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BACAd
Bioaugmentation with optimized in-situ culture propagation

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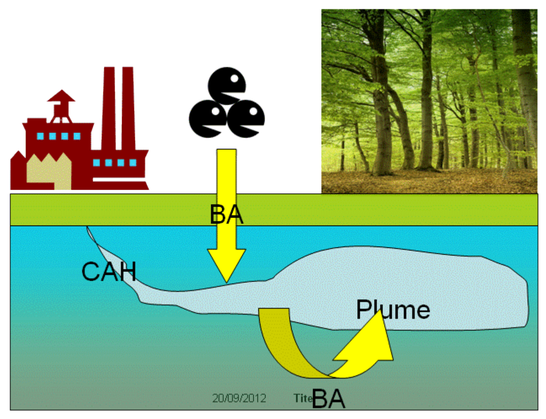
INTRODUCTION

A metal processing site in Flanders, Belgium is characterized by a groundwater contamination with chloro-ethenes that has migrated 1 km off-site and at a depth of 50 mbg. The groundwater has a high seepage velocity and is acidic. Laboratory feasibility tests have indicated that enhanced natural attenuation by addition of an organic substrate induces only partial dechlorination of PCE which stalls at cis-DCE. The main objective of the EU-LIFE+ sponsored project BACAd is to demonstrate that bioaugmentation can be achieved on full-scale in a cost efficient way by optimizing the propagation of injected cultures. This will be realized by on-site groundwater transfers.



SCREENING AND GROWTH OF MICROBIAL CULTURES

Laboratory microcosms

•Demonstrated complete dechlorination of PCE with bio-augmentation in the presence of the electron donors nutrolase (residue from potatoe processing) and glycerol (from biodiesel) .

•With only ED added and pH-buffering, dechorination stalled at cis-DCE after 6 months.

•Five cultures achieved complete dechorination of PCE to ethylene, with slightly different rates.

•Nutrolase (protamylasse) was slightly superior to glycerol because it causedlless acidification of the groundwater.

Microbial cultures

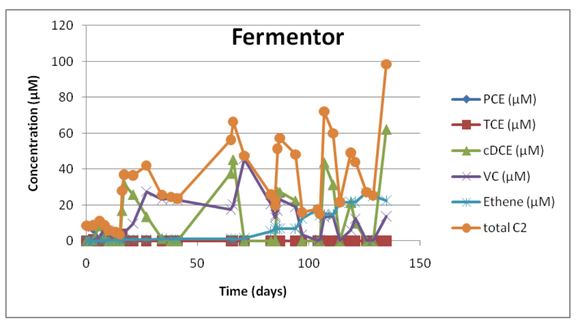
Two best cultures grown for field application:

2 x 50 L for 2 push-pull tests
Culture 1 + Nutrolase
Culture 2 + glycerol

1x 100 L for small scale test 1
grown on glycerol

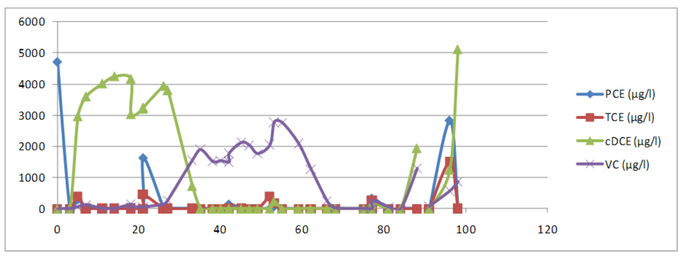
1x groundwater from other site for SCT2

First culture grown on the substrate nutrolase was contaminated by pathogenic bacteria. This contamination was caused by the nutrolase. The in-situ evolution of the pathogens in the field will be monitored.



A second culture has been grown successfully on glycerol for performing the second push-pull test.

Culture properties:
•Ethene production at day 90 = 220 µg/L/d
•Gene copies DHC : 2,8E+06 /mL
•Free of pathogens, except for SR-Clostridia



PILOT SCALE FIELD TESTS

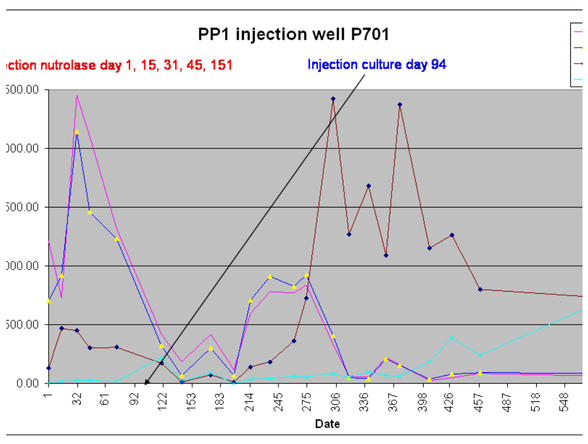
Push-pull tests

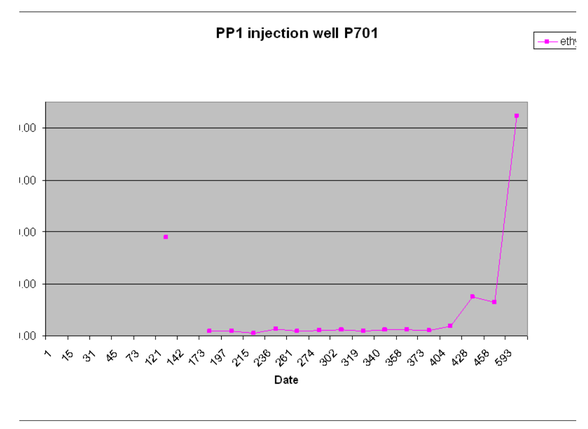
PP1 : Batch Injections of Nutrolase started in 12/2010 (5x in 6 months, 450L)
Injection bacterial culture 1: 09/03/2011

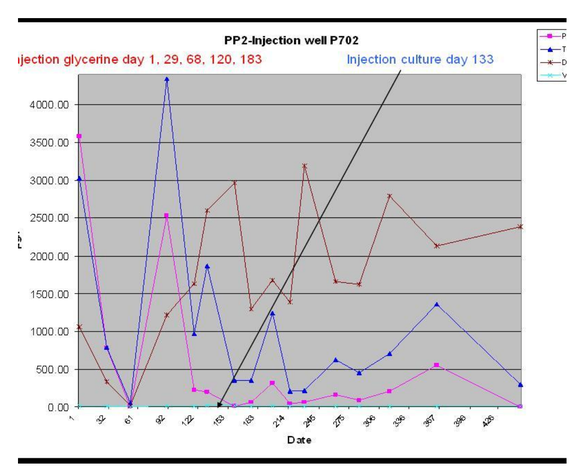
PP2: Batch injections of glycerol-NP started in 05/2011 (5x in 6 months, 76L)
Injection bacterial culture 2: 07/09/2011

Setup:
-1 IF and 1 MF in each test area
-Filter screens 16-22 mbg
-Extract & inject 1 m³ GRW (N2)

Challenges:
-High GRW-velocity (0,5 m/week)
-Low pH → Na2CO3 and NaHCO3 addition







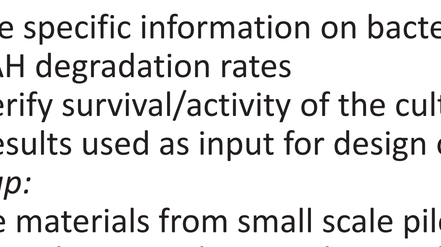
Sulfate reducing clostridia and fecal streptococci persist ed→ Evolution when TOC gets exhausted?

Sulfate reducing Clostridia "normal presence" in anaerobic soils? Microcosms from CAH-sites without BA : several were positive for Clostridia

Small scale field tests

Two larger scale field tests using glycerol as substrate

each pilot 4 injection wells and 7 monitoring wells



SCT1 with best culture of push-pull tests + glycerol
SCT2 with groundwater from other site with succesful remediation of CAH

Start injections of ED+bicarbonate/NP for SCT1: september 2011; culture injected in july 2012

Start injections of ED+bicarbonate/NP for SCT2: February 2012

MICROBIAL TRANSFER METHOD

Column test for design of microbial transfers

- Site specific information on bacterial migration and CAH degradation rates
- Verify survival/activity of the cultures that are injected
- Results used as input for design of full scale system

Setup:
•Site materials from small scale pilot test area
•Two columns with two cultures of push-pull field tests
•Migration of cultures in columns measured by Q-PCR

•Breakthrough of DNA measured by QPRC(vcrAB) for Inoculated columns C1 + C3 :
- for C1 after flushing with 1,5 Pore Volume
- for C3 after flushing with 2 PV

•Degradation of TCE in soil columns:
•Inoculated columns C1+C3 : PCE → ethene
•Control column C2 : PCE → cDCE (stall)

Groundwater transfer system

Transfer bacterial populations from small scale bioaugmentation test areas to other zones

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