Evaluation of Sulfate Reduction and TCE Reduction by the Evanite and Point Mugu Cultures

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Research and education focused on life below Earth's surface.

Introduction

Contamination of groundwater and soil by chlorinated compounds such as trichloroethylene (TCE) is a big issue in the United States. TCE is one of the most common soil contaminants and a suspected carcinogen. There are several methods to remove this compound such as pump and treat, purging and bioremediation. The method we focused on in this project was bioremediation. Anaerobic reductive dechlorination is a highly documented phenomenon for the remediation of TCE to the non-toxic compound ethene (ETH). The reduction steps are shown in Figure 1.

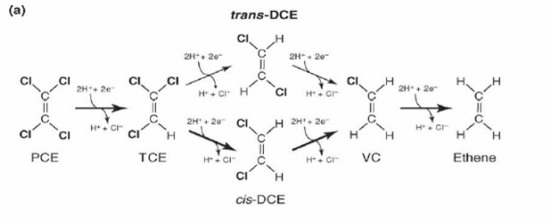

Figure 1: Reduction steps from PCE to ETH.

In each step, a Cl is replaced by an H in a reduction reaction, which requires the transfer of 2e⁻. The chlorinated compound is the electron acceptor and the hydrogen acts as the electron donor; however, this chlorinated compound competes for electrons with other substances such as ferric iron and sulfate. Sulfate and ferric iron are reduced in groundwater as well as on aquifer solids, consuming electron donors such as hydrogen. The competing electron acceptors are the chlorinated compounds PCE, TCE, cis-DCE, and VC, as well as sulfate and ferric iron. Figure 2 shows the reduction equation for sulfate, and Figure 3 shows a diagram of the reduction processes found at typical groundwater contamination sites.

$$SO_4^{2-}\ (Sulfate) + 4H_2 \ ===>\ S^{2-} + 4H_2O$$

Figure 2: Sulfate reduction equation

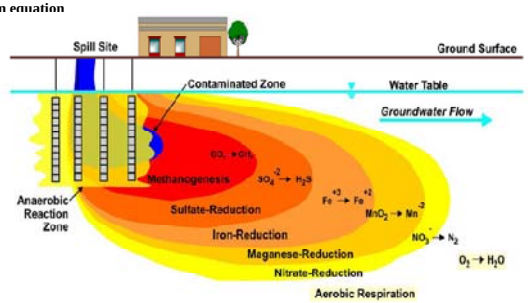

Figure 3: Contamination site reduction processes

As seen in the above diagram, there are a lot of reduction processes going on near a contamination site, and sulfate and iron pose the most competition for chlorinated compounds. This competition can lead to no, or very slow reduction of TCE.

The experiment performed determined if any of the bioremediation cultures in the ENVE lab have sulfate reducers, so that an experiment can be designed to evaluate sulfate competition with TCE. The two cultures being tested are The Evanite (EV) and The Point Mugu (PM). The EV and PM cultures are being grown differently in chemostats. The EV culture is being fed formate, which is a direct source of hydrogen, while the PM culture is being fed lactate, a fermenting substrate that produces hydrogen. The PM culture is expected to have a more diverse microbial community, so it has a higher chance of containing sulfate reducers.

ACKNOWLEDGEMENTS

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Method

Test 1: TCE Reduction

- EV and PM cultures were injected into an anaerobic batch reactor from chemostats
- Bottles were purged with N_2/CO_2 gas mixture
- TCE was added to all bottles with excess H_2 as an electron donor.
- Chlorinated compound concentrations were monitored over time using a gas chromatograph (GC). Between sampling times, the bottles were stored on a shaker table operating at 200 RPM.
- Bottles were purged for 15 minutes to remove all electron donors and acceptors.

Test 2: Sulfate Reduction

- Sulfate was added to each bottle to make a 0.1 mM solution. H_2 was added in excess as an electron donor.
- Liquid samples were extracted daily and analyzed by an ion chromatograph (IC) to determine the SO_4 concentrations. GC analyzed headspace samples were used to document the H_2 concentrations.
- Higher SO_4 concentrations were tested for the PM culture after initial reduction.
- PM soil samples from a previous column experiment were also tested for sulfate reducers. Soil was obtained from 0-2 cm, 2-4 cm, and 4-6 cm sections of the column.

Results

EV Culture

The EV bottles were injected with TCE to prove the culture reduced TCE to ETH. Figure 4 shows the reduction over time of the TCE to ETH.

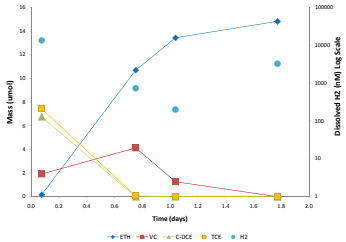

Figure 4: TCE reduction numbers for EV culture.

When aqueous hydrogen concentrations in the bottles fell near to 100 nM, H_2 was again injected into the bottles to keep concentrations far above the aqueous H_2 concentration threshold for both chlorinated compound and sulfate reduction, which are around 1-10 nM. Two bottles were injected with 100 uL of sodium sulfate, which achieved 0.1 mM. These bottles were monitored over approximately seventeen days and both bottles showed consistent sulfate concentrations. From analysis on the liquid samples taken from the EV bottles, there is no evidence of sulfate reduction. Figure 5 shows the plot of the average EV bottles sulfate data.

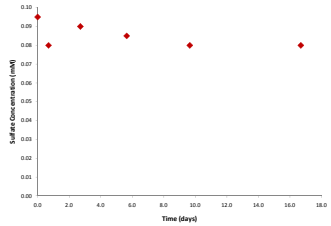

Figure 5: Plot of sulfate versus time for averaged EV bottles.

The results show sulfate reduction is not occurring in the EV bottles. Sulfate reducers in the EV culture never existed or have been flushed out of the chemostat since sulfate was not being added and formate was added as an easy source of H_2.

PM Culture

The PM culture was also tested for TCE reduction prior to testing for sulfate reduction. The TCE was not reduced as quickly as in the EV culture, but transformation to ETH was observed. TCE reduction was not as fast as in EV but the H_2 consumption was very similar. This is because there are other H_2 consuming processes occurring while the TCE is being reduced. After three days, the bottles were purged. The TCE reduction data is displayed in Figure 6.

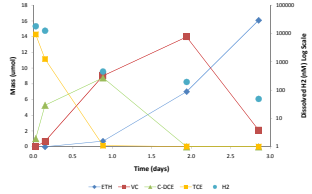

Figure 6: TCE reduction data for PM culture

100 uL of Na_2SO_4 was added to two PM bottles to test for sulfate reduction. The PM culture seemed to be reminiscent of the EV culture, because after approximately four days, little to no sulfate reduction occurred. After the eighth day, sulfate reduction was shown to be significant. The averaged concentrations from the two bottles are shown in Figure 7. After these bottle showed reduction, each bottle was injected with higher and varied amounts of sulfate. After liquid samples from various days were run through the IC, reduction was once again concluded. The second sulfate run for each bottle shown in Figure 8 shows continued sulfate reduction.

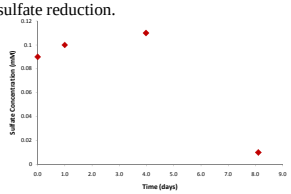

Figure 7: Plot of sulfate versus time for averaged PM bottles after the 1st injection

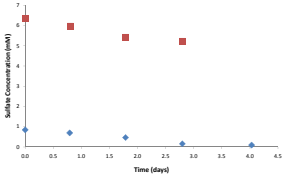

Figure 8: Plot of sulfate versus time for PM bottles after the 2nd injection

PM Soil

Of the three soil samples tested, two showed conclusive reduction of sulfate. The 0-2cm sample did not give conclusive data on sulfate reduction, but the 2-4 and 4-6 cm samples both showed good reduction. Figure 9 shows the concentration-over-time data for these samples.

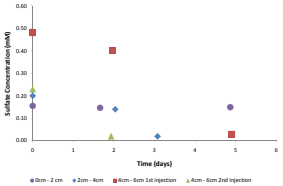

Figure 9: PM soil sulfate concentration versus time data

From the data presented above, it is obvious that the PM soil contains sulfate reducers. This was expected because in the past soil column experiment, these samples had shown proof of sulfate reduction.

Conclusion

This experiment was set up to determine if any of the cultures being grown in the lab contained sulfate reducers. The cultures tested were EV and PM. The EV culture showed no sulfate reduction which means that it contains no sulfate reducing bacteria or very low numbers of sulfate reducing bacteria. The PM culture samples as well as the PM soil samples showed sulfate reduction, meaning that this culture contains sulfate reducing bacteria. Both cultures showed TCE reduction to ETH.

The EV culture is fed with formate as the electron donor in the chemostat, whereas the PM culture is fed with lactate. Lactate is then fermented by the microorganisms into acetate, propionate, and hydrogen. The formate ferments only to carbon dioxide and hydrogen. Fermentation equations for lactate, propionate and formate are shown in Figure 10.

$$HCOO^-\ (Form) + H^+ \ ===>\ CO_2 + H_2$$
$$C_3H_5O_3^-\ (Lac) \ ===>\ 0.67\ C_3H_5O_2^-\ (Prop) + 0.33\ C_2H_3O_2^-\ (Acet) + 0.33\ HCO_3^- + 0.33H^+$$
$$C_2H_5COO^-\ (Prop) + 3H_2O \ ===>\ CH_3COO^-\ (Acet) + HCO_3^- + H^+ + 3H_2$$

Figure 10: Formate, lactate, and propionate fermentation equations

In the EV chemostat, formate simply ferments to CO_2 and H_2, while in the PM chemostat there are more fermentation processes. With these more complex fermentation processes, it seems that the PM culture contains a more diverse microbial community than the EV. It is likely that lactate fermenting bacteria may also be capable of sulfate reduction. Between the EV and PM cultures being grown in the lab, the PM culture appears to contain sulfate reducers due to its active fermentation processes, and shows the most potential to compete with the reduction of chlorinated compounds such, as TCE.