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Induced Polarisation (IP) Laboratory Measurements on Escherichia Coli (E. Coli)-Sand Mixtures

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Summary

For the characterization and monitoring of in-situ remediation of chlorinated hydrocarbon contamination, interdisciplinary approaches and geophysical methods are needed to secure water supply of sufficient quality and quantity. Geophysical methods, such as IP (induced polarisation) could be used to investigate bioremediation processes. However, to interpret geophysical field IP data, lab investigations with different kinds of bacteria are necessary to assess the sensitivity of the methods for these specific applications. Therefore, a first experiment was conducted with E. coli bacteria in sterilised Ottawa sand environment. These bacteria-sand-mixtures were harvested at different days and measured with e.g., SIP (spectral IP) under laboratory conditions.

A slight increase in phase and a decrease in resistivity were observed after several days of bacterial growth with sand, with a later decrease in phase appearing to coincide with die-off of the bacteria. Scanning electron microscope (SEM) images showed bacteria attached to the sand grains which could modify the grain surface (e.g. increasing the grain surface area and/or form a biofilm) and thus impact IP measurements. In future, the number of bacteria present in the sand will be determined using quantitative polymerase chain reaction (qPCR) to detect bacterial DNA (deoxyribonucleic acid).

Introduction

For the characterization and monitoring of in-situ remediation of chlorinated hydrocarbon contamination, interdisciplinary approaches and geophysical methods are needed to secure water supply of sufficient quality and quantity. One particular concern are sites contaminated with highly toxic chlorinated hydrocarbons (used by e.g. dry-cleaning facilities), as these compounds are heavy and thus prone to contaminate the groundwater. In order to reach the goals of remediating these sites within reasonable time frames and realistic costs, it is necessary to move away from “excavate-and-remove” in favour of in-situ remediation. The latter however requires verification of the remediation, by a suitable monitoring program, to avoid long-lasting chemical contamination of the groundwater (MIRACHL¹). Natural remediation can occur through processes where bacteria metabolize the chemicals, breaking them down into less harmful components. However, since this occurs below ground, the characterisation and monitoring of microbial in-situ remediation requires an interdisciplinary approach that combines microbiology with geophysical investigations.

Field investigations have already shown that microbial activity can affect the geophysical signature (Atekwana & Atekwana 2004, Atekwana et al, 2004). To interpret these geophysical field data, it is crucial to know the geophysical characteristics of geological materials and recognise the signature of different kinds of bacteria that interact with these materials. This will allow the sensitivity of the methods for these specific applications to be assessed. One promising tool for this is the spectral induced polarisation (SIP) technique. Direct-current resistivity method, together with measurement of induced polarization properties can give information about geochemical characteristics (e.g., Lesmes and Friedman 2005, Atekwana & Slater 2009). The studies from Abdel Aal et al (2004, 2009, 2010) have shown that this method has potential to detect microbially-induced signatures in the SIP signals.

In our study we observed *Escherichia coli* (*E. coli*) bacteria attached to sand to investigate the influence of these bacteria on the IP signals, including changes over time to follow bacterial growth. We have chosen to study a known *E. coli* strain, DSM 1116, that is suitable for laboratory studies, as a starting point for our geophysical measurement.

Experimental design

We designed an experiment including three test conditions:

- 1.) Measurement of 8 individual samples filled with sand, media (nutrient food) and *E. coli* bacteria (***E. coli* – sand samples**)
- 2.) Measurement of 8 individual samples filled with sand and media (**media – sand samples**)
- 3.) Measurement of 8 individual samples filled with sand and water (**water – sand samples**)

As the media, we used a rich source of nutrients (Luria-Bertani broth - LB). The bacteria were grown together with the media and mixed in different flasks with pre-sterilised Ottawa sand. These bacteria-sand-mixtures were continuously shaken (30°C, 80 RPM, *Figure 1*) until defined endpoints (within 21 days) when the mixtures were harvested and packed in a sample holder to measure the SIP in the frequency domain (FDIP) (frequency range 1 mHz – 10 kHz), time domain (TDIP) (1 s ON/OFF time) and SP (self-potential) under laboratory conditions. The same procedure was repeated with only the media-sand mixture to exclude any influences from just the nutrient and with water-sand mixtures.

As a sample holder we used a 4-point measuring cell, with plate electrodes for current injection and ring electrodes for potential measurements. FDIP, TDIP and SP was measured with the PSIP instrument by Ontash & Ermac. The individual samples were harvested at different days after incubation (at days 1, 2, 3, 6, 9, 13, 16, 21/63). The sand was filtered to separate the fluid and carefully packed in the sample holder and weighed. The weight range differed within 14% between all samples. Immediately after packing, the samples were measured and held in the sample holder for at least 24 h, during which the samples were measured repeatedly.

To confirm growth of *E. coli* in contact with the sand, liquid media separated from the sand was plated on LB agar, incubated at 37° C overnight and counted (*Figure 2a*). Fluid conductivity, pH and

¹ Website: <http://mirachl.com/>

temperature of the liquid removed by filtering were also measured. Portions of sand were frozen for future DNA analysis. SEM (scanned electron microscopy) pictures were taken to examine presence and distribution of bacteria (Lund University Bioimaging Centre).



Figure 1 Eight individual samples in the shaker.

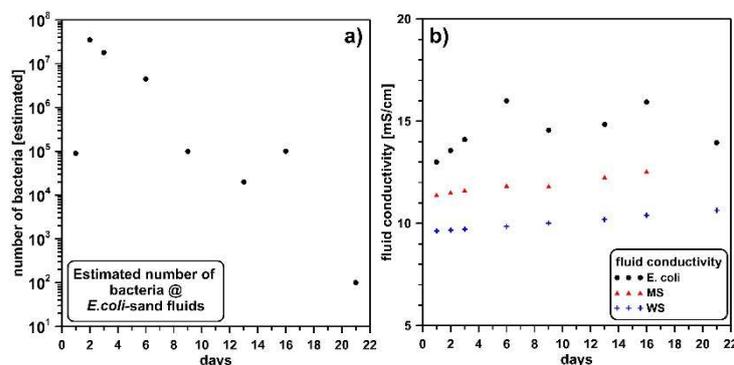


Figure 2 a) Number of bacteria in the filtered liquid over times (days). b) Comparison of fluid conductivity for all three test conditions (*E. coli*-sand, media-sand and water-sand samples).

Results

Comparing the fluid conductivity of the liquid from all samples (*E. coli* - sand, media – sand and water – sand, *Figure 2b*) differences between the three test conditions were observed. Fluid conductivity was higher (13 – 16 mS/cm) for samples containing bacteria, possibly due to the presence of bacteria and/or their degradation products. A slight increase in fluid conductivity with time for all samples can be explained by a (unavoidable) loss of fluid due to evaporation from the flasks, which must be aerated with shaking for bacterial growth. This would create an increasingly higher concentration of ions in the fluid, particularly over the longer time period of the study.

The measured pH value varied for all samples between pH 5 and 6 without any specific tendency. No clear differentiation could be made in the SP results (both data not shown), with all samples showing extensive scatter. Only the water – sand samples seems to be more homogenous in all, fluid conductivity, pH and SP.

The SIP measurements for the *E. coli* - sand samples showed very low resistivities ρ for all samples due to high NaCl concentration in the media (*Figure 3a*). During approximately the first two weeks, the resistivity values differed slightly, with only the last sample (day 21) showing a much higher resistivity than the others. In phase ϕ (*Figure 3b*), an increase within the first 6 days can be observed followed by a decrease in the phase shift. Reasons for this may be related to the number of bacteria (*Figure 2* **Error! Reference source not found.**). After two days, the maximum number of bacteria was counted in the fluid and then decreased, likely due to starvation. As these trends were only observed in the liquid, these population dynamics need to be confirmed by using DNA analysis to directly observe the number of bacteria that were being measure, in the sand.

In the SEM picture from the *E. coli* – sand sample day 6 (*Figure 3c*) the bacteria on the sand grain can be seen clearly. Biofilm formation (“spider-webs”) is also observed. By attaching to the sand grain, this bacterial biofilm increases the inner surface of the grain which results in an increase of the imaginary conductivity and thus in an increase in the phase shift signal (Abdel Aal et al. 2004, Ntarlagiannis et al. 2005, Davis et al. 2006).

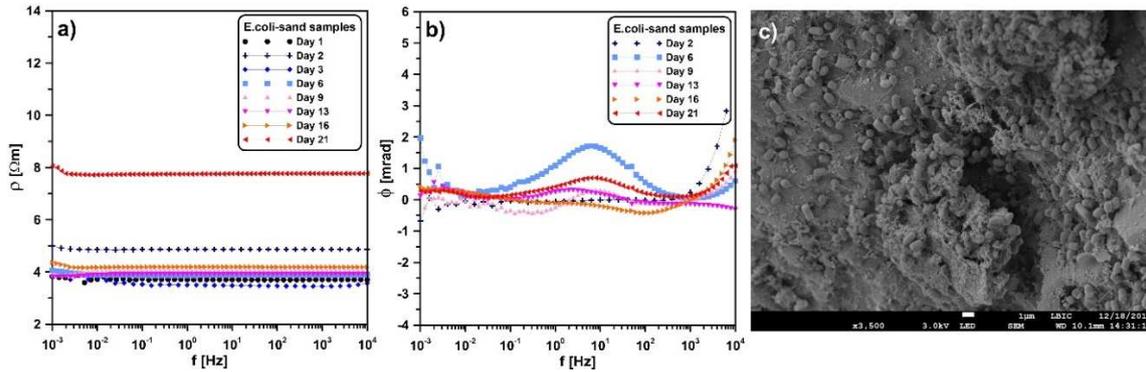


Figure 3 SIP measurement results for *E. coli* - sand samples from Day 1 to 21 (a) resistivity ρ , b) phase ϕ and SEM picture at Day 6 (c).

Comparing the SIP results for all samples at frequency $f = 1$ Hz (Figure 4) the most significant differences can be seen in the resistivity (a) resp. real conductivity σ' (c). According to the fluid conductivity of the filtered liquid, the presences of bacteria decreases the resistivity (resp. increase the real conductivity) of the sand samples. For later samples (> 6 days) the resistivity scattered more for all sample steps, which again may be explained by evaporation of the media. Due to the length of incubation it also cannot be ruled out that the resistivity scatter could be due to growth of contamination bacteria, and again, detailed DNA analysis to quantitate *E. coli* and total bacteria present in the sand will address this concern. In phase (Figure 4b) the rise in the first 6 days for the *E. coli* – sand samples can be clearly seen (compare Figure 3a). After that the phase decreases again. In imaginary conductivity σ'' a decrease in the first 6 days can be noticed, thereafter it increases again. So far, it is not fully understood how this behaviour can be explained. In contrast to the *E. coli* samples, the media – sand and water – sand samples show no significant rise but scatter around zero. As expected, neither the media nor the water is affecting the phase.

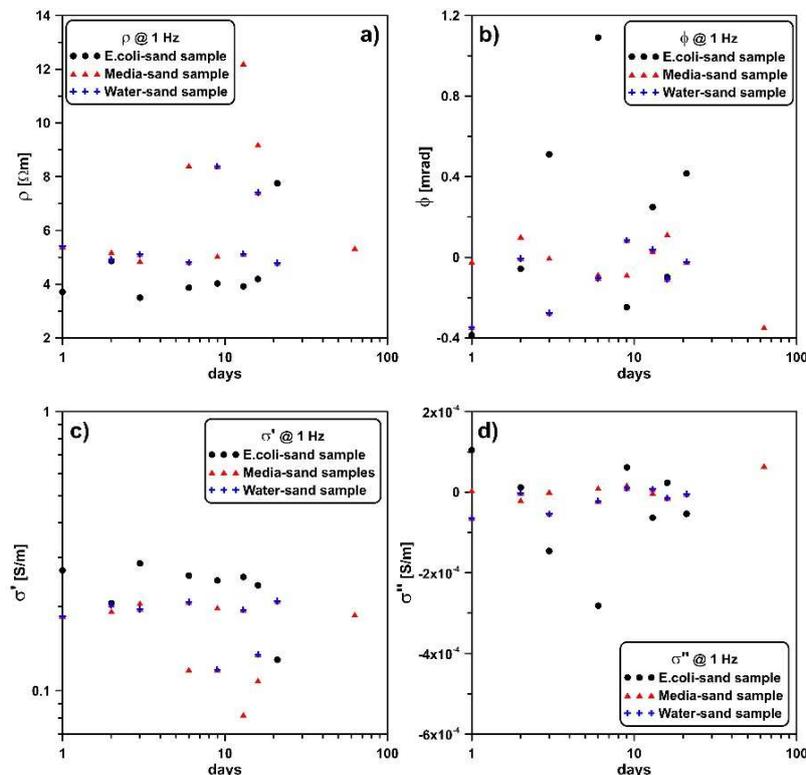


Figure 4 SIP comparison for *E. coli* - sand (black dots), media- sand (red triangles) and water – sand (blue crosses) samples at 1 Hz. a) resistivity ρ , b) phase ϕ , c) real conductivity σ' and d) imaginary conductivity σ'' .

Conclusion

In our first experiments with *E. coli* bacteria growing as biofilm in a sand environment, we could observe that the presence of bacteria affects the SIP signal. In contrast to the sample without bacteria the resistivities are generally smaller likely due to the presence of bacteria cells and/or their degradation products. With increasing incubation time, the phase starts to increase due to the increase of the inner surface by the attachment of the bacterial biofilm to the sand grains. Probably due to undercharged nutrient support for the bacteria, the number of bacteria start to decrease after 2 days in the liquid which therefore results in the decrease of the phase shift after 6 days. The decrease in imaginary conductivity within the first 6 days cannot be explained so far and needs further investigations.

A next step is the verification of the results by additional experiments measuring SIP in time and frequency domain. Furthermore, quantitative polymerase chain reaction will be carried out to determine the bacterial DNA density in the sand.

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