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Spatial & Temporal Geophysical Monitoring of Microbial Growth and Biofilm Formation

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1. ABSTRACT

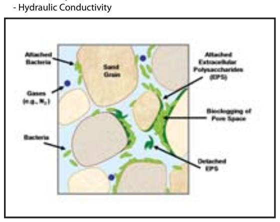
The spatiotemporal effect of microbial growth and biofilm formation was measured in sand columns using 2D acoustic wave and complex conductivity measurements. The biostimulated columns exhibited a high degree of spatial variability for both the amplitude and arrival times after 29 days. Furthermore, portions of the sample exhibited increased attenuation (~ 80%) with an increase in arrival times, while other portions exhibited decreased attenuation (~ 45%) with decreased arrival time. The acoustic amplitude and arrival times changed in the biostimulated column between days 5 and 7 of the experiment, consistent with a peak in the imaginary conductivity (σ") values. We propose that the observed σ" peak is related to the maximum biofilm thickness while the decreasing σ" is due to cell death or detachment resulting in an σ" response that corresponds to different stages of biofilm development. Microbial cell attachment to sand in the biostimulated columns was independently confirmed by environmental scanning electron microscope imaging, showing apparent differences in morphology of attached biomass between regions of increased and decreased attenuation, and indicate no mineral precipitation or biomineralization. Heterogeneity in the elastic properties may arise from differences in morphology/structure of attached biofilms. Combining acoustic and σ" techniques can provide additional information for assessing microbial growth or biofilm formation and associated changes in porous media, such as those that occur during bioremediation and microbial enhanced oil recovery.

2. BACKGROUND

- Previous studies have demonstrated that seismic methods are sensitive to microbial-induced changes in porous media, including:
  - Biogenic gases
  - Sulfide mineral precipitation
  - Microbially induced calcite precipitation (MICP)

- We need a mechanistic understanding of the processes resulting in biogeophysical signals

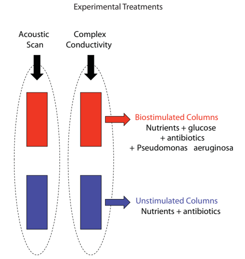
- Microbial growth can result in significant changes to the physical properties of a porous medium:
  - Porosity
  - Permeability
  - Hydraulic Conductivity



Bioclogging of pore space by bacteria cells, biomass (EPS), and biogenic gases

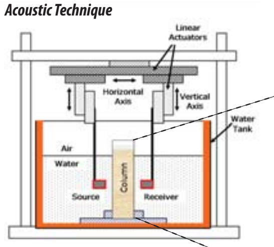
3. OBJECTIVE

Determine the effect of microbial growth and biofilm formation in porous media on acoustic wave propagation.

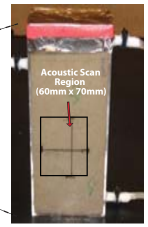


4. METHODOLOGY

Acoustic Technique

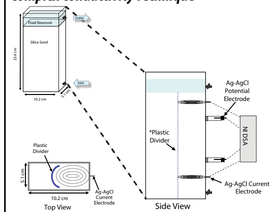


Acoustic imaging system and column placement in water tank



- Water-coupled transducers (1 MHz)
- Panametrics pulser / receiver
- Oscilloscope digitizes signal

Complex Conductivity Technique



- 0.1 – 1000 Hz
- 4-electrode technique
- NI 4551 DSA
- Measure impedance magnitude |σ| and phase shift ϕ
- Real (σ') and imaginary (σ") parts of σ* calculated

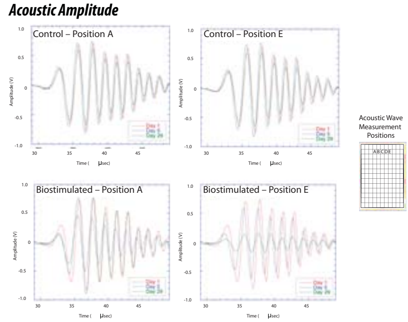
Additional Analyses

Geochemical:
- Fluid Samples
  - Fluid Conductivity
  - pH
- Measured using microelectrodes

Microbial:
- Sand samples
- Destructive sampling (sand sampled from the 2D acoustic scan region)
- Environmental scanning electron microscope (ESEM) imaging of sand surface characteristics

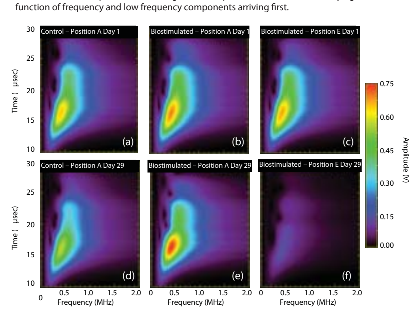
5. RESULTS

Acoustic Amplitude



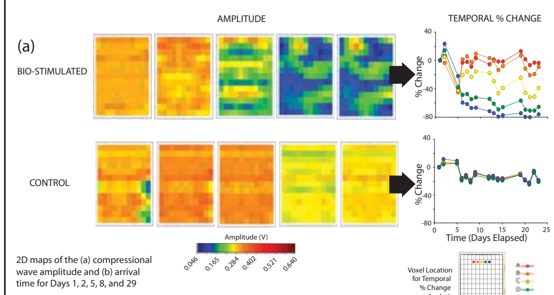
The acoustic amplitude scans presented for the control column at positions A & E did not significantly change in amplitude, arrival time, or phase throughout the experiment duration. The Day 1 biostimulated results for positions A & E are nearly identical. Day 5 shows both positions with a signal decreasing in amplitude as well as the presence of an observable phase shift.

The amplitude and arrival time vs. frequency contour plots below illustrate a maximum amplitude decrease of approximately14%. As well as a dramatic amplitude decrease observed in plots E, C & F. The maximum amplitude increased 7% for Position A and decrease 80% for Position B. While the transmitted compressional wave signals are dispersive, with arrival time varying as a function of frequency and low frequency components arriving first.



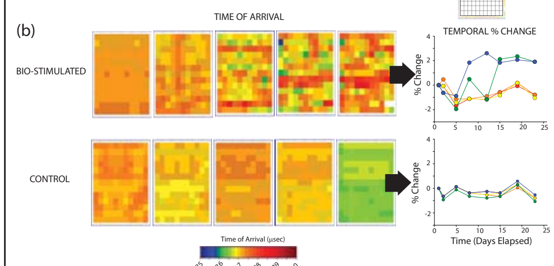
Day 1 & 29 amplitude and arrival times vs. frequency

2D Acoustic Scan Temporal Images & Analysis



2D maps of the (a) compressional wave amplitude and (b) arrival time for Days 1, 2, 5, 8, and 29

Voxel Location for Temporal % Change Analysis



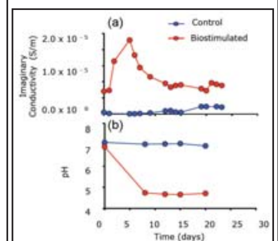
The control data set presented in the 2D acoustic scan sections above, shows a relatively uniform response in both the transmitted amplitude (a) and arrival time (b). The amplitudes generally become more spatially uniform over time, and they decrease slightly over the duration of the experiment. The measured amplitudes and arrival times became more spatially uniform over time.

The biostimulated data from Day 1, show the transmitted amplitude (a) and time of arrival (b) values are relatively uniform spatially over the scan region. By Day 5, spatial variations are observed over the scanned region, and relatively consistent spatial trend. Day 29 exhibits both increases in amplitude and time of arrival in some regions, while other regions show a decrease. The compressional waves transmitted through the biostimulated column showed decreases in amplitude as large as ~ 80% and increases in arrival time on the order of ~3%.

The percent change data is all presented relative to Day 1. The Day 5, biostimulated column transmitted amplitude and arrival time deviate sharply from the baseline (Day 1). The acoustic response from the control column remains relatively steady compared to baseline. Biostimulated column shows:
- Acoustic wave amplitudes are highly variable by Day 6, both increasing and decreasing in amplitude in the selected region
- Arrival times by Day 6 also vary in the selected region, though to a lesser degree.
- After Day 6, trend in amplitude and arrival times generally continued through the end of the experiment

The control column amplitude and arrival times remain relatively steady over time, and are consistent for all of the selected data points shown.

Complex Conductivity



Plots showing the temporal imaginary conductivity (at 10 Hz) and pH results.

- Biostimulated σ" (a) increased by ~220% to peak values (2.29×10^{-5} S/m) on Day 5 relative to pre-injection (Day -1) values then decreased to near baseline values by Day 14, and remained relatively consistent through Day 23
- Control column σ" magnitude small relative to the biostimulated column
- Control column remained steady near a pH of 7 throughout the experiment (b)
- Biostimulated column pH decreased from a pH value of 7 to near 4.4 on Day 8, then remaining at 4.4 to the end of the experiment

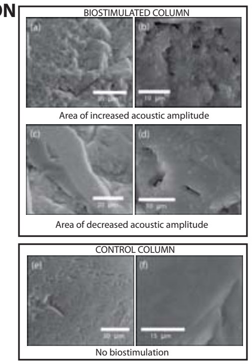
6. ESEM VERIFICATION

Environmental scanning electron microscope (ESEM) images from sand samples collected after acoustic column destruction.

- Bio Increased amplitude: (a and b) surface texture is rough with a patchy covering of 'bio-material" or EPS covering portions of the sand grain; other portions of the grain are not covered and the surface of the grain is visible

- Bio Decreased amplitude: (c and d) smooth biomaterial appears to completely cover the surface of the sand grain; several voids in the biomaterial covering the grain; rod-shaped bacteria present

- Control: (e and f) no bacterial cells or biomass are apparent in these images; surface of the silica sand grain is irregular but relatively smooth



Area of increased acoustic amplitude

Area of decreased acoustic amplitude

No biostimulation

7. DISCUSSIONS

Spatial Variability of Acoustic Properties

- Results suggest biostimulation of porous media alters the acoustic properties both spatially and temporally
- the observed amplitude variation in the biostimulated sample is attributed to the direct presence of biofilms
- increase in the transmitted wave amplitude is observed when a patchy covering of biomaterial is present on the grains
- this may be due to small amounts of biomaterial enhancing the coupling between grains either at grain contacts or directly coupling the grains together
- decreased amplitude regions occurred where biomaterial coated the grains and a smooth texture with numerous void spaces/pores or channels were present
- decreases in acoustic amplitude often result from biogenic gas production, weakening of grain contact/coupling, viscous losses from bio-altered pore fluid, or losses associated with skeletal frame complex moduli
- acoustic wave data from biostimulated column suggests multiple mechanisms may be responsible for the spatiotemporal variability in acoustic amplitude
- we infer that the spatial variations in acoustic amplitude and travel times result from the non-uniform distribution of biomass and variations in the biofilm structure in porous media, which affected the grain-to-grain coupling, pore geometry, fluid-solid coupling, and elastic/ viscoelastic response of the medium

Temporal Variability of the Complex Conductivity

- observed acoustic changes are consistent with a peak in the imaginary conductivity values on Day 5
- earlier work [Davis et al., 2006] demonstrated that imaginary conductivity measurements are uniquely sensitive to the physicochemical properties at grain fluid interfaces, and showed imaginary conductivity measurements may be used as a proxy indicator of microbial growth, attachment, and biofilm formation in porous media
- we suggest that the peak imaginary conductivity changes may reflect a peak in the concentration of attached biomass or biofilm thickness.
- we infer that variations in the acoustic amplitude that occurred between Day 5 and 7 are associated with the change in physical properties of the medium caused by peak microbial attachment and maximum biofilm thickness

Effect of Biofilm Development and Structure on Acoustic Properties

Davey et al. [2003] describes five different physiologies over the course of biofilm development by P. aeruginosa, including:

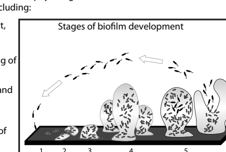
(1) initial reversible attachment,

(2) irreversible attachment,

(3) maturation through layering of bacterial cell clusters,

(4) maturation of cell clusters and maximum layer thickness, and

(5) dispersion of bacteria cells from within the inner portion of the biofilm



8. CONCLUSIONS

Our results suggest...
- Microbial growth and bifilm formation affects variability of acoustic wave amplitude & time of arrival
- Could be result of variatoins in biofilm distribution, stage of maturation / development, archetecture, texture
- Complex Conductivity is sensitive to different stages of biofilm development

Significance
- Bioremediation (i.e., subsurface biofilm barriers), bioclogging models
- Microbial enhanced oil recovery
- Ocean floor studies

9. ACKNOWLEDGEMENTS

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