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INTERSOL 2009

Accelerated bioattenuation of solvents. Feasability study by aquifer reconstruction.

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Layout

- Introduction
- Context of the study
- Experiments
- Results
- Conclusion/perspectives

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Nature of pollution

- 10 major pollutants observed in France

Hydrocarbons	**39.28 %**
Pb	**17.19 %**
PAH	**16.92 %**
Cr	**14.55%**
Halogenated solvents	**13.83 %**
Cu	**13.51 %**
As	**11.46 %**
Zn	**10.47 %**
Ni	**9.39 %**
Cd	**6.11 %**

From PhD thesis MC Guillot de Suduiraut (2005)

Attenuation alternatives

Attenuation Mechanisms

- Physical mechanisms
  - Dilution/dispersion
  - Sorption
  - Volatilization
- Chemical (abiotic) mechanisms
  - Oxidation
  - Mineralization
- Biological mechanisms = bioattenuation
  - Reductive dechlorination (adapted to chlorinated solvents)
  - Co-metabolism

Natural Bioattenuation

- Naturally occurring processes in soil and ground water that act without human intervention to reduce the mass, toxicity, mobility, volume or concentrations of contaminants

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Accelerated Bioattenuation

- Microbial ecology -- community of microbes adjusts to presence of pollutant and expresses enzymes to degrade the pollutant
- Microbes gain their energy through electron transfer
- MNA based on understanding hydrogeology, contaminant chemistry and degradation processes

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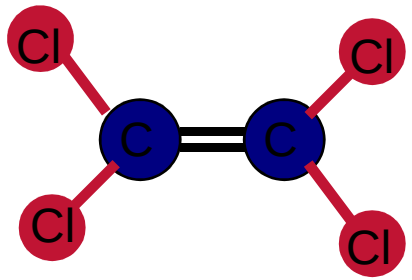
Requirements

- Site assessment - hydrogeology, geochemistry, microbiology
- High tech approaches - sampling, analytical, modeling techniques
- Prediction of plume behavior
- May be combined with source/hot spot control
- Containment of dissolved plume
- A risk management strategy

Compounds we'll focus on

- Synthetic organic hydrocarbons in which one or more hydrogen atoms have been replaced by chlorine atoms (a.k.a. chlorinated volatile organic compounds – CVOCs)

- Chlorinated Aliphatics
  - Perchloroethene (PCE)



PCE

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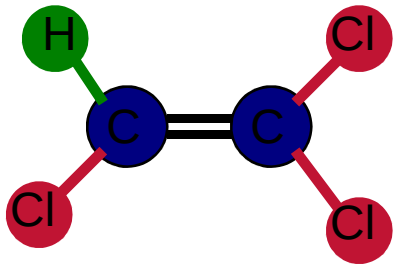
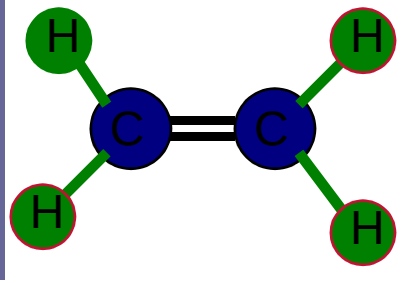
Compounds we'll focus on

- Chlorinated Aliphatics
  - Trichloroethene (TCE), cis-dichloroethene (DCE), vinyl cloride (VC),…

TCE can be "parent" solvent or a reductive dechlorination "daughter" of PCE





Ethene is a fully dechlorinated, non-toxic "daughter" of chlorinated ethenes

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Where do they come from?

- Chlorinated solvents (CVOCs) are commonly used as:
  - Industrial degreasers
  - Dry-cleaning agents
  - Septic system cleaners
  - Solvents to dissolve other substances (glue, paint)
  - Pesticide ingredients

- In common household products
  - Drain cleaners, printing inks, spot cleaners, stain repellents, shoe polishes, glues, and aerosol sprays

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Why do we care about them?

- CVOCs are persistent dense non-aqueous-phase liquids (DNAPLs) –
- CVOCs have varying degrees of toxicity
- Possible human exposure routes:
  - Direct contact (e.g., absorption through skin)
  - Ingestion (e.g., eating or drinking contaminated substance)
  - Inhalation (e.g., breathing CVOC vapors)

Disadvantages of CVOCs

(1)

- DNAPLs, which are the source of CVOCs:
  - Move with gravity rather than with groundwater flow
  - Tend to move in “blobs”
  - Bind to clay minerals
  - Dissolve very slowly into groundwater
  - Volatilize as vapors
  - Are subject to low groundwater cleanup criteria

Disadvantages of CVOCs
(2)

Therefore, DNAPLs

- Are difficult to fully characterize in the subsurface
- Persist in the environment for very long times
- Act as long-term sources for groundwater and vapor contamination
- Pose significant remediation challenges for currently available engineered cleanup technologies
- *Few, if any, DNAPL sites have been fully restored after 25+ years of cleanup*

Toxicity evaluation

- The drinking water limit for a common chlorinated solvent is 5 μg/L (5ppb) (Due in part to it's carcinogenic nature)

- **TCE:** suspect carcinogen. can affect kidneys, liver, and lungs, and can cause rapid and irregular heartbeat that can cause death. Causes skin, eye, and mucous membrane irritation.

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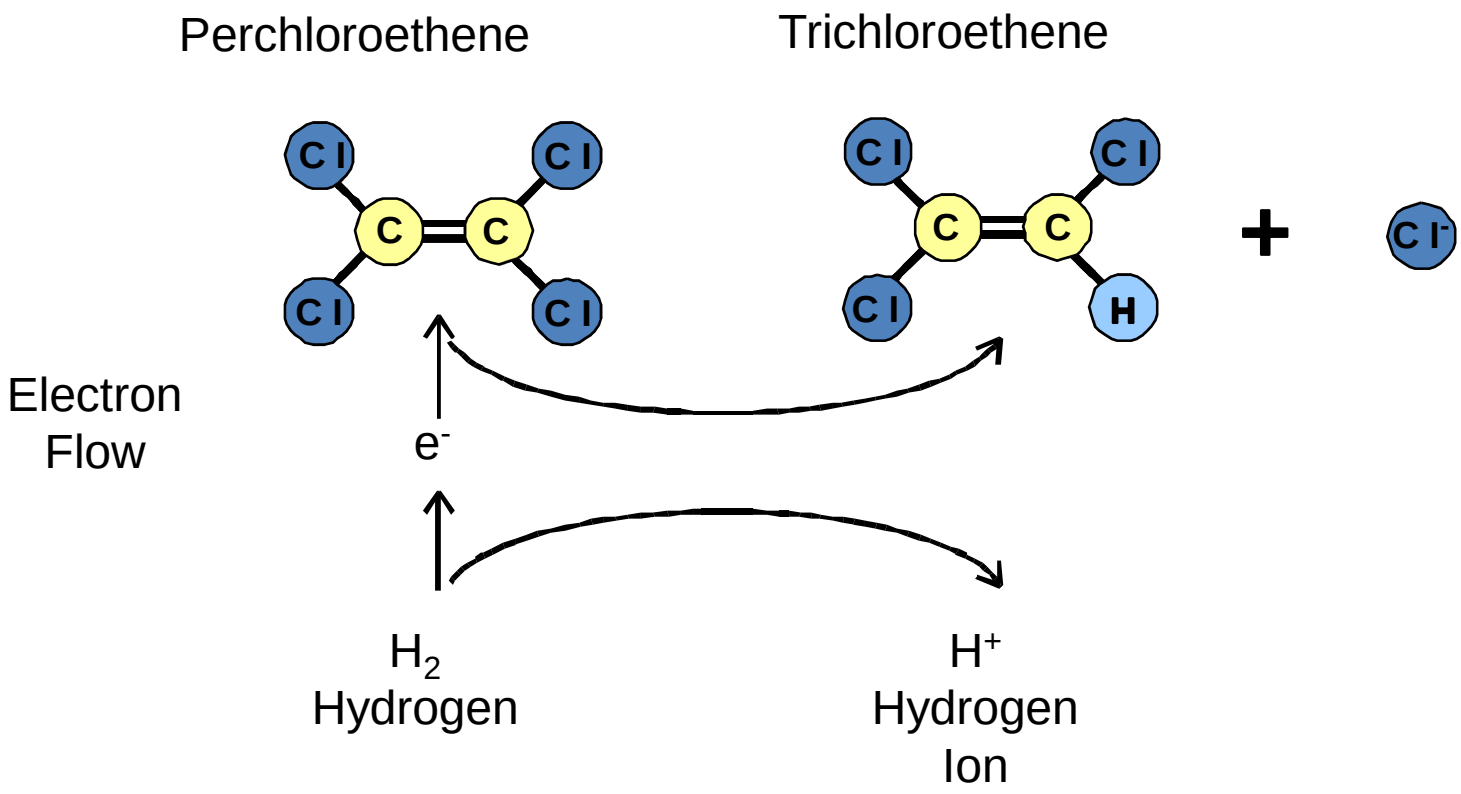
Biodegradation properties

- The common chlorinated solvents, PCE, TCE, carbon tetrachloride, predominantly biodegrade in the natural environment via a process called reductive dechlorination

Reductive dechlorination



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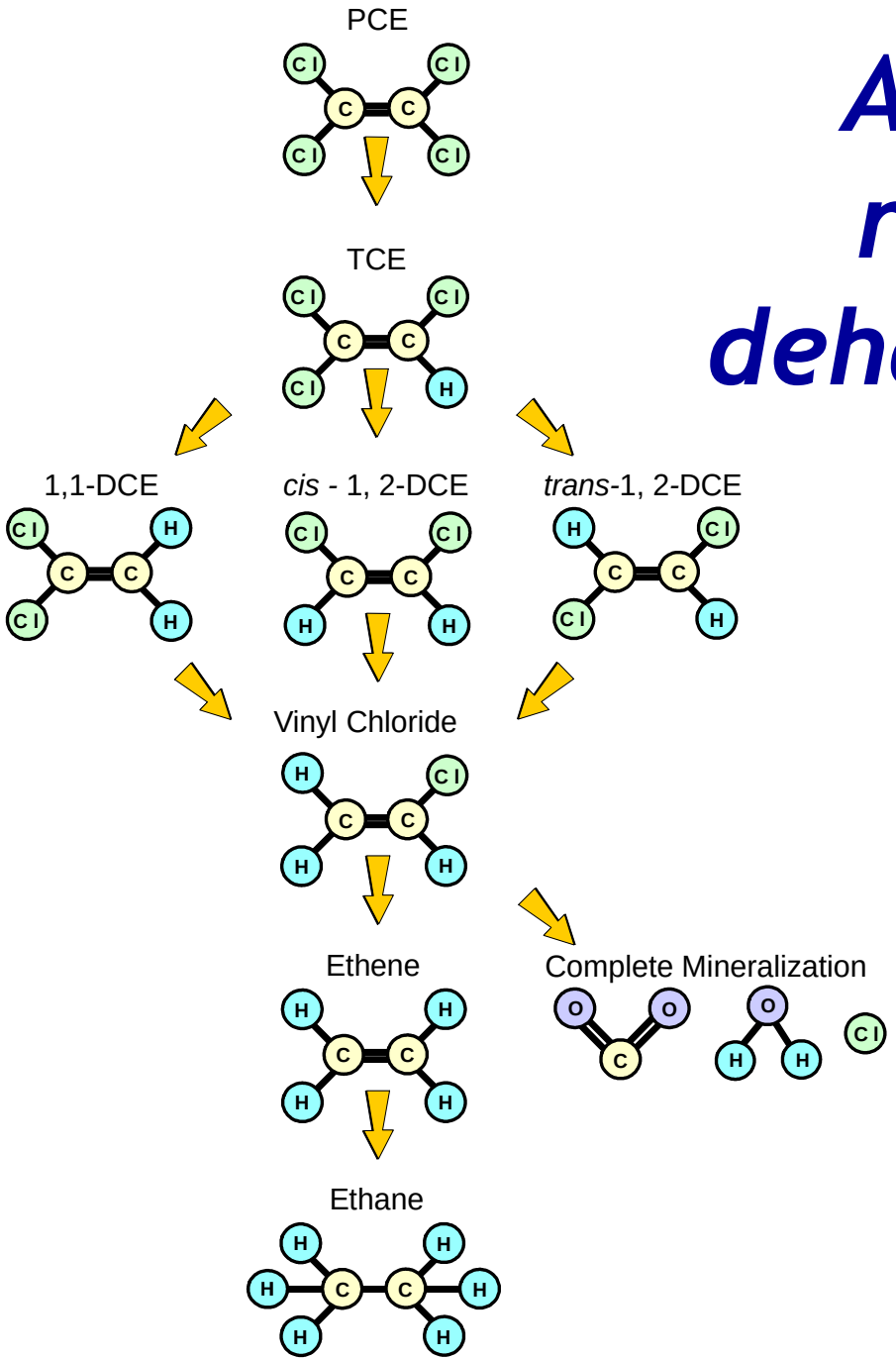
Anaerobic reductive dehalogenation



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Site conditions required to reductive dechlorination

- Biologically mediated dechlorination of solvents works best under certain subsurface site conditions
  - Anaerobic (low oxygen)
  - Reducing (low redox potential)
  - Neutral pH
  - Moderate temperature
  - The right microbial populations
  - A sufficient organic carbon source (substrate, electron donor source)

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Context and objectives of the study

This work is granted by ANR PRECODD, in collaboration with other academic and industrial partners. The role of Biobasic Environnement is to developp a method and tools able to translate biological biodegradation of chlorinated solvents in an aquifer at laboratory scale.

In this view, effects of some additives were investigated on anaerobic biodegradation of chlorinated solvents in laboratory conditions by using reactors simulating aquifer reconstruction

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Materials and methods

- Experiments realized with:
  - High Density PolyEthylen equipped columns
  - Peristaltic pump to ensure liquid matrice circulation
  - Nitrogen flushing in order to obtain anaerobic conditions

Column experiments

- Natural Bioattenuation without carbon source adding

- Carbon source adding

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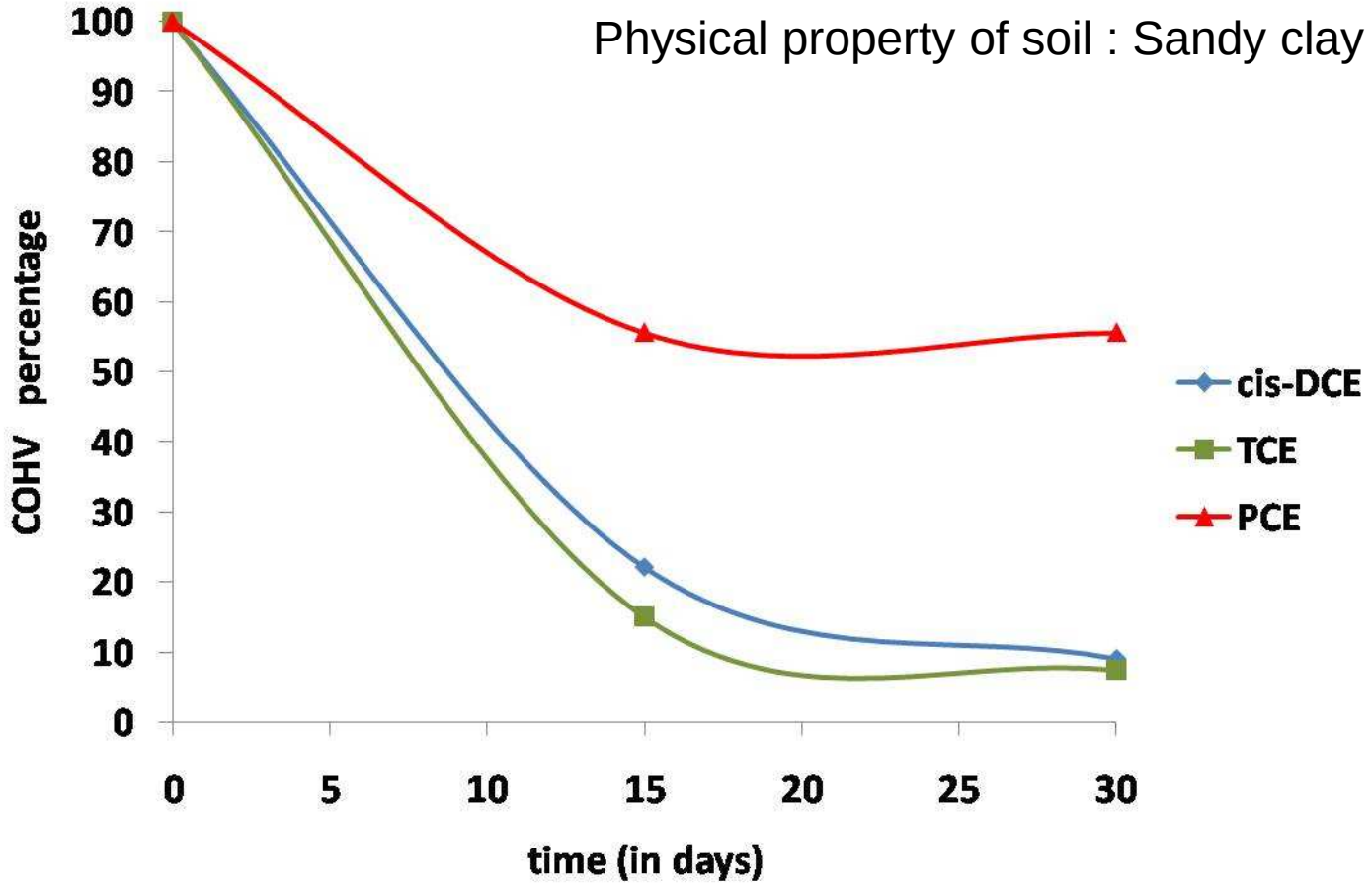
Parameters monitoring

- COHV concentrations
- Physico-chemical properties of matrices (Red Ox, granulometry,…)
- Microbial assessment

(Experiments in progress)

Results

- Natural attenuation assay



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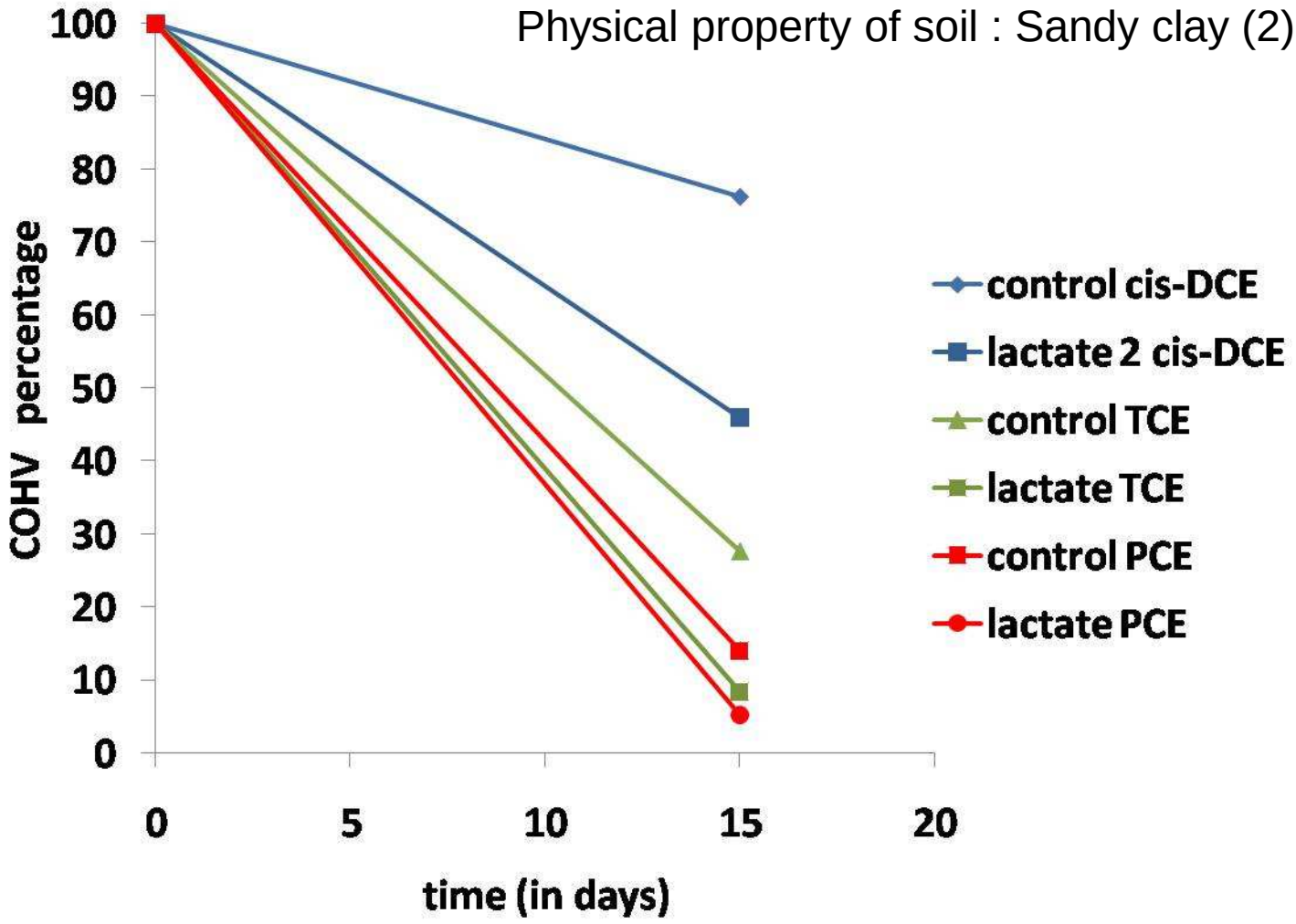
Natural attenuation vs carbon source adding



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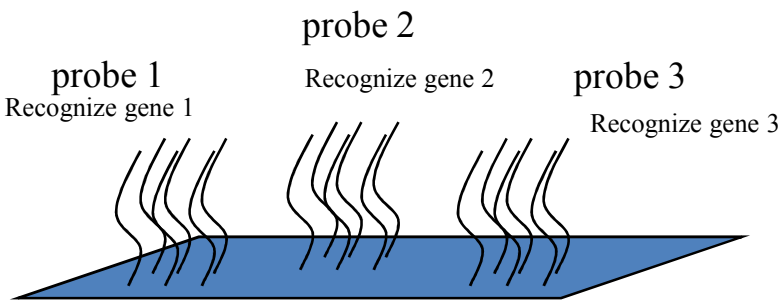
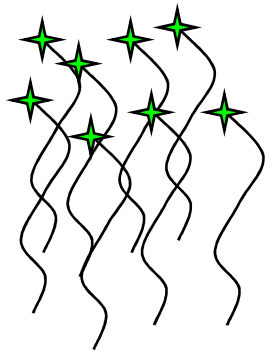
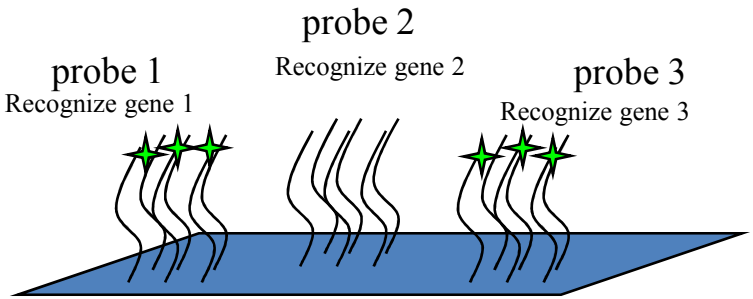
Microbial assessment : DNA microarray





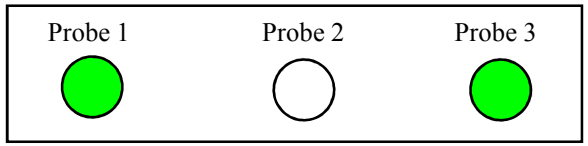
✓oligonucleotidic probes binding on glass slides

✓ extraction of DNA or RNA target of environnement and mark it with fluorochrome



✓ hybridization and scanner reading

✓ conclusion



genes 1 and 3 are present in the sample

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Phylogenetic microarray

- Goal : identification of microorganisms in specific environmental conditions
- Tools : probes targeted on 16s r RNA gene
- Software : « Phylarray »

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Functionnal microarray

- 3 probes directed on reductive dechloration (specific of *Dehaloccocoides*)
- Result : *Dehaloccocoides* absent of the assay
- Conclusion : may be others microorganisms responsible for chlorinated solvents biodegradation

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Conclusion

- Physico-chemical properties and hydrogeochemical properties of soil are fondamental in order to consider bioattenuation
- Anaerobic conditions adapted to chlorinated solvents bioremediation
- Adapted carbon source adding enhance reductive conditions and favor attenuation efficiency

Thank you for your attention

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