Induced Polarisation (IP) laboratory measurements on E. coli sand mixtures

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Induced Polarisation (IP) lab measurements on *Escherichia coli*-sand mixtures

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Motivation

The aim of the MIRACHL project is the **characterisation and monitoring of in-situ remediation of chlorinated hydrocarbon contamination** using an interdisciplinary approach. Geophysical methods, such as DCIP are used to investigate the remediation process. To interpret these geophysical field IP data, lab investigations with different kinds of bacteria are necessary to assess the sensitivity of the methods for these specific applications. For our first experiments we started with the **standard lab organism *Escherichia coli* (*E. coli*) strain DSM1116**, which has been isolated from soil in the

Material & Method

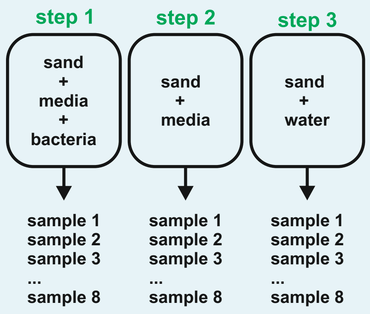

Fig. 1: Sketch of the experimental design. Step 2 and 3 are control measurements. Each step consists of 8 individual samples which are taken out at different days.

To investigate the geophysical IP signature of bacteria in a sand environment an experiment was designed which includes 3 steps (fig. 1):

- **Step 1**: 8 individual samples filled with sand, media (nutrient food) and *E.coli* bacteria (fig. 2a, b)
- **Step 2**: 8 individual samples filled with sand and media
- **Step 3**: 8 individual samples filled with sand and water

All material was sterilised before and the samples were **incubated in a temperature controlled shaker** at 30°C with gentle mixing at 100 rpm (fig.2c). To ensure *E.coli* growing, parts of the **fluid were put on agar plates**, incubated at 37°C overnight and counted (fig.2d). The samples were **taken out at different days** and measured.

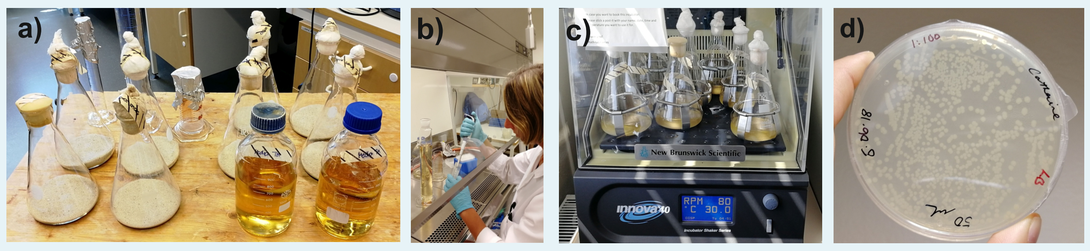

Fig. 2: Photos of the flasks filled with sand and media in bottles (a), inoculating the bacteria into the media (b), final samples in the shakers c). Plate with bacteria growing (d).

- The **sand was filtered to separate the fluid** and carefully **packed in the 4-point sample holder** (fig. 3a) and weighed. The weight range differed within 14% between all samples.
- Samples were measured immediately after packing and held in the sample holder for at least 24h, during which the samples **were measured repeatedly**
- Measurements of: **spectral IP (SIP)** (frequency range 1 mHz - 10 kHz), **time domain IP (TDIP)** (1 s ON/OFF-time) and **Self Potential (SP)** with PSIP instrument (fig. 4b)
- **Fluid conductivity, pH and temperature of the liquid** removed by filtering were also measured. Portions of sand were frozen for future **DNA analysis**
- SEM (scanned electrone microscopy) pictures were taken afterwards for bacteria confirmation aferwards

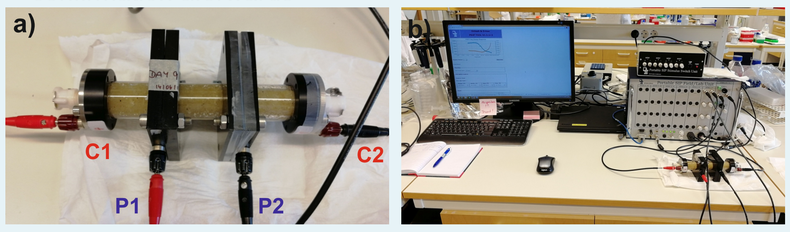

Fig. 3: Photos of the 4-point sample holder filled with the sand-bacteria mixture (C1, C2 = current electrodes, P1, P2 = potential electrodes) and PSIP (Ontash & Ermac) measurement instrument (b).

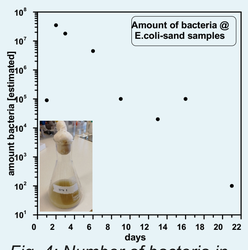

Fig. 4: Number of bacteria in the fluid.

Discussion

Comparing the results from ***E.coli*-sand, media-sand and water-sand samples** one can see that:

- **Fluid conductivity** is higher when (*E.coli*) bacteria are present (13 - 16 mS/cm, fig. 11a) due to the presence of bacterial cells and/or their degradation products. **pH** between 5-6 with water samples are most homogeneuos. **SP** scatter a lot for all samples
- **TDIP** was measured but showed no clear behaviour due to very small and unstable transmission current (and therefore not shown here)
- **SIP** resistivity values are smaller and more even for the *E.coli* than for the media and water samples (@ 1 Hz, fig. 12a) in accordance with the fluid conductivity (fig. 11a) and real conductivity (fig. 12c). For the media and water samples phase and imaginary conductivity are more even and smaller. In contrast, the results for *E.coli* are higher but after an increasing until Day 6, they decrease and scatter more

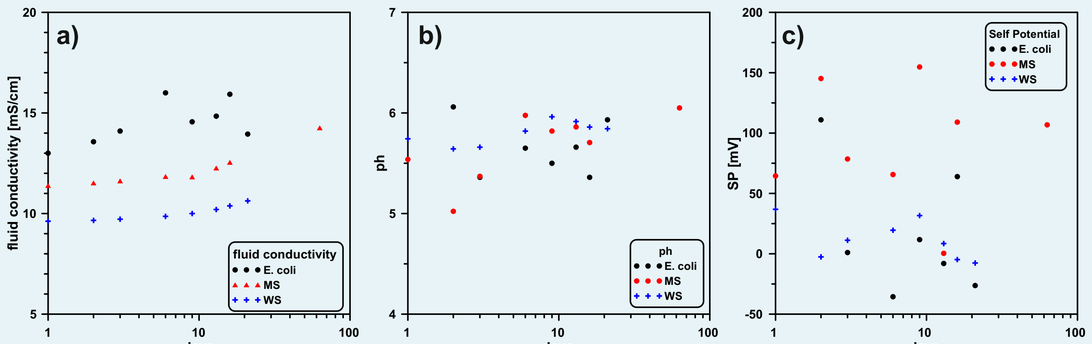

Fig. 11: Comparison of fluid conductivity (a), ph (b) and self potential (SP) for all samples.

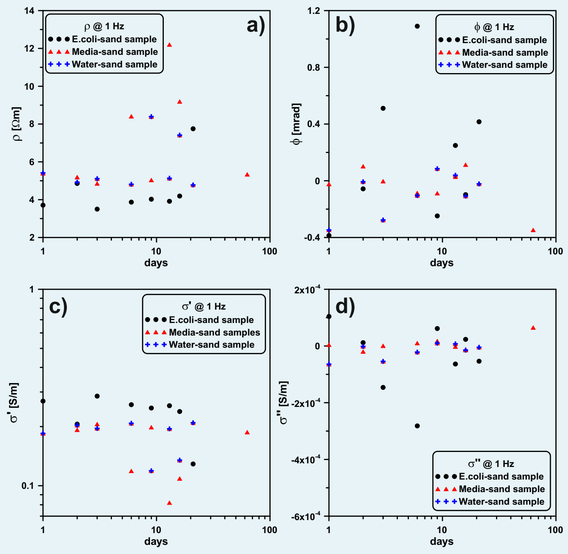

Fig. 12: SIP-comparison for E.coli-sand and media-sand samples for frequency at 1 Hz. a) resistivity ρ, b) phase ϕ, c) real conductivity σ' and D) imaginary conductivity σ''.

Conclusions:

- **SIP:** significant changes with time for *E. coli* samples and differences between the *E. coli*/media/water samples (but unclear positive phase effects). Very low resistivity values due to high salinity media
- **TDIP:** repetition and processing is needed
- **SP:** very inhomogenous data (sample holder effect?)
- **General:** the most interesting IP effects occurred within the first 7 days (probably as long as there was enough nutrients/food for the bacteria to grow)
- **SEM:** proof of bacteria contamination
- **DNA analysis** is necessary to determine the bacteria density in the sand (not only in the fluid)

Results

E.coli-sand mixtures

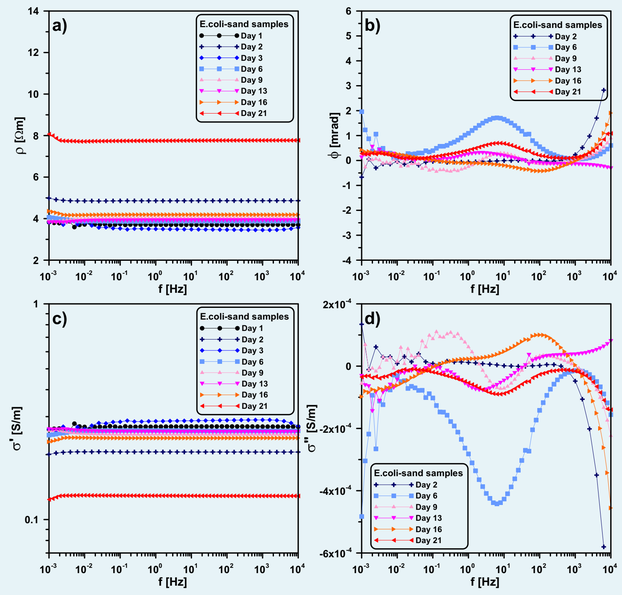

Fig. 5: SIP measurement results for E.coli-sand samples from Day 1 to 21. a) resistivity ρ, b) phase ϕ, c) real conductivity σ' and d) imaginary conductivity σ''.

The results from the *E.coli*-sand samples show:

- **SIP** results with very low resistivities (fig.5 a/c) and a (positive) phase maximum at day 6 (fig.5b)
- **SP** scattered, **TDIP** results unclear due to unstable current transmission
- **Fluid conductivity** increased until it reached a plateau, **pH** was quite stable (fig.6b)
- **SEM** picture (fig.6a) confirmed the presence of bacteria and the beginnings of biofilm formation

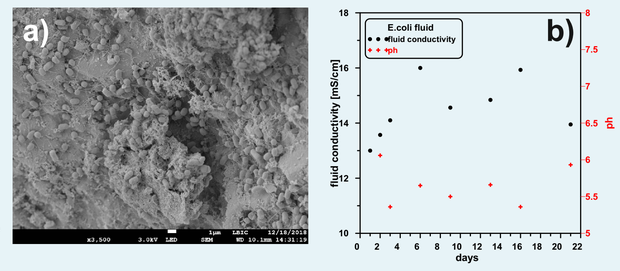

Fig. 6: SEM picture (a) as well as fluid conductivity and pH results for E.coli-sand samples (b).

Media-sand mixtures

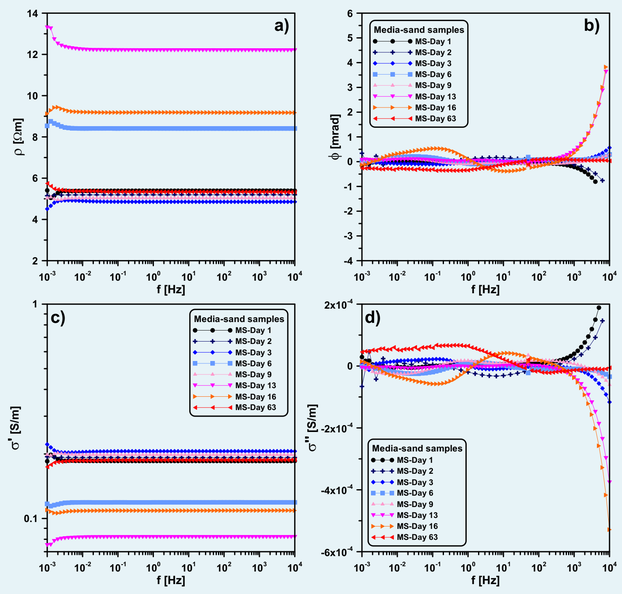

Fig. 7: SIP measurement results for media-sand samples from Day 1 to 63. a) resistivity ρ, b) phase ϕ, c) real conductivity σ' and d) imaginary conductivity σ''.

The results from the media-sand samples show:

- **SIP** results with low resistivities (fig.7 a/c) and very small phase effects (fig.7 b). Sample day 16 smelled very strong (other bacterial influence?)
- **SP** scattered, **TDIP** results unclear due to unstable current transmission
- **Fluid conductivity** increased continious (likely due to evaporation), **pH** was quite stable (fig.8b)
- **SEM** picture (fig.8a) confirmed no *E.coli* contamination

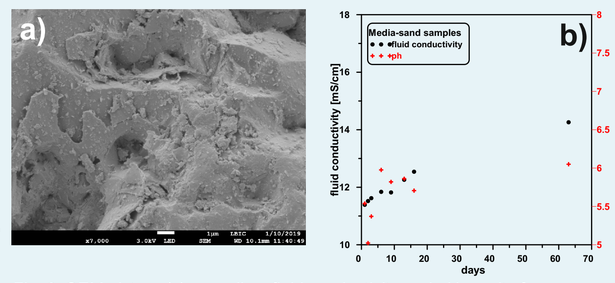

Fig. 8: SEM picture (a) as well as fluid conductivity and pH results for media-sand samples (b).

Water-sand mixtures

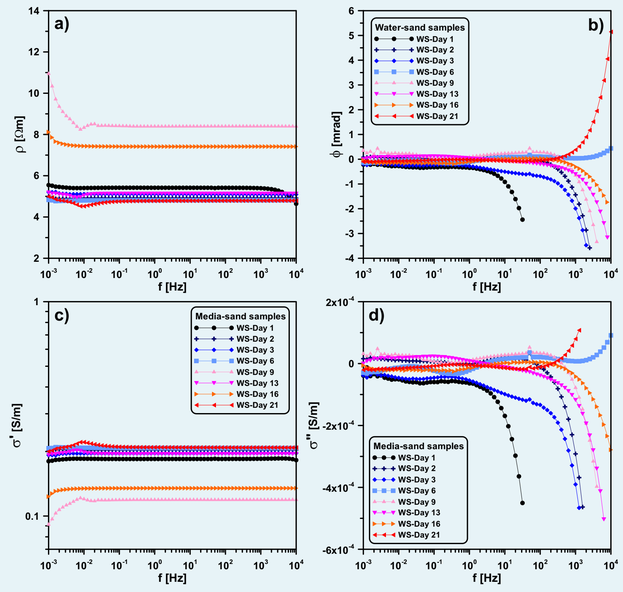

Fig. 9: SIP measurement results for water-sand samples from Day 1 to 21. a) resistivity ρ, b) phase ϕ, c) real conductivity σ' and d) imaginary conductivity σ''.

The results from the water-sand samples show:

- **SIP** results with low resistivities (fig.9 a/c) and almost no phase effect (fig.9 b).
- **SP** scattered, **TDIP** results unclear due to unstable current transmission
- **Fluid conductivity** increased continiously (likely due to water evaporation), **pH** very stable (fig.10b)
- **SEM** picture (fig.10a) confirmed no *E.coli* contamination

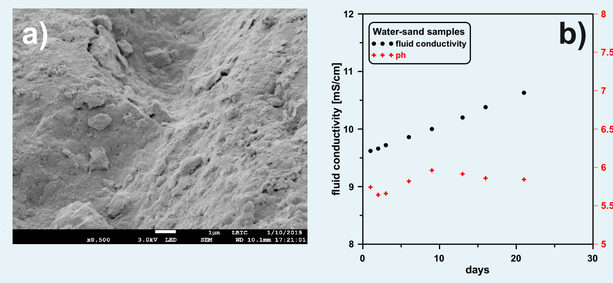

Fig. 10: SEM picture (a) as well as fluid conductivity and pH results for water-sand samples (b).

Outlook

These measurements are the beginning of a larger series of experiments on the effects of bacteria on the IP properties. We are planning further:

- to carry out DNA analysis
- improve the measurement technique and the experimental set up
- using of a better TDIP instrument and processing software
- set up an improved *E.coli* experiment focussing on the first 7 days and providing more nutrient
- set up the same panel of experiments only with natural sand communities (lab growing)
- investigations of biofilms
- investigations of natural contaminated soil samples

Acknowledgement

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