1. FINAL PUBLISHABLE SUMMARY REPORT

Halogenated aromatic compounds such as highly halogenated benzenes, dioxins and biphenyls (PCBs) are toxic, persistent and widely distributed organic pollutants strongly affecting environmental quality and economical value of many grounds and aquifers throughout Europe. As found for the transformation of perchloroethene (PCE) to benign products, these highly halogenated aromatics are exclusively transformed by anaerobic bacteria of the *Dehalococcoides* cluster. Although *Dehalococcoides* species have been isolated and physiologically described, many aspects of their physiology and a procedure for easy mass cultivation remains unknown. Central research objective of the proposed project is therefore the detailed description of physiological capacities of a *Dehalococcoides* strain (strain CBDB1) using isotopic and proteomic technologies. Strain CBDB1 is a highly remarkable bacterium as it was the first strain for which reductive dehalogenation of chlorinated benzenes and highly toxic chlorinated dioxins was shown. In addition, several other compounds such as chlorinated phenols and chlorinated biphenyls are used as terminal electron acceptor by the strain, thereby allowing the strain to grow and reducing the toxicity of the compounds.

During the ISPADEHAL project we have characterized strain CBDB1 in regard to the use of chloroethenes as electron acceptors. We observed that reductive dechlorination of perchloroethene (PCE) and trichloroethene (TCE) resulted in the accumulation of *trans*-dichloroethene (DCE) and *cis*-DCE in a ratio of ~3:1, which interestingly differs from the ethene production observed for most *Dehalococcoides* strains. The use of both PCE and TCE as electron acceptors was demonstrated after observing increasing cell numbers of strain CBDB1 by epifluorescence direct microscopy after different additions of either PCE or TCE. Strain CBDB1 failed to grow with other tested chloroethenes such as vinyl chloride, 1,2-dichloroethane; 1,2-dichloropropane, *trans*- and *cis*-DCE. We also studied the carbon isotope fractionation of PCE and TCE by strain CBDB1 since it provides valuable information to further distinguish between abiotic and biotic removal of chlorinated compounds in the field. Our results showed that dechlorination of PCE and TCE were accompanied by a change in the isotope composition of the chlorinated compounds. The enrichment factor of TCE (εC=-11.2) was within the range of previously reported values for TCE dechlorination by other *Dehalococcoides* species although the *tceA* gene responsible for ethene generation in the latter cultures was absent in strain CBDB1. On the contrary, the enrichment factor of PCE (εC=-1.6) was 3.8-times lower than that obtained for *Dehalococcoides* sp. strain 195 although both strains shared a high similarity in the *pceA* gene responsible for PCE dechlorination in strain 195. In addition, the product-related enrichment factors for TCE dehalogenation were calculated based on product isotope signature of the two accumulated products *cis*-DCE ($\varepsilon C_{TCE \rightarrow cis\text{-}DCE}$ = -11.0) and *trans*-DCE ($\varepsilon C_{TCE \rightarrow trans\text{-}DCE}$ = -15.9). We have performed a variety of biochemical tests to identify the key enzymes involved in PCE and TCE dechlorination including electrophoretic techniques (native gel electrophoresis) and gel-free shot gun

proteomics. However, several dehalogenases were expressed during growth with PCE and TCE and therefore it was not possible to assign the responsibility of dechlorination to one specific enzyme. A second part of the project was the use of protein-based stable isotope probing to investigate the carbon metabolism in strain CBDB1 by using two different analytical techniques: gas chromatography-mass spectrometry and LTQ-Orbitrap. The knowledge on carbon flux distributions is important for understanding and targeted optimization of growth in strain CBDB1 for future bioremediation purposes. The bacterium was cultivated either using ^{13}C-labeled sodium acetate ([1-^{13}C]- and [2-^{13}C]-acetate) with unlabeled bicarbonate or with unlabeled acetate and [^{13}C]-labeled bicarbonate. The analysis of protein amino acids by GC-MS resulted in the elucidation of the labeling pattern of 10 amino acids. However, there are some bottlenecks in the use of this analytical technique: i) the exclusion of some amino acids such as cysteine, tryptophan due to their degradation during hydrolysis of biomass, ii) histidine, arginine, lysine, and proline can not be measured precisely due to the very weak spectrum signals, and iii) asparagine and glutamine are converted to aspartate and glutamate during the protein hydrolysis. The analysis performed by LTQ-Orbitrap extent the number of amino acids capable of being identified.