Effects of Common Methods Used to Generate Anaerobic Water On Bioaugmentation Cultures Containing *Dehalococcoides sp.*

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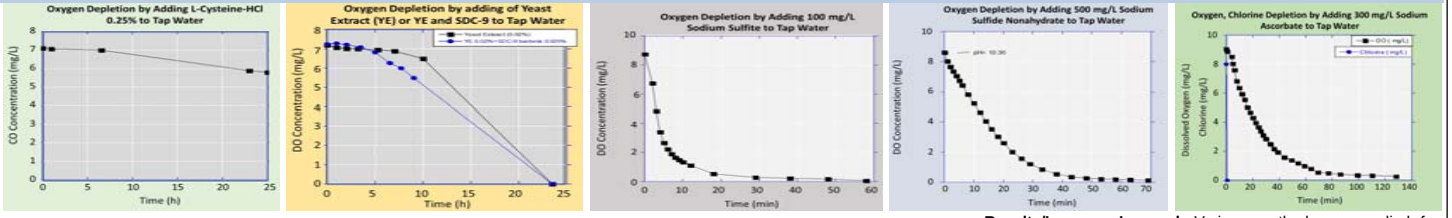
Background/Objectives. The distribution of microorganism to enhance in situ biologically mediated contaminant destruction (bioaugmentation) has become a common practice at many contaminated sites. Bioaugmentation cultures containing the dechlorinating microorganisms Dehalococcoides sp (Dhc) are commonly injected with a solution of organic substrates to enhance reductive dechlorination of chlorinated ethenes (CE) and other contaminants.

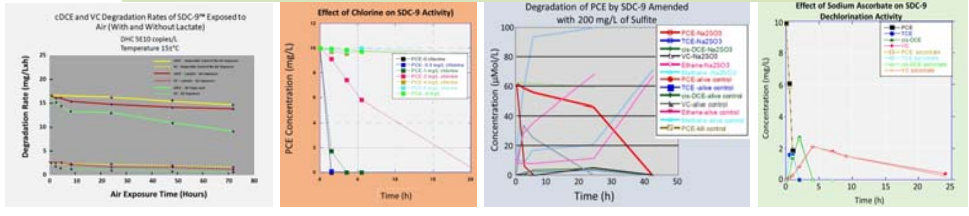
Dhc is an obligate anaerobe therefore, minimizing exposure to oxygen during distribution is optimal for successful treatment. To reduce exposure to oxygen, anaerobic water is usually generated in which the dechlorinating culture is added and distributed through the aquifer. Anaerobic water can be produced biologically or chemically; however, high substrate concentrations and chemical reductants which may be used to generate anaerobic water can be harmful to the culture. In addition, the water used for bioaugmentation solution may contain chemicals, such as chlorine, that are specifically included to inhibit biological activity. The objective of this study is to identify commonly used methods for generating anaerobic water for bioaugmentation and to evaluate the chemicals applied to generate anaerobic water on the effectiveness of Dhc.

Approach/Activities. This study initially evaluated commonly used field methods for generating anaerobic water for bioaugmentation. These methods typically involve either the biological or chemical reduction of dissolved oxygen (DO) to generate a bioaugmentation solution. Biological reduction usually involves the addition of an organic substrate to water containing microorganisms thereby removing the oxygen by aerobic respiration. Potable water is often used to produce this water however, potable water does not contain microrganisms which would biologically remove the oxygen. In such water, chemical reductants such as zero valent iron, sulfite, erythorbate, sorbate, ascorbic acid and sulfide compounds are often used to quickly remove oxygen. Bench tests were conducted to evaluate the effect of DO, high substrate concentration, chlorine, sulfide, sulfite and ascorbate compounds on dechlorination rates by Dhc. Also, the oxygen and chlorine depletion rates were determined for some of the typically used substrates.

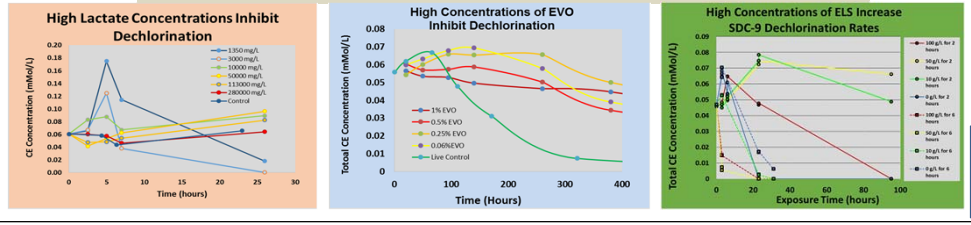
Oxygen Depletion by L-Cysteine-HCL, Yeast Extract, Sodium Sulfite, Sodium Sulfide, and Sodium Ascorbate (+Chlorine Removal)



Effect of Oxygen, Chlorine, Sulfite, and Sodium Ascorbate on SDC-9™



Effect of High Lactate, EVO and ELS® Concentration on SDC-9™



Results/Lessons Learned. Various methods are applied for generation of anaerobic water for bioaugmentation cultures. Large and often costly efforts have been made to eliminate the exposure of Dhc to DO. However, Dhc, has been shown to be moderately tolerant of DO for short periods of time questioning the need for some extreme efforts and potentially creating conditions more inhibitory to Dhc. The effects of exposure to oxygen can be mitigated by the presence of an organic substrate however, dechlorination by Dhc can be inhibited by high concentrations of some organic substrates. Chlorine, even at lower concentration, inhibits dechlorination activity and at concentration about 4 mg/L completely prevents it. There is no one method for generating anaerobic water for bioaugmentation cultures at all sites. The method selected will depend on site specific conditions including available space, time, and cost and other considerations. The most common methods are generally effective for generating and applying bioaugmentation cultures however, these methods can be improved by considering the effect of these parameters on the bioaugmentation culture when generating anaerobic water.

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