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## PRELIMINARY CHARACTERIZATION OF TCE AND TeCA CO-METABOLISING AEROBIC CULTURES UNDER SUSPENDED AND IMMOBILIZED FORM

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### Abstract

Chlorinated aliphatic hydrocarbons (CAHs) are toxic and environmental hazardous solvents increasingly detected in soil and groundwater for which new and most effective bioremediation approaches are needed. In this context, the European project MINOTAURUS is aiming to develop groundwater bioremediation robust and reliable strategies based on the use of immobilized biocatalysts. This work was focused on the enrichment of aerobic trichloroethylene (TCE) and 1,1,2,2-tetrachloroethane (TeCA) co-metabolizing microbial cultures from a contaminated site in Rho (MI, Italy), their preliminary characterization both as freely suspended and immobilized biomass on different carrier materials (Biomax<sup>®</sup>, Biopearl<sup>®</sup>, Biomech<sup>®</sup>, Cerambios<sup>®</sup>) and the selection of the best performing culture to be employed in a continuous Packed Bed Reactor (PBR) process.

The best performing culture was obtained through serial enrichment on filter sterilized site-water or a similar synthetic water in the presence of butane (2 mg/L) as carbon and energy source and TCE (10 mg/L) plus TeCA (3 mg/L). The structure and composition of the microbial population was carried out through denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA genes. The DGGE analysis outlined that the structure of the suspended microbial community changed remarkably throughout the enrichment with the exception of the most prominent phylotype. However, according to the Dice similarity coefficient, lower changes occurred during the last sub-culturing step, indicating a gradual stabilization of the community structure. When immobilized on different carriers, the most prominent phylotype previously detected represented a minor fraction of the immobilized community; conversely, several of the minor bands occurring in the suspended culture became dominant within the biofilm. Such a drastic change in the structure of the microbial community was evidenced by the separate clustering of the DGGE profiles from suspended cell cultures and biofilms. Furthermore, the high similarity indexes between the communities immobilized on different carriers (57.3% to 73.7%) suggest that the evolved consortium is quite stable and that the material and shape of the carrier do not affect remarkably its composition. Complementary information obtained from degradation kinetic tests showed that the selected culture immobilized on Biomax<sup>®</sup> retained the highest degrading activity. Identification of the community members via band sequencing and phylogenetic analysis is in progress and will allow to obtain key information on the potential TCE and TeCA co-metabolizing species both in the suspended and in the immobilized communities.

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