Session F

Risk Assessment of Soil and Groundwater

Chair: Mette Christophersen
Head of Department, Ph.D., Rambøll, Denmark

Assessment of in-situ natural and enhanced chlorinated ethenes degradation by use of isotopic and molecular biology techniques

Keynote Speaker Mette M. Broholm
Associate professor, DTU Environment
Technical University of Denmark

Direct radiotracer rate measurements of groundwater contaminants in intact cores – method and first results on cis-DCE dechlorination

Rasmus Jakobsen
Senior Researcher, Ph.D., GEUS, Denmark

Bacterial population dynamics in a groundwater plume from a heating oil spill quantified via 16S rRNA gene amplicon sequencing

Poul Larsen
R&D Project Manager, DMR, Denmark
Bacterial population dynamics in a groundwater plume from a heating oil spill quantified via 16S rRNA gene amplicon sequencing

Poul Larsen
Fagchef, Ph.d.

Per Loll & Claus Larsen - Dansk Miljørådgivning A/S
Jeppe Lund Nielsen & Nadieh de Jonge – Aalborg University
Morten Nørgaard Christensen - Oliebranchens Miljøpulje
Thomas Lehmann Hansen – Dansk Miljørådgivning A/S now DGE
A story from another world... Activated sludge

- Bacterial population dynamics in activated sludge plants gave us the idea behind this study.
- Facts:
  - Activated sludge is composed of billions of distributed on 1,000-1,500 different bacterial species but...
  - 63 of the most abundant bacterial groups/species make up about 70% of the total biomass in sludge.
  - The same species are important/abundant in different wastewater treatment plants.
  - Relative abundance can vary due to e.g. plant configuration or wastewater composition

Saunders et al., 2016; Nielsen et al., 2012
Activated sludge compared to oil polluted groundwater

- Ecological models: The diversity in unpolluted groundwater will be relatively high, but biomass low, due to low amount of nutrients.
- A heating oil spill will:
  - Cause a huge increase in media concentration.
  - Select for species which can grow on the new “media”.
  - Cause diversity to fall = few species will dominate.
  - Cause total biomass to increase due to growth.
- Compared to wastewater, heating oil is a simple and homogenous substrate – few different compounds and alike at different spills.
- Hypothesis: “As wastewater result in a limited core population- despite differences in e.g. wastewater composition and plant configuration. Heating oil was expected to result in an even more limited core population.”
Identification of core species – relevance?

• Why not just focus on measurement of degradation rates?
  – Tools for this is limited and/or expensive
  – Next generation DNA sequencing has reached a level which makes it costeffective.

• Relevance in a short timeframe:
  – Identification of indicator species for heating oil degradation – smoking guns
  – Allow application of conservative degradation rates
  – Easy, fast and cheap sampling
  – Price for analysis (sequencing and data analysis) has reached reasonable level about 700-1,000 kr. pr. sample

• Relevance in a longer timeframe:
  – Manageable to perform detailed in-situ studies on few relevant species
  – Species specific optimization of nutritional conditions to promote degradation
  – Isolate (if possible) and grow core species to perform bioaugmentation
  – Develop species specific methods to determine in situ degradation rate
Analysis of species composition

- Must be growth independent
- 16S rRNA gene as a phylogenetic marker gene – bacterial barcode
- Highly conserved (tips in figure) and variable (valleys) regions of 16S rRNA, makes it very useful for identification.
- Next generation sequencing has become a routine analysis and also relatively cheap choice
- Can manage large sample numbers (up to 300 pr. run)
- Species composition is achieved through random sequencing of >50.000 sequences in each sample

Ashelford et al. 2005
Purpose of this pilot study

- Test next generation sequencing to analyse bacterial species composition in groundwater samples

- Test the response from bacterial species composition in groundwater after a heating oil spill
  - One month after the spill
  - One year after the spill

- Test the effects of sampling and sample handling

- Test repeatability from analysis
Site with heating oil spill

- The tank was placed on the ground in a barn
- Leakage of about 1,000 L heating oil started in January 2016
- Heating oil spread to the groundwater table, which was found 0.8-1 m below surface
Groundwater concentration of heating oil

- 2016: 5 samples with TVOC-concentration above 9 µg/L and 4 below.
- TVOC content in B7 not characterized as heating oil.

- 2017: 5 samples with TVOC-concentration above 9 µg/L and 4 below.
Workflow

Sampling (2-4 samples from each well)

Up-concentration

DNA extraction

PCR

Sequencing

Sequence analysis Alignment with databases

GWT
Sampling and sample handling

- 2-4 groundwater samples (about 200 mL each) were taken from each well, after continuous prepumping of 20-30 L of groundwater and stored in cool boxes for about 24 hours

- The groundwater samples were stored in glasses for soil sampling = clean, but not sterile

- Each groundwater sample was filtered through a sterile filter (porediameter: 0.45 µm) and the filter was transferred to a sterile tube, and stored at -20°C until delivery to the lab at Aalborg University

- Filtration was done in a normal (not sterile) soil lab
Extractable DNA as a measure of biomass/growth

- In general more extractable DNA in polluted water samples

- In 2016 – one month after the spill – the extractable DNA fraction in polluted samples were 0.92-1.6 ng/ml and 0.15-0.52 ng/ml in unpolluted samples (<9 µg/L) – in average factor 4.4

- In 2017 – one year after the spill – the extractable DNA fraction in polluted samples were 0.28-13 ng/mL and 0.038-0.53 ng/mL in unpolluted samples (<9 µg/L) – in average factor 20

- Clear indication of growth after just one month and increased after one year
Effect of heating oil on species composition

- Replicates are grouped together – relative simple sampling and handling is sufficient to give reliable results
- Response (selection) from species composition can be measured one month after the spill
- Response is more clear after one year as polluted and unpolluted samples are more separated
- *Rhodaferax* seems to be highly selected for
Selection of heating oil degraders

- *Rhodoferax* is enriched from about 10 to 35% after one month
- *Rhodoferax* is also relatively abundant in unpolluted wells meaning that it is “ready to act”
- 3 species are enriched in polluted watersamples both years
- 11 species are enriched in polluted samples one year
- 7 species are enriched in unpolluted samples
- Remember – it is relative numbers!

<table>
<thead>
<tr>
<th>Bakterierarter</th>
<th>2016 Uforurenede boringer</th>
<th>2017 Uforurenede boringer</th>
<th>2016 Forurenede boringer</th>
<th>2017 Forurenede boringer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodoferax</em></td>
<td>10,2</td>
<td>2,8</td>
<td>34,9</td>
<td>25,2</td>
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<td>Acidovorax</td>
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<td>0,1</td>
<td>2,6</td>
<td>0,2</td>
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<td>0,1</td>
<td>0,1</td>
<td>2,5</td>
</tr>
<tr>
<td>Desulfobacteraceae</td>
<td></td>
<td></td>
<td>0,1</td>
<td></td>
</tr>
<tr>
<td><em>Rhodococcus</em></td>
<td>0,7</td>
<td></td>
<td>4,3</td>
<td></td>
</tr>
<tr>
<td><em>Massilia</em></td>
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<td></td>
<td>3,2</td>
<td></td>
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<tr>
<td><em>Simplicispira</em></td>
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<td>3,3</td>
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<tr>
<td>PL-11B10</td>
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<td></td>
<td>0,1</td>
<td></td>
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<tr>
<td>Microgenomates</td>
<td></td>
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<tr>
<td><em>Saccharibacteria</em></td>
<td></td>
<td></td>
<td>0,4</td>
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<tr>
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<td></td>
<td></td>
<td>0,1</td>
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<tr>
<td>Comamonadaceae 2</td>
<td>2,4</td>
<td>0,6</td>
<td>3,1</td>
<td>1,6</td>
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<tr>
<td><em>Comamonadaceae 1</em></td>
<td>0,9</td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td>Candidatus Planktophila</td>
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<td></td>
<td>0,6</td>
<td>1,6</td>
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<tr>
<td><em>Polaromonas</em></td>
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<td><em>Oxalobacteraceae</em></td>
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<td><em>Nocardioideae</em></td>
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<tr>
<td><em>Gallionellaceae</em></td>
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<td>6,4</td>
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<tr>
<td><em>B1-7BS</em></td>
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<td><em>Fernphelus</em></td>
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<tr>
<td><em>Gallionellaceae</em></td>
<td></td>
<td></td>
<td>2,4</td>
<td></td>
</tr>
</tbody>
</table>

21 species with most influence on separation in PCA-plot.
Diversity after heating oil spill

- Both the Shannon indeks and the inverse Simpson indeks decreases with increased groundwater pollution
- Diversity decreases = selection of few but numerous species, which can grow on heating oil
Conclusions

• Sampling and handling is possible with a very limited extra effort when routine groundwater sampling is performed on the case
• Samples can be stored/conserved at –20°C after filtration
• Extractable DNA concentration supplement sequencing as it gives a more direct measure of growth
• Sequencing is a reliable and growth independent method to investigate species composition in groundwater
• Heating oil spill in groundwater selected for relatively few species
• Fast selection of heating oil degrading bacteria – less than one month for some species

• Together, these data suggest that heating oil selects for a limited number of species (<15) on this location.

• Now we just need to show that it is the same few species that turn up every time.
Direct radiotracer rate measurements of groundwater contaminants in intact cores – method and first results on cis-DCE dechlorination

Rasmus Jakobsen – Christian Albers (GEUS)
Katerina Tsitonaki (Orbicon)
Mette M. Broholm (DTU Environment)
Liselotte Clausen (HOFOR)
Nina Tuxen (Capitol Region of Denmark) $$
Overview

• MOTIVATION
• INSPIRATION
• PRINCIPLE
• Endless details on the method development (condensed)
• The too few results
• Conclusions ?
MOTIVATION

DCE 10 mg/L
VC 1 mg/L

POC
75 m/yr
100 m

1 µg/l

Døssing Overheu et al. (2011)
F. Beulig, H. Røy, C. Glombitza and B. B. Jørgensen, Organic carbon degradation in the seabed, PNAS January 9, 2018
Rate = \frac{\text{Conc-Contam.} \times \text{Act-product}}{\text{Act-contam.} \times \text{time}}

**Products:** product-I / product-II

**Prerequisite:**
Extraction/separation with 100% separation of contaminant and products
**PRINCIPLE**

Product: Vinyl-chloride + ethene / CO₂

\[
\text{Rate} = \frac{\text{Conc-cisDCE} \times Act\text{-product}}{Act\text{-cisDCE} \times \text{time}}
\]

Prerequisite:
Extraction/separation with 100% separation of cis-DCE and products
Dry air → cis-DCE (Hayesep D) → GC-MS ads. sample tube → cis-DCE? (Carbotrap 217) → GC-MS ads. sample tube

Weak ads. (Hayesep D) → Strong ads. (Carbotrap 217)

First attempt

- Carbotrap 217, 22°C
- Hayesep D, 22°C
- non-adsorbed, 22°C
- Hayesep D, -1°C

Flow (L) vs. % cis-DCE

0 5 10 15 20
0 20 40 60 80 100 120
Second trial: trapping DCE but not VC

- Dry air → cis-DCE+VC GC-MS ads. sample tube
- cis-DCE? VC? GC-MS ads. sample tube

Weak ads. (Hayesep D) → cis-DCE+VC
Strong ads. (Carbotrap 217) → cis-DCE

GC-MS ads. sample tube

HayesepD at 0°C – cis-DCE

<table>
<thead>
<tr>
<th>% cis-DCE</th>
<th>Vol. (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

GC-MS ads. sample tube
STEP 1: Extraction and trapping of C-14

VC + Ethene as CO₂

NaOH

Cis-DCE

Air inlet

Cu

2-methoxy ethanol

Carbosorb

900°

org C oxidation

DCE-sorption

flow meter - 200 ml/min

nation tube membrane dryer

vacuum pump

liquids trap

12047 2 0

9%
1,2-cis-DICHLOOROETHYLENE or cis-DICHLOOROETHYLENE or DICHLOROETHYLENE, cis isomer or (Z)-1,2-DICHLOOROETHYLENE (156-59-2) \( \text{C}_2\text{H}_2\text{Cl}_2 \) Forms explosive mixture with air [explosion limits in air (vol %) 9.7 to 12.8; flash point 39°F/4°C cc; autoignition temp 860°F/460°C; Fire Rating: 3]. Violent reaction with copper and its alloys; strong bases (i.e., potassium hydroxide; sodium hydroxide); sodium. The reaction with copper, copper alloys, and strong bases can form highly toxic and spontaneously flammable chloroacetylene gas. Forms unstable peroxides in air; can polymerize unless inhibited. Polymerization or decomposition can be caused by air, moisture, peroxides and hydperoxides, strong sunlight, elevated temperatures, contact with oxidizers; decomposition products include hydrogen chloride gas. Incompatible with aluminum powder; alkali metals; chemically active metals;
CuO at 900 °C 3x2 hours ”warm”

0-2 hours: 15.3 % of total activity
2-4 hours: 3.6 % of total activity
4-6 hours: 2.2 % of total activity

CuO at 1000 °C 3x2 timer ”boiling” (reactor cracked)

0-2 hours: 22 % of total activity
2-4 hours: 1.6 % of total activity
4-6 hours: 0.8 % of total activity
Haldor Topsoe catalyst

% activity trapped in Carbosorb (oxidized)

CK-395 600°C – 3 hours (no sediment) 84%

CK-395 600°C – 3 hours - 2 ovens (no sediment) 83%

Activity left in reactor after extraction
at ~95 °C - without sediment

3 timer 3-4 %
2 timer 6%
extraction and trapping of C-14
VC + Ethene + CO2

activated C

cisDCE sorption

org C oxidation

2-methoxy ethanol
Carbosorb

HCl

60°C

nation tube membrane dryer x2

vacuum pump

liquids trap
extraction and trapping of C-14 CO₂

VC+cisDCE sorption

Air inlet
activating C

HCL

flowmeter - 100 ml/min

nafion tube membrane dryer x2

org C oxidation

600°

2-methoxy ethanol

Carbosorb

CK-395

vacuum pump

liquids trap

60°C
HCl
<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample ID</th>
<th>Depth</th>
<th>Ground-water meas. conc. µg/l</th>
<th>meas. conc. in sample µg/l</th>
<th>Inject. activity in DPM(^1)</th>
<th>Incubation days</th>
<th>rest activity in react. after extract. in DPM(^2)</th>
<th>Meas. reacted activity in DPM(^3)</th>
<th>reacted activity as fraction of injected activity</th>
<th>Calc. rate ng/l/d from groundw. Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skuldelev</td>
<td>SK2-A</td>
<td>8.5</td>
<td><strong>12000</strong></td>
<td>too high</td>
<td>159000</td>
<td>30</td>
<td>500</td>
<td>21264</td>
<td><strong>0.1337</strong></td>
<td><strong>53494(^4)</strong></td>
</tr>
<tr>
<td></td>
<td>SK2-B</td>
<td>8.65</td>
<td><strong>12000</strong></td>
<td>too high</td>
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<td>10</td>
<td>1000</td>
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<td><strong>0.0048(^5)</strong></td>
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<td>21</td>
<td>3100</td>
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<td><strong>4398</strong></td>
</tr>
<tr>
<td>Vassingerød</td>
<td>VA1-A</td>
<td>6.94</td>
<td>10</td>
<td>not meas.</td>
<td>176000</td>
<td>15</td>
<td>2328</td>
<td>2144</td>
<td><strong>0.0122</strong></td>
<td><strong>8</strong></td>
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<tr>
<td></td>
<td>VA1-B</td>
<td>6.82</td>
<td>10</td>
<td>not meas.</td>
<td>193000</td>
<td>25</td>
<td>2128</td>
<td>2762</td>
<td><strong>0.0143</strong></td>
<td><strong>6</strong></td>
</tr>
<tr>
<td></td>
<td>VA1-C</td>
<td>6.7</td>
<td>10</td>
<td>not meas.</td>
<td>201000</td>
<td>36</td>
<td>1153</td>
<td>6373</td>
<td><strong>0.0317</strong></td>
<td><strong>9</strong></td>
</tr>
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\(^1\)corrected for losses during injection an evaporation from the injection solution during the injection session

\(^2\)calculated from a subsample of the reactorfluid and a guesstimated fluidvolume

\(^3\)activity is not corrected for residual activity since VC+ethene+CO2 are more volatile than cis-DCE

\(^4\)the apparent high rate is probably due to an overloading of the adsorber tubes so 14C cis-DCE has made it to the oven

\(^5\)both fractions of reacted activity are of the same order of magnitude as the guesstimated limit of detection/ background
Sample from Skuldelev:

Used standard at same zoom level:
<table>
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^1 corrected for losses during injection and evaporation from the injection solution during the injection session

^2 calculated from a subsample of the reactor fluid and a guesstimated fluid volume

^3 activity is not corrected for residual activity since VC + ethene + CO2 are more volatile than cis-DCE

^4 the apparent high rate is probably due to an overloading of the adsorber tubes so 14C cis-DCE has made it to the oven

^5 both fractions of reacted activity are of the same order of magnitude as the guesstimated limit of detection/background

~ k = 0.2 yr⁻¹

~ k = 0.5 yr⁻¹
<table>
<thead>
<tr>
<th>Depth</th>
<th>Lithology</th>
<th>Incubation time</th>
<th>Injected activity</th>
<th>Inject. **cis-DCE</th>
<th>Reacted activity</th>
<th>Reacted fraction</th>
<th>Measured conc.</th>
<th>Calculated rate</th>
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<tbody>
<tr>
<td>Meter below surface</td>
<td>days</td>
<td>DPM</td>
<td>µg/l</td>
<td>DPM</td>
<td>fraction of injected</td>
<td>µg/l</td>
<td>ng/l/d</td>
<td></td>
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<tr>
<td>4.9-5.0</td>
<td>coarse sand</td>
<td>1/12*</td>
<td>210000</td>
<td>13</td>
<td>1034</td>
<td>0.0049</td>
<td>12.6</td>
<td>750</td>
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<tr>
<td>5.2-5.3</td>
<td>fine sand (dry)</td>
<td>1/12*</td>
<td>210000</td>
<td>13</td>
<td>15460</td>
<td>0.074</td>
<td>10.7</td>
<td>9500</td>
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<td>200000¹</td>
<td>12</td>
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<td>0.84</td>
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<td>760</td>
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<td>11</td>
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<td>0.024</td>
<td>8.7</td>
<td>18</td>
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<tr>
<td>6.1-6.2</td>
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<td>170000¹</td>
<td>11</td>
<td>2807</td>
<td>0.017</td>
<td>6.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*intended as blanks with incubation time = 0, but incubation time set to 1/12 d (2 hours) – the unusually high activity in the reacted fraction indicates reaction during freezing.

¹ precise activity not known injected activity may vary ±12%

**concentrationen from specific activity and assumed injected activity

¹ concentration calculated from cis-DCE in absorber tube and an assumed porosity of 30%

***calculated rate is a potential rate – there was no cis-DCE in the samples prior to injection
Conclusions

- Determination of cis-DCE concentrations in **highly polluted samples** needs to be made on a **separate sample** with a "robust" instrument/method.

- **Low rates** in samples with **high concentrations** may need extended incubation times to create enough "signal".

- **High rates** in samples with **low concentrations** need shorter incubation times.

- Samples with **very low concentrations** need smaller amounts of injected activity – to not have their concentration increased excessively by the injection.

  So - prior knowledge of the site is definitely helpful.

  – Otherwise incubation time and tracer amount needs to be varied.

Knowing degradation rates will help decide what to do with a contamination and therefore ...

**We are considering putting this analysis “in production” if there is an interest in the “remediation community”**

raj@geus.dk / +4591333579
Moleculite 600°C 3 hours (no sediment)                          72 %

Moleculite 1000°C 3 hours (no sediment)                          81 %
(oven quartz tube broke !)
Loss during incubation

Cis-DCE

$N_2$

Opti-phase

- 7 d, 0.7%
- 14 d, 3%
- 21 d, 3%
Assessment of in-situ natural and enhanced chlorinated ethenes degradation by use of isotopic and molecular biology techniques

Associate Professor Mette M. Broholm
NORDROCS keynote, September 2018

Co-authors see “publications” and “acknowledgements
Chlorinated solvents
Risk for water resources

- Dense non aqueous phase liquids (DNAPL)
- Long lasting w. high conc. in GW
- Degradation ➔ Mobile and toxic intermediates
- Degradation – essential in risk assessment
- Serious concern for Regions/Water supply

- Example: Rødekro 2006
- Source: PCE DNAPL (1-2 ton)
- Plume: > 2 km long, (1-2 ton)
- PCE & degr. prod. TCE, cDCE, a bit of VC
- Characterization of natural degradation
Biodegradation – Specific degraders

- Anaerobic conditions and donor
- Specific degraders
- Risk of cDCE or VC accumulation if no:
  - Dhc w. vcrA/bvcA
  - Dhg w. cerA

Organohalide-respiring bacteria:

- Dehalobacter (Dhb)
- Dehalospirillum
- Desulfitobacterium
- Desulfuromonas
- Dehalococcoides (Dhc)
- Dehalogenimonas (Dhg)

Some types of

- Dehalococcoides (Dhc)
- Dehalogenimonas (Dhg) (tDCE)

Dhc or Dhg with vinylchloride reductase gene

- Dhc (vcrA, bvcA)
- Dhg (cerA)

New qPCR based tools: Screen and quantify specific degraders, genes and activity
Compound Specific Stable Isotopes (CSIA)

- Document degradation
- Quantify degradation
- Identify processes
  - Biotic oxidation
  - Biotic reduction
  - Abiotic reduction
Rødekro Source remediation

Plume investigations

  - Chemical data, incl. Stable Isotopes
  - Microbial data
- Sample shipping
- Hydraulic data collection

Region of Southern DK, Orbicon, AAU/GEUS, U. Neuchatel
PCE in source area is 2 orders of magnitude lower
In the upper part of the aquifer a significant decrease in concentrations is observed to >750 m
Centrally in the plume (1050 m) DCE and VC has decreased
DCE continue to spread in downgradient direction
Development in plume composition and redox

In source, 2010:
- 100 mix
- no O2

2006:
- Concentration (μmol/L)
- Molar fraction (%)

2014:
- Concentration (μmol/L)
- Molar fraction (%)

2017:
- Concentration (μmol/L)
- Molar fraction (%)

Distance from source area (m)
- PCE
- TCE
- cDCE
- VC

Depth (m bgs)

Distance from source area (m)

2006 - 2007
- Aerobe
- Nitrate reducing
- Manganese - Iron reducing

2010: no O2
- Iron reducing

2014
- Sulphate reducing - Methanogen
- Manganese - Iron reducing

2017
- Manganese reducing
Specific degrader insights

- 2007: 2 detect nq Dhc only
- 2014: Several q Dhc and Dhc activity, Dhg in screening
- 2017: Several q Dhg and Dhc, q vcr<sub>A</sub> and bvc<sub>A</sub>, activity?
- cer<sub>A</sub>? – Role of Dhg w. cer<sub>A</sub>?
CSIA documentation and extend of degradation

- TCE and cDCE degradation near source caused by DOC release
- cDCE degradation at B34 increased
- CDCE plume dg little growth – degradation documented again 2017
- Degradation rates vary in space and time
- Halflives: PCE 3-6.3 yr, cDCE (fr) 11-33 yr
Degradation pathways – dual CSIA

- PCE and TCE slopes consistent with microbial reductive dechlorination
- cDCE slope not consistent with microbial aerobic oxidation or reductive dechlorination
  - But with abiotic reductive degradation
    - Consistent with low VC
  - B34 – specific degraders - biotic rd
Rødekro

Conclusion and perspective
• Mass much smaller and decreasing
• Reduced conditions induced by NVOC release
• Degradation increased
• Mixed degradation processes
  – Potential importance of Dhg w. cer\textsubscript{A}
• Risk decreased (not eliminated)
• Future evolution in conditions and degradation?
• Stimulation potential revealed for:
  – Biotic (ERD) degradation
  – Biotically induced (FeS) abiotic degradation (ISCR)

• **Monitored natural attenuation strategy**
  – Big savings

CSIA and molecular techniques
• CSIA has documented degradation of cDCE and VC
• Dual CSIA has identified abiotic reduction of cDCE as an important process
  – Little VC production
• Molecular biology tools has documented enhancement of biodegradation potential for all chlorinated ethenes
• These tools has played a critical role in the risk assessment of the plume
• And for the natural attenuation strategy
Vassingerødvej

- Cecilie Ottosen MSc-thesis, Orbicon, Capital Region
- Small low conc. TCE source zone
  - SW/sewage pond up gradient
  - DOC source
- Short plume
- w. relatively high VC and ethene/ethane

Details:
Tuxen et al. Nordrocs 2018
Vassingerødvej. Degradation potential - Redox conditions and specific degraders

Highest TCE degraders at source
Highest vcr/bvc just upgradient
highest ethene/ethane
Vassingerødvej: Documentation and quantification of degradation – Isotope fractionation

- Greatest extend of VC (17-24%) degradation near source correspond with high specific degraders and genes
- Transport (low flow) complicates interpretation from degradation products – highest conc. down gradient
- Variation or change over time in degradation – e.g. VC isotope depleted downgradient and enriched near source
- Plume is non-trivial – main degradation in the source today
- Lumped degradation rate: $2-8 \cdot 10^{-2} \text{ yr}^{-1}$, $t_{1/2} = 33-9$ years
- Apparently more effective natural attenuation than for Rødekor
Industrivej. Plume remediation. Liquid activated carbon - Plumestop

“Very fine particles of activated carbon (1-2μm) suspended in water through the use of unique organic polymer dispersion chemistry.” by Regenesis

Collaboration with: Rambøll, Regenesis, Capital Region

Harreklide et al. 2018
Nordrocs Poster
Industrivej.
Plume remediation

• **Scope:**
  - Create sorption barrier – cut off/retard plume
  - Facilitate growth of biodegradation culture in barrier for complete reductive dechlorination

• **Establishment:**
  - Injection in well sections – Plumestop (top down) – HRC donor and culture (bottom up)
Industrivej. Challenges and potential benefits

- **Monitoring/Analysis:**
  - Monitoring of CEs - inhibited by sorption to AC/Plumestop.
  - CE extraction from AC in sediment samples may be difficult. Use of strong solvents prevent analysis for degradation products.
    - Thermal desorption may be possible

- **Molecular biology:**
  - A way forward by documenting microbial growth and activity
    - changes in microbial composition can indicate degradation in the barrier
  - Help evaluate if donor transport cause risk of incomplete degradation down gradient?

- **Stable isotopes:**
  - Documentation of degradation and rate estimation
  - Does the potentially strong sorption influence isotope fractionation?
    - Equilibrium – time
  - How much is evaluation of degradation in downgradient wells influenced by compound selective retardation?
Industrivej. Microbial population - preliminary

- Initially TCE dominant
- At 3 and 6 months some degradation to cDCE apparent
- A small increase in Dhc (none in Dhg) abundance observed in the barrier at 3 months
- Increase in naturally occurring Dhbt
- Biostimulation of natural bacteria

Baseline: Nearly all TCE

M1: PlumeStop observed
Industrivej. CSIA degradation trends – preliminary

- Plumestop sorption in M1
- Degradation of TCE most significant in M1-2 and M5-3, and M6 (downgradient) at 6 months.
- Degradation of cis-DCE in M1-2 and M5-1 at 6 months.
- Extend of TCE degradation lower than cDCE fraction due to retarded transport
Industrivej. Learnings - preliminary

- Remediation technology
  - Distribution of Plumestop and bio-enhancement
    - Challenging in low and variable conductivity aquifer
  - Challenging
- Distribution
- Remediation technology
- Sorption effect
- Enhancement of bioaugmentation

- Monitoring
  - Challenging – aqueous and solid samples required if sorption
  - Degradation products
  - Degradation products
    - Assessment challenge
    - Modelling may be needed

- Molecular biology techniques
  - Distinguish biobarrier from enhanced natural attenuation
  - Verify biodegradation potential and activity in barrier and downgradient
  - Understand biodegradation processes
  - Destinguish biobarrier from enhanced natural attenuation
  - Verify biodegradation potential and activity in barrier and downgradient
  - Understand biodegradation processes
  - and dual-CSIA
  - Effect of strong sorption?
    - Initially – then equilibrates
    - Initial degradation
    - less influence for more degradation
    - Less influenced than concentrations
    - More influenced than concentrations
  - Degradation products
  - Degradation products
    - Modelling may be needed

- Lots of challenges
- Many ideas
- Some indications
- Still a ways to go
- Molecular biology and CSIA will help us
Conclusions

Natural and enhanced degradation:
- Monitored and enhanced natural attenuation are attractive technologies for plume-management and remediation
- Biotic and/or abiotic degradation is an essential element in NA
- Degradation processes and rates vary in time and space and may be strongly influenced by source zone remediation
- Stimulation of biodegradation and biotically induced abiotic degradation is feasible
- Enhancement through increased retardation and enhanced degradation in plumes complicates assessment of degradation with standard methods

Molecular biology and CSIA:
- Molecular biology and CSIA techniques are strong tools in the understanding and evaluation of degradation in plumes and source areas
- Documentation of degradation
- Potential for complete dechlorination
- Determination of extent and rate of degradation
- Evaluation of degradation processes and pathways
- Evaluation of potential for stimulation of degradation processes
- Evaluation of degradation in complex remediation schemes
Publications

• Orbicon (Vassingerødvej) and Rambøll (Industrivej) reports
• Ottosen, C., 2017. MSc thesis (Vassingerødvej)
• Skou, M., 2018. MSc thesis (Industrivej)
• Sammali, E., MSc thesis (Industrivej)

• Upcomming publications
• Ottosen, C., et al. 2018-20 (Vassingerødvej and Industrivej)
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