

## Sequential Anaerobic–Aerobic Bioremediation of Chlorinated Ethenes in a Complex Contaminated Site

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In Europe, approximately 650,000 sites require remediation, including about 2,600 in Italy. Chlorinated aliphatic hydrocarbons, particularly chlorinated ethenes, are among the most widespread pollutants, detected in 10% of contaminated groundwater. These compounds can be biodegraded anaerobically (organohalide respiration) or aerobically (metabolic and co-metabolic oxidation). Anaerobic degradation of highly chlorinated ethenes, such as tetrachloroethylene and trichloroethylene often results in the accumulation of intermediates like cis-1,2-dichloroethylene and vinyl chloride, which are more efficiently degraded under aerobic conditions.

In this study, a large-scale bioremediation project is implemented at a 33-hectare site affected by a petrochemical landfill, where chlorinated aliphatic hydrocarbons in groundwater exceeded legal limits by several orders of magnitude. The remediation strategy employs two sequential biobarriers—anaerobic and aerobic—installed perpendicular to groundwater flow. Bio-based substrates were injected to stimulate *in situ* microbial activity, with performance assessed through hydrochemical and microbiological monitoring over a two-year period.

Groundwater samples were collected from northern and southern transects, with piezometers located over the landfill, the anaerobic barrier, and the aerobic barrier. Microbial community analysis, using Illumina 16S rRNA sequencing and qPCR targeting total bacteria, archaea, *Dehalococcoides*, *Dehalogenimonas*, and functional biomarkers, confirmed active reductive dechlorination in the anaerobic zone and vinyl chloride degradation in the aerobic zone. The northern transect exhibited higher relative abundances of *Dehalogenimonas*, *Desulfitobacterium*, and *Desulfuromonas*, along with high presence of functional biomarkers and greater degradation efficiency.

Analysis by GC-MS of anaerobic microcosms set up with groundwater confirmed the microbial degradation of chlorinated ethenes, highlighting different efficiencies in the two transects. Ongoing microbial network analyses aim to assess potential competition between reductive dehalogenating bacteria and iron-reducing, sulfate-reducing bacteria, and methanogens.

These outcomes will contribute to a better understanding of microbial dynamics involved in the bioremediation process, reducing site uncertainties, informing remediation decisions and improving overall bioremediation efficiency.

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