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What's in Your Water?

Introduction

In 1990, Anne Anderson, a single parent, living in Woburn, Massachusetts took on the burden of suing Beatrice Food Company – one of the largest food processing businesses in America (1). She fought her case with such conviction because she believed that her nine year-old son had developed a very rare form of childhood cancer from a chemical compound called trichloroethene (TCE). The suit later revealed that more than 12 children had developed cancer in Woburn from TCE. Where could these children have possibly been exposed to this deadly chemical in high enough concentrations for them to develop cancer? It turned out that every time the residents took a sip of water, they were unknowingly being exposed to TCE. This small town later found out that their drinking water wells contained TCE concentrations that were 80,000 times greater than the allowed concentrations required by the law under the Environmental Protection Agency (EPA) (2). The company ended up having to pay over $64 million dollars to cleanup the town's drinking water, which is still recorded as the largest chemical cleanup site in the Northeastern United States. (2)

Starting in the early 1980's TCE was used as a metal degreasing agent in many manufacturing companies and military bases all across the nation from Massachusetts to California (3). It proved to be an excellent solvent for cleaning off dirt; however, its unknown effects on consumption would later show to be deadly. The combination of companies leaving little room in their budget for safe disposals

along with few government regulations led to large scale illegal dumping. This unregulated practice led to TCE seeping directly into the groundwater from deteriorating, metal barrels. Once TCE's fetal carcinogenic toxicity concerns were made public, many countries and companies abandoned using the degreasing agent; however, dealing with the already spilled hazardous waste largely went untreated (4). It has now been determined as a known carcinogenic compound that can cause: liver, kidney, prostate, breast, and lung cancer, arrhythmia, and lymphoma (5). Its widespread use made it such an issue that the EPA has set a maximum contaminant level(MCL) of 5 micrograms per liter to ensure safe drinking water (6). For reference, in the shallow wells of Woburn, Massachusetts TCE levels were recorded at over 400,000 micrograms per liter.

One of the most effective and natural ways of cleaning up chemical toxins such as TCE is through a method called in-situ bioremediation. This method uses bacteria that are able to biodegrade toxic compounds into more manageable and benign substances. The one and only bacterium that can biodegrade TCE completely to the harmless compound ethene is called *Dehalococcoides* (7). For the Spring 2013 semester, I have been researching this bacterium and ways to enhance and maintain its TCE biodegradation rates in water.

One of the most important factors in understanding how bacteria thrive is to understand the environment that is best suited for their growth. The environment of concern for my research is groundwater, which has the unique property of hosting anaerobic pockets underground (8). Anaerobic means that the environment is oxygen-free, unlike the atmosphere we breathe. *Dehalococcoides* is one of the few

bacteria that can survive in an anaerobic environment and utilize other gases to obtain electrons. In fact, *Dehalococcoides* has only five basic, but essential requirements to survive in groundwater, which are: a carbon source, an electron donor, an electron acceptor, vitamin B12, and an oxygen-free environment. All of these five key elements combined enable the microorganism *Dehalococcoides* to biodegrade TCE and clean up the contaminated drinking water. To understand how to make the process more efficient, each of these key requirements must be specified and understood.

Nutrient Requirement For Dehalococcoides

A carbon source is considered the main nutrient or food source for the bacteria. The most common carbon source for *Dehalococcoides* cited in the literature is acetate (9). This compound is usually added in a different form called lactate, which can be broken down into acetate and hydrogen. (10) Because *Dehalococcoides* cannot single-handedly perform every task needed to obtain its nutrient and energy sources, it must rely on the other microorganisms that can perform those processes (11). For example, homoacetogens are a common group of bacteria found with *Dehalococcoides* because they can break down lactate into acetate and hydrogen, which provides an accessible carbon source to *Dehalococcoides* (2).

The electron donor for *Dehalococcoides* is hydrogen, which is produced as a byproduct of lactate fermentation to acetate and hydrogen. The electron acceptor is TCE, which is degraded into dichloroethene and vinyl chloride before being converted to the harmless compound ethene. (7) It is important to note that hydrogen is the only electron donor that *Dehalococcoides* can obtain energy from to

survive. In research, hydrogen is either added by using lactate as the carbon source and having it biodegraded to acetate and hydrogen or by adding it directly (12).

Similar to how humans need trace minerals for growth, bacteria also grow much faster with certain minerals present. In fact, vitamin B12 has been shown to be a vital nutrient in maintaining rapid TCE biodegradation rates (13). Without vitamin B12, *Dehalococcoides* is not able to biodegrade TCE as fast or completely to the harmless compound ethene.

An anaerobic environment is considered the top priority in maintaining a healthy groundwater population of *Dehalococcoides* both in the lab and in the field. In the lab, scientists use an anaerobic glove box, which is a plastic container that vacuums oxygen out and replaces it with a gas mixture of nitrogen and carbon dioxide, to maintain an oxygen-free environment (14). Also, specific chemicals, such as hydrogen sulfide are added to the water to help reduce any oxygen that seeps into the bottle. In most subsurface environments, other bacteria and chemicals naturally reduce oxygen in the groundwater without any outside interference, which provides a conducive environment for engineers and scientists to design an enhanced anaerobic bioremediation system in the field.

Experimental Design

My experiment focused on how sulfate concentrations in groundwater may impact *Dehalococcoides'* ability to biodegrade TCE. Sulfate is commonly found in groundwater and can range from 0 to 630 milligrams per liter (mg/L). The EPA has set the drinking water MCL standard, at 250mg/L because levels greater than that cause laxative health problems (5). MCL's are determined based upon the effects

that chemicals have on humans. This experiment explores whether or not the sulfate MCL present in groundwater has a negative impact on the TCE biodegradation rates of *Dehalococcoides ethenogenes* strain 195. This concentration was chosen because it is the maximum value allowed in drinking water and is a reasonable average that is mostly likely to be present in groundwater.

The experiment used 120 milliliter (ml) serum bottles for the microcosms that contained 90ml of medium and 30ml of headspace. The bottles were each inoculated with 10ml of *Dehalococcoides ethenogenes* strain 195 from a previous reactor that had been maintained for several weeks. Two types of reactors were created; one with 250 mg/L (MCL) of sulfate and one without sulfate. Each reactor was set up as duplicates to provide biological replicates in order to achieve valuable statistical analyses of the data. The four serum bottles were stored in 34°C incubators. Every 5 days the degradation rates of TCE to ethene were measured on a gas chromatograph, which uses a flame to separate the gas particles by weight into distinguishable peaks. These peaks are identified as different compounds and their levels can be marked as the bacteria continue to grow. One hundred microliters (uL) of gas is withdrawn from the serum bottles using a micro syringe and the needle and Teflon stopper are flamed for sterilization purposes. The microcosm bottles were evaluated for 20 days to ensure enough time for the bacteria to reach a plateau in their growth without having significant biomass decay.

Results

Figures 1 and 2 show the chlorinated solvents, TCE and byproducts cis-1,2-dichloroethene (cDCE), vinyl chloride (VC) and ethene versus time in the presence

and absence of sulfate using the microbial consortium *Dehalococcoides ethenogenes* strain 195 (DHC), respectively . The microcosm results indicate that *Dehalococcoides ethenogenes* st. 195 was able to degrade TCE at similar rates regardless of the sulfate level. In other words, the data demonstrated that if sulfate concentrations were at the MCL or lower *Dehalococcoides* were not negatively affected.

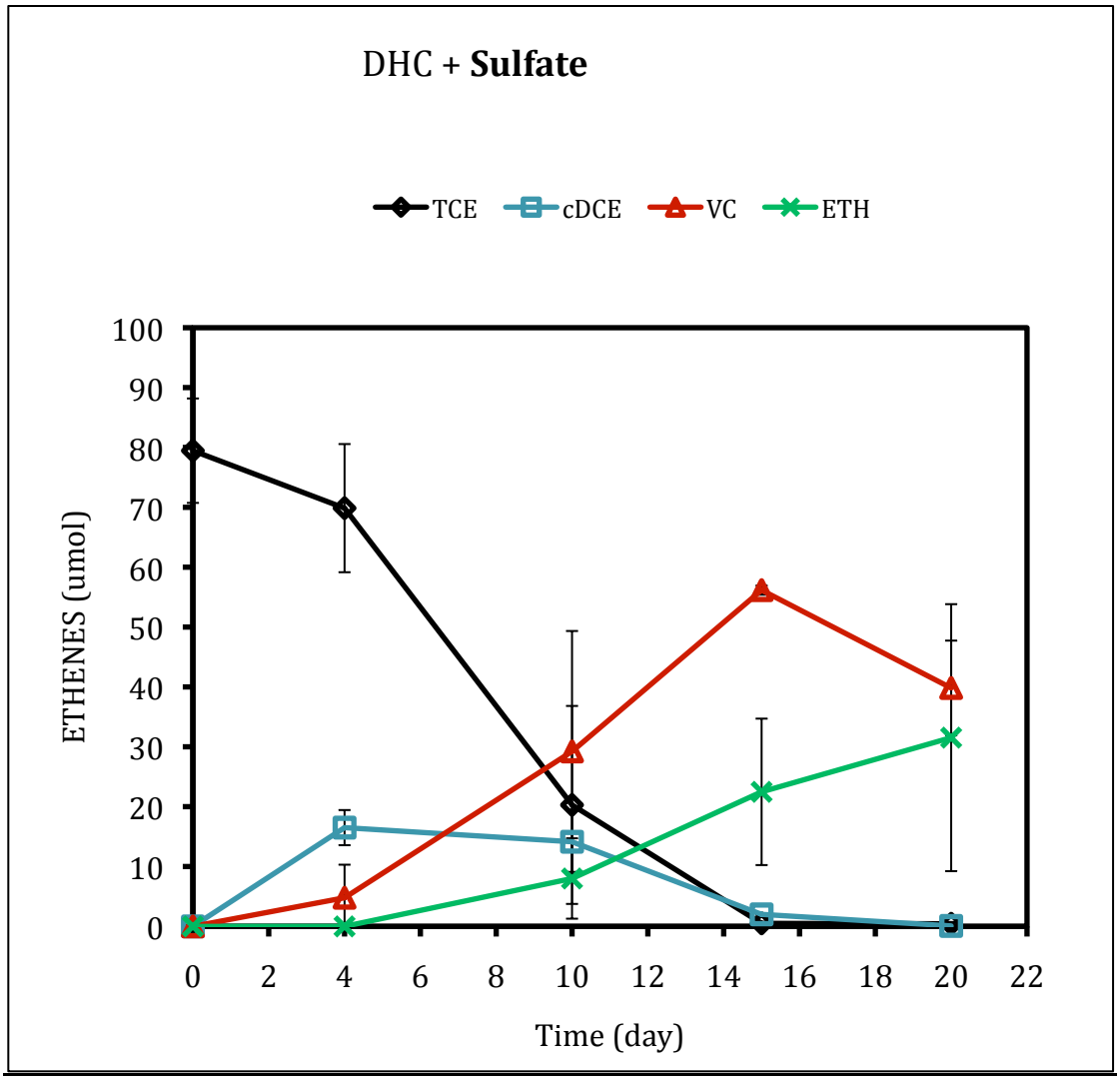

Figure 1. TCE and byproducts cDCE, VC and Ethene versus time in presence of Sulfate

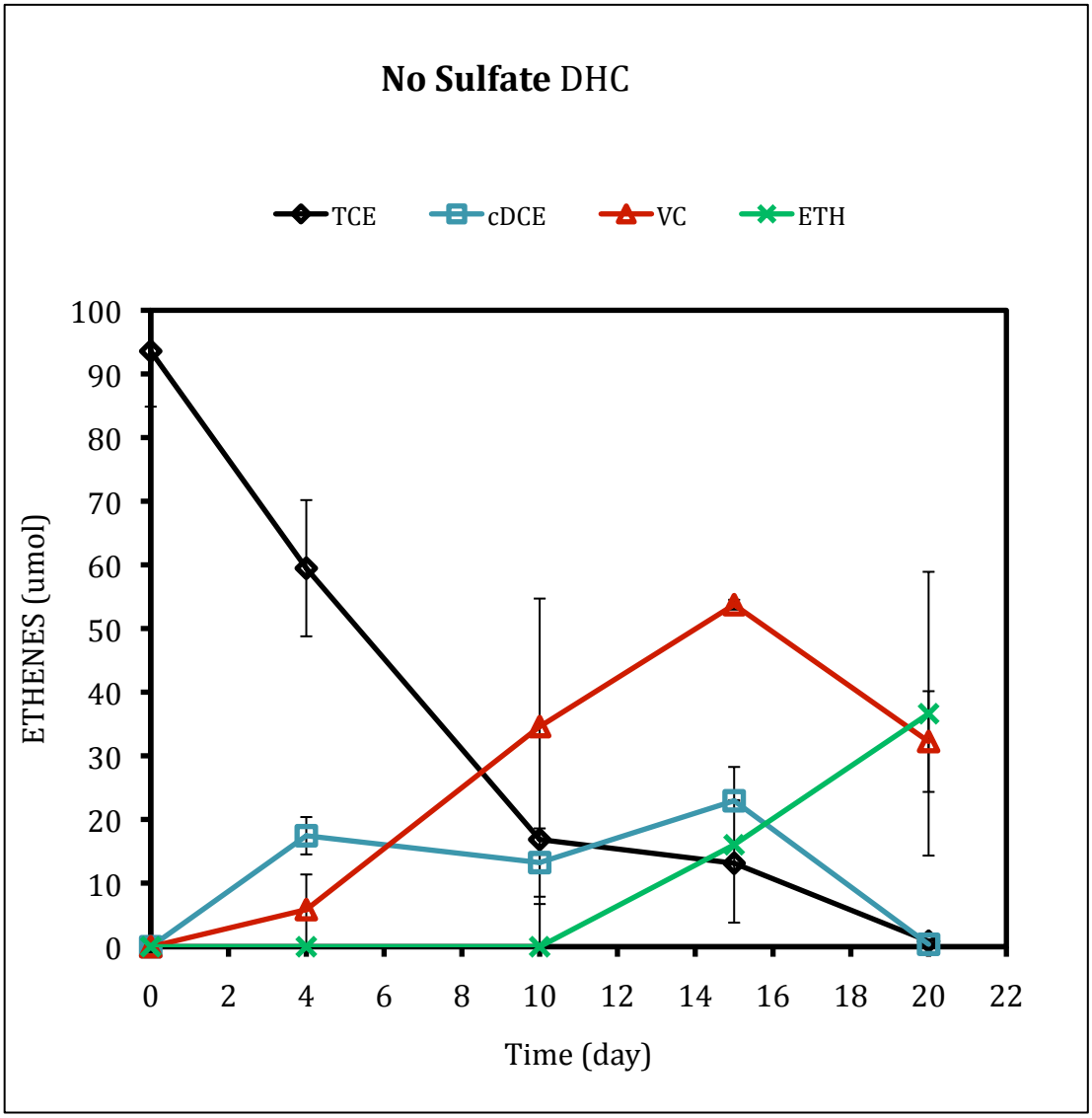

Figure 2. TCE and byproducts cDCE, VC and Ethene versus time in absence of Sulfate

Conclusion

Because the data demonstrate that *Dehalococcoides* is not harmed by 250mg/L sulfate, future designers of remediation systems do not have to worry about decreasing the effectiveness of in-situ bioremediation of TCE if sulfate levels are at the MCL standard. This research helps companies and the government save

money that may have been spent trying to reduce the sulfate concentrations below the MCL when it is actually unnecessary. Knowing how sulfate concentrations impact *Dehalococcoides* helps scientists become one-step closers to fully understanding how to optimize this process.

Future Research

Future research for this project could entail increasing the sulfate concentrations above the MCL. Just because *Dehalococcoides* wasn't impacted by 250 mg/L of sulfate doesn't mean higher concentrations will not have a negative impact. If a site had both, high concentrations of sulfate and TCE, depending on how high the sulfate concentrations were, degradation of TCE may be inhibited. A future experiment could be to set up maintaining four microcosms that all contained TCE and *Dehalococcoides*; two or more would contain sulfate concentrations higher than 250 mg/L and two would again not contain any sulfate. This would provide information as to whether or not sulfate concentrations need to be addressed at a site before TCE degradation can efficiently occur.

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