Compound-Specific Isotope Analysis and Microbial Molecular Data for Effective Monitoring of a Bioremediation Pilot Trial at a Heavily Contaminated 1,2-DCA Area: Laboratory and Field Results

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Background/Objectives: The monitoring of remedial processes has to be carefully evaluated as target contaminant concentrations might vary for a number of reasons linked not only to degradation processes. During bio-stimulation treatment the injection of significant amount of amendment solution can dilute or displace the contaminants. The use of compound-specific isotope analysis (CSIA) may be used for both qualitative and quantitative estimation of degradation processes. Moreover, CSIA can benefit by the coupling with other powerful tools, such for example microbial molecular analysis. This combined approach was utilized to assess 1,2-DCA degradation in the proximity of a source area at a heavily contaminated site undergoing a pilot scale bioremediation treatment.

Approach/Activities: The proposed approach combined extensive microbiological characterization of the impacted site by microbial molecular methods and CSIA. Site-specific enrichment factors were estimated experimentally on cultures derived from pore water samples from the contaminated area. Monitoring activity during the actual bioremediation period was based on regular sampling (28 campaign over a two-year period) and analysis by gene amplification of the levels of 1,2-DCA dehalogenases and Carbon CSIA. The extension of the 1,2-DCA removal was assessed by the use of target contaminants and byproducts concentration and by C-CSIA data.

Results/lessons learned: Initial levels of contamination by 1,2-DCA at the site reached up to 4 g/L. Pre-treatment CSIA data in the area support natural attenuation processes active at the site, as shown by significant fractionation on ^{13}C at some locations together with a decrease for 1,2-DCA concentration over time. Microcosms with indigenous population resulted in good 1,2-DCA removal over time and an average enrichment factor of -10 ‰.

Microbial population profiling (based on population 16S rDNA metagenomic sequencing and discovery of functional genes involved in dehalogenation of 1,2-DCA) and microbial analysis after cultivation of active dehalogenating consortia were utilized to identify anaerobic species involved in the process of attenuation. While *Dehalogenimonas sp*. was shown to be present at low levels, the major factor in the process was identified as belonging to the *Geobacter* genus. The reaction characteristics of biodegradation correlate in vitro with the activity of a reductive dehalogenase belonging to *rdh* (reductive dehalogenases) group VI. specific qPCR assays were designed to identify and monitor the levels of this gene.

CSIA analysis of 1,2-DCA before and after the injection of amendments has shown specific trends of isotopic enrichment with pattern specific for different areas in the treated zone. The integration of the qPCR data, the analytical data on the levels of contamination and the isotopic data has been utilized to guide treatment timing and modification and to construct a solid background for the interpretation of the results of the treatment in the area of influence. An average contaminant removal from 80 to 100 kg was estimated by 1,2-DCA and ethylene concentration data while more conservative but consistent estimation between 70 to 90 kg where confirmed by C-CSIA data.