## **Carbon + Nutrients = Stronger Bacteria = Faster Remediation**

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**Background/Objectives.** Enhanced reductive chlorination (ERD) through bioremediation is an effective and affordable approach to remove chlorinated compounds such as perchloroethylene (PCE) and trichloroethylene (TCE) from groundwater. ERD is typically achieved by injecting a carbon substrate to stimulate growth of dechlorinating bacteria, either native or added through bioaugmentation. While this approach is successful at some sites, lower-than-expected dechlorination rates and undesirable accumulation of intermediate daughter products such as cis-dichloroethylene (cDCE) and vinyl chloride (VC) can occur if other macro- and micronutrients are not present in adequate amounts. In this presentation, we will review the importance of maintaining optimal amount of nutrients to achieve complete ERD and promote conversion of cDCE and VC to ethene.

Approach/Activities. This presentation covers prior research on the effect of nutrient limitations on ERD and successful application at an industrial site. Diverse laboratory studies have demonstrated the importance of nutrients, such as vitamin B12 and fixed nitrogen, for efficient conversion of cDCE to ethene. Kaya et al. (2019) have shown that *Dehalococcoides* (Dhc) species, the bacteria primarily responsible for degrading cDCE to produce non-hazardous ethene, depends on fixed nitrogen (e.g.  $NH_4^+$ ) as a nitrogen source. Likewise, He et al. (2007) and Yan et al. (2021) have shown that Dhc are not able to synthesize vitamin B<sub>12</sub> and low concentrations of this vitamin can slow cDCE conversion to ethene. This knowledge was applied to a field site where prior substrate addition had stimulated PCE reduction to cDCE, but further conversion to ethene was very limited partially due to aquifer conditions such as low pH and low total Kjehldahl nitrogen (<0.15 mg/L). Nutrient addition in a pilot test resulted in a rapid decline in cDCE and VC, with Dhc levels increasing from 3 to 40,000 copies/mL and VC reductase increasing from 0.2 to 1,460 copies/ml in 2 months, showing that this strategy could contribute to remediation of accumulated daughter products. Therefore, the site was amended with additional nutrients (nitrogen, phosphorus, yeast extract and vitamin B<sub>12</sub>) to achieve complete ERD.

**Results/Lessons Learned.** Full scale injection of nutrients reduced cDCE and VC levels by over 99% and ethene was detected at the source zone, providing evidence that complete ERD was achieved and demonstrating that nutrient addition is sometimes required to remove chlorinated compounds. Thus, supplementing a carbon source with optimal quantities of nutrients to promote growth conditions for Dhc can be an effective strategy for complete, efficient removal of chlorinated compounds.