## Spatial & Temporal Geophysical Monitoring of Microbial Growth and Biofilm Formation

Caroline A. Davis<sup>1</sup>, Laura J. Pyrak-Nolte<sup>2</sup>, Estella A. Atekwana<sup>3\*</sup>, D. Dale Werkema Jr.<sup>4</sup> and Marisa E. Haugen<sup>2</sup>

- 1. Missouri University of Science and Technology, Rolla, MO 65409
- 2. Purdue University, West Lafayette, IN 47907
- 3. Oklahoma State University, Stillwater, OK 74078
- 4. U.S. Environmental Protection Agency, Las Vegas, NV 89119

Previous studies have examined the effect of biogenic gases and biomineralization on the acoustic properties of porous media. In this study, we investigated the spatiotemporal effect of microbial growth and biofilm formation on compressional waves and complex conductivity in sand columns. A control column (non-biostimulated) and a biostimulated column were studied in a 2D acoustic scanning apparatus, and a second set of columns were constructed with Ag-AgCl electrodes for complex conductivity measurements. At the completion of the 29-day experiment, compressional wave amplitudes and arrival times for the control column were observed to be relatively uniform over the scanned 2D region. However, the biostimulated sample exhibited a high degree of spatial variability within the column for both the amplitude and arrival times. Furthermore, portions of the sample exhibited increased attenuation ( $\sim 80\%$ ) concurrent with an increase in the arrival times, while other portions exhibited decreased attenuation ( $\sim 45\%$ ) and decreased arrival time. The acoustic amplitude and arrival times changed significantly in the biostimulated column between Days 5 and 7 of the experiment and are consistent with a peak in the imaginary conductivity ( $\sigma$ ") values. The  $\sigma$ " response corresponds to different stages of biofilm development. That is, we interpret the peak  $\sigma$ " with the maximum biofilm thickness and decreasing  $\sigma$ " due to cell death or detachment. Environmental scanning electron microscope (ESEM) imaging confirmed microbial cell attachment to sand surfaces in the biostimulated columns, showed apparent differences in the morphology of attached biomass between regions of increased and decreased attenuation, and indicated no mineral precipitation or biomineralization. The heterogeneity in the elastic properties arises from the differences in the morphology and structure of attached biofilms. These results suggest that combining acoustic imaging and complex conductivity techniques can provide a powerful tool for assessing microbial growth or biofilm formation and the associated changes in porous media, such as those that occur during bioremediation and microbial enhanced oil recovery. Furthermore, this study suggests microbial growth and development can vield a detectable geophysical response without biofilm biomineralization effects.

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