

# **Characterization of the Intrinsic Biodegradation Potential of an Aquifer Contaminated with Chlorinated Ethenes and Implementation of a Field-Scale Biostimulation Test**

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**Background/Objectives.** The aim of this project was to assess the intrinsic bioremediation potential of an aquifer located in an industrial site in Barcelona (Spain) contaminated with perchloroethylene (PCE) and its breakdown products (trichloroethylene [TCE], cis-dichloroethylene [DCE] and vinyl chloride [VC]) using laboratory microcosm studies, carbon isotope analysis, and Illumina sequencing analysis of 16S rRNA genes. Based on these results, a strategy to enhance reductive dechlorination was selected and implemented to clean up the groundwater through a pilot test.

**Approach/Activities.** Prior to the implementation of the *in situ* pilot test, two sampling campaigns (May and October 2016, 14 monitoring wells in total) were carried out to check whether the aquifer had geochemical and hydrogeological conditions suitable for reductive dechlorination. Intrinsic biodegradation of chlorinated ethenes in the aquifer was investigated by means of carbon isotopic fractionation ( $\delta^{13}\text{C}$ ) along the contaminated groundwater plume. In addition, laboratory microcosms were prepared to evaluate whether native bacteria could completely dechlorinate the chlorinated ethenes present in the groundwater samples, and how different fermentable organic substrates (sodium lactate, a mixture of methanol and ethanol, and a commercial fermentable product) could enhance this biodegradation. Finally, the presence of *Dehalococcoides* and other organohalide-respiring bacteria were assessed using Illumina sequencing. Based on these results, a pilot-scale lactate-injection test was conducted to evaluate the efficacy of biostimulation for remediation of the contaminated groundwater.

**Results/Lessons Learned.** Significant differences in the  $\delta^{13}\text{C}$  of PCE, TCE, and cis-DCE (greater than 2‰) across the different monitoring wells, and closed isotopic balances among these compounds, indicated biological reductive dechlorination processes taking place in the field by autochthonous bacteria. Laboratory microcosms studies show that microcosms amended with fermentable organic substrates exhibited higher dechlorination rates of chlorinated ethenes to ethene. Illumina sequencing was used to confirm the presence of *Dehalococcoides mccartyi* species in groundwater. Following the above-mentioned results, sodium lactate was selected as organic fermentable substrate to perform *in situ* biostimulation. The pilot test started on the 25th October 2016 with the injection of an aqueous solution of sodium lactate containing sodic fluorescein as a conservative tracer through one of the most contaminated monitoring wells. The results showed that addition of lactate lowered the redox potential of the aquifer and dramatically decreased the concentration of oxygen. This promoted transformation of chlorinated ethenes to ethene and provoked a concomitant enrichment in  $^{13}\text{C}$  in the residual fraction of the chlorinated ethenes. After 200 d, ethene was the major product detected in the monitoring well where lactate was injected.