR	5 mg/L
24	Lactate	40mM	RYR	5 mg/L
25	Lactate	40mM	RYR	20 mg/L
26	Lactate	40mM	RYR	20 mg/L
27	Lactate	40mM	RYR	20 mg/L
28	Lactate	40mM	RYR	50 mg/L
29	Lactate	40mM	RYR	50 mg/L
30	Lactate	40mM	RYR	50 mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
31	Lactate	40mM	RYR	100 mg/L
32	Lactate	40mM	RYR	100 mg/L
33	Lactate	40mM	RYR	100 mg/L
34	ELS	40mM	RYR	1 mg/L
35	ELS	40mM	RYR	1 mg/L
36	ELS	40mM	RYR	1 mg/L
37	ELS	40mM	RYR	5 mg/L
38	ELS	40mM	RYR	5 mg/L
39	ELS	40mM	RYR	5 mg/L
40	ELS	40mM	RYR	20mg/L
41	ELS	40mM	RYR	20mg/L
42	ELS	40mM	RYR	20mg/L
45	ELS	40mM	RYR	50mg/L
48	ELS	40mM	RYR	100mg/L
102	Lactate	40mM	RYR	150mg/L
103	Lactate	40mM	RYR	150mg/L
104	Lactate	40mM	RYR	150mg/L
105	ELS	40mM	RYR	150mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
106	ELS	40mM	RYR	150mg/L
107	ELS	40mM	RYR	150mg/L
49	Lactate	40mM	RYR, GO#1	1mg/L, 50mg/L
50	Lactate	40mM	RYR, GO#1	1mg/L, 50mg/L
51	Lactate	40mM	RYR, GO#1	1mg/L, 50mg/L
57	Lactate	40mM	RYR, GO#1	20mg/L, 50mg/L
58	Lactate	40mM	RYR, GO#1	50mg/L, 50mg/L
59	Lactate	40mM	RYR, GO#1	50mg/L, 50mg/L
60	Lactate	40mM	RYR, GO#1	50mg/L, 50mg/L
61	Lactate	40mM	RYR, GO#1	100mg/L, 50mg/L
62	Lactate	40mM	RYR, GO#1	100mg/L, 50mg/L
63	Lactate	40mM	RYR, GO#1	100mg/L, 50mg/L
108	Lactate	40mM	RYR, GO#1	150mg/L, 50mg/L
109	Lactate	40mM	RYR, GO#1	150mg/L, 50mg/L
110	Lactate	40mM	RYR, GO#1	150mg/L, 50mg/L
138	Lactate	40mM	RYR, GO#1	300mg/L, 50mg/L
139	Lactate	40mM	RYR, GO#1	300mg/L, 50mg/L
140	Lactate	40mM	RYR, GO#1	300mg/L, 50mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
141	Lactate	40mM	RYR, GO#1	500mg/L, 50mg/L
142	Lactate	40mM	RYR, GO#1	500mg/L, 50mg/L
143	Lactate	40mM	RYR, GO#1	500mg/L, 50mg/L
150	Lactate	40mM	RYR, GO#2	300mg/L, 50mg/L
151	Lactate	40mM	RYR, GO#2	300mg/L, 50mg/L
152	Lactate	40mM	RYR, GO#2	300mg/L, 50mg/L
153	Lactate	40mM	RYR, GO#2	500mg/L, 50mg/L
154	Lactate	40mM	RYR, GO#2	500mg/L, 50mg/L
155	Lactate	40mM	RYR, GO#2	500mg/L, 50mg/L
64	Lactate	40mM	GO#1	1mg/L
65	Lactate	40mM	GO#1	1mg/L
66	Lactate	40mM	GO#1	1mg/L
67	Lactate	40mM	GO#1	50mg/L
70	Lactate	40mM	GO#1	100mg/L
129	Lactate	40mM	GO#1	50mg/L
130	Lactate	40mM	GO#1	50mg/L
131	Lactate	40mM	GO#1	50mg/L
132	Lactate	40mM	GO#1	300mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
133	Lactate	40mM	GO#1	300mg/L
134	Lactate	40mM	GO#1	300mg/L
135	Lactate	40mM	GO#1	500mg/L
136	Lactate	40mM	GO#1	500mg/L
137	Lactate	40mM	GO#1	500mg/L
111	Lactate	40mM	GO#2	150mg/L
112	Lactate	40mM	GO#2	150mg/L
113	Lactate	40mM	GO#2	150mg/L
144	Lactate	40mM	GO#2	300mg/L
145	Lactate	40mM	GO#2	300mg/L
146	Lactate	40mM	GO#2	300mg/L
147	Lactate	40mM	GO#2	500mg/L
148	Lactate	40mM	GO#2	500mg/L
149	Lactate	40mM	GO#2	500mg/L

Table E.1: Experimental List for Preliminary Experiments

Appendix F

SDC-9 Bioaugmentation Study Experiment List

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
1	Unamended			
2	Unamended			
3	Unamended			
4	Lactate	5mM		
5	Lactate	5mM		
6	Lactate	5mM		
7	Lactate	10mM		
8	Lactate	10mM		
9	Lactate	10mM		
10	Lactate	40mM		
11	Lactate	40mM		
12	Lactate	40mM		
13	Acetate	5mM		
14	Acetate	5mM		
15	Acetate	5mM		
16	Acetate	40mM		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
17	Acetate	40mM		
18	Acetate	40mM		
73	ELS	5mM		
74	ELS	5mM		
75	ELS	5mM		
106	ELS	40mM		
107	ELS	40mM		
108	ELS	40mM		
76	ELS	50mM		
77	ELS	50mM		
78	ELS	50mM		
79	ELS	100mM		
80	ELS	100mM		
81	ELS	100mM		
91	BOS100	100mg/L		
92	BOS100	100mg/L		
93	BOS100	100mg/L		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
82	BOS100, Lactate	1mg/L, 40mM		
83	BOS100, Lactate	1mg/L, 40mM		
84	BOS100, Lactate	1mg/L, 40mM		
85	BOS100, Lactate	50mg/L, 40mM		
86	BOS100, Lactate	50mg/L, 40mM		
87	BOS100, Lactate	50mg/L, 40mM		
88	BOS100, Lactate	100mg/L, 40mM		
89	BOS100, Lactate	100mg/L, 40mM		
90	BOS100, Lactate	100mg/L, 40mM		
118	EHS	100mg/L		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
119	EHS	100mg/L		
120	EHS	100mg/L		
109	EHS, Lactate	1mg/L, 40mM		
110	EHS, Lactate	1mg/L, 40mM		
111	EHS, Lactate	1mg/L, 40mM		
112	EHS, Lactate	50mg/L, 40mM		
113	EHS, Lactate	50mg/L, 40mM		
114	EHS, Lactate	50mg/L, 40mM		
115	EHS, Lactate	100mg/L, 40mM		
116	EHS, Lactate	100mg/L, 40mM		

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
117	EHS, Lactate	100mg/L, 40mM		
19	Lactate	40mM	RYR	1 mg/L
20	Lactate	40mM	RYR	1 mg/L
21	Lactate	40mM	RYR	1 mg/L
22	Lactate	40mM	RYR	5 mg/L
23	Lactate	40mM	RYR	5 mg/L
24	Lactate	40mM	RYR	5 mg/L
25	Lactate	40mM	RYR	20mg/L
26	Lactate	40mM	RYR	20mg/L
27	Lactate	40mM	RYR	20mg/L
28	Lactate	40mM	RYR	50mg/L
29	Lactate	40mM	RYR	50mg/L
30	Lactate	40mM	RYR	50mg/L
31	Lactate	40mM	RYR	100mg/L
32	Lactate	40mM	RYR	100mg/L
33	Lactate	40mM	RYR	100mg/L
94	Lactate	40mM	RYR	150mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
95	Lactate	40mM	RYR	150mg/L
96	Lactate	40mM	RYR	150mg/L
34	ELS	40mM	RYR	1 mg/L
35	ELS	40mM	RYR	1 mg/L
36	ELS	40mM	RYR	1 mg/L
37	ELS	40mM	RYR	5 mg/L
38	ELS	40mM	RYR	5 mg/L
39	ELS	40mM	RYR	5 mg/L
40	ELS	40mM	RYR	20mg/L
41	ELS	40mM	RYR	20mg/L
42	ELS	40mM	RYR	20mg/L
43	ELS	40mM	RYR	50mg/L
44	ELS	40mM	RYR	50mg/L
45	ELS	40mM	RYR	50mg/L
46	ELS	40mM	RYR	100mg/L
47	ELS	40mM	RYR	100mg/L
48	ELS	40mM	RYR	100mg/L
97	ELS	40mM	RYR	150mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
98	ELS	40mM	RYR	150mg/L
99	ELS	40mM	RYR	150mg/L
49	Lactate	40mM	RYR, GO#1	1 mg/L, 50mg/L
50	Lactate	40mM	RYR, GO#1	1 mg/L, 50mg/L
51	Lactate	40mM	RYR, GO#1	1 mg/L, 50mg/L
52	Lactate	40mM	RYR, GO#1	5 mg/L, 50mg/L
53	Lactate	40mM	RYR, GO#1	5 mg/L, 50mg/L
54	Lactate	40mM	RYR, GO#1	5 mg/L, 50mg/L
55	Lactate	40mM	RYR, GO#1	20 mg/L, 50mg/L
56	Lactate	40mM	RYR, GO#1	20 mg/L, 50mg/L
57	Lactate	40mM	RYR, GO#1	20 mg/L, 50mg/L
58	Lactate	40mM	RYR, GO#1	50 mg/L, 50mg/L
59	Lactate	40mM	RYR, GO#1	50 mg/L, 50mg/L
60	Lactate	40mM	RYR, GO#1	50 mg/L, 50mg/L
61	Lactate	40mM	RYR, GO#1	100 mg/L, 50mg/L
62	Lactate	40mM	RYR, GO#1	100 mg/L, 50mg/L
63	Lactate	40mM	RYR, GO#1	100 mg/L, 50mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
142	Lactate	40mM	RYR, GO#1	300 mg/L, 300 mg/L
143	Lactate	40mM	RYR, GO#1	300 mg/L, 300 mg/L
144	Lactate	40mM	RYR, GO#1	300 mg/L, 300 mg/L
145	Lactate	40mM	RYR, GO#1	500 mg/L, 500 mg/L
146	Lactate	40mM	RYR, GO#1	500 mg/L, 500 mg/L
147	Lactate	40mM	RYR, GO#1	500 mg/L, 500 mg/L
100	Lactate	40mM	RYR, GO#2	150 mg/L, 50 mg/L
101	Lactate	40mM	RYR, GO#2	150 mg/L, 50 mg/L
102	Lactate	40mM	RYR, GO#2	150 mg/L, 50 mg/L
130	Lactate	40mM	RYR, GO#2	300 mg/L, 50 mg/L
131	Lactate	40mM	RYR, GO#2	300 mg/L, 50 mg/L
132	Lactate	40mM	RYR, GO#2	300 mg/L, 50 mg/L
133	Lactate	40mM	RYR, GO#2	500 mg/L, 50 mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
134	Lactate	40mM	RYR, GO#2	500 mg/L, 50 mg/L
135	Lactate	40mM	RYR, GO#2	500 mg/L, 50 mg/L
64	Lactate	40mM	GO#1	1 mg/L
65	Lactate	40mM	GO#1	1 mg/L
66	Lactate	40mM	GO#1	1 mg/L
67	Lactate	40mM	GO#1	50mg/L
68	Lactate	40mM	GO#1	50mg/L
69	Lactate	40mM	GO#1	50mg/L
70	Lactate	40mM	GO#1	100mg/L
71	Lactate	40mM	GO#1	100mg/L
72	Lactate	40mM	GO#1	100mg/L
136	Lactate	40mM	GO#1	300 mg/L
137	Lactate	40mM	GO#1	300 mg/L
138	Lactate	40mM	GO#1	300 mg/L
139	Lactate	40mM	GO#1	500 mg/L
140	Lactate	40mM	GO#1	500 mg/L
141	Lactate	40mM	GO#1	500 mg/L
121	Lactate	40mM	GO#2	50mg/L

Bottle Number	Electron Donor	Electron Donor Concentration	Methane Inhibitor	Methane Inhibitor Concentration
122	Lactate	40mM	GO#2	50mg/L
123	Lactate	40mM	GO#2	50mg/L
103	Lactate	40mM	GO#2	150mg/L
104	Lactate	40mM	GO#2	150mg/L
105	Lactate	40mM	GO#2	150mg/L
124	Lactate	40mM	GO#2	300 mg/L
125	Lactate	40mM	GO#2	300 mg/L
126	Lactate	40mM	GO#2	300 mg/L
127	Lactate	40mM	GO#2	500 mg/L
128	Lactate	40mM	GO#2	500 mg/L
129	Lactate	40mM	GO#2	500 mg/L

Table F.1: Experimental list for SDC-9 Bioaugmentation Experiments

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