IN FIELD ACTIVITIES OF CHLORINATED ETHENE BIODEGRADATION: SPATIAL EVOLUTION OF THE CONTAMINATED PLUME MICROBIAL COMMUNITY AFTER BIOSTIMULATION

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ABSTRACT

Background information

Chloroethenes are ones of the main contaminants worldwide due to their intensive used in industrial sector as solvents, in particular tetrachloroethene (PCE) and trichloroethene (TCE). These compounds are hardly degraded by chemical and physical treatments, whereas they are efficiently dechlorinated in anaerobic and reductive conditions through organohalide respiration (OHR) pathway by organohalide respiring bacteria (OHRB). These bacteria use chloroethenes as electron acceptors and hydrogen as electron donor to replace each chlorine atom with hydrogen atoms forming ethene as final product. OHR keystone bacteria are *Dehalococcoides* (*Dhc*) and *Dehalogenimonas* belonging to Chloroflexi, and *Geobacter* belonging to Desulfuromonadia. Only *Dehalococcoides mccartyi* strains BTF08 and 195 and *Candidatus* Dehalogenimonas etheniformans are able to completely dechlorinate chloroethenes, indeed, *cis*-dichloroethene (cis-DCE) and vinyl chloride (VC) are hardly dechlorinated and hence are accumulated in contaminated plumes. However, these compounds are efficiently mineralized in aerobic condition by different bacteria: *Mycobacterium*, *Nocardioides*, *Pseudomonas, Rhodococcus* and *Ralstonia* (Mattes et al., 2010).

Bioremediation treatments exploit these two transformation pathways to remediate chloroethenes contaminated sites through their biostimulation. Addition of reducing substrates and air and nutrients enhances OHR and aerobic biodegradation, respectively, thus improving the remediation efficacy (Dolinova et al., 2017).

Main results

In this study, a contaminated aquifer interested by anaerobic and aerobic bio-stimulation treatments was characterized for bioremediation activity and evolution of microbial community along the path of the plume. Reducing substrates derived from food wastes and biorefinery by-products were tested for their ability to enhance OHR.

The OHR enhancement efficacy of three reducing substrates (engineering molasse, by-product of lycopene extraction and whey) was tested in groundwater microcosm set up using landfill water of two piezometers (Pz25 and Pz22). In microcosms with Pz25 water, the presence of substrates increased the dechlorination activity of all chloroethenes after 2 months. After 6 months, the dechlorination of PCE improved whereas the other chloroethenes accumulated in all microcosms. Accumulation of all chloroethenes, except for PCE, persists after 10 months of incubation. Comparison of concentrations after 6 months and after 10 months of incubation showed a slight recovery of OHR activity for the dechlorination of cis-1,2-DCE and VC in microcosms amended with molasse.
In Pz22 microcosms, after 2 months of incubation, all chloroethenes were efficiently dechlorinated except for cis-1,2-DCE in microcosms amended with molasse and control ones. TCE dechlorination showed an improvement in microcosms amended with molasses, instead, VC was faster dechlorinated in microcosms with the addition of by-product of lycopene extraction.

Consequently, for the remediation of the aquifer (17 ha) affected by multiple contamination (hydrocarbons and chlorinated solvents, 5'850 and 219'487μg L^{-1}, respectively) far exceeding law limits (Directive 2000/60/EC), one anaerobic permeable reactive bio-barrier (500 meter long) was established and supplemented by molasse. After about 4 months of operation, TCE and PCE showed a decrease of one

order of magnitude about from 800 μg L^{-1} to 70 μg L^{-1}, but accumulation of DCE and VC occurred (3'700 and 45'000 μg L^{-1}, respectively) at the distal part of the contaminated plume (350 meter apart). The construction of an aerobic bio-barrier (enriched with N, P and air, through injection of 1.97 to 2.05 kg/day of urea, 1.09 to 1.12 kg/day of ammonium phosphate and 1.0-1.8 m^3/h of air) led to the decrease of DCE and VC of one order of magnitude reaching 470 and 4'100 μg L^{-1}, respectively.

Phylogenetic and functional biomarkers, characteristic of dechlorination and aerobic biodegradation pathways, were quantified through qPCR in situ to monitor the effects of biostimulations. OHR biomarkers (*Dehalococcoides*, *Geobacter*, *tceA* and *vcrA*) were present in the order of 10^5-10^7 gene copies L^{-1} also in aerobic biobarrier, underlining the heterogeneity of conditions in the aquifer. Gene copies L^{-1} of aerobic degradation functional gene (alkene monooxygenase, *etnC*), present in the order of 10^6-10^7, increased during the time in aerobic biobarrier, in concomitance with the formation of VC.

Illumina sequencing of 16S rRNA of the aquifer showed that each OHRB were present with a relative abundance of about 1%, except for *Dehalogenimonas* that is present with a relative abundance of about 4%. OHRB and bacteria involved in VC oxidation were correlated with ORP and electric conductivity. OHRB relative abundance increased with more negative value of ORP, instead VC oxidizing bacteria increased in combination with ORP value. On the other hands, OHRB and VC oxidizing bacteria were positively and negatively correlated with electric conductivity, respectively. Beta diversity of bacterial and archaeal community were positively correlated with Mn concentrations. Biostimulation treatments affected the composition of bacterial community. Indeed, the similarity of piezometers bacterial community was influenced more by the biostimulation treatment to which they are subjected than their position in the site. In anaerobic biobarrier, the addition of molasses decreased the presence of methanogens. The treatment determined a modification of the microbial community, which returned to the original composition at the distal part of the contaminated plume, being then primed afterward by the aerobic treatment.

Conclusions

The present study demonstrated that the use of food industry wastes as bio-stimulants is a feasible approach for the enhancement of dechlorination, in the prospective of sustainable bioremediation techniques and in frame with circular economy concepts.

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