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EFFECTS OF REDUCING SUBSTRATES ADDITION ON CONTAMINATED AQUIFER MICROBIAL POPULATION AND ON DECHLORINATION ACTIVITY

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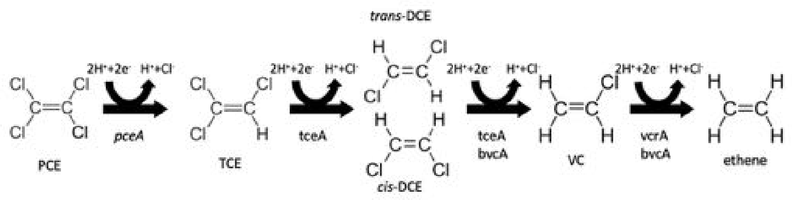
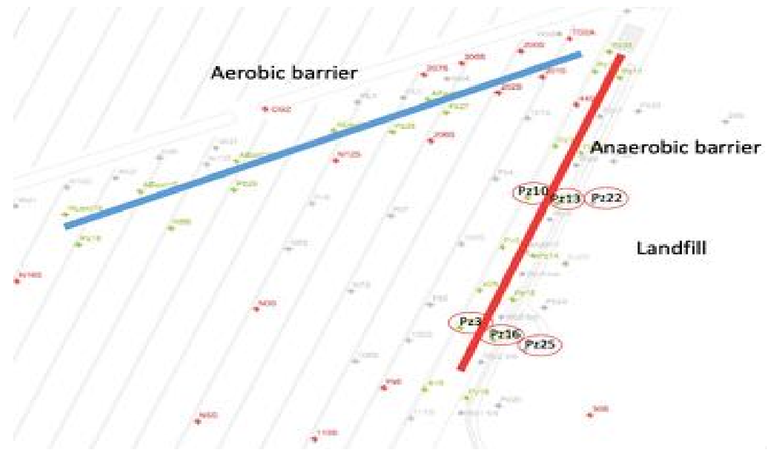
Chlorinated solvents are widely found as contaminants in groundwater worldwide. Tetrachloroethene (PCE) and trichloroethene (TCE) are intensively used in industrial sector and for dry-clean products. These compounds are recalcitrant to traditional chemical and physical remediation treatments. But they are efficiently dechlorinated by bacteria through organohalide respiration (OHR). OHR needs reductive conditions that can be created through bioremediation technique of biostimulation by the addition of reducing substrates that can be developed from agri-food wastes.



AIMS

- To test the effects of two reducing substrates (molasse and tomato extract) on OHR activity in contaminated groundwater microcosms.
- To monitor at filed scale the effect of addition of molasse in a contaminated aquifer near a landfill.



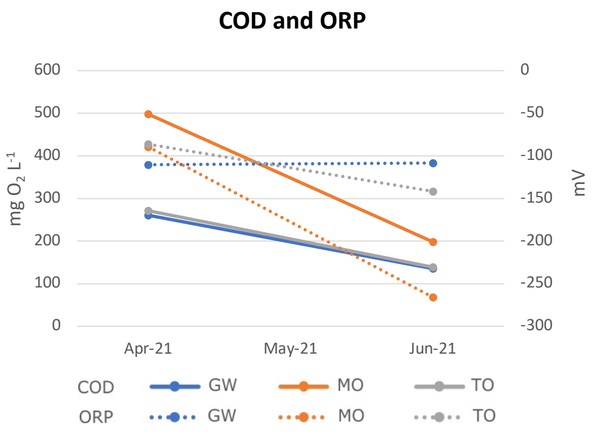
METHODS

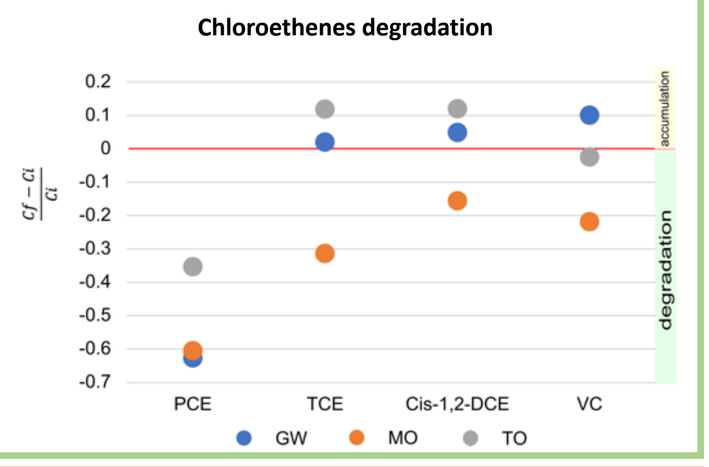
MICROCOSM SCALE: anaerobic microcosms were set up with the groundwater supplemented or not with molasse and tomato extract (with a final COD of 160, 498 and 271, respectively for no integration, with molasse and with tomato extract). Chloroethenes were analyzed by GC-MS.

FIELD SCALE: chloroethenes concentrations were analyzed through GC-MS. Real Time q-PCR of phylogenetic (*Dehalococcoides* and *Geobacter*) and functional biomarkers (two reductases, *tceA* and *vcrA*, involved in the dechlorination of highly and lower chlorinated ethenes, respectively) was applied to DNA extracted from the landfill (Pz22 and Pz25) and the biobarrier (Pz13, Pz16, Pz10, Pz3). Illumina sequencing was used to assess the microbial community composition.

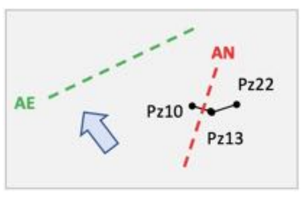
RESULTS

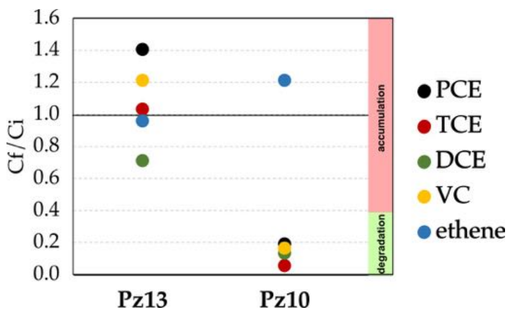
In all the three thesis, after two months of incubation, COD decreased. ORP decreased efficiently only in the microcosms with the addition of molasse.
PCE was efficiently dechlorinated in all microcosms. Molasse enhanced dechlorination of TCE, *cis*-1,2-DCE and VC, whereas tomato extract slightly affected dechlorination of VC.
Molasse was then chosen for field experiments.

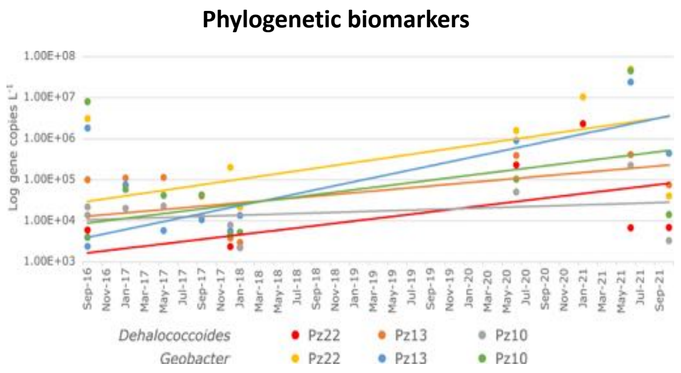


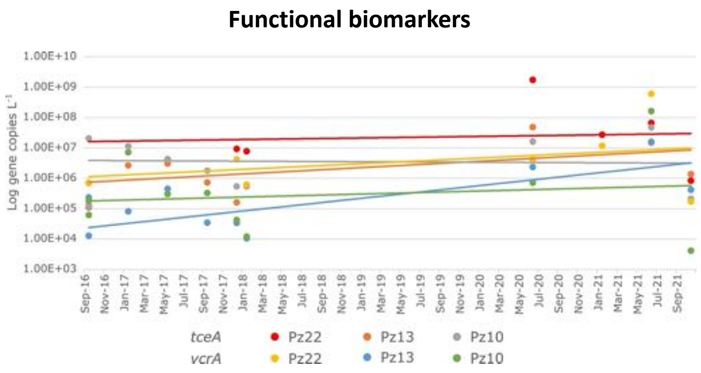


At field scale, the addition of molasse increased dechlorination of all chloroethenes (Pz10) with an accumulation of ethene.
Phylogenetic and functional biomarkers in anaerobic bio-barrier showed an upward trend. Functional biomarkers (*tceA* and *vcrA*) were higher of about 1 order of magnitude than phylogenetic biomarkers.

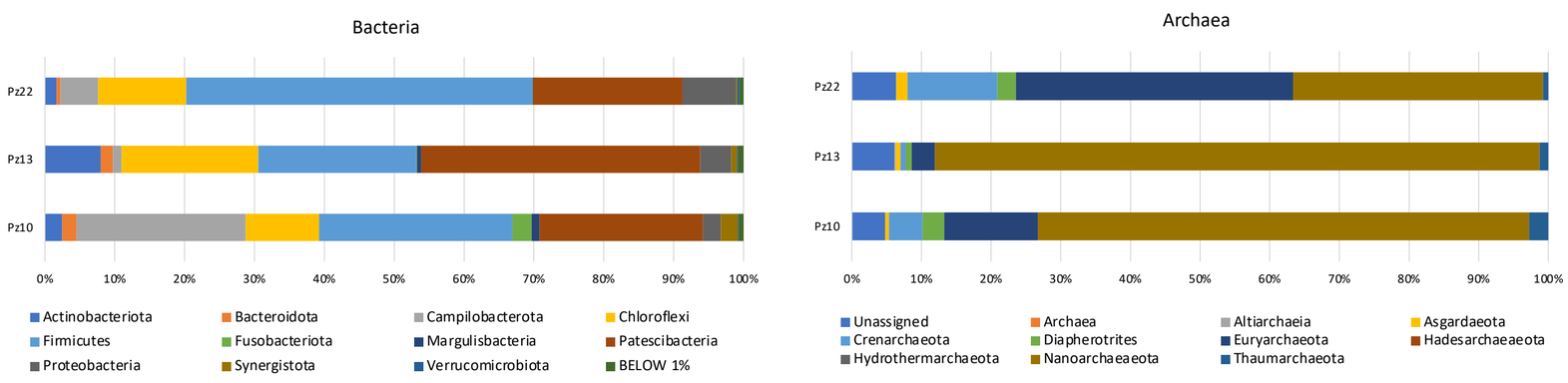








Bacterial community present in the aquifer was characterized by *Chloroflexi* and *Firmicutes,* OHRB phyla, that were the most abundant phyla (~14% and 33%, respectively). Known OHRB relative abundance was lower than 1%. In addition, *Patescibacteria,* suggested fermentative bacteria, were present with a relative abundance of 28%.
Nanoarchaeaeota, in particular *Woesearchaeia* order, was the prevalent phylum in Archaeal community present in anaerobic barrier groundwater (79%).
Woesearchaeia are poorly characterized, they belong to DPANN superphylum and have a potentially fermentative metabolism. In the landfill, *Nanoarchaeaeota* showed a similar relative abundance (39%) of *Euryarchaeota*, known methanogens Archaea. Instead, this phylum had a relative abundance low of 14% in the biobarrier.



CONCLUSIONS

A native organohalide respiration activity was already present in the aquifer, but the addition of reducing substrates enhanced the dechlorination.
The positive effects of molasse addition on organohalide respiration activity were observed at microcosms scale but also at field scale.
The addition of molasse enhanced OHR biomarkers.
In the bacterial community *Chloroflexi*, *Firmicutes* and *Patescibacteria* were the predominant phyla. The addition of reducing substrate increased the presence of fermentative microorganisms.
The low presence of known OHR bacteria suggests that other dechlorinating microorganisms might be present at the site. Further analyses are ongoing.

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