

Chlorinated compounds are among the contaminants mainly found in groundwater. The removal of chloride groups is efficiently carried out by anaerobic bacteria through organohalide respiration. However, dechlorination of vinyl chloride is a limiting step of this pathway. A number of aerobic bacteria are able to perform VC degradation, although, with the exception of the first two enzymatic steps, this pathway, which also leads to ethene degradation, remains poorly characterized.

In this study, an aquifer undergoing a bioremediation process for the removal of chlorinated compounds was considered. After a period of unsuccessful chemical and physical treatments, the combination of two multi-permeable reactive biobarriers was set up. Within a first anaerobic biobarrier, the addition of a reducing substrate aimed at increasing organohalide respiration. Within a subsequent aerobic biobarrier, the addition of air and mineral nutrients (phosphate and nitrogen) enhanced biodegradation of VC.

The presence of native VC-degrading bacterial populations was examined by enrichment cultivation using site groundwater as inoculum and ethene as sole carbon and energy source. Moreover, an environmental genomics approach was applied for *in situ* quantification of *etnC* genes (coding for one subunit of alkane monooxygenase) by qPCR. The ability of the selected bacterial enrichment cultures to degrade ethene (as determined by GC-FID analysis) was differently distributed along the reactive bio-barrier, indicating that aquifer site conditions were variable, as confirmed by physico-chemical characterization. Five ethenotrophs bacterial strains belonging to *Mycobacterium*, *Bacillus*, *Ralstonia* and *Simplicispira* were isolated from the most promising ethene-degrading enrichment culture. The quantification of *etnC* copy number at bio-barrier aquifer evidenced that *etnC*-carrying bacteria were present in the order of  $10^7$  genes  $L^{-1}$ , and increased after one year-long addition of air and mineral nutrients at the site. According the *in situ* chemical data, VC concentration downstream the biobarrier decreased after one year of treatment of one order of magnitude, from 40000 to 2000  $\mu g L^{-1}$ .

These outcomes revealed that the autochthonous microbial populations contributed to a native VC/ethene degradation, which is efficiently stimulated by air and mineral nutrients addition. The isolation of previously uncharacterized bacteria able to use VC/ethene as carbon source encourage future efforts to elucidate the mechanisms involved in VC degradation.