ISCR-Enhanced Bioremediation
**Summary**

*In Situ* Chemical Reduction (ISCR) enhanced bioremediation is a remediation approach that combines zero valent iron (ZVI), an organic hydrogen donor, and contaminant-degrading microbes to degrade contaminants in soil and groundwater. This approach is most commonly used for chlorinated contaminants including chlorinated ethenes. ISCR-enhanced bioremediation is particularly effective because it stimulates anaerobic biological degradation by rapidly creating a reducing environment favorable to reductive dechlorination. Furthermore, ISCR-enhanced bioremediation may limit the formation of toxic daughter products such as cis-1,2-dichloroethene (cis-DCE) and vinyl chloride (VC) by degrading parent compounds abiotically, or via direct chemical reduction. This tech bulletin describes this remedial approach in more detail and showcases the performance of S-MicroZVI® a sulfated zero-valent iron amendment developed by REGENESIS.

**Background**

*In situ* bioremediation is an established and cost-effective option for managing chlorinated groundwater contaminants. Traditionally, contaminants are treated by adding an organic hydrogen donor (e.g., fatty acids) and allowing anaerobic microbes (native or augmented) to convert the contaminants into harmless end-products. This strategy can be greatly enhanced by the addition of strong reducing agents like ZVI, which create favorable aquifer conditions for contaminant-degrading bacteria as well as directly reacting with many chlorinated compounds. This approach is referred to as ISCR-enhanced bioremediation. Regenesis offers S-MicroZVI® a sulfated ZVI, which facilitates ISCR-enhanced bioremediation and owing to the sulfidation, is longer-lived and more reactive than standard ZVI. S-MicroZVI is a colloidal suspension containing 40% sulfidated ZVI (S-ZVI) by weight with < 5 µm iron particles suspended in food grade glycerol. S-MicroZVI is formulated to be easily injected, transport well in the subsurface during application and be long-lasting.
Enhanced reductive dechlorination (ERD) describes the bioremediation of contaminants by anaerobic bacteria that are supported by the molecular hydrogen produced by fermentation of organic hydrogen donors. The biological degradation pathway for perchloroethene (PCE) and trichloroethene (TCE) is provided in Figure 1. This pathway, also known as hydrogenolysis, involves the sequential replacement of a chlorine atom with a hydrogen atom and is always accompanied by the formation of chlorinated intermediates. Many common anaerobic bacteria can transform PCE to TCE and then to cis-DCE, but only Dehalococcoides ethenogenes (DHC) is known to transform cis-DCE and VC to ethene.

Supplementing dechlorinating bacteria with zero-valent iron and organic hydrogen donors can enable more rapid and complete biodegradation. ZVI quickly deoxygenates groundwater and provides an electrochemically reducing environment that is highly fertile for the microbes involved in anaerobic bioremediation. In many situations this favorable environment can be sustained for several years.

**Figure 1.** Reductive dechlorination sequentially replaces chlorine atoms with hydrogen atoms. The intermediates cis-DCE and VC are more toxic than parent compounds PCE and TCE.

Abiotic Degradation

Beyond the benefits of accelerated bioremediation, ZVI provides an abiotic degradation mechanism involving the direct reaction of ZVI with groundwater contaminants. The abiotic, beta-elimination pathway for chlorinated ethenes is shown in the bottom track of Figure 2. The beta-elimination pathway involves short-lived dichloroacetylene and chloroacetylene intermediates and bypasses the formation cis-DCE and VC intermediates. An ISCR-enhanced bioremediation approach can utilize both the reductive dechlorination and the beta-elimination pathways and reduce the observed concentrations of cis-DCE and VC relative to an approach using ERD alone.
**Abiotic Degradation - Continued**

**Figure 2.** ISCR-enhanced bioremediation allows the degradation of chlorinated contaminants by reductive dechlorination (single-line arrows) or beta-elimination (double-line arrows). Beta-elimination avoids the formation of cis-DCE and VC.

**When to Use ISCR-Enhanced Bioremediation**

ISCR-enhanced bioremediation can be used to treat contaminants such as chlorinated solvents, haloalkanes, and chlorinated pesticides. Contaminants that are resistant to abiotic degradation (e.g., 1,2-dichloroethane, dichloromethane) and compounds that can inhibit bioremediation (e.g., 1,1,1-trichloroethane, chloroform) may be effectively treated by ISCR-enhanced bioremediation. ISCR-enhanced bioremediation can be used for source zones, plumes, and barrier applications.
Column Study Demonstrating ISCR-Enhanced Bioremediation

Study Objective:
The objective of this study was to demonstrate that the use of the combination of S-MZVI, dechlorinating bacteria, and an organic electron donor results in a more complete degradation of TCE with less formation of cis-DCE and VC compared to an approach using only dechlorinating bacteria and an electron donor.

Experimental Setup:
Three Omnifit™ columns, 25 mm in diameter and 500 mm in length, were dry-packed with medium-fine sand (200-500 µm), purged with carbon dioxide for 15 minutes, and filled with deoxygenated tap water. The column conditions were:

- **Sterile TCE control**: Column was sterilized with one pore volume (90 mL) of 200 mg/L sodium azide.
- **Biotic treatment**: One pore volume (90 mL) of deoxygenated lactate/nutrient solution (1000 mg/L sodium lactate, 10 mg/L nutrients) was flowed through the column. Next, an additional pore volume of dechlorinating bacteria solution (10^9 cells/L *Dehalococcoides ethenogenes*, 1000 mg/L lactate, 10 mg/L nutrients, prepared in deoxygenated water) was flowed through the column. The column flow was turned off for approximately 20 hours to allow the bacteria to acclimate.
- **ISCR-enhanced bioremediation treatment**: One pore volume (90 mL) of S-MicroZVI was flowed through the column as a dilute aqueous solution (1 % as iron). The column was then flushed with deoxygenated tap water until the effluent appeared clear. After this S-MicroZVI treatment, the column was prepared in the same manner as the Biotic control column described above.

After the conditioning, TCE was continuously flowed through all three columns as a 2 mg/L solution at a rate of one pore volume (90 mL) per week. The influent for the sterile control contained TCE as well as 200 mg/L sodium azide. The influent for the biotic control column and the ISCR-enhanced bioremediation column contained TCE as well as 100 mg/L lactate and 1 mg/L nutrients. Effluent samples from each column were collected weekly and analyzed by GC-MS for their TCE, cis-DCE, and VC concentrations.

Results & Discussion
The effluent concentration data from the columns are depicted in Figure 3.

The concentration of TCE in the sterile control trended upward for the first 10 pore volumes with no daughter products produced. The biotic column displayed conversion of TCE from the influent to cis-DCE and VC in the effluent. The ISCR-enhanced bioremediation column facilitated the complete removal of TCE from the effluent solution throughout the experiment. Some cis-DCE and VC were eluted during the first 7 pore volumes with a cumulative elution about 40% of the TCE eluted in the sterile column. After 7 pore volumes, no chlorinated ethenes were detected in the effluent solution. These results demonstrate the effectiveness of ISCR-enhanced bioremediation in promoting the complete degradation of TCE and limiting the formation of cis-DCE and VC.
ISCR-enhanced bioremediation combines multiple degradation pathways to promote the rapid removal of chlorinated contaminants from solution. While chlorinated compounds can be slowly degraded using only an electron donor and dechlorinating bacteria, the addition of S-ZVI generates strongly anaerobic and reducing conditions that further enhance biologically-mediated ERD. The presence of S-ZVI also provides a secondary abiotic, beta-elimination pathway. The availability of multiple pathways allows the removal of parent compounds and lessens the potential for the formation of more toxic daughter products.