Journal of Applied Geophysics 75 (2011) 558–564

Contents lists available at SciVerse ScienceDirect

Journal of Applied Geophysics

journal homepage: www.elsevier.com/locate/jappgeo

Complex conductivity response to microbial growth and biofilm formation on phenanthrene spiked medium

Remy Albrecht [a,*], Jean Christophe Gourry [b], Marie-Odile Simonnot [c], Corinne Leyval [a]

[a] *Laboratoire des Interactions Microorganismes-Minéraux-Matière Organique dans les Sols UMR7137, Nancy Université, CNRS, Faculté des Sciences, B.P. 70239, 54506 Vandoeuvre-les-Nancy Cedex, France*
[b] *Bureau de Recherches Géologiques et Minières, ARN, BP6009, F-45060 Orléans, France*
[c] *Laboratoire Réactions et Génie des Procédés UPR3349, Nancy Université INPL-CNRS, 1 rue Grandville, BP 20451, 54001 Nancy Cedex, France*

ARTICLE INFO

Article history:
Received 14 April 2010
Accepted 2 September 2011
Available online 12 September 2011

Keywords:
Bacterial biofilm
Complex conductivity
Microbial processes
PAH

ABSTRACT

Several laboratory studies have recently demonstrated the utility of geophysical methods for the investigation of microbial-induced changes over contaminated sites. However, it remains difficult to distinguish the effects due to the new physical properties imparted by microbial processes, to bacterial growth, or to the development of bacterial biofilm. We chose to study the influence of biofilm formation on geophysical response using complex conductivity measurements (0.1–1000 Hz) in phenanthrene-contaminated media. Biotic assays were conducted with two phenanthrene (PHE) degrading bacterial strains: *Burkholderia sp* (NAH1), which produced biofilm and *Stenophomonas maltophilia* (MATE10), which did not, and an abiotic control. Results showed that bacterial densities for NAH1 and MATE10 strains continuously increased at the same rate during the experiment. However, the complex conductivity signature showed noticeable differences between the two bacteria, with a phase shift of 50 mrad at 4 Hz for NAH1, which produced biofilm. Biofilm volume was quantified by Scanning Confocal Laser Microscopy (SCLM). Significant correlations were established between phase shift decrease and biofilm volume for NAH1 assays. Results suggest that complex conductivity measurements, specifically phase shift, can be a useful indicator of biofilm formation inside the overall signal of microbial activity on contaminated sites.

1. Introduction

Subsurface soil contamination by polycyclic aromatic hydrocarbons (PAHs) represents a major concern for human health and environment (Wilcke, 2000). Some PAHs are toxic, mutagenic and carcinogenic (White and Claxton, 2004). Various remediation technologies have been developed from ex situ thermal desorption to in situ bioremediation, but large areas are still contaminated. Bioremediation using the ability of soil microorganisms to degrade PAHs is a cost effective and environmental friendly technique for in situ treatment of soil PAH contamination (Liebeg and Cutright, 1999). However, in situ monitoring of bioremediation efficiency is difficult. Several contributions have shown the utility of geophysical methods for the investigation of microbial-induced changes in geologic porous media (Abdel Aal et al., 2004; Atekwana et al., 2004a; Naudet and Revil, 2005; Ntarlagiannis et al., 2005a,b; Williams et al., 2005). However, the direct contribution of microbial growth and microbial activity remained unclear.

Among geophysical methods, complex conductivity was used to detect changes in the chemical properties of pore solutions caused by microbial growth and metabolism. Ntarlagiannis et al. (2005a) showed an enhanced polarization associated with the direct presence of dormant alive bacterial cells in silica sands. Ntarlagiannis et al. (2007) showed that electrically conductive appendages in metal reducing bacteria called bacterial nanowires were directly associated with electrical potentials. In the same way, Davis et al. (2006) investigated the effect of microbial growth and biofilm formation on the electrical properties of porous media. They suggested that dynamic changes in the imaginary conductivity arise from the growth and attachment of microbial cells and biofilms to sand surfaces. These authors concluded that complex conductivity techniques, specifically imaginary conductivity measurements are a proxy indicator for microbial growth and biofilm formation in porous media. However, it remains difficult to distinguish the effects due to microbial growth, to microbial activity and to biofilm development. Also, previous works were carried out in columns on mineral support, such as sand. But it is well known that mineral grains are at origin of a spectral polarization (e.g. Chelidze and Gueguen, 1999; Chelidze et al., 1999, Scott, 2006). It is easier to distinguish microorganism polarization effect, which is time dependent and mineral polarization effect, which is not. Ntarlagiannis et al. (2007) have demonstrated that the

---

* Corresponding author. Tel.: +33 3 83 68 42 83; fax: + 33 3 83 68 42 84.
E-mail address: remyalbrecht@gmail.com (R. Albrecht).

0926-9851/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jappgeo.2011.09.001

existence of nanowires connecting bacterial cells have an impact on self-potential signals. As a consequence they increase the surface conductance and thus could enhance the capacitance effect (Davis et al., 2006).

From these findings, we chose to examine the influence of biofilm formation on geophysical response (spectral induced polarization — SIP) using two bacterial strains, producing biofilm or not, but in absence of mineral substrate in order to examine the only influence of bacterial growth. Biofilm structure can be described as complex three-dimensional structures where cells are embedded in a thick matrix of extracellular polymeric substances. They are crossed by fluid-filled channels that enable nutrient transport to the interior parts of the biofilm and the removal of waste products (Hall-Stoodley et al., 2004).

Our objectives were to study microbial growth in hydrocarbon-contaminated media using a non-invasive geophysical investigation technique: spectral induced polarization (SIP), (i) to determine if the geophysical responses are sensitive to biofilm formation and (ii) to validate our interpretation of the geophysical responses using Scanning Confocal Laser Microscopy (SCLM) and elaborate an accurate correlation between geophysical response and biofilm formation. For that purpose, it was necessary to develop an original technique to measure SIP variations at bacteria cell surface.

2. Methods

2.1. Bacterial strains and culture conditions

Assays were conducted with two phenanthrene (PHE) degrading bacterial strains isolated from PAH-contaminated soils of a former gas works site in France (Leglize et al., 2008). Strains used were *Burkholderia sp* (NAH1), which produces biofilm and *Stenophomonas maltophilia* (MATE10), which does not. Three experimental assays were conducted in Petri plates (15.2 cm long) during 1 month at 24 °C: two biotic assays with pure cultures of NAH1 (with biofilm) and MATE10 (without biofilm), and one abiotic assay (control). Petri plates were filled with the sterile mineral nutrient Bushnell Hass (BH, 3.27 g L^{-1}) medium (*Fluka)* and phenanthrene (PHE, 200 mg L^{-1}). Bacteria were pre-cultured as described in Leglize et al. (2008). Cells were then washed twice with sterile NaCl solution (8.5 g L^{-1}). Bacterial cell densities were measured under a microscope using a Thoma numeration cell (Polylabo, France). 10^6 cells were added in each assay at the beginning of the experiment. In order to estimate the validity of our results, we conducted the three assays in three replicates.

2.2. Scanning Confocal Laser Microscopy (SCLM)

To observe biofilm structure formation over the duration of the experiment, 15 to 20 glass beads (2 mm diameter) were added in each Petri plate. The beads were collected along time of the experiment. Biofilm was stained using acridine orange (AO) buffer as described by Moller et al. (1996). SCLM observations of biofilm were performed on a Biorad confocal laser microscope (Radiance 2100) with a Nikon optic (TE2000 U Eclipse, 4 and 20×). To estimate biofilm formation with time, some glass beads added in each assay were observed. Spatial recovery and biofilm volume were measured on a band of 200 μm thickness on 16 glass beads.

2.3. Complex conductivity measurements

Complex conductivity (also called spectral induced polarization) method is widely used for mining exploration, for locating disseminated metallic ore bodies (e.g. Marshall and Madden, 1959; Sumner, 1976). In the presence of metallic ores, it has been demonstrated that electrochemical processes are responsible of variations of phase difference between an applied varying electrical current and the measured potential (Pelton et al., 1978; Wong, 1979). Olhoeft (1985), Börner et al. (1993) and more recently Atekwana and Slater (2010) have also shown that porous media could create induced polarization phenomenon. Most works were carried out on soil samples in columns where experimental protocol is widely discussed (Abdel Aal et al., 2010; Slater et al., 2009).

As we expect to measure complex conductivity variations due to microbial growth without mineral support, electrodes are installed directly at the surface of the medium.

Two thin Ag–AgCl current injection electrodes (4 mm diameter, Warner Instruments) were installed in the diagonal of the Petri plates, and two Ag–AgCl potential electrodes (3 cm apart) were installed between the current electrodes (Fig. 1). This configuration represents a micro Wenner-alpha electrical resistivity array. Transmitter is the Zonge LAB-2, which provides a square-wave current, with fundamental frequency f_0. Current intensity is as low as possible (around 0.2 mA), in order to have a low current density to avoid non-linear effects, but a sufficient measured potential difference (above 100 mV).

Spectral induced polarization (SIP) measurements (1–100 Hz) were obtained by using a four-electrode technique based with a Zonge GDP-32 Multi-Function Receiver. Impedance magnitude Z (or resistance) and the phase shift ϕ (between a measured voltage and an injected current) of the sample were measured relative to a high-quality resistor. Impedance magnitudes and phase shifts were measured at square-wave current fundamental frequency, odd integer harmonics, up to 9th harmonics.

For each frequency a 2-min repetition cycle (e.g: 128 cycles at 1 Hz and 2048 cycles at 16 Hz) was used in order to improve signal/noise ratio. Impedance precision was lower than 1% and phase shift precision was 0.1 mrad.

Geometric factor was calculated for this electrodes configuration, taking into account boundaries of Petri plates, by modeling using the ERTLAB 3-Dimensions Electrical Resistivity Tomography software (Morelli and Labrecque, 1996). Potential difference was calculated for a 1 Ω.m medium surrounded by a 1 MΩ.m insulating medium. Then resistivity magnitude ρ and phase shift ϕ between current and potential could be represented as complex resistivity ρ^* or complex conductivity components σ^*:

$$\sigma^* = \rho^{*-1} = |\sigma| e^{i}$$
$$\sigma^* = \sigma + i\sigma'' = |\sigma| \cos\varphi + i|\sigma| \sin\varphi$$

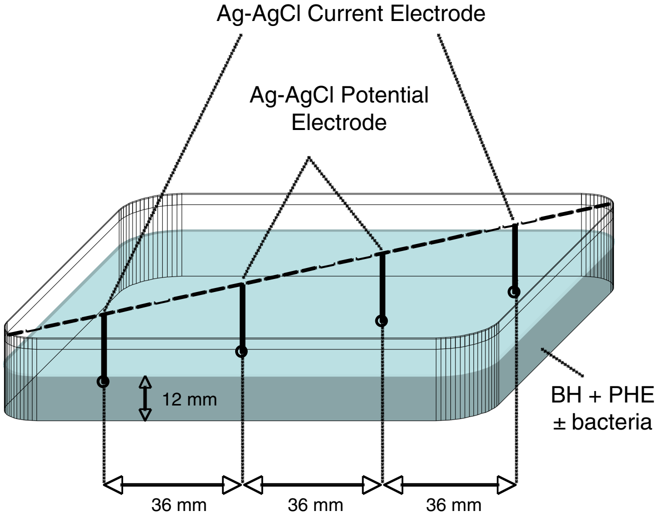

Fig. 1. Schematic diagram showing the experimental set up.