BiogROUT
Microbially induced carbonate precipitation in sand
Flow and Transport aspects
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Overview

• What is BioGrout?
• Research objectives and approach
• What flow and transport phenomena?
• Experiment at large labscale: 1m³
• Further research
Grout?

Grout is a construction material, which is generally composed of a mixture of water, cement, sand, and applied as a thick liquid and hardens over time. (wikipedia.com).

Applications
• Foundations
• Tunneling
• Filling cracks
Grout as soil improvement method

**Limitations**
- Circular injection distance ($\approx 1 \text{ m}$)
- High pressure $\rightarrow$ can lead to soil instability
- Low permeability $\rightarrow$ full clogging of the pore spaces and restriction of water flows
- Expensive $\sim 400$ Euros per $\text{m}^3$ (2m diameter column = 38,000 Euros)
Biogrout

using natural biochemical processes for in situ soil improvement

S(t)imulating natural diagenesis from sand to sandstone

Only within short time instead of millions of years
Microbially induced carbonate precipitation?

- Producing CO₂ (e.g. by respiration, hydrolysis, etc)
- Increasing pH (e.g. by consuming CO₂)
- Acting as nucleation site in oversaturated solution

Stromatolites (layered domes of rock) formed by microbially induced precipitation of CaCO₃

Cyanobacteria, Purple sulfur bacteria
General Biogrouting process

1. Bacteria are grown in- or ex situ.
2. Reagents, nutrients and bacteria are transported through the soil.
3. Bacteria cause an increase of dissolved carbonate.
4. In presence of e.g. dissolved calcium, carbonate minerals will precipitate and form crystals.
5. The newly formed crystals change the micro-properties the soil.
6. Consequently the macro-properties of the soil are changed.
Current Biogrouting process

Enzymatic catalysis of urea hydrolysis in presence of dissolved calcium results in CaCO₃

\[
\text{Urease} \\
\text{CO(NH}_2\text{)}_2 + \text{Ca}^{2+} + 2\text{H}_2\text{O} \rightarrow 2 \text{NH}_4^+ + \text{CaCO}_3 (\text{s})
\]

Sporosarcina pasteurii

Urea
Potential applications

Railway stabilisation  Erosion control

Dike stability  Liquifaction prevention
Research objectives and approach
Practical objectives

BiogROUT must be a commercially competitive and reliable soil improvement method

- Long injection distance (meters) with low pressure
- (Relatively) homogeneous results
- Retention of permeability
- Low cost
- **Engineering model for prediction and control**
Engineering (black box) model

INPUT
- Flow rate
- Concentrations
- Pumping time

OUTPUT

road embankment
application by (multiple) injections

INPUT
- Flow rate
- Concentrations
- Pumping time
- Injection strategy

OUTPUT
INPUT
• Flow rate
• Concentrations
• Pumping time
• Injection strategy

OUTPUT
• Strength
• Stiffness
• Permeability
• Porosity
• Costs
What is in the black box?
Empirical approach: experiments

\[ q = -K \frac{dh}{dx} \]

\[ \frac{d}{dt} (\theta C_i) = \frac{\partial (D \partial C)}{\partial x^2} - \frac{d}{dx} \left( u L \theta C_i \right) + r_i \theta \]

- 1D column experiments
- 2D experiments
- 3D/2D axial symmetry experiments
Fundamental approach: identify all relevant phenomena

at all scales!
Pulse seems plug flow

- Darcy flow
- Convection and reaction of bacteria, crystals and solutes
Column diameter: 66 mm

- Darcy flow
- Convection, dispersion and reaction of bacteria, crystals and solutes
Column wall

1 centimeter ($10^{-2}$ m)

Interface effects

Heterogeneous permeability
Fine sand (125 – 250 um)

- Hydrodynamic dispersion by different flow paths
- Multiphase flow (at the front)
- 100 micrometer $10^{-4}$ m
- Fine sand grain (125 um)
- Silt grain (50 um)
- Navier-Stokes flow
- Filtration, attachment, detachment and reaction of bacteria, crystals and solutes
100 micrometer \((10^{-4} \text{ m})\)

Pore blocking by crystals and bacteria
Dense bacterial suspension contains $10^9$ cells/L.

Crystals and bacteria can attach to and detach from:

- grains
- each other
- themselves

Depending on fluid and solid properties and flow regime.
Crystallisation causes concentration gradients.
Fluxes of substrate and products cause local gradients.

- Ureum
- Ammonia
- Carbonate

Extracellular enzymes

100 nanometers (10^{-7} m)

Intracellular enzymes

Location of the enzyme determines specific urease activity

Excretion of enzymes causes decay of activity

Cytoplasm
Diffusive or convective of molecules transport through cell membrane
Membrane permeability or presence of specific transporters determines urease activity
Transport through the enzyme:
Enzyme folding structure determines specific enzymatic urease activity
1 nanometer ($10^{-9}$m)

Nickel atom

Enzyme active site

1 nanometer ($10^{-9}$m)
CO(NH₂)₂ + 2H₂O → 2 NH₃ + H₂CO₃

Concentrations of substrates and products define urease kinetics
# Multi-scale

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<th>nm</th>
<th>μm</th>
<th>mm</th>
<th>m</th>
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<td>10^-9</td>
<td>10^-6</td>
<td>10^-3</td>
<td>10^-1</td>
</tr>
<tr>
<td>10^-8</td>
<td>10^-5</td>
<td>10^-2</td>
<td>10^-0</td>
</tr>
<tr>
<td>10^-7</td>
<td>10^-4</td>
<td>10^-1</td>
<td>10^-1</td>
</tr>
</tbody>
</table>

- Molecules
- Enzymes
- Membranes
- Crystal nuclei
- Bacteria
- Crystals
- Grains
- Cores
- Sandbodies

Solute and particle transport at all scales!
Examples of transport related questions
What controls the urease activity during growth?

Growth parameters:
- Amount of bacteria
- Type of bacteria
- Growth phase
- Nutrient medium

High activity
Small cells

Low activity
Large cells
What controls urease kinetics during batch cementation conditions?

Batch kinetic parameters:

- urea affinity
- calcium inhibition
- pH
- carbonate yield
- Bacterial decay in time
- Temperature
- Diffusion and Convection

\[ r = v_0 \cdot f(C_{\text{urea}}, C_{\text{Ca}}, C_{\text{CaCO}_3}, t, T) \]

\[ f_{\text{urea}} = \frac{C_{\text{urea}}}{K_{m,\text{urea}} + C_{\text{urea}}} \]
What controls the crystal morphology, size and spatial distribution?

- High bacterial concentration
- High urease activity

- Spherical vaterite
- Spherulite with fibrous surface texture

- Low bacterial concentration
- Low urease activity

- Rhombohedral calcite

In unstirred liquid
hollow spheres with crystalline material in the interior dissolution and recrystallisation effects?

On a microscope slide

© Salwa Al-Thawadi, Murdoch
Vaterite spheres with amorphous CaCO$_3$ inside and calcite outside

Continuously flushed sand column

Inlet, vaterite

Outlet, calcite
How far away from bacteria do crystals form?

bacterial activity and crystallisation cause local concentration gradients
What is the effect of the formation of crystals on engineering properties at pore scale....

Calcite crystals on glass beads

Acc.V  Spot Magn  Det  WD  100 µm
20.0 kV  4.0  250x  BSE 10.2  1.0 Torr glassbeads & crystals
at core scale (1D) after treatment....

Measurements at core scale lead to empirical correlations.
at core scale (1D) during treatment....

Experiment → model
at 2D lab scale....
and at small pilot scale: a 1m³ experiment
Objective

Test feasibility of the BioGrout process in a controlled 3D environment using techniques and methods, which are similar to those used in a practice.
Set-up

Box dimensions:
Height: 1 m
Width: 1.1 m
Depth: 0.9 m

Tube is centered in the box
Tube dimensions:
• Diameter: 3 cm
• 2 injection zones: 1 above and 1 below the center of the box: tube wall is perforated with 0.2 mm slots, which have 3 cm length along the tube axis

Boundary conditions:
1. Top and bottom of the box and the tube (excluding the injection zones) are impermeable (slip/symmetry)
2. The sides of the box have a constant water level (constant hydrostatic pressure).
3. The injection zones have a constant normal flow rate.
Set-up

Monitoring:
- Influent: Pressure
- Effluent: Electrical conductivity/pH

Constant water level at the sides of the box

Clay plug to prevent shortcutting along tube wall

Injection tube
Set-up

Drainage mats along the sides at the sides of the box

4 effluent tubes at the bottom (1 in each corner) are connected before going to the effluent pump and the monitoring cell.
Liquids

• Bacterial suspension:
  • Activity = 4.8 mS/min (2.3 mS/min in supernatant)
    -> total activity of approx 3.3 M urea/hr

• Fixation fluid ≈ 0.05M CaCl$_2$

• Cementation solution ≈ 0.5M CaCl$_2$/urea
  (1 bag urea/2 bags CaCl$_2$)
### Flushes

<table>
<thead>
<tr>
<th>date</th>
<th>liquid</th>
<th>start</th>
<th>end</th>
<th>time [h]</th>
<th>volume [L]</th>
<th>flow rate [L/h]</th>
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</thead>
<tbody>
<tr>
<td>07-Sep</td>
<td>bacterial suspension</td>
<td>13:00</td>
<td>13:37</td>
<td>0.62</td>
<td>50</td>
<td>81.08</td>
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<td>18:00</td>
<td>8.00</td>
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<td>85.60</td>
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<td>18:00</td>
<td>8.00</td>
<td>685</td>
<td>85.60</td>
</tr>
<tr>
<td>10-Sep</td>
<td>cementation fluid</td>
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<td>17:30</td>
<td>8.00</td>
<td>320</td>
<td>40.00</td>
</tr>
<tr>
<td>11-Sep</td>
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<td>09:30</td>
<td>17:10</td>
<td>7.67*</td>
<td>307</td>
<td>40.00</td>
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<td>8.00</td>
<td>304</td>
<td>38.00</td>
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<tr>
<td>17-Sep</td>
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<td>8.00</td>
<td>304</td>
<td>38.00</td>
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<tr>
<td>2-Okt</td>
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<td>17:00</td>
<td>8.00</td>
<td>304</td>
<td>38.00</td>
</tr>
</tbody>
</table>

* 20 minutes pumping air
Bacterial fixation

**Graphical Representation:**

- **X-axis:** Flushed volume [L]
- **Y-axis 1:** Activity [mM urea/hr]
- **Y-axis 2:** Optical density (600nm)

The graph shows the relationship between flushed volume and activity, as well as optical density, with distinct markers for bacterial suspension, fixation fluid, and cementation fluid.
Bacterial fixation

<table>
<thead>
<tr>
<th>Volume [L]</th>
<th>Activity [mM urea/hr/L]</th>
<th>Total activity [M urea/hr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN 50</td>
<td>3300</td>
<td>165</td>
</tr>
<tr>
<td>OUT 1000</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>LEFT 350</td>
<td>443 (13%)</td>
<td>155 (94%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume [L]</th>
<th>OD600 [1/L]</th>
<th>Total OD600 [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN 50</td>
<td>6</td>
<td>300</td>
</tr>
<tr>
<td>OUT 1000</td>
<td>0.25</td>
<td>250</td>
</tr>
<tr>
<td>LEFT 350</td>
<td>0.14 (2.4%)</td>
<td>50 (16%)</td>
</tr>
</tbody>
</table>

Expected activity in sand box:

- based on activity loss: 440 mM urea/hr
- based on OD600 loss: 78 mM urea/hr
Measured ammonium in time
Cumulative ammonium in time

Expected activity in sand box:
- based on activity loss: 440 mM urea/hr
- based on OD600 loss: 78 mM urea/hr

Actually measured
- 2 mM urea/hr decreasing within days to 0.2 mM urea/hr
Measured ammonium with volume

- Ammonium concentration [M]
- Flushed volume [L]
Total CaCO₃: 200 mol in 50 days using 4 m³ 0.5 M urea/CaCl₂ (of maximum 2000 mol: 10% conversion!)
Hypothesis for activity loss

- Coarse sand – flush out
- Low affinity for attachment of bacteria
- Temperature – lower activity
- Heterogeneous flow patterns
- Heterogeneous distribution of bacteria
- Activity in supernatant
- Loss of bacteria during cementation flushes
- Substrate quality
- Natural decay of activity
- Inhibiting compounds in sand
- ......
Monitoring EC and Ammonium

- EC [mS/cm]
- Ammonium concentration [M]

- EC 13 sep
- EC 2 okt
- EC 25 okt
- Amm 13 sep
- Amm 2 okt
- Amm 25 okt

Time [hours after start injection]
Modelling effluent (2D axial)
Monitoring inlet pressure

![Graph showing flushed volume vs pressure and flow rate](image-url)
Conclusions 1st 1m3

- Successful Injection bacterial suspension, fixation fluid and cementation fluid without significant pressure increase.

- Much Lower in situ urease activity 2 instead of 78 mM urea/hr

- 200 mol of CaCO3 in 50 days with 10% efficiency.

- An exponential decay of the in situ urease activity in time is observed.

- Electrical conductivity (EC) is a useful tool for continuous monitoring. After the front has passed it directly relates to % of conversion within the effluent.

- EC and ammonium measurements indicate non-homogeneous distribution of bacteria
2nd 1m3 experiment
Differences with 1st 1m3

To prevent activity loss

- More bacteria: 50L -> 100L
- Finer sand 125- 250 um (more filtration)
- Different sand surface chemistry (more attachment)
- Lower flow rate (less detachment)
- Smaller fixation pulse
## Flushes

<table>
<thead>
<tr>
<th>Date</th>
<th>Liquid</th>
<th>start</th>
<th>end</th>
<th>time</th>
<th>volume</th>
<th>flow rate</th>
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<tbody>
<tr>
<td>1</td>
<td>Bacterial suspension</td>
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<td>10:30</td>
<td>2.5</td>
<td>100</td>
<td>50</td>
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<tr>
<td>1</td>
<td>fixation fluid</td>
<td>10:30</td>
<td>11:30</td>
<td>1.0</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>cementation fluid</td>
<td>11:30</td>
<td>19:30</td>
<td>8.0</td>
<td>320</td>
<td>40</td>
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<tr>
<td>2</td>
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<td>320</td>
<td>40</td>
</tr>
</tbody>
</table>

* 20 minutes pumping air
Bacterial fixation

No results yet.....
Measured ammonium in time

- cum ammonium [mol]
- flow rate [L/h]
Cumulative ammonium in time

Cumulative ammonium [mol]

time [days]
Measured ammonium with volume

flushed volume [L]
Ammonium concentration in effluent [mM]

02-Nov
6-nov
7-nov
08-Nov
09-Nov

November 29, 2007
Total CaCO$_3$: 300 mol in 3 days using 1 m$^3$ 0.5 M urea/CaCl$_2$ (of maximum 500 mol: 60% conversion!)
Monitoring EC

![Graph showing EC monitoring over time after start injection. The graph plots EC in mS/cm against time after start injection in hours. The data points indicate a significant increase in EC starting around 12:00, with a peak around 15:00.]

November 29, 2007
Monitoring pressure

![Graph showing overpressure at inlet tube over time after start injection. The graph includes data from 2-Nov, 6-Nov, 7-Nov, 08-Nov, and 09-Nov with different symbols and colors representing each date. The x-axis represents time after start injection in hours, ranging from 0 to 1,400. The y-axis represents overpressure at inlet tube in meters of water, ranging from 0 to 7.](image)

- **2-Nov**
- **6-Nov**
- **7-Nov**
- **08-Nov**
- **09-Nov**
Preliminary conclusions 2nd 1m3

- Full conversion within one day
- Conversion rate > 20 mM urea/hr
- 300 mol CaCO₃ formed in 3 days of flushing
  - with 60% conversion efficiency
- Pressure at inlet increased to 5 m water pressure caused shortcutting leakage along inlet tube, which ended the experiment.
- Working in mid november outside in a tent is not a good idea!
Further research

- Perform real pilot scale experiment: 100 m$^3$
- Model experimental results at macro-scale
- Include effects of variable density, viscosity, porosity and permeability
- Study heterogeneity effects
- Link the effects at the different scales
Further research

Other microbial carbonate precipitation processes:

Calcium acetate + calcium nitrate -> Biomass + CaCO$_3$(s) + N$_2$(g)