On the α-polarization of bacterial suspensions: SIP measurements on four bacterial strains

Tamara PILAWSKI[(1)*], Wolfgang TAPPE[(2)], Egon ZIMMERMANN[(3)], Johan Alexander HUISMAN[(2)], Frank DELVIGNE[(4)], Frédéric NGUYEN[(1)]

[(1)]Applied Geophysics Research Unit, Department ArGEnCo, Faculty of Applied Sciences, University of Liège, Allée de la Découverte 9 (B52), 4000 Liège, Belgium; [(2)]Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany; [(3)]Central Institute for Engineering, Electronics and Analytics (ZEA-2), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany; [(4)]Microbial Processes and Interactions, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

Introduction

This experiment is part of a PhD thesis aiming at a better understanding of complex conductivity measurements during hydrocarbon biodegradation. The goal is to quantify different mechanisms proposed to explain the influence of bacteria on the electrical properties with lab experiments of increasing complexity.

This experiment was designed to study **direct bacterial polarization** on bacterial suspensions at frequencies **below 10 kHz**, so-called **α-polarization**. To our knowledge, only a **few studies have reported α-polarization measurements** and they were performed by **dielectric spectroscopy techniques relying on two electrodes and models to correct for electrode polarization at low frequencies.**

Objectives

- Measuring the **complex conductivity of bacterial suspensions** from 1 Hz to 10 kHz with an **impedance spectrometer based on a four point measurement method** as described in Zimmermann et al. (2008) and Huisman et al. (2015)
- Determining the **α-polarization** associated with bacterial cells in this frequency range.

Bacterial strains

Four bacterial strains were chosen for their different surface electrical properties.

Two gram-positive bacteria

Bacillus subtilis GA1

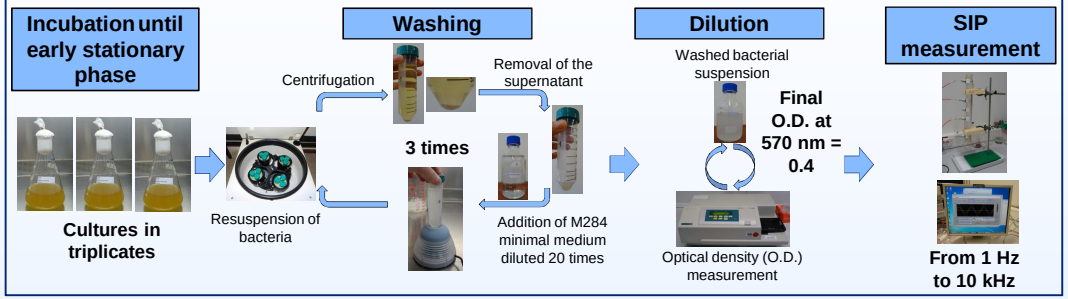
Rhodococcus erythropolis T902.1

Two gram-negative bacteria

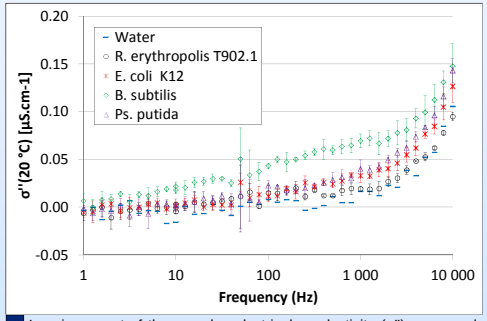
Escherichia coli K12

Pseudomonas putida KT2420

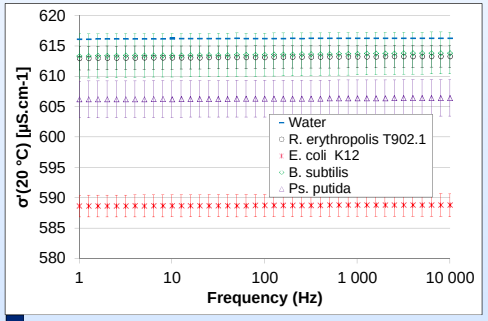
Experimental protocol



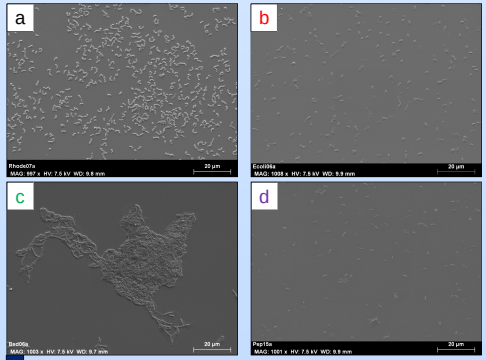
Results



1 Imaginary part of the complex electrical conductivity (σ'') measured on the 4 bacterial suspensions. The vertical error bars represent the standard deviation of the data from 3 independent measurements. For comparison, σ'' of the water used to suspend the bacteria is also shown on the graph. Only *B. subtilis* shows a small polarization below 10 kHz.



2 Real part of the complex electrical conductivity (σ') measured on the four bacterial suspensions. They were obtained by averaging triplicates. The vertical error bars represent the standard deviation of the data.



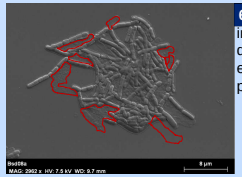
3 Scanning electron microscope images of (a) *R. erythropolis*, (b) *E. coli*, (c) *B. subtilis* and (d) *Ps. putida*. Only B. subtilis forms large aggregates.

R. erythropolis	*E. coli*	*B. subtilis*	*Ps. putida*
$(9.0\pm0.2)*10^7$	$(6.3\pm0.2)*10^8$	$(5.5\pm2.9)*10^7$	$(6.7\pm1.0)*10^8$

4 Colony-forming units per millilitre (CFU/ml) of bacterial suspensions. These values give an estimate of the number of viable cells in each sample.

Water	*R. erythropolis*	*E. coli*	*B. subtilis*	*Ps. putida*
7.2	7.0	6.6	7.0	7.0

5 pH of the bacterial suspensions and of the water used to suspend the bacteria. The bacteria produced organic acids because of limited oxygen during the SIP measurements.



6 Scanning electron microscope image of B. subtilis. Red curves delineate some of the extracellular polymeric substance produced by the bacteria.

Conclusions

α-polarization has been postulated to explain changes in the complex electrical conductivity observed in geological media affected by bacterial activity below 10 kHz. To date, α-polarization measurements on bacteria remain rarely reported expect in a few dielectric spectroscopy studies (e.g. Bot and Prodan, 2009; Zhang *et al.*, 2013) because of the magnitude of the correction resulting from electrode polarization that occurs in the kHz frequency range and below (Asami, 2014).

We performed complex electrical conductivity measurements on four bacterial suspensions using an impedance spectrometer at frequencies from 1 to 10 kHz. We observed **small polarization for *B. subtilis* from 100 to 10 kHz** (corresponding to a **phase shift of 0.1mrad**). SEM observations showed that *B. subtilis* was the only strain to form **aggregates of tens of µm** and to produce some **extracellular polymeric substance**, like in biofilms. Whether the cause of the polarization could be attributed to the size of the aggregates and/or to the extracellular polymeric substance is still to be determined.

Future experiments will focus on bacterial growth and attachment, and biofilm formation in silica sands.

References

- Asami, K., 2014, Low-frequency dielectric dispersion of bacterial cell suspensions: Colloids and Surfaces B: Biointerfaces, 119.
- Bot and Prodan, 2009, Probing the membrane potential of living cells by dielectric spectroscopy: European Biophysics Journal, 38 (8), 1049-1059.
- Huisman ,J.A., Zimmermann, E., Esser, O., Haegel, F.-H., Treichel, A., and Vereecken, H., 2015. Evaluation of a novel correction procedure to remove electrode impedance effects from broadband SIP measurements: Journal of Applied Geophysics.
- Zhang, C., Slater, L. and Prodan, C., 2013, Complex Dielectric Properties of Sulfate-Reducing Bacteria Suspensions: Geomicrobiology Journal, 30 (6), 490-496.
- Zimmermann, E., Kemna, A., Berwix, J., Glaas, W., Münch, H.M., and Huisman, J.A., 2008, A high-accuracy impedance spectrometer for measuring sediments with low polarizability: Measurement Science and Technology, 18, 105603.

Acknowledgements

This work was supported by a *Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture* (F.R.I.A.) grant funded by the Belgian National Fund for Scientific Research (*Fonds National de la Recherche Scientifique*, FNRS) and attributed to T. Pilawski.

*tamara.pilawski@ulg.ac.be