

2-1-2009

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Recommended Citation

G. Z. Abdel Aal et al., "Effect of Bacterial Adsorption on Low Frequency Electrical Properties of Clean Quartz Sands and Iron-Oxide Coated Sands," *Geophysical Research Letters*, vol. 36, no. 4, American Geophysical Union (AGU), Feb 2009.

The definitive version is available at <https://doi.org/10.1029/2008GL036196>

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Effect of bacterial adsorption on low frequency electrical properties of clean quartz sands and iron-oxide coated sands

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Received 1 October 2008; revised 22 December 2008; accepted 26 December 2008; published 27 February 2009.

[1] Low frequency electrical measurements (0.1–1000 Hz) were conducted to investigate the adsorption effect of *Pseudomonas aeruginosa* cells onto clean quartz sands and iron-oxide coated sands. The clean quartz sands showed a gradual increase in the microbial adsorption to mineral grains, concurrent with an increase of 13% in the imaginary conductivity component (σ''). However, iron-oxide coated sands (20–100% by weight) showed a rapid increase in microbial adsorption with σ'' reaching a maximum of 37 % for the 80–100% iron coated sands. No significant changes were observed in the real conductivity component (σ') due to microbial adsorption. A power law dependency was observed between the adsorbed cells and σ'' . We suggest that the polarization results from the increase in the surface roughness and surface area of the grain due to bacteria sorption. These results suggest that low frequency electrical measurements can play an important role in assessing microbial transport in subsurface environments. **Citation:** Abdel Aal, G., E. Atekwana, S. Radzikowski, and S. Rossbach (2009), Effect of bacterial adsorption on low frequency electrical properties of clean quartz sands and iron-oxide coated sands, *Geophys. Res. Lett.*, *36*, L04403, doi:10.1029/2008GL036196.

1. Introduction

[2] At Earth's surface and in the subsurface, some microbial cells are free living, but most exist attached to surfaces [e.g., Hazen *et al.*, 1991]. Studies of bacterial attachment in ground water systems are essential to accurately predict and quantify the extent of bacterial transport in aquifers and for the development of bioremediation strategies. There are several factors which potentially influence the adhesion of bacteria onto mineral surfaces, including bacterial species, the chemistry and surface area of the geological media, and the chemistry (ionic strength), pH, flow rate and temperature of the pore water [e.g., van Loosdrecht *et al.*, 1989; Mills *et al.*, 1994; Yee *et al.*, 2000; Jiang *et al.*, 2007].

[3] Microbial colonization of mineral surfaces can have a profound effect on the chemical and physical properties of their environment potentially measurable by geophysical techniques. Several laboratory and field studies have documented changes in geophysical signals associated with microbial growth and enzymatic activity in geologic media. Proposed mechanisms for geophysical changes in biostimu-

lated geologic media include: (1) enhanced mineral weathering [e.g., Atekwana *et al.*, 2004a, 2004b]; (2) growth of microbial cells attached to sediment grains in biofilms [e.g., Abdel Aal *et al.*, 2004, 2006; Davis *et al.*, 2006]; (3) biomineralization [e.g., Ntarlagiannis *et al.*, 2005a; Williams *et al.*, 2005; Personna *et al.*, 2008]; (4) pore clogging [e.g., Ntarlagiannis *et al.*, 2005b]; and (5) redox processes [e.g., Naudet and Revil, 2005]. Clearly, there is a knowledge gap in our understanding of the mechanism(s) resulting in the electrical/geophysical response of microbial interactions with geologic media and many questions remain, including (1) does the direct presence of microbial cells in porous media cause changes in electrical properties? (2) What is the source mechanism for the polarization response in biological systems? (3) What is the magnitude of polarization of cells sorbed to mineral surfaces?

[4] The work described in this letter advances the work of Ntarlagiannis *et al.* [2005b]; Abdel Aal *et al.* [2006], and Davis *et al.* [2006] by investigating the polarization response resulting from the direct adsorption of the gram-negative bacterium *Pseudomonas aeruginosa* on minerals including clean quartz sands and iron coated sands. The information obtained in this study is of fundamental importance for the interpretation of microbial-induced polarization (IP) signals and should have some important implications for the application of geophysics to microbial transport studies. Here we show for the first time that the imaginary conductivity was directly correlated to the magnitude of cell sorption to mineral surfaces and conclude that the enhancement in polarization previously observed in other studies was in part the direct response of the sorbing/attaching of cells to mineral surfaces.

2. Methods

2.1. Bacteria and Growth Conditions

[5] The bacterial strain used for the microbial attachment experiments performed throughout the study was *Pseudomonas aeruginosa*, which is known to be a model organism in microbial attachment and motility studies. *P. aeruginosa* is a gram negative, 1 $\mu\text{m} \times 3 \mu\text{m}$ rod-shaped bacterium with a flagellum and an estimated surface area of 25–30 μm^2 . The strains were obtained from the University of Denmark in Lyngby, Denmark where previous studies were conducted with the bacteria [Pamp and Tolker-Nielsen, 2007]. The specific strain used was PAO1 Tn7-Gm-gfp, having no structural mutations to hinder microbial attachment. The cells were grown in 30% Bushnell Haas broth, (0.41 mM magnesium sulfate, 0.05 mM calcium chloride, 1.84 mM monopotassium phosphate, 1.89 mM diammonium hydrogen phosphate, 2.47 mM potassium nitrate, 0.08 mM ferric chloride), with the addition of 30 mM

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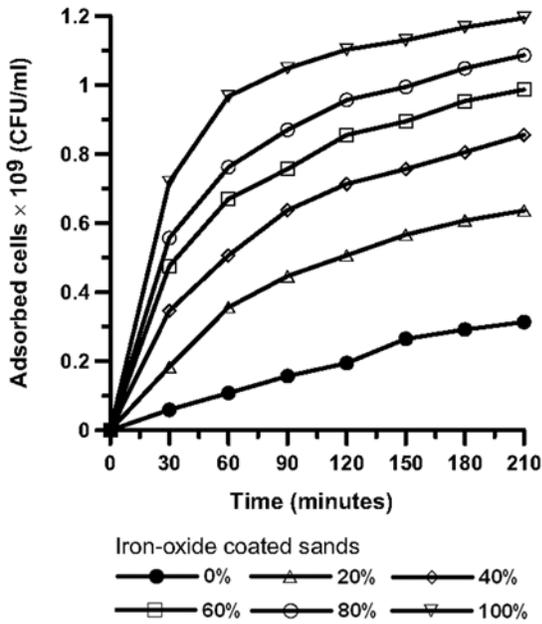


Figure 1. Temporal change in adsorption of *Pseudomonas aeruginosa* cells onto different percentages of iron-oxide coated sands.

glucose as a carbon source. 50 ml of cells were incubated for 24 – 48 hours at 37°C on a rotary shaker at 150 rpm. To remove the cells for laboratory use, the culture was centrifuged at 2000 rpm for 50 minutes, the supernatant was removed, and the cells were washed with Artificial Ground Water (AGW; see below) before being re-suspended in a final sterile AGW solution (1000 $\mu\text{S}/\text{cm}$) for experimental use. Optical density (OD) measurements were used to approximate the number of cells in solution at any given point in time using a spectrophotometer. To compare the OD to the actual number of cells, serial dilutions and plate counts were used to determine the colony forming units (CFU/ml). CFU/ml were plotted against the corresponding OD readings, resulting in a highly correlated linear function (cell counts (CFU/ml) = $5.40 \times 10^9 \cdot \text{OD}_{600} + 1.46 \times 10^8$ with $R^2 = 0.98$) which was used to convert OD_{600} to cell counts (CFU/ml).

2.2. Iron-Oxide Coating Procedure

[6] Iron coated sands were prepared following a procedure similar to that used by *Joshi and Chaudhuri* [1996]. A fraction of the pretreated sands were mixed with a 2 M ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \times 9\text{H}_2\text{O}$) solution (adjusted to pH 11 using 10 M sodium hydroxide). The mixture was placed in a drying oven at 110°C for 14 hours. After drying, the iron coated sands were washed several times with deionized water (DIW) until the runoff was clear, then the sands were oven dried at 105°C. Different percentages (0, 20, 40, 60, 80, and 100%) of dry weight of iron coated sands to clean quartz sands were prepared and used for the experiments.

2.3. Artificial Groundwater

[7] The solution used for the attachment experiments was an artificial groundwater (AGW) similar to that used by *Mills et al.* [1994]. The formulation for the AGW used in this study was as follows: KNO_3 , 0.75 mM; MgSO_4 ,

7.00 mM; CaSO_4 , 5.10 mM; NaCl , 1.70 mM; NaHCO_3 , 7.00 mM. The AGW solution was diluted with distilled deionized water (DIW) to yield a fluid conductivity of 1000 $\mu\text{S}/\text{cm}$.

2.4. Bacteria-Mineral Adsorption in Sand Columns

[8] Six identical columns made of polyvinyl chloride (PVC), 10 cm long and with an inner diameter of 3.8 cm were used. Two Ag-AgCl current injection electrode coils were installed 10 cm apart in the column, and two Ag-AgCl potential electrodes (3 cm apart) were installed between the current electrodes. A schematic representation of the IP setup used for the experiment can be found elsewhere [e.g., *Davis et al.*, 2006]. All columns, tubing, and accessories were disinfected by rinsing with ethanol. The columns were dry packed with the different percentages of iron coated sands. Several pore volumes of the background solution (AGW) were pumped through the column until the pH and specific conductance of inlet and outlet solutions were the same. To start the experiment, a peristaltic pump was used to continuously feed the media (filter sterilized AGW with 1.63×10^9 (CFU/ml) of initial bacteria concentration) through the sand columns at a flow rate of 0.5 ml/min. IP measurements (0.1–1000 Hz) and fluid samples of both the media being pumped into the sand columns (influent) and media coming out of the sand columns (effluent) were taken every 30 minutes. The samples were analyzed for the cell counts (CFU/ml) by obtaining the OD at 600 nm using a spectrophotometer and the obtained values were converted into cell counts (CFU/ml) using the pre-derived equation described above. The number of cells sorbed to the mineral surfaces was determined by subtracting the number of cells at each sampling time from the number of cells in the initial suspension. In addition, the fluid conductivity, pH and temperature of the sampled media were measured immediately after sampling using microelectrodes.

2.5. Low Frequency Electrical Measurements

[9] Low frequency electrical measurements (0.1–1000 Hz) were obtained by using a four-electrode technique as described by *Davis et al.* [2006] based around a National Instruments (NI) 4551 dynamic signal analyzer. The impedance magnitude $|\sigma|$ and the phase shift ϕ (between a measured voltage sinusoid and an impressed current sinusoid) of the sample were measured relative to a high-quality resistor. The real ($\sigma' = |\sigma| \cos \phi$) and imaginary ($\sigma'' = |\sigma| \sin \phi$) parts of the sample complex conductivity were then calculated. The real conductivity is an energy loss term that contains an electrolytic (σ_{el}) and interfacial (σ'_{surf}) component [e.g., *Lesmes and Frye*, 2001]. The σ'_{surf} results from surface conduction via the formation of the electrical double layer (EDL) at the grain-fluid interface [*Revil and Glover*, 1998]. The σ'' is an energy storage or polarization term, which at low frequencies (<1000 Hz) results primarily from the polarization of the ions in the EDL at the mineral-fluid interface [*Lesmes and Frye*, 2001].

3. Results

[10] Figure 1 shows the temporal change in number of cells (CFU/ml) being adsorbed onto different percentages of iron-oxide coated sands. It is evident that *P. aeruginosa* displays a higher affinity for iron coated sand surfaces than

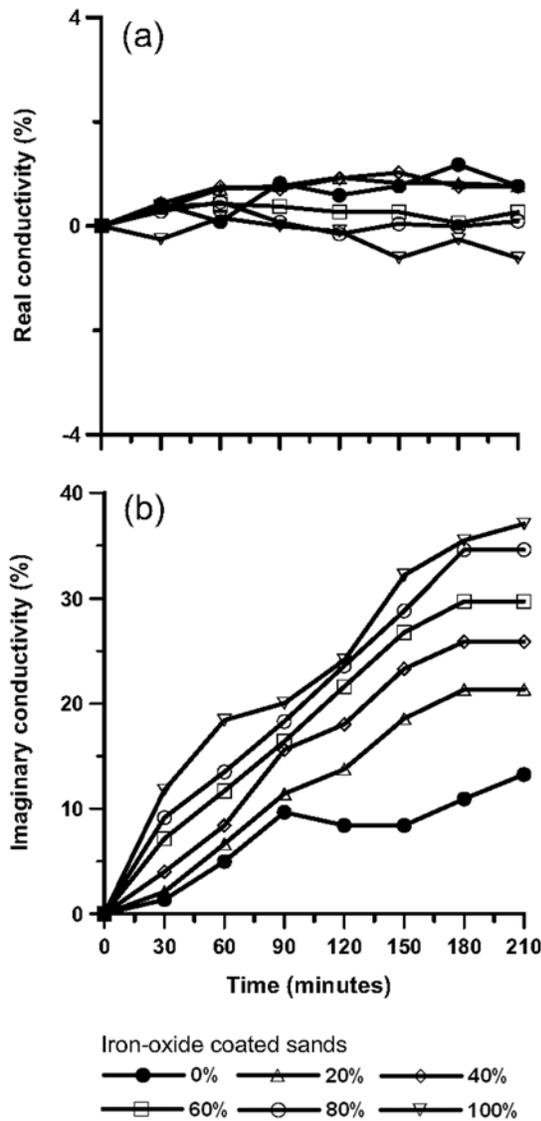


Figure 2. Temporal percent change in (a) real conductivity and (b) imaginary conductivity due to adsorption of *Pseudomonas aeruginosa* cells onto different percentages of iron-oxide coated sands.

for clean quartz surfaces, and that the extent of bacterial adsorption onto mineral surfaces as a function of time increases with percentage of iron coated sands. The highest magnitude of bacterial cell adsorption occurred with the highest percentage of iron coated sands and the lowest magnitude was obtained for the clean quartz sands. Even small amounts of iron oxide coated sand influenced the extent of adsorption of bacterial cells. Figure 1 indicates that for the iron coated sands, adsorption proceeded rapidly with equilibrium reached within the first initial 1 hour and with minimal subsequent changes to the extent of adsorption.

[11] Figure 2 shows the temporal percent changes in measured low frequency electrical parameters (real and imaginary conductivity) due to adsorption of *P. aeruginosa* cells onto mineral surfaces of different percentages of iron-oxide coated sands. We present the electrical data at 10 Hz as this was the frequency at which our measurement error was lowest. There is no clear relationship between the σ'

and cell adsorption (Figure 2a) or significant changes in the σ' due to cell adsorption on sands with different iron content. In contrast, the polarization represented by the σ'' is sensitive to cell adsorption. The σ'' increased with cell adsorption to mineral surfaces as a function of time and percentages of iron coated sands (Figure 2b). The magnitude of the σ'' response of iron coated sands was greater, increasing with increasing percentages of iron coated sands (up to 37% for 80–100% Fe coated sands and ~13% for clean quartz sands). Thus the greater amount of bacterial cell adsorption on iron oxide coated sands is reflected in the σ'' . Nonetheless, we note that although maximum adsorption of cells to mineral surfaces occurred within the initial 1 hour of the experiment, this is not reflected in the σ'' data. Instead we observe that the polarization continued to increase reaching a threshold at ~140 minutes beyond which no subsequent significant changes in polarization occurred. This threshold point is reached much earlier (~90 min) for the clean quartz sand.

4. Discussion

[12] In this study, we tested the effect of adsorption of bacterial cells on low frequency electrical properties of clean quartz sands and iron coated sands. The greater affinity of bacterial adsorption to iron coated sands compared to clean quartz sands can be explained by the fact that the sorption of negatively charged bacterial cells [e.g., *van Loosdrecht et al.*, 1989; *Mills et al.* 1994] to positively charged Fe oxide coating [e.g., *Mills et al.*, 1994; *Jiang et al.*, 2007] is clearly favored by electrostatic attraction. The reason for the lesser amount of *P. aeruginosa* adsorption on clean sands is also due to the more negative charges on the quartz sands which exert stronger repulsion forces for *P. aeruginosa*. Our results are consistent with previous studies that suggest that the electrostatic properties of mineral surfaces play a vital role in the adsorption of bacteria to mineral surfaces [e.g., *Mills et al.*, 1994; *Yee et al.*, 2000; *Jiang et al.*, 2007] and that bacterial adsorption is dramatically enhanced in the presence of Fe coated quartz grains relative to pure quartz grains.

[13] The increase in cell adsorption to mineral surfaces with the increase in iron content is reflected in the polarization magnitude represented by σ'' (Figure 2b) consistent with the high sensitivity of σ'' to changes on the interface between the solid and fluid phase in sediments [*Börner et al.*, 1996]. However, σ' shows no dependence on bacterial adsorption onto mineral surfaces (Figure 2a). A linear correlation between σ'' and cell adsorption is revealed and we document a power law relation to cell adsorption

Table 1. Regression Coefficients and Power Law Exponent Values Derived From the Regression Analysis of Imaginary Conductivity (S/m) Versus Adsorbed Cells (CFU/ml)^a

Iron-Oxide Coated Sands	Regression Coefficient (R ²)	Power Law Exponent
0 %	0.87	0.06
20 %	0.89	0.14
40 %	0.94	0.23
60 %	0.91	0.28
80 %	0.87	0.33
100 %	0.78	0.39

^aConductivity is in S/m, and adsorbed cells are in CFU/ml.

(Table 1) with this power law exponent increasing from 0.06 for clean quartz sands to 0.39 for 100% Fe coated sands. This power law relationship suggests that σ'' is dramatically enhanced by the sorption of bacteria cells to mineral surfaces.

[14] The bacterial cell surface is suggested to be charged (due to dissociation and association of ionizable surface groups) and highly complex, analogous to colloidal particles. During bacterial adhesion, a heterogeneous redistribution of charges on the bacterial cell surface reduces the EDL repulsion enhancing attachment [Poortinga *et al.*, 2002]. In the frequency range of our measurements σ'' results primarily from the polarization of the ions in the EDL at the bacteria-mineral-fluid interface. The magnitude of this polarization depends on the mobility of the counterions in the stern layer, surface charge density, and the size of the grains (surface area) [Revil and Glover, 1998; Lesmes and Frye, 2001]. We suggest that the increase in the surface roughness of the mineral grains due to the progressive sorption of bacteria to the mineral surfaces causes the increase in polarization magnitude during microbial adhesion. The large surface area of the bacteria accentuates this effect. A recent study by Leroy *et al.* [2008] modeled the effect of surface roughness on the spectral induced polarization response of glass beads. They observed that second order heterogeneities such as surface roughness (with first order heterogeneities associated with the grain size distribution) creates an additional peak in the phase spectrum typically in the frequency range 1–100 Hz. Hence, it is conceivable that the sorption of the bacteria on the grain surfaces creates similar second-order heterogeneities.

[15] Although previous geophysical investigations have speculated that the microbial-induced polarization observed in porous media is due to cell interactions with mineral surfaces [e.g., Ntarlagiannis *et al.*, 2005b] or to cell growth and attachment [Davis *et al.*, 2006] no data has been provided to support this. We believe the data presented in this study provide evidence to support the above speculations and provide important insights useful for interpreting low frequency electrical measurements from active microbial systems. Hence, as far as know, this is the first research study presenting bacterial adsorption on minerals and their induced polarization response.

[16] Bacterial attachment onto mineral surfaces has been the focus of many microbial transport studies [e.g., Mills *et al.*, 1994; Yee *et al.*, 2000; Jiang *et al.*, 2007]. This is because the interactions between bacteria and soil particles play an important role in the fate and transport of a wide variety of contaminants including heavy metals [e.g., Jiang *et al.*, 2007]. For example, bacterial adsorption onto mineral surfaces retards bacterial mobility and the mobility of contaminants adsorbed onto the bacterial surfaces [Yee *et al.*, 2000]. On the other hand, motile bacteria with adsorbed contaminant can also significantly enhance contaminant transport through the subsurface [e.g., McCarthy and Zachara, 1989; Yee *et al.*, 2000]. Thus, quantifying the extent of bacterial adsorption onto mineral surfaces is essential for the accurate modeling of contaminant transport in groundwater systems [Yee *et al.*, 2000]. Given the fact that chemical changes induced in aquifers due to treatment programs can lead to the precipitation of certain chemical species including microbial induced precipitation of metal-

lic minerals (e.g., FeS or ZnS), the ability of IP to detect these mineralized zones [e.g., Ntarlagiannis *et al.*, 2005b; Personna *et al.*, 2008] and the enhancement in polarization resulting from the attachment of bacteria to these minerals suggest that low frequency electrical measurements can also play an important role in assessing the transport of microorganisms in subsurface environments.

5. Conclusion

[17] The results from this study provide evidence that low frequency electrical measurements, specifically the imaginary conductivity component, can be used as a proxy indicator of microbial adsorption to porous geologic media. We surmise that the observed polarization (σ'') response arises from the direct sorption of microbial cells on mineral grain surfaces and the subsequent increase in mineral surface roughness accentuated by the large surface area of the bacteria. This was supported by the strong power law dependency of σ'' on cell adsorption to mineral surfaces with the power law exponent increasing with increasing percentages of iron coated sands. These results further our understanding of the direct effect of microbial attachment to mineral surfaces. Since most microbial adsorption studies are laboratory/model based and have not been applied to field-scale efforts, our work suggests that geophysical techniques may have significant implications for microbial transport studies at field sites.

[18] **Acknowledgments.** This material is based in part on work supported by the National Science Foundation under grant OCE-0729642 and grant EAR 0525316. We thank S. Pamp and T. Tolker-Nielsen for providing the *Pseudomonas* strain to S. Rossbach. We further thank A. Revil and an anonymous reviewer for their constructive comments that helped improve the manuscript.

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