

Integrated Anaerobic–Aerobic Bioremediation of Chlorinated Aliphatic Hydrocarbons in Groundwater

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Chlorinated aliphatic hydrocarbons (CAHs), particularly chlorinated ethenes, are among the most prevalent and persistent groundwater contaminants worldwide. These compounds migrate through aquifers as dense non aqueous phase liquids (DNAPLs), forming long lasting contamination plumes. Their presence is mainly due to improper disposal practices associated with industrial activities. Chlorinated ethenes are toxic, and some of their degradation products, such as trichloroethene and vinyl chloride, are known carcinogens. Biodegradation, both anaerobic (via organohalide respiration) and aerobic (via metabolic or co-metabolic pathways), offers a sustainable and effective alternative to traditional remediation strategies.

This work presents a large-scale bioremediation project at a 33 hectares site in Northern Italy impacted by a historical petrochemical landfill. Groundwater monitoring showed chlorinated ethenes concentrations exceeding legal Italian thresholds by several orders of magnitude (150–300 mg/L). To address this, two sequential biobarriers anaerobic and aerobic (length of 390 meters) were installed perpendicular to the groundwater flow direction to promote stepwise dechlorination.

Prior to implementation, laboratory scale microcosm experiments evaluated different biostimulation strategies: addition of reducing substrates (agro-industrial byproducts), injection of hydrogen, groundwater mixing, and aerobic bioaugmentation. Among these, the addition of molasse as reducing substrate proved to be the most effective in enhancing microbial dechlorination.

At field scale, the anaerobic biobarrier was implemented using molasses, as a carbon and electron donor. Monitoring was carried out every three months over two years and every six months thereafter, using both hydro chemical and molecular analyses.

Groundwater was sampled across northern and southern transects, including piezometers located above the landfill and upstream and downstream of both biobarriers. Microbial community structure was analyzed through Illumina 16S rRNA gene sequencing, while quantitative PCR (qPCR) targeted total bacteria, archaea, *Dehalococcoides*, *Dehalogenimonas* and functional genes involved in reductive dechlorination.

Results confirmed active anaerobic degradation in the anaerobic barrier and aerobic degradation of VC in the downstream aerobic barrier. The northern transect, positioned in parallel to the contamination plume, exhibited higher relative abundances of key organohalide respiring bacteria such as *Dehalogenimonas*, *Desulfitobacterium*, and *Desulfuromonas* after the treatment. These findings are correlated with stronger biomarker quantifications and more efficient dechlorination.

This case study demonstrates the feasibility and long term effectiveness of sequential anaerobic–aerobic bioremediation in a highly contaminated site. The integration of molecular analysis allowed to monitor the microbial response over time and assess treatment efficacy. These findings contribute to improving the design and monitoring of bioremediation strategies for complex groundwater contamination scenarios and emphasize the importance of understanding microbial community dynamics *in situ*.

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