DETERMINATION OF A BACTERIAL CONSORTIUM WITH REDUCTIVE DECHLORINATION.

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Introduction. Some bacterias converted tetrachloroethene (PCE) to trichloroethene (TCE), to *cis*-1,2-dichloroethene (cis-DCE), to vinyl chloride (VC) and finally the nontoxic final product ethane by their anaerobic respiration (dehalorespiration) using the compounds as the terminal electron acceptors[(1)]. The PCE and TCE are used as solvents in the dry-cleaning and as metal degreasing agents those are groundwater contaminants and affect human health; cis-DCE is used for obtained VC that are used for make plastics. In other way the use of this knowledge is the bioremediation.
This work has the intention to identified the bacterias from a consortium and try to explaine how the consortium works.

Methods. From samples of bioreactors with marine sediments from a hydrothermal vents from "Punta Mita" Nayarit, Mexico, RNA were extracted using the Tripure Reagent Kit (Roche applied sciece), the design of the initiators for the 16S rDNA and the deshalogenases in GENBANK (http://www.ncbi.nlm.nih.gov/genbank/), the sequences were amplified with RT-PCR, the amplicons were cloned into $CaCl_2$ competent TOP-10 *E. coli* and were sequenced.

Results. The amplicons sequenced by Crustal W, have more than 98% of similitude with the sequences of the bacterias that are reported with dehalorespiration. The analysis of the sequences by Crustal W., only identified the genus of the bacterias, the four bacterias that are identified in the consortium are:

Desulfomicrobium sp,
Clostridium sp.,
Sulforospirillum sp.,
and *Desulfovibrio sp.*

Conclusions. In the consortium, the bacterias have different capability of reduction, in the Fig1, the reductive dechlorination pathways are showed. *Clostridium sp*., and *Sulforospirillum sp*., only reduce until *cis*-DCE.

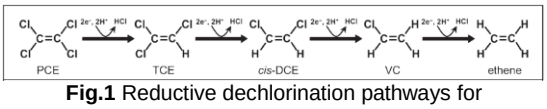

Fig.1 Reductive dechlorination pathways for chloroethenes by the dehalorespiring bacteria (Johnson et al., 2005) [(1)]

Another important difference between the bacterias is the different enzymes that they used for catalyzed the reductive dechlorination. There are information available of the dehalogenases which are monomeric enzymes with molecular masses around 50 kDa or 60 kDa[(2)], an exception is the *Clostridium bifermentas*[(3)] with a subunit molecular mass of 35 kDa. In this work the new step is found the enzymes that will give information of the reductive dechlorination.

References.

1. Taiki Futagami, et al. (2007), *Biochemical and Genetic Bases of Dehalorespiration*, The chemical record, Vol. 8, Issue 1. Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Japan. pag 1-12.
2. Maillard J., Schumacher W., Vazquez F., Regeard C., Hagen W. R., and Hollinger C. (2003) *Characterization of the Corrinoid Iron-Sulfur Protein Tetrachloroethene Reductive Dehalogenase of Dehalobacter restrictus*. Applied and Env. Micrrobiology. ENAC-Laboratory for Environmental Biotechnology, Swiss Federal Institute of Technology Lausanne, The Netherlands. 4628-46382.
3. Okeke, B. C., Y. C. Chang, M. Hatsu, T. Suzuki, and K. Takamizawa. (2001). *Purification, cloning, and sequencing of an enzyme mediating the reductive dechlorination of tetrachloroethylene (PCE) from Clostridium bifermentans*. DPH-1. Can. J. Microbiol. Department of Bioprocessing, Gifu University, Japan. 47:448–456.
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