Reduction Analysis Using the Colorimetric Method

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Goal:

Develop a method to determine the reduction capacity of a continuous flow column bioreactor packed with non-sterile aquifer solids from the Hanford DOE site (Washington) by employing the Colorimetric method and Spectra Suite software.

Figure 1: The column setup above was used in this experiment.

Abstract:

PCE and TCE and other chlorinated solvents were widely used for mechanical and electrical parts degreasing. This widespread use has led to these compounds being a major source of ground water contamination. These compounds have significantly impacted the ground water in many areas of the world; primarily due to the chemical characteristics of the compounds. Both TCE and PCE are soluble in water, this is due to a fairly high solubility of 1100 mg/L for TCE and 200 mg/L for PCE. These compounds are also very persistent and take a very long time to degrade in nature. So, when these compounds contact ground water they are transported away from the initial contamination site and will remain in the ground water for a long period of time.

The EPA has deemed chlorinated solvents to be toxic to humans and the environment and has set rules and regulation regarding the use of these compounds. However, widespread contamination already exists with these compounds. Therefore, it is necessary to remediate the ground water and the soils that have been contaminated with these compounds.

Fortunately there are many different ways to remediate PCE and TCE. Some of those techniques include air stripping, soil vapor extraction and through microbial activity. However, we are concentrating our research on the degradation of PCE and TCE through microbial activity.

Microorganisms in the soil are able to consume chlorinated solvents via the electron transport chain and by co-metabolizing the pollutants. Microbes break down PCE by reducing it to TCE then to cis-DCE, followed by the reduction to Vinyl Chloride, then it is reduced to harmless Ethene gas as see below.

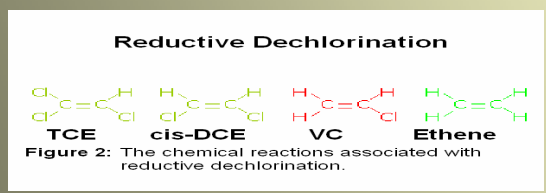

Figure 2: The chemical reactions associated with reductive dechlorination.

The reduction capacity, is an estimate of the ability of the microorganisms in the soil to consume chlorinated solvents such as PCE and TCE. Generally, microbial reductive dechlorination of chlorinated solvents occurs under a highly reduced conditions when all other electron acceptors such as Fe(III) and NO3- have been depleted.

Method:

The method involves spectrophotometer monitoring of the reaction of a known amount of a colored redox indicator in solution with an anoxic aqueous sample. Based on the amount of indicator employed and the fraction of indicator reduced, an effective concentration of reductants, termed reductive capacity (RC), is determined.

The RC of effluent samples was measured with the redox indicator THI during the course of the experiment. THI has a formal redox potential at pH 7 of 52 mV and will be reduced significantly only if O2 is absent and there is a sufficient concentration of reductants with redox potentials near or below the THI formal potential. Species known to reduce THI include Fe(II) (~100 µM) and S(-II) (~1 µM) and may include reduced species on cell membranes of anaerobic bacteria.

The reductive capacity is measured in this experiment by monitoring the indicator Thionine (THi) with a spectrometer at the wavelengths of 600 nM and 750 nM. In general, the Thionine (THi) works in a manner that when the indicator is in the oxidized form it is colored and when it is in the fully reduced state it is basically colorless.

Procedure:

Prepare the following solutions.

Mixture of EtOH (20% v/v) pH 7 buffer
THI 2mM,

- Add the 2.5 mL of the EtOH solution to a cuvette and use this as the standard for the test.
- Reference the light and dark settings to the standard.
- Inject 25 uL of the THI indicator solution to the cuvette.
- Sparge the cuvette with Nitrogen gas for 45 minutes.
- Inject the 100-300ul of sample from the column into the cuvette.
- Monitor the experiment with a spectrometer at wavelengths of 600 nM and 750 nM until the sample is fully reduced.
- Once fully reduced, Oxidize the sample by injecting air into the system.

Calculations:

Based on the following.

Reductive Capacity = [(1-Amin/Amax)*Cind*Vind]/ Vsample

Where Amin = Minimum absorbance of indicator after adding sample
Amax = maximum absorbance of indicator after reoxidation
Cind = concentration of indicator
Vind = volume of indicator in cuvette
Vsample= sample volume

Analysis:

Using Spectra Suite software program

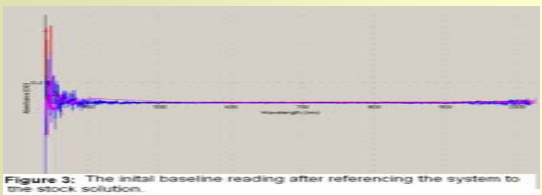

Figure 3: The inital baseline reading after referencing the system to the stock solution.

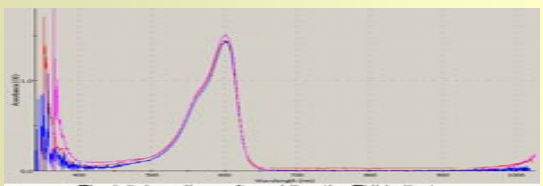

Figure 4: The inital readings after adding the THI indicator.

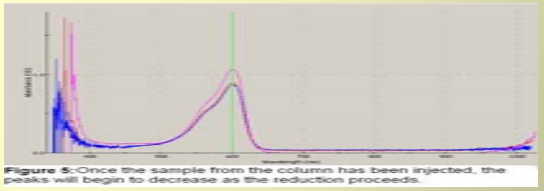

Figure 5: Once the sample from the column has been injected, the peaks will begin to decrease as the reduction proceeds.

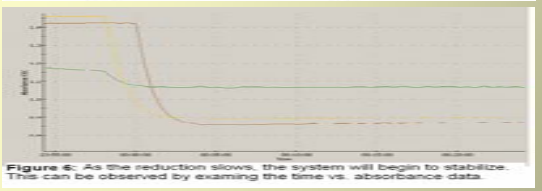

Figure 6: As the reduction slows, the system will begin to stabilize. This can be observed by examing the time vs. absorbance data.

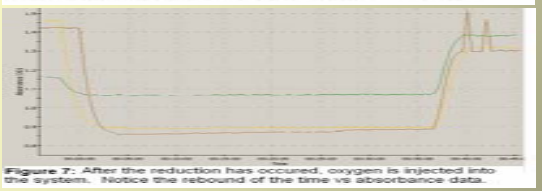

Figure 7: After the reduction has occured, oxygen is injected into the system. Notice the rebound of the time vs absorbance data.

Results:

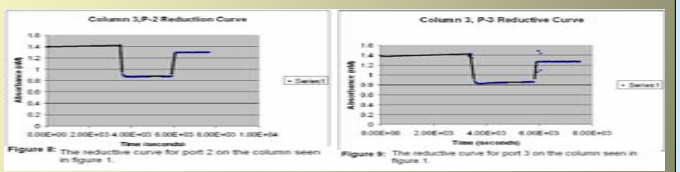

Figure 8: The reductive curve for port 2 on the column seen in figure 1.

Figure 9: The reductive curve for port 3 on the column seen in figure 1.

Reductive capacity Port-2=168 uM; Reductive capacity Port-3=184 uM

Conclusion:

The highly reduced state within the column (168-184 uM) indicates that the reactions within the columns are occurring as we expect. The reductive capacity achieved in the column indicates a high level of sulfide, iron and nitrogen reducing activity occurring. Since TCE and PCE generally become degraded in an anaerobic conditions and when all other electron accepters are depleted, we expect the TCE and PCE that is injected into the column to be reduced to Ethene gas by following the reductive dechlorination reactions associated with these compounds.

Acknowledgments:

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References:

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