Title:

Self-Assembling Sup-porosity: The Effect On Fluid Flow And Seismic Wave Propagation

Final Report

Reporting Period Start Date: 12/01/2005

Reporting Period End Date: 11/30/2009

Laura J. Pyrak-Nolte

April 2013

DOE Award: DE-FG02-97ER14785

Department of Physics

1396 Physics Building, Room 166

525 Northwestern Avenue

West Lafayette, Indiana 47907-1396

Table of Contents

1. Project Summary	2
2. Introduction	3
3. Results of Work (Summary of Publications)	3
3.1 Effect of a Sub-Porosity on Wave Propagation Across a Fracture	3
3.2 Monitoring Biofilm Growth in Sediments	6
3.3 Seismic Illumination Scale on Interpreting Fracture Specific Stiffness	7
4. List of Publications	8
5. Attachments: Selected Published Articles	9

1. Project Summary

Fractures and joints in the field often contain debris within the void spaces. Debris originates from many different mechanisms: organic and/or inorganic chemical reactions/mineralization, sediment transport, formation of a fracture, mechanical weathering or combinations of these processes. In many cases, the presence of debris forms a “sub-porosity” within the fracture void space. This sub-porosity often is composed of material that differs from the fracture walls in mineralogy and morphology. The “sub-porosity” may partially fill voids that are on the order of hundreds of microns and thereby reduce the local porosity to lengths scales on the order of sub-microns to tens of microns. It is quite clear that a sub-porosity affects fracture porosity, permeability and storativity. What is not known is how the existence/formation of a sub-porosity affects seismic wave propagation and consequently our ability to probe changes in the subsurface caused by the formation or alteration of a sub-porosity. If seismic techniques are to be developed to monitor the injection and containment of phases in sequestration reservoirs or the propping of hydraulically induced fracture to enhance oil & gas production, it is important to understand how a sub-porosity within a fracture affects macroscopic seismic and hydraulic measurements. A sub-porosity will directly affect the interrelationship between the seismic and hydraulic properties of a fracture.

This reports contains the results of the three main topics of research that were performed (1) to determine the effect of a sub-porosity composed of spherical grains on seismic wave propagation across fractures, (2) to determine the effect of biofilm growth in pores and between grains on seismic wave propagation in sediment, and (3) to determine the effect of the scale of observation (field-of-view) on monitoring alteration the pore space within a fracture caused by reactive flow. A brief summary of the results for each topic is contained in the report and the full details of the research and approach are contained in the publications found in the Attachment section of this report. A list of presentation and publications of all work associated with this grant is also provided.

2. Introduction

Knowledge of the scale dependence of seismic wave propagation and fluid flow through fractures and other porous media is necessary to extrapolate experiments performed in the laboratory on small-scale core samples towards the field scale. A goal of laboratory experiments is to illuminate fundamental physical behavior that can be used on the laboratory scale as well as on the field scale. To achieve this goal, our approach to understanding the interrelationship between the hydraulic and seismic properties of fractures is based on determining the relevant geometric length scales that define a fracture and how these length scales are altered through time dependent processes (Figure 1). In addition, our ability to seismically measure and quantify physical changes in fractures also depends on the length scales associated with seismic measurements.

Most research on fluid flow through, and seismic wave propagation in, fractures in rock has involved the use of “clean” fractures, i.e., fractures with void space relatively free of “debris”. However,

observation of fractures or joints (as well as pores in the matrix) in the field have shown that fractures can contain pieces of the fracture walls, organic and inorganic particles, mineralization, etc, just to name a few. In this project, we investigated the effect of “particles”, biofilms and scale of the seismic illumination on seismic wave propagation and interpretation. Specifically, we performed research (1) to determine the effect of a “sub-porosity” formed from spherical grains within a fracture on seismic waves, (2) to determine the ability to monitor biofilm growth in sediment over time, and (3) to determine the effect of scale of measurement on the interpretation of fracture specific stiffness.

Figure 1. The geometric scales associated with a fracture and the processes that alter these geometric scales over time. The ability to detect and monitor changes in fractures is a function of the seismic scales

3. Results of Work (Summary of Publications)

In Section 3, we provide a summary of the research results for the work on granular sub-porosities (section 3.1), biofilm growth (section 3.2) and scales effects related to the size of region probed (section 3.3). Additional details on the work performed can be found in the attachments to this report.

3.1 Effect of a Sub-Porosity on Wave Propagation Across a Fracture

The objective of this study was to explain and predict the effects of sub-porosity in a fracture on seismic wave propagation through fractured rock. The principal focus was on

experimentally determining the link between the frequency-dependent seismic properties of fractures and length scales associated with the presence, morphology and spatial distribution of a sub-porosity that affect the hydraulic properties of a fracture. Laboratory experiments were performed to examine acoustic wave scattering from packings of spherical beads used to create a sub-porosity within a synthetic fracture. The sub-porosity in the fracture was created by using spherical beads with a range of diameters from 500 μm to 7.79 mm. Compressional waves were transmitted across the fracture using contact piezoelectric transducer. The analysis was performed for a fixed aperture with different sub-porosity created by single and multiple layers.

The acoustic response depends critically on the length scales associated with the layers (bead diameter, layer thickness), the aperture of the fracture, and seismic wavelengths. The structure of the sub-porosity affected the time-frequency response of the transmitted seismic waves. For layers formed by successive deposition of beads smaller than a wavelength, the spectral response showed strong stop-band behavior. Good agreement was found between the information provided by the stop-band behavior (e.g., size of the grain in the fracture, the impedance contrast between the grains and fluids in the fracture) and the experimental parameters, suggesting that the spectral response of an in-filled fracture provides information about the infill material. Effective stiffness as a function of frequency was used to determine the dominant scattering regime produced by the sub-porosity (Figure 2). When the grain size is comparable to a wavelength, a frequency-independent effective stiffness is observed. Sub-wavelength grains produced a divergence of effective stiffness at low frequencies because the signals were not attenuated. For short-wavelengths, sub- and multiple wavelength bead sizes produced a linear dependence of fracture specific stiffness on frequency, essentially approaching the ray regime.

The detection of multiple layers of debris within a fracture is possible by understanding wave interference, dispersion and the reflections in the waveform. The complete description of the experimental approach, analysis methods and findings can be found in the publication entitled “*Effects of the Sub-porosity on Wave Propagation Across a Fracture*” found in the Attachment Section of this report.

Figure 2. The dependence of effective fracture stiffness on frequency enables the delineation of the scale of the structure associated with a sub-porosity, i.e. grain size and layering relative a wavelength.

3.2. Monitoring Biofilm Growth in Sediments

A laboratory study was performed to determine the effect of micro-scale phenomena, such as microbial growth and biofilm formation, on macro-scale seismic wave measurements, and to determine the relevant seismic attributes that indicate a sub-porosity has been formed/ destroyed. Evolution of a sub-porosity within a porous medium affects length scales associated with the spatial distribution of elastic and visco-elastic properties of a porous medium. The size of the geometric length scales relative to the seismic wavelength determine the scattering response caused by a non-uniform distribution of microbial growth and/or biofilm formation. Seismic imaging and induced polarization (IP) techniques were used to quantify the spatial and temporal changes in the geophysical response of water-saturated sand caused by bio-stimulation using *Pseudomonas aeruginosa*, a PAO1 wild type bacteria culture. This experimental approach was used on a bio-stimulated and a control (non-bio-stimulated) sand columns over a 29-day period.

We examined the effect of biofilm formation on compressional wave attenuation. Over a 29-day period, compressional wave amplitudes (Figure 3 *top*) for the control sample were observed to be relatively uniform over the scanned area. However, the bio-stimulated sample exhibited a high degree of spatial variability in amplitude (Figure 3 *bottom*). Environmental scanning electron microscopy (ESEM) confirmed microbial cell attachment to sand surfaces and showed apparent differences in the morphology of attached biomass between regions of increased and decreased attenuation. The heterogeneity in the elastic properties arises from the differences in the morphology and structure of attached biofilms. The density and the porosity of a biofilm varied with the growth and evolution of the microbial colony. Biofilms can affect the sediment matrix stiffness and the elastic and/or visco-elastic moduli of a porous medium by altering grain contact coupling, decreasing the size of pores and/or altering the relative motion between the bio-alter pore fluid and the solid grains (i.e., viscous losses). If the microbially-induced alterations are not uniformly distributed throughout the sediment, then the sediment textural and mechanical properties can vary spatially and lead to seismic scattering.

Figure 3. Two dimensional acoustic maps (60 mm by 70 mm) of the compressional wave amplitude for the (*top*) control sample and (*bottom*) bio-stimulated sample from Day 1 and Day 29 of the experiment.

The complete description of the experimental approach, analysis methods and findings can be found in the publications entitled “*Microbial-Induced Heterogeneity in the Acoustic Properties of Porous Media*” and “*Acoustic and electrical property changes due to microbial growth and biofilm formation in porous media*” found in the Attachment Section of this report.

3.3 Seismic Illumination Scale on Interpreting Fracture Specific Stiffness

The scaling behavior of the hydraulic and seismic properties of a fracture determines how properties observed on the laboratory size (typically less than tens of centimeters) relate to the same properties measured at larger sizes. To understand the scaling behavior of fracture properties, the length scales (Figure 1) of the fracture geometry (apertures, contact areas, spatial correlations) and the fluid phase distribution (wetting and non-wetting phase areas, interfacial areas) must be characterized and compared to the length scales associated with the seismic probe (wavelength, beam size, divergence angle, field-of-view).

For seismic wave propagation measurements across a fracture there are two length scales involved in addition to the length scale inherent in a fracture specific stiffness. One is the wavelength contained in the seismic signal and the second is the size of region probed by the acoustic measurements. Both are extrinsic scales that we controlled in our acoustic measurements. The wavelength of the signal affects the seismic regime under study (i.e., the Rayleigh regime, resonant regime or ray regime (Nolte et. al., 2000)). While in laboratory experiments, the lateral size of the acoustic lobe pattern at the fracture plane determines the region sampled with traditional contact transducers, which is a function of the diameter of the piezoelectric crystal. These two extrinsic length scales affect interpretation of fracture properties from seismic measurements.

For this study, we developed an acoustic lens system that enabled us to control the size of the region sampled by the acoustic wavefront for a range of wavelengths. We examined the effect of field-of-view on the seismic interpretation of fracture specific stiffness for fractures subjected to chemical alteration. Hydraulic and seismic measurements were made prior to and after the invasion of a hydrochloric acid in an initially water-saturated fracture in limestone. Seismic measurements were collected before, during and after the reactive fluid flow. Two methods for monitoring the seismic response of the sample were used: 1) a detailed compressional-wave transmission map of the sample; and 2) seismic measurements as a function of scale. In the second method, the acoustic lens system (Figure 4) was used to vary the seismic beam from 15 mm to 30 mm to 60 mm to determine the effect of scale in interpreting the changes in

Figure 4. An acoustic lens system was developed to expand the beam waist 15, 30 and 60 mm.

fracture properties.

Analysis showed that multiple measurements on the local scale cannot be averaged to obtain the same seismic response of a fracture as on the global scale. We observed that a non-uniform spatial distribution of fracture specific stiffness resulted in a scale-dependent seismic response of fracture properties, i.e., interpretation of fracture specific stiffness depends on the frequency content of the signal and field-of-view (i.e. illuminated area). This study showed that 21 measurements over three scales (i.e., fields of view) were needed to unravel the competing effects of spatial correlations and probability distributions in fracture specific stiffness. Measurements obtained from different fields of view enable the estimation of the spatial correlation length in fracture specific stiffness.

The complete description of the experimental approach, analysis methods and findings can be found in the publication entitled “*Laboratory-Scale Study of Field-of-View and the Seismic Interpretation of Fracture Specific Stiffness*” found in the Attachment Section of this report.

4. Publications & Presentations from this Work

A list of publications and presentations of the work associated with this grant period is provided in this section.

4.1 Journal Publications & Conference Proceedings

Acosta-Colon, A. A., Nolte D. D. and L. J. Pyrak-Nolte, Reactive Flow in a Fracture: Scale Effects in the Interpretation of Seismic Measurements, *Proceedings of the 7th International Workshop on Geophysics Applied to Rock Engineering*, Lisbon, Portugal, July 8, 2007 (2007).

Acosta-Colon, A. A. and L. J. Pyrak-Nolte, Effects of the Sub-porosity on Wave Propagation Across a Fracture, in the *Proceedings of the 42nd US Rock Mechanics Symposium and 2nd U.S.-Canada Rock Mechanics Symposium*, San Francisco, June 29-July 2, 2008, paper number ARMA 08-330 (2008).

Acosta-Colon, A. and L. J. Pyrak-Nolte, Seismic Wave Propagation across Partially-Infilled Fractures, *Extended Abstracts of the 2008 Annual Meeting of the Society of Exploration Geophysicists*, Las Vegas, Nevada, Nov 9-14, 2008.

Acosta-Colon, A., Nolte, D.D. and Pyrak-Nolte, L. J., Laboratory-scale study of field of view and the seismic interpretation of fracture specific stiffness, *Geophysical Prospecting*, 57, 209-224, (2009)

Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen, Microbial-Induced Heterogeneity in the Acoustic Properties of Porous Media, *Geophysical Research Letters*, doi:10.1029/2009GL039569 (2009).

Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen, Acoustic and electrical property changes due to microbial growth and biofilm formation in porous media, *Journal of Geophysical Research – Biogeosciences* 115, doi:10.1029/2009JG001143 (2010).

Li, W. Petrovitch, C. and L. J. Pyrak-Nolte, The effect of fabric-controlled layering on compressional and shear wave propagation in carbonate rock, *International Journal*

of the Japanese Committee for Rock Mechanics, V. 4, no. 2, Nov 2009, pp79-85 (2009).

Pyrak-Nolte, L. J., Fracture anisotropy: The role of fracture-stiffness gradients, *The Leading Edge*, Society of Exploration Geophysicists, September, p1124-1127, (2007).

4.2 Presentations

Acosta-Colon, A. and L. J. Pyrak-Nolte, The Effect of Scale on Seismic Monitoring of Fracture Alteration by Reactive Flow, 5–9 December 2005, San Francisco, CA, USA, Eos Trans. AGU, 86(52), 2005 Fall Meet. Suppl., Abstract H41B-0410

Acosta-Colon, A. A. and L J Pyrak-Nolte, 2006, Interpretation of the seismic response of a fracture as a function of the scale. Eos Trans. AGU, 87(36), Joint Assembly Suppl., Abstract NS32A-01.

Acosta-Colon, A. A. and L J Pyrak-Nolte, 2006, Alteration of the Hydraulic and Seismic Properties of a Fracture by Mineral Precipitation and Sand Deposition, Eos Trans. AGU, 87(52), Fall Meet. Suppl., Abstract H31B-1432.

Acosta-Colon, A. and L. J. Pyrak-Nolte, 2007, AGU, Detecting the Presence of a Sub-Porosity in an Open Fluid-Filled Fractures, Eos Trans. AGU, 88(52), Fall Meet. Suppl., Abstract H11B-0485.

Davis, C., Atekwana, E. A., Pyrak-Nolte, L. and D. D. Werkma, Investigating the Effects of Biofilm Development in Porous Media on Seismic Wave Propagation, Spring 2009 Meeting of the American Geophysical Union, Eos Trans. AGU, 90(22), Jt. Assem. Suppl., Abstract H12A-05, Toronto, Ontario, Canada. May 24-27, 2009.

Davis, C. A., Pyrak-Nolte, L. J., Atekwana, E. A., Werkema, D. D. and M. E. Haugen, Spatial & Temporal Geophysical Monitoring of Microbial Growth and Biofilm Formation, Fall 2009 Meeting of the American Geophysical Union, Eos, Vol. 90, Number 52, 29 December 2009, Fall Meet. Suppl., Abstract NS23A-1125, San Francisco U.S.A. December 2009.

Pyrak-Nolte, L. J., Imaging Physical Processes in Fractures, Lassonde Institute *Distinguished Lecture*, Lassonde Institute, University of Toronto, Canada, April 6th, 2006.

Pyrak-Nolte, L. J., Fractures as Time-Dependent Structures: Characterizing Alteration & Changing Length Scales, *Colloquium*, Department of Physics, Western Michigan University October 30, 2006

Pyrak-Nolte, L. J., Fractures as Time-Dependent Structures: Characterizing Alterations & Changing Length Scales, in the Proceedings of the Special Session on Geophysics Applied to Rock Engineering at the 11th International Congress on Rock Mechanics, held in Lisbon, Portugal, July 9-13th, 2007.

Pyrak-Nolte, L.J., Seismic Interpretation of Fracture Specific Stiffness: Spatial Distributions and Non-Uniform Stress, invited presentation at the EAGE/SEG Research Workshop, September 3 -6, 2007, Perugia, Italy.

Pyrak-Nolte, L. J., Scaling & Fractures, DOE Basic Energy Sciences workshop “Basic Research Needs for Geosciences: Facilitating 21st Century Energy Systems.” Feb. 21-23, 2007, in North Bethesda, Maryland

Pyrak-Nolte, L. J. & A. Acosta-Colon, Effect of a sub-porosity on seismic wave propagation across a fracture, Basic Research Relevant to Geological CO₂

Sequestration, March 12- 14, 2008, Geosciences Research Program, Office of Basic Energy Sciences, Gaithersburg, Maryland.
Pyrak-Nolte, L. J., Fractures as Time-Dependent Structures: Characterizing Alterations & Changing Length Scales, Fall 2008 Meeting of the American Geophysical Union, invited presentation, NS41A-03, San Francisco U.S.A. December 2008.

5. Attachments of Selected Publications

The attachment section contains the following selected publications:

- Acosta-Colon, A. A. and L. J. Pyrak-Nolte, Effects of the Sub-porosity on Wave Propagation Across a Fracture, in the *Proceedings of the 42nd US Rock Mechanics Symposium and 2nd U.S.-Canada Rock Mechanics Symposium*, San Francisco, June 29-July 2, 2008, paper number ARMA 08-330 (2008).
- Acosta-Colon, A., Nolte, D.D. and Pyrak-Nolte, L. J., Laboratory-scale study of field of view and the seismic interpretation of fracture specific stiffness, *Geophysical Prospecting*, 57, 209-224, (2009)
- Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen, Microbial-Induced Heterogeneity in the Acoustic Properties of Porous Media, *Geophysical Research Letters*, doi:10.1029/2009GL039569 (2009).
- Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen, Acoustic and electrical property changes due to microbial growth and biofilm formation in porous media, *Journal of Geophysical Research – Biogeosciences* 115, doi:10.1029/2009JG001143 (2010).

Effects of the Sub-porosity on Wave Propagation Across a Fracture

Acosta-Colon, A.

Earth and Atmospheric Sciences Department, Purdue University, West Lafayette, IN, USA

Pyrak-Nolte, L. J.

Physics Department, Purdue University, West Lafayette, IN, USA

Earth and Atmospheric Sciences Department, Purdue University, West Lafayette, IN, USA

Copyright 2008, ARMA, American Rock Mechanics Association

This paper was prepared for presentation at San Francisco 2008, the 42nd US Rock Mechanics Symposium and 2nd U.S.-Canada Rock Mechanics Symposium, held in San Francisco, June 29-July 2, 2008.

This paper was selected for presentation by an ARMA Technical Program Committee following review of information contained in an abstract submitted earlier by the author(s). Contents of the paper, as presented, have not been reviewed by ARMA and are subject to correction by the author(s). The material, as presented, does not necessarily reflect any position of ARMA, its officers, or members. Electronic reproduction, distribution, or storage of any part of this paper for commercial purposes without the written consent of ARMA is prohibited. Permission to reproduce in print is restricted to an abstract of not more than 300 words; illustrations may not be copied. The abstract must contain conspicuous acknowledgement of where and by whom the paper was presented.

ABSTRACT: Natural fractures are often filled with debris. This debris originates from many different mechanisms: organic and/or inorganic chemical reactions (such as mineralization), sediment transport, formation of the fracture, mechanical weathering or combinations of these processes. In many cases, the presence of debris forms a sub-porosity within the fracture void space. In this study, we investigate how the existence of a sub-porosity affects seismic wave propagation and consequently our ability to probe changes in the fractures properties caused by the formation or alteration of a sub-porosity. Laboratory experiments were performed to examine acoustic wave scattering from packing of spherical beads used to create a sub-porosity within a synthetic fracture. The sub-porosity in the fracture was created by using spherical beads with a range of diameters from 500 μ m to 7.79 mm. Compressional waves were transmitted across the fracture using contact piezoelectric transducer. The analysis was performed for a fixed aperture with different sub-porosity created by single and multiple layers. Interpretation of the acoustic response depends critically on understanding the length scales associated with the layers (bead diameter, layer thickness), the aperture of the fracture and seismic length scales (wavelengths, field of view). The detection of multiple layers of debris within a fracture is possible by understanding wave interference, dispersion and the reflections in the waveform.

1. INTRODUCTION

Fractures and joints in the field often contain debris within the void spaces (aperture). These debris originate from many different mechanisms: organic and/or inorganic chemical reactions (such as mineralization), sediment transport, formation of the fracture, mechanical weathering or combinations of these processes. In many cases, the presence of debris forms a sub-porosity within the fracture void space. The material that composes this sub-porosity can differ or be the same in chemical and physical properties as that from the fracture walls. The “sub-porosity” may partially fill voids thereby reducing the local porosity to lengths scales on the order of sub-microns to tens of microns. It is known that the sub-porosity affects fracture porosity, permeability and storativity.

Several laboratory studies have measured the acoustic properties of spherical beads in fluids. Most of these measurements are obtained using seismic reflection methods [1] to simulate marine field measurements. Other experiments consist of simulating sediment transport where the spherical beads are submerged in a fluid and free to move [2,8]. These and other studies have shown that beads produce elastic and acoustic scattering signatures that depend on the relative

size of the bead to the wavelength, λ , of the acoustic signal. There are different scattering regimes based on the size of the scatterer, a , and the wave number, k ($k=2\pi/\lambda$) of the signal that exhibit different frequency-dependent attenuation coefficients. For Rayleigh scattering ($ka < 1$), the attenuation coefficient is proportional to the fourth power of frequency. For resonance (Mie) scattering, the attenuation coefficient depends on the first power of frequency when the wavelength approaches the size of the scatterer. Mie scattering is commonly observed in monodispersions of glass beads in fluidized beds [2].

The characterization and understanding of the mechanical, seismic and hydraulic properties of fractures is affected by the size of the sediments filling a fracture or a mechanical discontinuity. The size of the material that creates a sub-porosity inside a fracture or other mechanical discontinuity varies in natural systems. Depending on the site geology, fractures (joints, pipes, caves, faults, etc.) and the filling can vary in size from microns to millimeters to meters. For example, in a tunnel on the Runcorn Peninsula, UK [9] infilled fractures with apertures as large as 35 mm were found to be filled with fine to medium grain sands (0.125 – 0.5 mm). A field study of sediment fissures in soils in

Chihuahuan Desert of Texas, USA observed apertures that ranged from 25 mm to 65 mm with sediment infill that ranged from clay-size particles to clast-supported gravel (< 80%), a size range of approximately 5 microns to 10 mm [10]. From a study of ancient karsts environments [11], mechanical discontinuities, such as fractures, pipes and caves, ranged in size from a few millimeters to several meters and are often filled with sediment that range from clays (a few microns) to boulders (~meter).

Thus, in addition to the length scales associated with fracture geometry (aperture, surface roughness, contact area distributions, spatial correlation lengths), the length scales associated with the infilling material must also be considered. When characterizing fractures, joints and other mechanical discontinuities, these geometric length scales must be considered in terms of the seismic scales (wavelength and field of view) to interpret seismic waves propagated across these features. The interpretation of the seismic measurements is affected by the scattering from the fracture and potentially by scattering from the infill material.

In this study, we used a synthetic fracture to investigate the effect of grain size and layer thickness on the wave propagation across the fracture.

2. EXPERIMENTAL SET UP

2.1 Acoustic Measurements

Acoustic measurements were performed to investigate the effect of a sub-porosity on the wave propagation across an open fluid-filled fracture. Figure 1 shows a sketch of the experimental set up. A synthetic fracture was created by the separation of two acrylic (Lucite) right cylinders. The thicknesses of the cylinders were 25.4 mm (top cylinder) and 35 mm (bottom cylinder). The bottom cylinder contained an 18 mm deep recess, which is filled with water and the sub-porosity.

The aperture of the synthetic fracture was controlled by using a computer-controlled linear actuator to increment the aperture in 50 μm steps. The fracture was filled with water and beads to create the sub-porosity (see section 2.2). The acoustic signal of the fractures was measured using compressional wave 1 MHz (central frequency) piezoelectric contact transducers for the source and receiver. The contact transducers were coupled to the acrylic cylinders with honey. The honey provided a stable coupling between the transducer and the acrylic cylinder. A 200 gram mass was placed on top of the transducer to prevent movement of the transducer during measurements.

Figure 1. Experimental set up to measure the effects of the sub-porosity on the wave propagation across a fracture. The top and bottom acrylic cylinders create the fracture.

2.2 Creating the sub-porosity

The sub-porosity in the fracture was created by using spherical beads (either Lucite or sodium silicate glass) with a range of diameters from 500 μm to 7.79 mm. A list of the size and properties of the spherical beads is given in Table 1. The fracture was saturated with water and a single layer or multiple layers of the spherical beads were added. The seismic response for the fracture was measured first with only water and then with different bead packings. The water only data set is used as a reference. In this paper, we present a comparison of the effect of bead size and layer thickness on acoustic wave propagation across a fixed aperture fracture. The aperture used for this paper is 7.79 mm (i.e., the size of the largest bead).

Table 1. Spherical beads used to create the sub-porosity in the fracture and its description: material, acoustic impedance (Z) and its size and the relationship between the wavelength (λ) at 1 MHz and its size.

Sample Name	Material	Size (mm)	Z ($10^6 \text{ Kg/m}^2\text{s}$)	Ratio Size/ λ (1MHz)
G0.1	Glass	0.5	13.4	0.08
G0.1	Glass	0.91	13.4	0.14
2 layers				
G0.1	Glass	1.72	13.4	0.63
4 layers				
R0.5	Lucite	1.47	3.22	0.53
R0.5	Lucite	2.67	3.22	0.98
2 layers				
R0.5	Lucite	3.87	3.22	1.41
3 layers				
R0.5	Lucite	5.07	3.22	1.85
4 layers				
R1	Lucite	3.05	3.22	1.11
R2	Lucite	5.41	3.22	1.98
R3	Lucite	7.79	3.22	2.85

2.3 Case Experiments

The experiments explored the acoustic response of the fracture filled with spherical beads to create sub-porosity within the fracture. For R1 (3.05 mm), R2 (5.41

mm) and R3 (7.79 mm) the acoustic response was measured for a single layer (ring type). For R0.5 (1.47 mm), the acoustic signature was measured for single layer and multiple layers for an equivalent size of 2.67 mm (2 layers), 3.87 mm (3 layers) and 5.07 mm (4 layers) due to the packing of the layers. Additionally, the acoustic measurements were performed in a single layer and multiple layers of G0.5 to create an equivalent size of 0.91 mm (2 layers) and 1.72 mm (4 layers).

The experimental results are presented as four separate cases in sections 3.2 through 3.5. For all cases the aperture was held constant at 7.79 mm and the fracture was water saturated. The cases are defined as follows:

- I. *Grain Size*: This case examines the effect of grain size on seismic wave propagation across a fixed aperture water-filled fracture. This study was performed on single layers of spherical acrylic beads (R05 - 1.47 mm, R1 - 3.05 mm, R2 - 5.41 mm and R3 - 7.79 mm) as shown in Figure 2 (a).
- II. *Layer Thickness*: This case examines the effect of increasing number of layers for a fixed grain size on the seismic wave propagation across a fixed aperture water-filled fracture. A grain size of 1.47 mm (R05) was used to create a single layer as well as layers composed of 2, 3 and 4 grains in height as shown in Figure 2 (b).
- III. *Comparison of equivalent size*: This case compares the seismic response from a single layer of spherical beads to that from 2 layers of spherical beads with a similar layer thickness. Two examples are shown: a) a single layer of R1 (3.05 mm) & 2 layers of R0.5, which creates an equivalent size of 2.67 mm, and b) a single layer of R2 (5.41 mm) and 4 layers of R0.5, which creates an equivalent size of 5.07 mm.
- IV. *Small (sub-wavelength) beads*: The case is similar to case II on the effect of layer thickness but the layers are created with sub-wavelength grains (G0.5-500 μ m). A single layer, two layers and four layers of G0.5 were seismically probed.

Figure 2. An illustration of spherical beads layers experiments, for case I (a), case II (b).

3. RESULTS & DISCUSSION

3.1 Data Analysis

Compressional waves were used to investigate the effect of a sub-porosity on wave propagation across a fracture. Data analysis on the measured signals included a time-frequency analysis [6] to obtain dispersion and wave interference information from the first arriving compressional wave (P-wave). The time-frequency analysis (wavelet transform) gives the variation in frequency content of the signal as a function of arrival time.

The seismic data were also used to calculate an effective fracture stiffness for the fracture containing a sub-porosity. The spectrum from the wavelet analysis at the arrival time of dominant energy is used to examine the variation in effective fracture stiffness using the displacement discontinuity theory [3,4,5]. A transmission coefficient, $T(\omega)$, is calculated by taking the ratio of the spectrum from the fracture with a sub-porosity to that from the water-filled fracture. Using equation (1), an effective fracture stiffness, κ , is calculated as a function of frequency (resolution of 0.02MHz), for a known acoustic impedance (phase velocity*density).

$$\kappa(\omega) = \frac{\omega Z}{2\sqrt{\left(\frac{1}{T(\omega)}\right)^2 - 1}} \quad (1)$$

The stiffness is an effective stiffness because the fracture aperture and asperity spacing are much greater than a wavelength. The displacement discontinuity theory applies when the asperity spacing is smaller than a wavelength. However, it is still instructive to calculate an effective fracture stiffness to classify behavior even in situations where the technical requirements are asperity

spacing and thickness are not strictly satisfied. This approach removes the need for multi-parameter models such as multi-layer models where thickness and layer properties would have to be defined. The multiple parameters are replaced by a single parameter, i.e., effective stiffness whose frequency dependence captures the complexity of the substructure [12]. The use of broadband transducers enables us to examine both short-wavelength and long wavelength regimes, i.e. the results span the limits of the theory. The variation in effective fracture stiffness with frequency is used to determine when the fracture is in or deviates from displacement discontinuity like behavior versus short-wavelength behavior.

In our analysis, we used a phase velocity of 2730 m/s and a density of 1180 kg/m³ for the acrylic. The acoustic impedance for the acrylic used in this study was 3.22×10^6 kg/m²s.

3.2 Case I: Grain Size

Figure 3a shows the received waveforms for case I, that is, the seismic response as a function of grain size for a single layer. Case I experiments simulate the effect of increasing grain size. In Figure 3, the seismic signature of the fracture as a function of grain size is shown as well as the signal from the water-filled fracture with no grains for comparison.

Figure 3. Seismic response for a single layer of spherical beads (R3 =black, R2=green, R1=blue and R0.5=red) and the water saturated gap (pink). The seismic signals had been dc shifted for comparison.

Examination of the signals in Figure 3 shows that the acoustic response is affected by the size of the grain. The wavelength in Lucite λ_L at 1 MHz is 2.73 mm and Table 1 gives the size of the grains as well as layers in terms of wavelengths. When the grain size is

greater than a wavelength (5.41 mm & 7.79 mm), (around 19 μ s) the first arrival (or prompt wave) is low in amplitude and frequency-content, and is the portion of the wave that traveled mainly through the bead. The main energy arrives later around 21 μ s and is associated with travel through the water. In contrast, for a grain size less than the wavelength (1.47 mm), the signature of the layer of beads is given by the “shoulder wave”, i.e. an interference wave arriving around 20.5 μ s in Figure 3. Neither the prompt wave nor the shoulder wave is observed when the bead size is comparable to a wavelength. The arrival time of the signal for bead R1 (3.05 mm) is delayed relative to the signals from water and the other bead sizes. This delayed is caused by strong interference between the prompt wave and the portion of the wave that is dominated by travel through the water.

Figure 4. Dispersion curves extracted from the time-frequency analysis (wavelets). The curves show the difference in the arrival time of the frequency for the different size of the beads (R0.5=red, R1=blue, R2=green and R3=black) and water-saturated fracture for comparison.

The dispersion and interference nulls caused by wave interference of the “shoulder wave” and “prompt waves” are clearly evident in the dispersion shown in Figure 4 and the group wavelet transformations shown in Figure 5. From the dispersion curve (Figure 4) and the wavelet transformations (Figure 5), the low frequency components of the signal arrive later for beads size similar (3.05 mm) and smaller (1.47 mm) than λ_L than the high frequency components of the signal.

Figure 5. Time-frequency analysis (wavelets) results for the first arrival of the seismic signal of the fracture filled with a) water, and single layers of b) R0.5, c) R1, d) R2 and e) R3.

Just the opposite behavior is observed for the beads sizes larger than λ_L . In addition, the interference null

associated with the shoulder wave (Figure 5b) for bead size 1.47 mm is observed to arrive after the dominant energy. However, for a bead size of 7.79 mm the interference null arrives prior to the dominant energy (Figure 5e).

3.3 Case II: Layering Effect

The effect of increasing layer thickness for a fixed grain size on the acoustic signals is shown in Figure 6. Acoustic measurements were obtained for the fracture filled with a single beads layer and multiple beads layers of 1.47 mm bead size (R1). The layers create an equivalent size of 2.67 mm (2 layers), 3.87 mm (3 layers) and 5.07 mm (4 layers). From case I, it was observed that the bead caused wave interference that was dependent on the size of the bead relative to a wavelength. In case II, the interference wave creates a shoulder wave as that observed for a single layer of R0.5. For the multiple layers, the interference between the waves traveling through the bead and the water interferes. This interference is observed in the group wavelet transformation for the 3 layer and 4 layer signals (Figure 7 c & d) as the start of the formation of A stop-band between the frequencies 0.3 MHz and 0.45 MHz.

Figure 6: Layering effect in the first arrival of the seismic response of the fractures with a single layer of R0.5 (red) and multiple layers (2 layers=blue, 3 layers=green and 4 layers=black).

The seismic measurements presented in Figure 6 show that the layers affect velocity and produce interference effects on the seismic response of the fracture. The wavelet transformation for the signals shown in Figure 6 are given in Figure 7. Again, the structure of the wave packet indicates wave interference when beads are present in the fracture. In addition, an interference null is observed in the wavelet

transformations (Figure 7). The arrival time of the interference null is observed to be affected by the number of layers within the fracture. For example, the difference, Δt , in the arrival time of the interference null for the signals from the single layer and 2 layers samples is $0.58 \mu s$, i.e. a time equivalent to a distance of 1.5 mm in Lucite (velocity of Lucite is 2730 m/s). That length is very similar to the difference in layer thickness (1.2 mm). Comparing the arrival time of the interference null for the single layer and 4 layers samples, a $\Delta t = 1.56 \mu s$ was observed, a time equivalent to a distance of 4.2 mm which is close to the difference in the thickness of the layers (3.6 mm). An arrival time analysis of the interference nulls can potentially be used to the detection of layers created by successive sediment deposition over time.

Figure 7: Wavelet transformation for the multiple layers signals. The interference null is observed in the wavelets (dark blue region, where the amplitude is 0). The wavelets were obtained from the signal: a) single layer of R0.5 and multiple layers: b) 2 layers, c) 3 layers and d) 4 layers.

Additionally, the dispersion obtained from the wavelets measuring the layers effect is presented in Figure 8. The dispersion curves had similar frequency dependence. But the dispersion (arrival time of the frequency) decreases with decreasing number of layers. This is another effect of the layering.

Figure 8. Dispersion curves for the seismic measurements of the fracture filled with a single layer of R0.5 (red), 2 layers (blue), 3 layers (green), 4 layers (black) and water saturated (pink).

3.4 Case III: Comparison of Equivalent Size.

A comparison is made of the seismic response from two sub-porosity that are similar in height but differ in substructure, i.e., a comparison of the signal from a single layer of large beads to the signal from several layers of small beads but with a thickness equivalent to that of the single layer. As observed in the data in the previous section, the seismic response to a subporosity within a fracture is controlled by the bead

size and number of layers. This raises the question, can the difference in substructure of a sub-porosity be delineated from seismic or acoustic data? Case III investigates these questions for 2-layers of R0.5 (equivalent size of 2.67 mm) and the bead size of 3.05 mm (R1) (Figure 9 lower graph). Figure 9 (upper graph) also shows the signals for 4-layers of R0.5 (equivalent of 5.07 mm) and a single bead layer of 5.41 mm (R2). Inspection of the first arrival of the seismic measurements shows that the arrival times, phase and frequency content of the signals of single layer of large beads and an equivalent layer composed of several layers of small beads are very different for both cases. For both comparisons, the signals from the 2 & 4-layer samples arrive earlier than the signal from the single layer sample.

Figure 9. Comparison of the signals for the fracture filled with single bead (red) and equivalent size composed of multiple layers (blue).

The wavelets and the dispersion curves of the signals were presented earlier in this paper. The wavelets of the single layer of 3.05 mm and the single layer of 5.41 mm are shown in Figure 5 (c) and (d) respectively. The dispersion curves from these wavelets are shown in Figure 4, blue and green symbols respectively. For the multiple layers wavelets, the transforms for the 2 layer and 4 layer samples are shown in Figure 7 (b) and (d) respectively, and the dispersion curves are given in Figure 8, blue symbols (2 layers) and black symbols (4 layers).

From a comparison of the wavelets, the interference nulls are observed in the wavelets for the multiple layer cases (2 & 4 layers) indicating strong interference. For the multiple layers the low frequencies arrive later than the single bead ones. The dispersion curves for an equivalent layer sample and a single layer

sample are very different especially for the 5.41 mm bead size and equivalent layer of 5.07 mm. The signal layer of large beads exhibits increasing frequency with increasing arrival time while the equivalent layer exhibits a decreasing arrival time with increasing frequency.

Substructure of the sub-porosity affects the seismic response of the fracture because the scattering response depends on the size of the grain/bead relative to a wavelength as well as the spacing between of layers as a function wavelength. For example, the stop-band behavior observed for the 4 layer sample composed of grains of 1.47 mm occurs when the bead spacing is approximately a quarter of a wavelength, i.e. approximately a frequency of 0.5 MHz. This stop band behavior is not observed for a single layer of equivalent size.

3.5 Case IV: Small (sub-wavelength) Beads

In nature, fractures can be filled with a material that differs in composition from the matrix of the rock. The case IV experiments are similar to Case II, but with glass beads of 0.5 mm (G0.1). The glass beads simulate a material with different acoustic impedance than the synthetic fractured sample (acrylic). The relationship between the bead size and the wavelength of sodium silicate glass is given in Table 1 and it was 0.08 (ANGEL: is this relative to glass wavelength or Lucite wavelength?).

Figure 10 shows the received waveform for compressional waves propagated across case IV sample fractures. A calculated dispersion shows that as the number of layers increased, the arrival time of the compressional wave decreased. In Figure 10, the signals were aligned to show that the phase components of the signals from the samples with layers are exactly the same as that for water and themselves in the first arrival, i.e. no significant change in the frequency content of the first arrival is observed. None of the interference waves (shoulder or prompt) measured in previous cases were observed for case IV. Only the speed of the wave was affected by the presence of the layers composed of sub-wavelength beads. The inset in Figure 10 is an enlargement of the portion of signal showing signals that were multiply reflected within the sample. The wavelet transformations are identical in shape and the dispersion curves have similar slopes because the first arrival does not change in frequency with respect to each other. However, an analysis of the multiply reflected components of the signals (inset in Figure 10) enables discrimination of the presence of layers, i.e. the small differences in arrival times are accentuated by wave components that have longer travel paths. Three main reflections are easily observed (R1, R2, and R3). The reflections provide information on the thickness of

water-filled portion of the fracture as well as the presence of sub-wavelength grains.

Figure 10: Peak-align signals obtained for case IV, in which 0.5 mm single layer (red), 2 layers (blue) and 3 layers (green). Inset: a closer look of the reflection of the signals.

3.6 Fractures Properties: effective fracture stiffness

An effective stiffness was calculated from the seismic measurements using the method described in the data analysis section (see section 3.1) and by equation 1. Figure 11 shows the effective fracture stiffness as a function of frequency the case 1 fracture samples.

Figure 11: Fracture effective stiffness obtained from the frequency spectrum of the seismic measurements for the water saturated fracture and single layer of beads with different sizes: R0.5=red, R1=blue, R2=green, R3=black.

As stated earlier, the variation in effective fracture stiffness with frequency is used to classify the seismic response of a fracture containing a sub-porosity. A linear dependence on frequency of effective fracture stiffness occurs when the layer behaves as a welded contact or is in the geometric ray regime. A frequency-independent effective stiffness occurs when the layer behaves as a non-welded contact (displacement discontinuity) with a uniform “stiffness”. A linear-dependence of effective fracture stiffness is observed for R0.5, R2 and R3. For R3, the linear behavior is observed above 0.4 MHz and occurs because the wave is traveling through Lucite, while for R0.5 the wave is mainly dominated by travel through the water layer. R1 exhibits an effective stiffness that is independent of frequency, i.e. like non-welded contact with a uniform stiffness.

4. SUMMARY

The ability to distinguish a sediment-filled or partially sediment-filled fracture from a water-saturated fracture depends on the size of the sediment grains relative to a wavelength. In this paper, a study of the seismic measurements effects produced by spherical beads that crates a sub-porosity (and can simulate debris or sediments) in a synthetic fracture was presented. In this study we observed that dispersion, interference nulls and wave speed were the prime indicators of the presence of a sub-porosity created by grains (beads) within a fracture.

In Case I when the bead diameter, $d \leq \lambda_L$, negative time dispersion was observed and the converse occurred for $d > \lambda_L$. Also different types of interference waves appeared depending the relationship between the bead size and λ_L . In Case II, the difference in layer thickness for layers composed of the same bead size can be detected by examining the arrival time of the interference nulls. Interference nulls indicate that two waves are destructively interfering. In Case III, for sub-wavelength layers composed of sub-wavelengths beads, we observed that multiply reflected signals enabled the delineation of the presence of layers because a longer path length enables differentiation of small time differences. In Case IV, for layers with approximately the same thickness but composed of different bead sizes, the dispersion and the seismic properties differed depending on the bead size.

Single layer and multiple layers of beads in a fixed aperture fracture affect the acoustic response of the fracture. Interpretation of the acoustic response depends critically on understanding the length scales associated with the layers (bead diameter, layer thickness), the aperture of the fracture and seismic length scales

(wavelengths, field of view). The detection of multiple layers of debris within a fracture is possible by understanding the wave interference, dispersion and/or the reflections in the waveform.

5. ACKNOWLEDGMENTS

The authors wish to acknowledge support of this work by the Geosciences Research Program, Office of Basic Energy Sciences US Department of Energy (DEFG02-97ER14785 08).

6. REFERENCES

1. Y. Le Gonidec, D. Gilbert and J. Proust. 2002. Multiscale analysis of waves reflected by complex interfaces: Basic principles and experiments. *Journal of Geophysical Research*. 107(B9): 2184:2199
2. Cowan, M.L., K. Beaty, J.H. Page, Z. Liu and P. Sheng. 1998. Group velocity of acoustic waves in strongly scattering media: Dependence on the volume fraction of scatters. *Physical Review E*. 58(5): 6626-6636
3. Schoenberg, M., 1980. Elastic wave behavior across linear slip interfaces. *Journal of the Acoustical Society of America*. 5(68): 1516-1521.
4. Gu, B.L., K.T. Nihei, L.R. Myer, and L.J. Pyrak-Nolte. 1996. Fracture interface waves. *Journal of Geophysical Research-Solid Earth*. 101(B1): 827-835.
5. Pyrak-Nolte, L.J., L.R. Myer, and N.G.W. Cook. 1990. Transmission of Seismic-Waves across Single Natural Fractures. *Journal of Geophysical Research-Solid Earth and Planets*. 95(B6): 8617-8638.
6. Nolte, D.D., L. J. Pyrak-Nolte, J. Beachy and C. Ziegler. 2000. Transition from the displacement discontinuity limit to the resonant scattering regime for fracture interface waves. *International Journal of Rock Mechanics and Mining Sciences*. 37(1-2): 219-230.
7. Pyrak-Nolte, L.J. and D.D. Nolte. 1992. Frequency-Dependence of Fracture Stiffness. *Geophysical Research Letters*. 19(3): 325-328.
8. Y. Le Gonidec and D. Gilbert. 2006. The wavelet response as a multiscale characterization of scattering processes at granular interfaces. *Ultrasonics*. 44: 381-390.
9. Wealhall, G. P., Steel, A., Bloomfield, J. P., Moss, R. H. and D. N. Lerner. 2001. Sediment filled fractures in the Permo-Triassic sandstones of the Cheshire Basin: observations and implications for pollutant transport. *Journal of Contaminant Hydrology*, 50: 41-51.
10. Scanlon, B. R. 1992. Moisture and solute flux along preferred pathways characterized by fissured sediments in deserts soils. *Journal of Contaminant Hydrology*. 10:19-46.
11. Juhasz, E., Korpas, L. and A. Balog., 1995. Two hundred million years of karst history, Dachstein Limestone, Hungary. *Sedimentology*. 42: 473-489.
12. Acosta-Colon, A. A., Pyrak-Nolte, L. J. and D. D. Nolte. 2008. Laboratory-Scale Study of Field-of-View and the Seismic Interpretation of Fracture Specific Stiffness. Submitted to *Geophysical Prospecting*.

Laboratory-scale study of field of view and the seismic interpretation of fracture specific stiffness

Angel Acosta-Colon¹, Laura J. Pyrak-Nolte^{1,2*} and David D. Nolte²

¹*Department of Earth and Atmospheric Sciences, Purdue University, 525 Northwestern Avenue, West Lafayette, IN 47907-2036, USA, and*

²*Department of Physics, Purdue University, 525 Northwestern Avenue, West Lafayette, IN 47907-2036, USA*

Received January 2008, revision accepted May 2008

ABSTRACT

The effects of the scale of measurement, i.e., the field of view, on the interpretation of fracture properties from seismic wave propagation was investigated using an acoustic lens system to produce a pseudo-collimated wavefront. The incident wavefront had a controllable beam diameter that set the field of view at 15 mm, 30 mm and 60 mm. On a smaller scale, traditional acoustic scans were used to probe the fracture in 2 mm increments. This laboratory approach was applied to two limestone samples, each containing a single induced fracture and compared to an acrylic control sample. From the analysis of the average coherent sum of the signals measured on each scale, we observed that the scale of the field of view affected the interpretation of the fracture specific stiffness. Many small-scale measurements of the seismic response of a fracture, when summed, did not predict the large-scale response of the fracture. The change from a frequency-independent to frequency-dependent fracture stiffness occurs when the scale of the field of view exceeds the spatial correlation length associated with fracture geometry. A frequency-independent fracture specific stiffness is not sufficient to classify a fracture as homogeneous. A nonuniform spatial distribution of fracture specific stiffness and overlapping geometric scales in a fracture cause a scale-dependent seismic response, which requires measurements at different field of views to fully characterize the fracture.

INTRODUCTION

The scaling behaviour of the hydraulic and seismic properties of a fracture determines how properties observed on the laboratory size (typically less than tens of centimetres) relate to the same properties measured at larger sizes. To understand the scaling behaviour of fracture properties, the length scales of the fracture geometry (apertures, contact areas and spatial correlations) and the fluid phase distribution (wetting and non-wetting phase areas and interfacial areas) must be characterized and compared to the length scales associated with the seismic probe (wavelength, beam size, divergence angle and field of view).

For fractures in rock, measurements on the laboratory scale encompass several different length scales that include the size of the sample and fracture. A single fracture can be viewed as two rough surfaces in contact that produce regions of contact and open voids in a quasi two-dimensional fashion. This fracture geometry has many length scales that are described as contact area and its spatial distribution, as well as by the size (aperture) and spatial distribution of the void space. From laboratory measurements, Pyrak-Nolte, Montemagno and Nolte (1997) found that the aperture distribution of natural fracture networks in whole-drill coal cores were spatially correlated over 10 mm to 30 mm, i.e., distances that were comparable to the size of the core samples. However, asperities on natural joint surfaces have been observed to be correlated over only about 0.5 mm from surface roughness measurements (Brown, Kranz and Bonner 1986). These two quoted values for

*E-mail: ljpn@purdue.edu

correlation lengths vary by up to two-orders of magnitude. The spatial correlation lengths are likely to be a function of rock type but this needs to be verified experimentally. The observation that correlation lengths are smaller or on the same order as the sample size may explain why core samples often predict different hydraulic-mechanical behaviour than is observed in the field. If a fracture on the core scale is correlated over a few centimetres, the same fracture on the field scale may behave as an uncorrelated one.

An additional length scale that is necessary to consider, when investigating the scaling behaviour of seismic wave propagation across single fractures, is the spatial variation in fracture specific stiffness. Fracture specific stiffness is defined as the ratio of the increment of stress to the increment of displacement caused by the deformation of the void space in the fracture. As stress on the fracture increases, the contact area between the two fracture surfaces also increases, raising the stiffness of the fracture. Fracture specific stiffness depends on the elastic properties of the rock and depends critically on the amount and distribution of contact area in a fracture that arises from two rough surfaces in contact (Kendall and Tabor 1971; Hopkins, Cook and Myer 1987; Hopkins 1990). Kendall and Tabor (1971) showed experimentally and Hopkins *et al.* (1987, 1990) have shown numerically that interfaces with the same amount of contact area but different spatial distributions of the contact area have different stiffnesses. Greater separation between points of contacts results in a more compliant fracture or interface.

The geometric length scales of a fracture affect the length scales involved in the flow of multiple fluid phases in a fracture partially saturated with gas and water. Recently, Johnson, Brown and Stockman (2006) showed experimentally and numerically that for intersecting fractures fluid-fluid mixing in intersecting fractures is controlled by the spatial correlations of the aperture distributions in the fractures. Pyrak-Nolte and Morris (2000) found that spatial correlations of the fracture apertures control the relationship between fluid flow through a fracture and fracture specific stiffness. Furthermore, the fracture void geometry is sensitive to stress (Pyrak-Nolte *et al.* 1987) as well as chemical alteration through precipitation and dissolution (Gilbert and Pyrak-Nolte 2004) and also controls the distribution of multiple fluid phases (e.g., gas and water) leading to interfacial area per volume (Cheng *et al.* 2004, 2007), which is an inverse length scale. These physical processes have the potential to alter the geometric length scales of a fracture.

All of these geometric length scales can be compared to the wavelength of the seismic probe, as well as to the scale sam-

pled by the seismic probe. In terms of seismic monitoring of fractures, the role of the size and spatial distributions of fracture stiffness distributions are determined by the wavelength of the signal and by the size of the region probed. Pyrak-Nolte and Nolte (1992) showed theoretically that, for a single fracture, different wavelengths sample different subsets of fracture geometry. They calculated dynamic fracture stiffness based on the displacement discontinuity theory (Schoenberg 1980; Pyrak-Nolte, Myer and Cook 1990a,b; Gu *et al.* 1996) for wave transmission across a fracture. Transmission was based on local stiffnesses. A uniform distribution results in a frequency-independent dynamic fracture specific stiffness. A bimodal distribution results in a dynamic stiffness that depends weakly on frequency. However, a strongly inhomogeneous distribution of fracture specific stiffness results in a frequency-dependent fracture specific stiffness.

The theoretical work of Pyrak-Nolte and Nolte (1992) only explored the effect of spatially uncorrelated distributions of fracture specific stiffness on seismic wave transmission. However, one must also consider the effect of spatial correlations on the interpretation of seismic measurements. On the laboratory scale or the field scale, seismic measurements only probe a portion of a fracture (local measurement). The area illuminated by the wavefront is a function of the wavelength as well as the source-receiver configurations. In laboratory studies, the lateral size of the acoustic lobe pattern at the fracture plane determines the region sampled with traditional contact transducers. The first-order effect of spatial correlations of fracture specific stiffness is that local seismic measurements sample different fracture specific stiffnesses in different regions of the fracture. For instance, the experimental work of Olinger, Nolte and Pyrak-Nolte (2003) demonstrated seismic focusing caused by spatial gradients in fracture specific stiffness. The second-order effect of a spatially varying fracture specific stiffness is scattering caused by a heterogeneous distribution of fracture specific stiffness. The strength of the scattering depends on the spatial correlation length of the variation in fracture specific stiffness relative to a wavelength and on the field of view of the seismic measurements. A fundamental question is whether the size of the region probed is sufficient to capture high-angle scattering losses outside of the detection angle. If scattering angles are high, this raises the important question of how many seismic measurements are needed to fully characterize a fracture. To begin to answer such questions, the effect of field of view on the interpretation of fracture specific stiffness from seismic measurements needs to be explored. This paper presents results of a laboratory study that examines the effect of field of view on the

interpretation of fracture specific stiffness from seismic measurements.

EXPERIMENTAL METHODS

Two experimental approaches were used to measure the seismic signature of a fracture. These methods are: 1) an acoustic lens system and 2) an acoustic mapping system. With the acoustic lens system, the area illuminated by the seismic wavefront is varied to change the field of view (i.e., the region probed by the wavefront). This approach enables us to investigate the effect of field of view on the interpretation of fracture properties from seismic measurements. In the next section, a brief discussion of the design and characterization of the lens system is provided. The second approach, acoustic mapping, produces a high-resolution map of the local variations in fracture properties over a two-dimensional region. Both experimental approaches were used to explore the interpretation of fracture specific stiffness from seismic measurements as a function of field of view and as a function of seismic frequency.

Acoustic lens system

For this study, an acoustic lens system was designed to produce pseudo-collimated beams with adjustable probe diameters, as shown in Fig. 1. The area illuminated by the seismic wavefront was varied to change the field of view (i.e., the region probed by the wavefront). The acoustic lens was designed using geometric optics of elliptical lenses. An elliptical surface (Fig. 2) was chosen to eliminate on-axis aberration (Dunn *et al.* 1980). The side of the lens facing the transducer had a concave ellipsoidal surface (concave meniscus) and the

Figure 2 Sketch of the geometry to create the acoustic lens.

side of the lens facing the sample was flat. The geometrical shape of the ellipsoid is determined by the semi major axis (a), semi minor axis (b) and the centre of the ellipsoid (c). For machining purposes, the ellipsoid was represented by a sphere with a curvature matched to the ellipsoid. The sphere has a radius R_l and centred at a point such that the sphere surface matches the apex of the elliptical surface, as shown in Fig. 2. The lens dimensions are the diameter (d) and the length (l) from the apex of the lens to the flat surface. Because the lens is submerged in water during data acquisition, the acoustic impedance and the matching impedance of water were used for the design. This avoids large dispersion and large angles of refraction created by the different material properties.

The material for the lens was acrylic (Lucite). Table 1 lists the properties of Lucite and water. The lenses were right cylinders of acrylic measuring 80 mm in diameter (d) by 25.4 mm in length (l). The diameter of the lens was chosen to be 80 mm because the maximum diameters of the collimated wavefront

Figure 1 Sketch of the experimental set-up to create pseudo-collimated acoustic beams with beam waists measuring approximately 60 mm (solid line), 30 mm (dashed line) and 15 mm (dotted line) using a spherically-focused source transducer and an acoustic lens. The distance between the source transducer and the acoustic lens is adjusted to vary the beam size at the surface of the sample.

Table 1 Acoustic properties of water and Lucite (acrylic)

	Water	Lucite
Acoustic Velocity (m/s)	1480	2730
Acoustic Impedance ($\times 10^6$ kg/m ²)	1.48	3.22

are limited to the diameter of the lens. The 25.4 mm length was chosen for ease of machining. The spherical radius R_1 depends on a and b . Calculation of the radius of curvature R_1 is obtained by using the equation for an ellipse,

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1. \quad (1)$$

By taking the second derivative of the ellipse equation with respect to y , the radius of the curvature is:

$$R_1 = \frac{b^2}{a}. \quad (2)$$

The focal length of the lens used for the experiments was chosen in order to accept the largest beam width of 60 mm and to collimate the beam through the fracture. The focal point of the lens was set by:

$$f = \frac{D}{2 \tan \alpha}, \quad (3)$$

where D is the desired diameter of the collimated wavefront and α is the half angle of the divergence of the wavefront from the transducer at its focal point (not the lens). The divergence angle (α) of the transducer is 5° . For a beam diameter of 60 mm, the focal length is $f = 310$ mm. This focal length determines R_1 using the index of refraction of water (n_w) and Lucite (n_l) as:

$$f = \frac{n_w}{n_l - n_w} R_1 = \frac{R_1}{n - 1}, \quad (4)$$

where the index of refraction in acoustics is the inverse of the velocity of sound in the material. The radius is then:

$$R_1 = (n - 1) f, \quad (5)$$

where $n = 0.54$ for water and Lucite. The lens radius for the 60 mm beam diameter was 143 mm (see Table 2 for the value of the other parameters).

The lens that was designed to collimate the 60 mm diameter beam was also used to produce a 30 mm and a 15 mm pseudo-collimated beam diameter at the fracture plane. These

Table 2 Values for the acoustic lens design

Design	Value
R_1	143.02 mm
a	202.52 mm
b	170.19 mm
l	25.4 mm
d	80 mm
Material	Lucite

smaller beam diameters were produced by moving the transducer closer to the lens. The lens intersects the diverging beam when the beam is smaller and directs it towards the fracture. The distances L of the transducer from the lens to produce these beam diameters, as shown in Fig. 1, were 302 mm (for 60 mm), 195 mm (for 30 mm) and 110 mm (for 15 mm). The beam diameters were verified by scanning a transducer across the beam at a distance equivalent to the distance of the fracture from the lens. The beam profile measurements are shown in Fig. 3. The beam diameters are taken as the full width at half maximum.

The collimation of these smaller beams was not exact because the transducer is moved toward the lens from the focal point for each of these cases and hence the beams were diverging at the location of the fracture. The degree of non-collimation for the 30 mm and 15 mm beam diameters can be estimated by considering the depth of the focus (twice the Rayleigh range) of the beam passing through the lens. If the distance of the source point to the lens is within a Rayleigh range, then the beam leaving the lens will be nearly collimated. The Rayleigh range of the beams is given by:

$$Z_R = \frac{4}{\pi} \frac{f^2 \lambda}{D^2}, \quad (6)$$

where D is the beam diameter. The Rayleigh ranges Z_R for the three beam diameters are equal to 124 mm (for 60 mm), 496 mm (for 30 mm) and 2000 mm (for 15 mm). The Rayleigh ranges for the 30 mm and 15 mm diameter probe beams are much larger than the source-to-lens distance (because the beam diameters are no more than ten times the wavelength). Therefore, the use of the lens designed to collimate the 60 mm diameter beam also produces pseudo-collimated beams in the other two cases of 30 mm and 15 mm. This near-collimation is confirmed by the small variation of the arrival time across the beam at the location of the fracture as listed in Table 3.

Figure 3 Beam profiles measured 65 mm from the surface of the acoustic lens for source - lens separations of a) 110 mm, b) 195 mm and c) 302 mm. Using the full width at half max, the diameters of the beam waists are a) 15 mm, b) 30 mm and c) 60 mm. The variation in arrival time across the beam waist is listed in Table 3.

Table 3 Distance between source and lens (L), the resulting field of view, the variation in arrival time across the pseudo-collimated wavefront and the percent error in measured transmitted amplitude

L (mm)	Field of View (mm)	Variation in Arrival Time (μ s)	Error (%)
110	15	0.20	4.1
195	30	0.19	6.4
302	60	0.37	5.0

Two acoustic lenses were used in the acoustic system, one on the source side and one symmetrically on the receiver side to collect the transmitted waves, as shown in Fig. 4. The source transducer was a compressional-mode water-coupled spherically focused piezoelectric transducer (central frequency of 1 MHz) and the receiver was a water-coupled plane-wave transducer (central frequency of 1 MHz) with a 2 mm pinhole on the receiver face. The pinhole was used to obtain a ‘point’ measurement. The water coupling ensured reliable coupling between the sample and transducer for the acoustic mapping method. The receiver transducer location was matched to the source locations.

Because the locations of the source and receiver transducers were not at the focal planes of the lenses in our experimental configuration for the 30 mm and 15 mm beam diameters, it is necessary to assess if the field of view is restricted relative to the probe beam size by vignetting. Vignetting would be significant in the ray optics regime for Fresnel numbers much larger than unity. The Fresnel number for the beams is given by:

$$N_F = \frac{D^2}{L\lambda}, \quad (7)$$

where L is the distance from the lens to the receiver. The Fresnel numbers N_F are equal to 1.1 (for 60 mm), 1.8 (for 30 mm) and 4.4 (for 15 mm). These Fresnel numbers are comparable to unity, demonstrating that no significant vignetting (restriction on the field of view) occurs in our system design despite the locations of the transducers off the focal planes. The fact that the Fresnel numbers are all near unity indicates that the laboratory lens system is operating in the transition between the near field and the far field. Fresnel numbers near unity also indicate that diffraction effects are strong and the ray approximation cannot be used.

Figure 4 Sketch of the acoustic lens system experimental set-up to obtain the seismic measurements as a function of field-of-view. The distance between the transducers and the lens were the same for the source side of the sample and the receiver side of the sample. The distances used are listed in Table 3.

A related analysis calculates the spatial blurring (Fresnel length) at the fracture plane as viewed by the receiver transducer through the collecting lens. If the Fresnel length is larger than or comparable to the beam size, then the field of view is set by the beam size and little or no vignetting occurs. The Fresnel length observed by the receiving transducer is given by:

$$\xi_F = \sqrt{\frac{Lf\lambda}{f-L}} \quad (8)$$

In the three cases, the Fresnel lengths are 77 mm (for 60 mm), 33 mm (for 30 mm) and 17 mm (for 15 mm). The Fresnel lengths are comparable to the beam sizes, confirming that vignetting is not significantly reducing the field of view relative to the beam size. Furthermore, even if a small amount of vignetting is occurring, the similarity of the ratios of the Fresnel length to the beam diameter for all three cases indicates that each is affected almost equally. Therefore, for all three beam diameters, the field of view observed by the pin-hole at the receiving transducer is set approximately by the designed probe beam sizes of 60 mm, 30 mm and 15 mm.

For the desired beam diameters of 15 mm, 30 mm and 60 mm, the sampling pattern shown in Fig. 5 was used. For the 60 mm field of view, one measurement was made at the centre of the sample. For the 30 mm field of view, four measurements were made that covered the same approximate region. For the 15 mm field of view, 16 measurements were made, as shown in Fig. 5. Computer-controlled linear actuators were used to move the sample to collect the data for the 15 mm and 30 mm probe sizes.

Acoustic mapping method

The second approach used to probe fracture properties was an acoustic mapping method. Acoustic mapping (C-scans) probed the same 80 by 80 mm area of the fracture in 2 mm increments. Figure 5 shows the region (square area) over which the acoustic method mapping was applied to the sample relative to the measurements made for the three probe sizes. Computer-controlled linear actuators were used to move the source and receiver in unison. In the text and in the figures, we refer to data obtained from the acoustic map as the 2 mm scale, because the receiving transducer used a 2 mm aluminium pin-hole plate. The transducers were oriented perpendicular to the surface of the sample and were coaxially aligned. The distance of the acoustic mapping transducers from the face of the sample was 30 mm. The experimental setup was similar to that of the acoustic lens system but instead of the lenses the transducers were located where the acoustic lenses are located in Fig. 4. The acoustic mapping datasets consist in a 20-microsecond window of 1600 waveforms that contain the compressional wave (first arrival) to obtain the local variations in the seismic response of the fracture.

Sample preparation

Two limestone rock samples (Rock 1 and Rock 2), each containing a single induced fracture and one acrylic control sample (intact) were used in this study. The control sample (intact) did not contain any fractures and was used to measure systematic trends. All three samples were right cylinders with a diameter of 156 mm. The height of samples Rock 1,

Figure 5 A sketch of the regions probed for different field-of-views relative to the sample diameter (156 mm). Sixteen regions were probed for the 15 mm (dotted-edged circles) field-of-view; four regions for the 30 mm (dashed-edged circles) field-of-view; and one region for the 60 mm (solid-edged circle) field-of-view. The square region represents the area probed using the acoustic mapping method, i.e., measurements were made in 2 mm increments over an 80 mm by 80 mm area.

Rock 2 and intact were 72 mm, 76 mm and 68 mm, respectively. A fracture was induced in Rock 1 and Rock 2 using a technique similar to the Brazil testing (Jaeger and Cook 1972). After fracturing of the limestone samples, inlet and outlet ports were attached to the sample for flow measurements and for the injection of reactive fluids as well as sand transport (silica beads). Rock 1 had two ports (180° apart), while Rock 2 was fitted with eight ports (45° apart). The samples were sealed with marine epoxy to avoid geochemical interaction between the surface of the rock and the water in the acoustic imaging tank. The same seismic measurements were performed on the intact sample as were performed on Rock 1 and Rock 2. For the initial measurements (i.e., initial condition) of the limestone samples, the samples were vacuum-saturated with water.

Alteration of sample

Two different processes were used to alter the fractures in the rock samples to change the fracture specific stiffness through non-mechanical processes: 1) reactive flow that produced chemical alteration of the fracture and 2) simulated sand transport by using silica beads. The reactive flow altered the fracture geometry by etching and/or precipitating minerals in the limestone. Sand transport was used to deposit and/or erode the fractures. Seismic and flow-rate measurements were made before and after each alteration. A falling-head method was used to obtain the flow rates through the fracture plane. The measurements for the initial water-saturated fracture condition without any alterations are referred to as 'initial'. For Rock 1, an aqueous solution of 30% hydrochloric acid (HCl) was used, which was the final condition for Rock 1. For

Rock 2, a reactive solution of HCl and sulphuric acid (H_2SO_4) solution was used followed by a silica bead flow. For Rock 2, the seismic and flow measurements after the chemical flow are referred to as 'reactive' and after the silica bead flow as the 'final' condition.

Chemical alteration and sand transport

The limestone-fractured samples (Rock 1 and Rock 2) were subjected to chemical alteration. Limestone is a sedimentary rock composed primarily of the mineral calcite. For Rock 1, the aqueous HCl solution resulted in the dissolution of calcite (calcium carbonate, CaCO_3) and the production of calcium chloride (CaCl_2):

For Rock 2, the chemical solution consisted of a combination of 0.24 M HCl and 0.36 M H_2SO_4 (Singurindy and Berkowitz 2003). The sulphuric acid (H_2SO_4) reacts with the limestone producing the mineral gypsum (CaSO_4) and carbon dioxide and water,

Additionally, the products (gypsum) of this reaction can react with the hydrochloric acid (HCl) to produce calcium chloride and sulphuric acid, creating a continuous interaction between the acids and the rock until equilibrium is obtained:

The sulphuric acid solution and hydrochloric acid were injected into separate ports. The reactions of the sulphuric/hydrochloric acid solutions occurred in flow paths where the two solutions mixed. The acidic solutions reacted in their own path until they reached a common path/channel. The dissolution and the precipitation were the factors expected to alter the geometry of the fracture, i.e., to affect the mechanical and hydraulic properties of the fracture.

For Rock 2, after the chemical alteration, a solution of solid spherical silica beads (average diameter of 25 microns) was flowed through the fracture. The bead solution consisted of 0.23 grams of silica beads per 100 ml of water. The aqueous solution of beads was injected into the sample using the same method as that used for the chemical flow but with a higher head (height). This solution simulated sand transport in fractures.

Fluid flow measurements

To understand the relationship between the seismic and hydraulic properties of the fracture, flow rates were measured. A falling head method was used to measure flow rates through the fracture plane. The flow rates were measured using distilled water. A burette (4000 ml) was filled with water and connected to the fracture sample by Tygon tubing. The output of the fracture was measured in grams using a Mettler PM6100 electronic scale and in millilitres using burettes. The outflow was measured as a function of time. From the flow measurements, an average aperture can be calculated. Brown (1987) showed that hydraulic conductivity is locally proportional to the cube of the aperture. The 'cubic law' that relates aperture to volumetric flow rate is:

$$b_{ap}^3 = \frac{12\nu}{\rho g} \frac{Q \Delta L_{fp}}{w \Delta h}, \quad (9)$$

where Q is the flow rate, g is acceleration due to gravity, ν is the viscosity, ρ is the density of the water, Δh is the head drop in the burette drop, ΔL_{fp} is the length of the flow path inside the fracture (port to port distance), w is the diameter of the flow ports and b_{ap} is the average fracture aperture. Based on equation (9), volumetric flow rate data were used to estimate the average aperture of the fracture.

DATA ANALYSIS

Seismic data

A coherent sum of the signals at each probe scale was used to determine if measurements from a small-scale result in the same interpretation of fracture properties as those made on a larger scale. The coherent sum (C) consists in summing all the signals ($S(t)$) for a given scale and then dividing by the number of signals, N :

$$C(t) = \frac{1}{N} \sum_i^N S_i(t). \quad (10)$$

For example, for the 2 mm scale ($N = 1600$ signals), all of the signals were summed and divided by the number of signals. For the probe scale of 15 mm, $N = 16$ signals were used and $N = 4$ signals were used for the 30 mm scale. The 60 mm probe scale used only one signal, therefore a coherent sum was not used. To make the comparison, the coherent sum at each scale was shifted in time to remove system delay differences and to align the first peak.

The dominant frequency of the signals was extracted using a wavelet transformation analysis (Pyrak and Nolte 1995; Nolte *et al.* 2000). The dominant frequency is the frequency

at which the maximum amplitude of the group wavelet transform occurs. The error in choosing the dominant frequency is ± 0.05 MHz (step-size in the frequency analysis). Transmission coefficients as a function of frequency were also determined from the information provided by the wavelet analysis. The signal spectrum at the arrival time that coincides with the maximum amplitude was determined for each signal for each sample. The spectra from the rock samples were normalized by the spectrum from the intact sample to produce the transmission coefficient. The transmission coefficient, $T(\omega)$, was used in equation (11) to calculate an effective fracture specific stiffness, κ , as a function of frequency, ω :

$$\kappa(\omega) = \frac{\omega Z}{2\sqrt{\left(\frac{1}{T(\omega)}\right)^2 - 1}}, \quad (11)$$

where Z is the acoustic impedance defined by the product of the phase velocity and density. For our analysis, we used a phase velocity of 4972 m/s (measured velocity in the laboratory for non-fractured limestone) and a density of 2360 kg/m³. The acoustic impedance for the limestone samples used in this study is 11.73×10^6 kg/m²s.

RESULTS

Intact sample results

The intact acrylic sample was used as a control sample because it is homogeneous and contains no fractures or micro-cracks. The intact sample was used to quantify the repeatability of the seismic measurements of transmitted amplitude made using the lens system. The error in the measured transmitted amplitude across the fracture as a function of the field of view is listed in Table 3 and is on the order of 5%.

Figure 6 shows the coherent sums for the three field of view datasets as well as that from the acoustic mapping dataset for the intact sample. The signals were shifted in time to align the first peaks for comparison. By comparing the period of the first cycle, it is observed that the frequency content of the signal is approximately the same on all scales for the intact sample (see also Fig. 11b). From the wavelet analysis, the coherent sums from the 15 mm, 30 mm and 60 mm field of views exhibited a maximum frequency of 0.71 MHz (± 0.02 MHz) and at the 2 mm scale a frequency of 0.73 MHz (± 0.02 MHz). Therefore, the acoustic lens system does not affect the frequency content of the signal within the experimental error. However, the amplitude of the signals is affected. These systematic effects are accounted for in the analysis by

Figure 6 Coherent sum of received compressional waves for the intact acrylic sample. The acoustic map (2 mm) signal is in black. The seismic measurements as a function of field-of-view are given for: 60 mm (red), 30 mm (blue) and 15 mm (green).

normalizing the data from the rock samples by the data from the intact sample.

Results from rock samples

Flow rates

Volumetric flow rates were measured for the fractures in Rock 1 and Rock 2 (Table 4). Rock 1, which had two ports (180° apart), exhibited an increase in flow rate after the HCl solution flowed through the fracture. The flow rate through the fracture increased 32%. This suggests that the acidic solution enlarged the aperture of the fracture. Assuming a cubic relationship (equation (9)) between flow rate and aperture, the average aperture increased by 60 microns from 430 to 490 microns.

For Rock 2, which had eight ports (45° apart), the volumetric flow rates were measured for a combination of diametrically opposite ports. The combinations of ports are referred to as (inlet to outlet): 5–1, 6–2, 7–3 and 8–4. The sulphuric acid solution was introduced into the fracture through port 5 and the hydrochloric solution was introduced into port 3 to create the HCl and H₂SO₄ solution. During the chemical invasion, ports 7, 8 and 1 were left open to allow CO₂ produced by the reaction to escape. The sand solution was injected through port 8 and the only outlet was through port 4. After the two alteration processes (reactive flow and sand transport), the flow rates from (a) ports 5–1 and 7–3 were similar to that

Table 4 Volumetric flow rates for sample Rock 1 and Rock 2. Initial flow rates were measured for the water saturated condition for both samples. Reactive flow rates were measured for both samples, but Rock 1 is shown in the final condition. For Rock 2 the final condition is after the sand transport

	Initial Flow Rate (m ³ /s)	Reactive Flow Rate (m ³ /s)	Final Flow Rate (m ³ /s)
Rock 1	$4.09 \pm 0.16 \times 10^{-8}$		$5.39 \pm 0.16 \times 10^{-8}$
Rock 2 Ports 5–1	$3.12 \pm 0.02 \times 10^{-6}$	$3.34 \pm 0.02 \times 10^{-6}$	$3.50 \pm 0.07 \times 10^{-6}$
Rock 2 Ports 6–2	$1.78 \pm 0.02 \times 10^{-6}$	$4.23 \pm 0.02 \times 10^{-6}$	$3.26 \pm 0.03 \times 10^{-6}$
Rock 2 Ports 7–3	$1.00 \pm 0.02 \times 10^{-6}$	$1.26 \pm 0.02 \times 10^{-6}$	$0.95 \pm 0.03 \times 10^{-6}$
Rock 2 Ports 8–4	$2.64 \pm 0.02 \times 10^{-6}$	$4.62 \pm 0.02 \times 10^{-6}$	$1.13 \pm 0.08 \times 10^{-6}$

Table 5 The average aperture calculated by using the flow rates given in Table 3 and equation (9) for samples Rock 1 and Rock 2

	Initial Average Aperture (mm)	Reactive Average Aperture (mm)	Final Average Aperture (mm)
Rock 1	0.430		0.490
Rock 2 Ports 5–1	4.64	4.75	4.84
Rock 2 Ports 6–2	3.84	5.19	4.72
Rock 2 Ports 7–3	3.16	3.41	3.10
Rock 2 Ports 8–4	4.38	5.30	3.88

of the initial condition, (b) port 6–2 increased relative to the initial condition and (c) port 8–4 were smaller than the initial flow rates (Table 4). The reactive flow tended to increase the flow rate through all port combinations, while sand transport decreased the flow rate through all port combinations except 5–1.

The 6–2 port combination was perpendicular to the sand deposition process and was not used for the chemical invasion of the reactive solutions. Using the cubic law (equation (9)), the average apertures for all port combinations are listed in Table 5. The 6–2 port combination was found to have increased by roughly 1 mm after all alterations. The proximity of ports 6 and 2 to the ports used for the reactive flow (ports 5 and 3) may have caused the large increase of the flow rates for this port combination. The reactive flow altered the average aperture for all port combinations. Based on the flow measurements, the chemical solutions etched the fracture. The decrease in the 8–4 port combination was due to the sand transport process, i.e., the silica beads filled the voids in this flow path because these ports were used for the injection of the beads. Only for the 5–1 port combination did sand transport result in an increase in the flow rate and an increase in aperture.

Acoustic mapping results

The two-dimensional acoustic maps obtained using the acoustic mapping method described in Section 2.2 provided information on the local variations in fracture specific stiffness for the fractured rock samples. Figures 7 and 8 show the acoustic transmission maps for Rock 1 and Rock 2, respectively. The acoustic transmission maps are the ratio of the signal amplitude for the fractured samples normalized by the signal amplitude from the Intact sample. The colour scales in Figs 7 and 8 are proportional to the transmission coefficients. Figures 7(a) and 7(b) show the transmission maps for the initial and final conditions for Rock 1. By comparing Figs 7 and 8, the effect of the reactive flow on the local fracture properties contains both local increases and local decreases in transmission. Reduced transmission is caused by chemical erosion of the fracture, while enhanced transmission is caused by the deposition of the end products of the reaction, i.e., calcium chloride.

Figure 8 shows the acoustic transmission maps for the initial and final conditions for Rock 2. After the reactive flow followed by sand transport, transmission across the fracture increased across the entire area that was mapped. The

Figure 7 Transmission as a function of position for Rock 1 for (a) the initial condition and (b) the final condition. The total area scanned was 80 mm by 80 mm in a 2 mm increment. The colour scale to the right indicates the ratio of the transmitted compressional wave amplitude through the fractured sample to that through the acrylic sample.

Figure 8 Transmission as a function of position for Rock 2 for (a) the initial condition and (b) the final condition. The total area scanned was 80 mm by 80 mm in a 2 mm increment. The colour scale to the right indicates the ratio of the transmitted compressional wave amplitude through the fractured sample to that through the acrylic sample.

transmission coefficients range between 1% to 5% for Rock 2 for both the initial and final conditions, which are much smaller than those observed for Rock 1, which ranged between 10%–80%. The low transmission coefficients exhibited by Rock 2 are consistent with the higher flow rates (i.e., larger apertures) observed for Rock 2 compared to Rock 1 (see Tables 4 and 5). Low transmission is associated with low fracture specific stiffness and high flow rates (Pyrak-Nolte and Morris 2000).

Coherent sum signals and frequency content

Rock 1

Figure 9 is an example of seismic data obtained as a function of field-of-view by using the acoustic lens system on Rock 1 in the initial condition. One signal represents the 60 mm

scale (Fig. 9c), while 4 and 16 signals represent the 30 mm (Fig. 9b) and 15 mm (Fig. 9a) scales, respectively. These signals were collected in the same region but probed different subsets of the region (see Fig. 5). Differences in arrival times, amplitudes and frequency content depend on the sub-region that was probed. One trend is the decrease in the amplitude from the large field-of-view to the small field-of-view, because the collection area decreases as the field-of-view decreases.

The coherent sums of the signals from Rock 1 shown in Figs 9(a) and 9(b) are given in Fig. 10(a) as a function of field of view. The signals have been shifted in time to align the first peaks to enable a direct comparison of the amplitude and frequency content of the signal. For Rock 1 in the initial condition (Fig. 10a) the coherent sums have approximately the same frequency for the 2 mm, 15 mm and 60 mm field of view scales. This was confirmed by the wavelet analysis, which found that the frequencies for these field of views ranged

Figure 9 Received compressional waves transmitted through sample Rock 1 in the initial condition for field-of-views (a) 15 mm, (b) 30 mm, and (c) 60 mm. The systematic time delay associated with the acoustic lens method is not included in the time base.

between 0.51 MHz to 0.59 MHz (Fig. 11b). Only the coherent signal from the 30 mm scale exhibited a significantly different frequency (0.41 MHz). From the histogram (Fig. 11a) of the dominant frequency obtained from the 1600 signals on the 2 mm scale, it is inferred that the fracture in Rock 1 is relatively uniform, i.e., the probabilistic distribution of the dominant frequency is very narrow.

After the reactive flow, the signals from Rock 1 in the final condition (Fig. 10b) decreased in amplitude and the dominant frequency also decreased (Fig. 11a). The histogram of the dominant frequency for Rock 1 (final condition) is observed to shift to slightly lower frequencies and the width of the distribution decreased compared to that from Rock 1 in the initial condition (Fig. 11a). The decrease in the width of the distribution indicates that the fracture has become more uniform.

The narrowing of the width of a frequency distribution was observed by Gilbert and Pyrak-Nolte (2004) for single fractures in a granite rock in which calcium carbonate was precipitated by the mixing of two solutions within a fracture. They observed a shift of the frequency distribution to high frequencies as well as a decrease in the width (variance) of the frequency distribution. In Gilbert and Pyrak-Nolte (2004), the homogenization of the fracture (i.e., with mineral precipitation), coincided with a decrease in flow rate. In our current study, the decrease in the width of the frequency distribution for Rock 1 also indicates homogenization of the fracture

plane but the flow rates increased after the reactive flow and the probabilistic distribution shifted to a lower frequency. Equation (11) was used to determine the specific stiffness of the fracture in Rock 1 prior to and after the reactive flow. Fracture stiffness was calculated as a function of frequency by using the coherent sum signals for the 2 mm, 15 mm, 30 mm and 60 mm scales (Fig. 12a). At the 2 mm scale, the fracture specific stiffness decreased after the reactive flow. This is consistent with the observed increase in flow rate and the shift in the probabilistic distribution of the dominant frequency to lower values after the reactive flow.

A frequency-dependent stiffness indicates the heterogeneity (or lack of homogeneity) of the probability distribution of fracture specific stiffness (Pyrak-Nolte and Nolte 1992). When the field-of-view is small (2–15 mm), the fracture specific stiffness in Fig. 12(a) is relatively constant with frequency, i.e., the fracture is behaving as a displacement discontinuity (Pyrak-Nolte *et al.* 1990a). As Pyrak-Nolte and Nolte (1992) demonstrated theoretically, a frequency-independent fracture specific stiffness arises when the fracture has a uniform probabilistic distribution of stiffness. However, as the field of view increases to 60 mm, the fracture specific stiffness becomes frequency dependent, which occurs when a fracture contains a nonuniform probabilistic distribution of stiffnesses. The change in the functional relationship between fracture specific stiffness and the frequency change in the field of view is caused by a spatial distribution of fracture specific stiffness. When the

Figure 10 A comparison of the coherent-sum signals for (a) Rock 1 in the initial condition, (b) Rock 1 in the final condition, (c) Rock 2 in the initial condition, and (d) Rock 2 in the final condition for four field-of-views. The signals have been shifted in time so that the first positive peak of the signal at each field-of-view is aligned. The acoustic map (2 mm) signal is in black. The seismic measurements as a function of field-of-view are given for: 60 mm (red), 30 mm (blue) and 15 mm (green).

field of view is small, the wavefront illuminates only a small region of the fracture. In this small region, the fracture specific stiffness may be relatively uniform. However, as the field of view increases to 60 mm, the beam encounters a spatial distribution of fracture specific stiffness, resulting in a fracture specific stiffness that increases with increasing frequency. The frequency dependence is a good indicator of the degree of homogeneity of the fracture specific stiffness. A change in the frequency dependence with field-of-view is a good indicator of a spatial distribution in fracture specific stiffness. From the analysis, we estimate a spatial correlation length of the fracture specific stiffness that is on the order of 30 mm, for the fracture in Rock 1, which is where the fracture specific stiffness changes from being relatively frequency-independent to being frequency-dependent (Fig. 12).

Rock 2

Rock 2 is a weakly-coupled fracture that has a larger average aperture than Rock 1 and exhibits a lower fracture specific stiffness (Table 5 and Fig. 12). The frequency distribution for the fracture in this sample is not as homogeneous as for Rock 1. The histogram of the dominant frequency from the 2 mm scale is tri-modal for Rock 2 (Fig. 11a), while the frequencies of the 15 mm, 30 mm and 60 mm scales increase with increasing field-of-view (Fig. 11b). The high-frequency components of the signal were scattered out of the field of view for the 15 mm and 30 mm scales. On the other hand, the scattered signal components are captured on the 60 mm scale because the lens is collecting signals from larger scattering angles. The scattering losses are most likely associated

Figure 11 (a) Histogram of dominant frequency for Rock 1 and Rock 2 in the initial and final conditions from the 1600 waveforms collected on the 2 mm scale. (b) The dominant frequency obtained from the coherent signals as a function of scale (i.e., field-of-view).

Figure 12 Effective fracture specific stiffness as function of frequency for samples (a) Rock 1 and (b) Rock 2 as a function of field-of-view for initial (solid lines) and final (dashed lines) conditions. The acoustic map (2 mm) result is shown in black, and the field-of-view results are shown in red (60 mm), blue (30 mm) and green (15 mm).

with diffraction from the fracture. For Rock 2, the seismic measurements from the small-scale cannot be simply scaled up to the large-scale (60 mm) because of the change in the frequency content of the signals (Figs 10c, 10d and 11) with scale.

The volumetric flow rates through Rock 2 (Table 4) are consistent with fracture apertures on the order of 1–5 mm. At 1 MHz, the wavelength of the signal is comparable to some of the apertures in the fracture, resulting in strong scattering. This is confirmed from an examination of the fracture specific

stiffness as a function of frequency for Rock 2 in Fig. 12(b). As noted earlier, when the seismic response of a fracture is within the displacement discontinuity regime, the fracture exhibits either a fracture specific stiffness that is frequency independent (if the stiffness distribution is uniform) or the fracture specific stiffness increases with increasing frequency (if there is a probabilistic distribution of fracture stiffness and if the asperity spacing is smaller than a wavelength). For Rock 2 at field-of-view scales of 2 mm, 15 mm and 30 mm, the fracture specific stiffness decreases with increasing frequency for frequencies

up to 0.5 MHz. Above this frequency, the curves flatten out. On the other hand, for the 60 mm scale, the stiffness increases slightly with frequency. The stiffness distribution for the fracture in Rock 2 produces a seismic response that is a mixing of regimes. Rayleigh scattering causes the stiffness to decrease with increasing frequency because the high-frequency components of the signal are scattered out of the collecting field of view. The narrower angles for the smaller fields of view eliminate the high frequencies. Therefore, the fracture specific stiffness is not frequency dependent at frequencies higher than 0.5 MHz for this sample. On the other hand, the part of the fracture response behaving as a displacement discontinuity causes the fracture specific stiffness to increase slightly with increasing frequency. A balance between these two regimes is only observed on the 60 mm scale because it is the only scale able to collect the scattered energy. It is only by comparing the frequency-dependent behaviour of fracture specific stiffness as a function of field of view that enables the discrimination of the existence of the two scattering regimes, i.e., Rayleigh scattering and displacement discontinuity behaviour. Identification of multiple scattering regimes provides information on the geometric properties of the fracture relative to the wavelength and improves seismic characterization of the mechanical and hydraulic properties of a fracture.

CONCLUSIONS

The ability to interpret fracture properties from seismic data is intimately linked to an understanding of the role of probabilistic and spatial distributions in fracture specific stiffness. Fracture specific stiffness is a function of the asperity distribution within a fracture as well as the size of the fracture apertures. Both of these geometric properties prevent single-point measurements on the small-scale to be used to interpret fracture properties over a large-scale. For example, Rock 1 was found to have a relatively uniform fracture specific stiffness when the field of view (the portion of the fracture illuminated by the wavefront), was small (for 2 mm and 15 mm). Only on the larger scales (60 mm) was a spatial and probabilistic distribution of fracture specific stiffness inferred. Also, as observed for Rock 2, a range of geometric scales cause a mixed seismic response because of overlapping scales. Parts of the fracture may behave as a displacement discontinuity (seismic wavelength (λ) > asperity spacing or aperture) while other areas of the fracture may produce strong scattering (seismic wavelength (λ) $\leq$ asperity spacing or aperture). In our experiments, it was our ability to change the field of view that enabled us to determine that the fracture response was a mix

of scattering regimes. Understanding the effect of overlapping length scales on seismic wave propagation across fractures is important for correctly interpreting fracture properties.

The results from this study have important implications for interpreting seismic data on the laboratory scale as well as on the field scale. For example, in the laboratory, measurements made on the 15 mm to 30 mm scale are compatible with the diameter of the piezoelectric crystal in the transducers. If measurements are only made at these scales, the interpretation of fracture properties or bulk properties may be difficult or misleading if the sample produces strong scattering. In turn, many small-scale local measurements of the seismic response of a fracture cannot be directly summed and averaged to predict the global (large-scale) response of the fracture because of scattering losses outside the field of view. The key to understanding the seismic response on any scale is to examine the fracture specific stiffness both as a function of frequency and as a function of the field of view. As observed in our experiments, how fracture specific stiffness changes or remains constant with frequency helps determine if a uniform or nonuniform probabilistic distribution of fracture specific stiffness is present and also if scattering regimes are involved. In the characterization of a fracture from seismic measurements, frequency independent fracture specific stiffness is not sufficient to establish the homogeneity of the fracture. This study showed that 21 measurements over three scales (i.e., fields of view) were needed to unravel the competing effects of spatial correlations and probability distributions in Rock 2. Measurements obtained from different fields of view enable the estimation of the spatial correlation length in fracture specific stiffness.

Quantifying fracture specific stiffness using seismic data is important for remotely sensing fracture properties and monitoring alteration in these properties from time-dependent processes. The connection between fluid flow and fracture specific stiffness is an important interrelationship because measurements of seismic velocity and attenuation can be used to determine remotely the specific stiffness of a fracture in a rock mass. If this relationship holds, seismic measurements of fracture specific stiffness can provide a tool for predicting the hydraulic properties of a fractured rock mass. Currently, no analytic solution exists to link flow and fracture specific stiffness, and the link is most likely statistical in nature (Jaeger, Cook and Zimmerman 2007). However, it has been shown numerically (Pyrak-Nolte and Morris 2000) that the relationship between fluid flow and fracture specific stiffness arises directly from the size and spatial distribution of contact area and void space within a fracture. The acoustic lens method for

adjusting the field of view demonstrates that information on spatial distributions in fracture properties is achievable from seismic measurements.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of this work by the Geosciences Research Program, Office of Basic Energy Sciences, US Department of Energy (DEFG02-97ER14785 08). Also Angel Acosta-Colon acknowledges the Rock Physics Group at Purdue University for their help during this work.

REFERENCES

- Brown S.R. and Scholz C.H. 1985. Closure of random surfaces in contact. *Journal of Geophysical Research* **90**, 5531.
- Brown S.R., Kranz R.L. and Bonner B.P. 1986. Correlation between surfaces of natural rock joints. *Geophysical Research Letters* **13**, 1430–1434.
- Brown S.R. 1987. Fluid flow through rock joints: The effect of surface roughness. *Journal of Geophysical Research* **92**, 1337–1347.
- Chen D., Pyrak-Nolte L.J., Griffin J. and Giordano N.J. 2007. Measurement of interfacial area per volume for drainage and imbibition. *Water Resources Research* **43**, W12504. doi:10.1029/2007WR006021.
- Cheng J.-T., Pyrak-Nolte L.J., Nolte D.D. and Giordano N.J. 2004. Linking pressure and saturation through interfacial areas in porous media. *Geophysical Research Letters* **31**, L08502. doi:10.1029/2003GL019282.
- Dunn F. and Fry F.J. 1980. Acoustic elliptic lenses – an historical note. *Journal of the Acoustical Society of America* **68**, 350–351.
- Gilbert Z. and Pyrak-Nolte L.J. 2004. Seismic monitoring of fracture alteration by mineral deposition. *Proceedings of the 6th North American Rock Mechanics Symposium*. Houston.
- Gu B.L., Nihei K.T., Myer L.R. and Pyrak-Nolte L.J. 1996. Fracture interface waves. *Journal of Geophysical Research-Solid Earth* **101**, 827–835.
- Hopkins D.L. 1990. *The Effect of Surface Roughness on Joint Stiffness, Aperture and Acoustic Wave Propagation*. University of California, Berkeley.
- Hopkins D.L., Cook N.G.W. and Myer L.R. 1987. Fracture stiffness and aperture as a function of applied stress and contact geometry. *Proceedings of the 28th US Symposium on Rock Mechanics*. A.A. Balkema, Tucson.
- Jaeger J.C. and N.G.W. Cook. *Fundamentals of Rock Mechanics*. 1972. London. Methuen & Co, LTD.
- Jaeger J.C., Cook N.G.W. and Zimmerman R. 2007. *Fundamentals of Rock Mechanics*. 4th edn. Wiley-Blackwell.
- Johnson J., Brown S.R. and Stockman H.W. 2006. Fluid flow and mixing in rough-walled fracture intersections. *Journal of Geophysical Research* **111**. B12206. doi:10.1029/2005JB004087.
- Kendall K. and Tabor D. 1971. An ultrasonic study of the area of contact between stationary and sliding surfaces. *Proceedings of the Royal Society London, Series A* **323**, 321–340.
- Nolte D.D., Pyrak-Nolte L.J., Beachy J. and Ziegler C. 2000. Transition from the displacement discontinuity limit to the resonant scattering regime for fracture interface waves. *International Journal of Rock Mechanics and Mining Sciences* **37**, 219–230.
- Olinger A., Nolte D.D. and Pyrak-Nolte L.J. 2003. Focusing of seismic waves by a single fracture. *Geophysical Research Letters* **5**, 1203. doi:10.1029/2002GL016264.
- Pyrak-Nolte L.J., Myer L.R., Cook N.G.W. and Witherspoon P.A. 1987. Hydraulic and mechanical properties of natural fractures in low permeability rock. In: *Proceedings of the Sixth International Congress on Rock Mechanics* (eds G. Herget and S. Vongpaisal), pp. 225–231. A.A. Balkema, Rotterdam, The Netherlands.
- Pyrak-Nolte L.J., Myer L.R. and Cook N.G.W. 1990a. Transmission of seismic-waves across single natural fractures. *Journal of Geophysical Research-Solid Earth and Planets* **95**, 8617–8638.
- Pyrak-Nolte L.J., Myer L.R. and Cook N.G.W. 1990b. Anisotropy in seismic velocities and amplitudes from multiple parallel fractures. *Journal of Geophysical Research* **95**, 11 345–11 358.
- Pyrak-Nolte L.J., Montemagno C.D. and Nolte D.D. 1997. Volumetric imaging of aperture distributions in connected fracture networks. *Geophysical Research Letters* **24**, 2343–2346.
- Pyrak-Nolte L.J. and Morris J.P. 2000. Single fractures under normal stress: The relation between fracture specific stiffness and fluid flow. *International Journal of Rock Mechanics and Mining Sciences* **37**, 245–262.
- Pyrak-Nolte L.J. and Nolte D.D. 1992. Frequency-dependence of fracture stiffness. *Geophysical Research Letters* **19**, 325–328.
- Pyrak-Nolte L.J. and Nolte D.D. 1995. Wavelet analysis of velocity dispersion of elastic interface waves propagating along a fracture. *Geophysical Research Letters* **22**, 1329–1332.
- Schoenberg M. 1980. Elastic wave behavior across linear slip interfaces. *Journal of the Acoustical Society of America* **5**, 1516–1521.
- Singurindy O. and Berkowitz B. 2003. Evolution of the hydraulic conductivity by precipitation and dissolution in carbonate rock. *Water Resources Research* **39**, 1016.

Microbial-induced heterogeneity in the acoustic properties of porous media

Caroline A. Davis,¹ Laura J. Pyrak-Nolte,² Estella A. Atekwana,³ D. Dale Werkema Jr.,⁴ and Marisa E. Haugen²

Received 10 June 2009; revised 25 August 2009; accepted 13 October 2009; published 13 November 2009.

[1] It is not known how biofilms affect seismic wave propagation in porous media. Such knowledge is critical for assessing the utility of seismic techniques for imaging biofilm development and their effects in field settings. Acoustic wave data were acquired over a two-dimensional region of a microbial-stimulated sand column and an unstimulated sand column. The acoustic signals from the unstimulated column were relatively uniform over the 2D scan region. The data from the microbial-stimulated column exhibited a high degree of spatial heterogeneity in the acoustic wave amplitude, with some regions exhibiting significant increases in attenuation while others exhibited decreases. Environmental scanning electron microscopy showed differences in the structure of the biofilm between regions of increased and decreased acoustic wave amplitude. We conclude from these observations that variations in microbial growth and biofilm structure cause heterogeneity in the elastic properties of porous media with implications for the validation of bioclogging models. **Citation:** Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen (2009), Microbial-induced heterogeneity in the acoustic properties of porous media, *Geophys. Res. Lett.*, 36, L21405, doi:10.1029/2009GL039569.

1. Introduction

[2] Bioclogging caused by biofilm development is a phenomenon that can cause significant changes in the physical properties of porous media including porosity and permeability changes that influence fluid flow and transport properties [e.g., Baveye *et al.*, 1998] and remediation efforts. Numerical models and simulations have been developed to qualitatively forecast the change in hydraulic properties of a porous medium from bioclogging [e.g., Brovelli *et al.*, 2009]. Bioclogging processes are dynamic and are influenced by many phenomena including initial heterogeneities in biomass distribution as well as the physical properties of the porous medium [e.g., Brovelli *et al.*, 2009]. A major difficulty inherent with experimental modeling approaches is that in situ quantitative information from direct observation of biological growth and clogging from field data is difficult to obtain at the appropriate spatiotem-

poral scales needed for model validation [Dupin and McCarty, 2000]. Minimally invasive diagnostic techniques are needed to provide near real-time information of the spatiotemporal distribution of biofilms in porous media for validating predictive models and for monitoring microbial growth in situ. Although several studies have investigated the rheological properties of biofilms in laboratory settings [e.g., Stoodley *et al.*, 1999], it is not known how biofilms affect seismic wave propagation in porous media. Such an understanding is critical for assessing the utility of seismic techniques for imaging biofilm spatial heterogeneity and their effects on porous media in field settings.

[3] To date, most biogeophysical investigations have focused on geoelectrical techniques [Atekwana *et al.*, 2006]. Apart from a few studies [e.g., Williams *et al.*, 2005; DeJong *et al.*, 2006], less attention has been given to the effects of microbial interactions with geologic media on elastic properties, hence questions remain about the effect of microbial growth and biofilm formation in porous media on acoustic wave propagation in the absence of biomineralization. The work described in this letter investigates the influence of biofilm formation on the spatiotemporal seismic properties of porous media. We show for the first time that variations in biofilm structure/texture cause heterogeneity in acoustic wave attenuation of porous media.

2. Materials and Methods

[4] Prismatic experimental columns, measuring 102 mm by 51 mm by 254 mm (width × depth × height), were fabricated using 3.2 mm thick clear acrylic. The columns were wet-packed with coarse grain (0.6–1.18 mm) ASTM 20/30 silica sand (Ottawa, IL). Prior to packing, the sands were washed with deionized (DI) water and disinfected by autoclaving. Columns and accessory equipment were also disinfected by rinsing with a 70% ethanol solution. Prior to saturation with the experimental fluids, the sand-packed columns were saturated with sterile 25% Bushnell Haas (BH) nutrient broth (Becton Dickinson) and baseline acoustic measurements were recorded. After initial background measurements, microbial growth was stimulated in one sand column (biostimulated column) by saturating with 25% BH nutrient broth, 30 mM glucose, *Pseudomonas aeruginosa* PAO1 wild type bacteria culture, and 30 µg/mL Gentamicin antibiotic. The bacteria strain (specifically PAO1 Tn7-Gm-gfp) was obtained from the University of Denmark (Lyngby, Denmark), where previous studies with this bacteria strain have been conducted [e.g., Pamp and Tolker-Nielsen, 2007]. The other column (unstimulated column) was used for background measurements and was saturated with 25% BH and Gentamicin antibiotic. The Gentamicin antibiotic

¹Geological Sciences and Engineering, Missouri University of Science and Technology, Rolla, Missouri, USA.

²Department of Physics, Purdue University, West Lafayette, Indiana, USA.

³Boone Pickens School of Geology, Oklahoma State University, Stillwater, Oklahoma, USA.

⁴U.S. Environmental Protection Agency, Las Vegas, Nevada, USA.

Figure 1. The 2D acoustic wave amplitude scans (at 0.5 MHz) from the (a) biostimulated and (b) unstimulated columns for Days 1, 2, 5, 8, and 29. Black letters (A,B,C) on Day 29 of the 2D scans denotes location of ESEM images shown in (c): location A is from the biostimulated column from an area of increased amplitude; location B is from an area of decreased amplitude; and location C is from the unstimulated column with no apparent attached biomass. Note the differences in the texture of the attached biomass between location A's having a rough texture and location B's smooth texture with the grain surfaces completely covered by biomaterial.

was added to both the biostimulated and unstimulated columns to inhibit the growth of microorganisms other than the *P. aeruginosa* in the biostimulated column.

2.1. Acoustic Wave Measurements

[5] A full-waveform acoustic wave imaging system was used to obtain two-dimensional point-by-point maps of the acoustic response of the samples [e.g., *Acosta-Colon et al.*, 2009]. The acoustic imaging system used two water-coupled plane-wave transducers (1 MHz central frequency) for the source and receiver. The columns were placed in a water tank to a depth 2/3 the length of a column, and remained in situ at laboratory temperature (22–24°C) for the duration of the experiment. Using the acoustic mapping mode (C-scan), computer-controlled linear actuators (Newport 850-B4 and Motion Master 2000) were used to move the source and receiver in unison over a 60 mm by 70 mm region in 5 mm increments. A pulse generator (Panametrics PR1500) was used to excite the source and to receive the transmitted signal from the receiver. At each point in the 2D scan region, a 100 microsecond window of the transmitted signal was recorded and digitized with an oscilloscope (Lecroy 9314L). The entire 2D region was scanned 2–3 times per week for the 29 day duration of the experiment.

2.2. Sampling and Analyses

[6] Fluid samples were collected 1–2 times per week from the bottom valve of the columns. The pH was measured using a bench-top probe immediately after fluids were withdrawn. Upon termination of the experiment, the columns were destructively sampled by withdrawing cores of the wet sand (core diameter ~ 6 mm) in a grid-like fashion (15 mm by 15 mm grid) from the acoustic scan region. The sand cores were used for environmental scanning electron microscopy (FEI Quanta 600 ESEM) to image and characterize the surfaces of the sand grains.

3. Results

3.1. Acoustic Wave Monitoring

[7] A time-frequency analysis [*Nolte et al.*, 2000] was performed to determine the amplitude of the compressional signal at a frequency of 0.5 MHz, i.e., the most probable or dominant frequency of the signals. The 2D acoustic scan images of the transmitted compressional wave amplitude obtained from the biostimulated and unstimulated columns are shown in Figure 1, and the temporal percent change in acoustic wave amplitude relative to Day 1 is shown in

Figure 2. Graphs showing the temporal percent change in acoustic wave amplitude (at 0.5 MHz) relative to Day 1 for the (a) biostimulated and (b) unstimulated columns. Note the significant increase in attenuation ($\sim 80\%$) for the biostimulated compared to the unstimulated sample ($\sim 20\%$).

Figure 2. The 2D scans obtained from the biostimulated column on Day 1 reveal relatively uniform compressional wave amplitude over the scan region. However, by Day 5 significant changes were observed in the biostimulated column and the average amplitudes varied spatially over the scan region. For the biostimulated column, the 2D image obtained on Day 29 exhibited an increase in amplitude in some regions (i.e., Figure 1a, location A), while other regions showed a decrease in amplitude (i.e., Figure 1a, location B). This is clearly observed in Figure 2a. Unlike the unstimulated column (Figure 2b), the change in amplitude as a function of time varied with location for the biostimulated column (Figure 2a). Compared to Day 1, locations A-C in the biostimulated column (Figure 2a) initially show a decrease in amplitude of $\sim 40\%$ to Day 5. Thereafter, the amplitudes at locations A and B in the biostimulated column increased reaching initial baseline amplitudes by Day 10. The amplitude at location C also increased after Day 5 but did not return to baseline values. Locations D and E (Figure 2a) show a decrease in amplitudes of $\sim 80\%$ by Day 14 and remain relatively steady to the end of the experiment. The relatively small overall variation in amplitude ($<20\%$) relative to Day 1 observed from the unstimulated column is consistent for all of the select data points plotted (Figure 2b, locations A–E).

3.2. Geochemical Monitoring

[8] The pH values from the biostimulated column steadily decreased from a baseline pH of 7 to near 4.4 on Day 12, and remained at a pH of 4.4 through Day 20 (data not shown). From the unstimulated column, a pH of 7 was consistent throughout the duration of the experiment.

3.3. Sand Surface Imaging

[9] Representative ESEM images from the columns sampled at the end of the experiment are shown on Figure 1c. Samples from an area of increased acoustic amplitude

(location A, Figure 1a) in the biostimulated column shows a rough textured surface which appears to have a patchy covering of ‘biomaterial’ over some portions of the sand grain, while on other portions of the image the silica sand surface is clearly visible (Figure 1c, location A). Rod-shaped bacterial cells are present in this biomaterial, but not clearly distinguishable in this image. The ESEM images of sand sampled from an area of decreased acoustic amplitude (location B, Figure 1a) in the biostimulated column show the surface of a sand grain which appears to be completely covered in a smooth biomaterial, with several holes and void-spaces (Figure 1c, location B). Figure 1c (middle) also shows the presence of attached rod-shaped bacteria embedded in this biomaterial. In contrast, the ESEM images of samples obtained from the unstimulated column (location C, Figure 1b) show the irregular or hummocky surface of a silica sand grain with no apparent attached bacteria cells or biomass (Figure 1c, location C).

4. Discussion and Conclusions

[10] In this study the compressional wave amplitude was observed to differ both temporally and spatially, between the biostimulated and unstimulated columns (Figure 1). Compressional wave amplitudes in the biostimulated column became more spatially variable while the acoustic response of the unstimulated column homogenized over time. While the changes observed in the unstimulated column ($<20\%$) are not insignificant (Figure 2b), they are far less than the measured changes from the biostimulated column and consistent for all of the select data points. Hence we attribute the changes in the latter to particle settling. Except for a few locations (e.g., location A, Figures 1a and 2a) that showed an increase, most locations in the biostimulated column showed a decrease in the compressional wave amplitudes over time with some regions decreasing to $\sim 80\%$ of Day 1 values (e.g., location E, Figure 2a). Microbial growth was active in the biostimulated column as evidenced by the decrease in pH (from 7 to 4.4) and ESEM images that confirm microbial cell colonization of sand surfaces (Figures 1c, locations A and B). No microbial growth was observed in the unstimulated column (Figure 1c, location C). The decrease in pH most likely resulted from the accumulation of metabolic byproducts such as organic acids [e.g., Silverman and Munoz, 1974], which eventually inhibited continued microbial growth in the columns.

[11] The bacteria culture used in this study (*P. aeruginosa*) is capable of producing different types of biofilms, depending on the environment [e.g., Friedman and Kolter, 2004], and formation of these biofilms is documented to occur in different stages [e.g., Davey et al., 2003]. Initial stages in the formation of *P. aeruginosa* biofilms are characterized by the attachment of planktonic cells to solid surfaces. Initial attachment is followed by colonization of the surfaces followed by the production of extrapolymer substances (alginate - a viscous gum) that embed the reproducing cells allowing them to form microcolonies and build thick biofilms. One characteristic of the *P. aeruginosa* biofilms described in the literature [e.g., Davey et al., 2003; Pamp and Tolker-Nielsen, 2007] is the presence of macrocolonies surrounded by large void spaces or open, dark fluid-filled channels, through which the lower levels of bacteria in the

biofilm are thought to dispose of accumulating waste products (e.g., Figure 1c, location B). The ESEM images (Figure 1c, location A and B) obtained from the biostimulated column show apparent qualitative differences in the texture of the attached biofilm between areas of increased and decreased amplitude. We hypothesize that differences in the measured amplitudes reflect differences in the structure/texture of the attached biofilms in the biostimulated column.

[12] Acoustic properties of porous media are generally dependant on the bulk modulus of the saturating fluid [e.g., Knight and Nolen-Hoeksema, 1990], the elastic moduli of the solid media [e.g., Ecker et al., 1998], and the solid-fluid interactions [e.g., Clark et al., 1980]. Energy loss mechanisms for fluid-saturated porous media fall into three categories: viscoelastic loss, fluid-solid surface physiochemical loss, and scattering loss [Li et al., 2001]. Generally, decreases in acoustic amplitude result from biogenic gas production or the weakening of grain contacts (physical/chemical alteration of surfaces and/or grain contacts [e.g., Murphy et al., 1984; Clark et al., 1980]) in porous media, both of which reduce the elastic moduli and are manifested by delays and attenuation of acoustic waves. We observed no gas bubble formation in the biostimulated column. Hence, we hypothesize that the presence of biofilms caused the changes in elastic properties of the sample which is consistent with studies that suggest that soft and patchy structures like biofilm surfaces result in the effective attenuation of sound [e.g., Janknecht and Melo, 2003].

[13] Increases in acoustic amplitude may result from increases in the bulk modulus of the solid media [e.g., Li et al., 2001] through the stiffening of grain contacts. Hence it is possible that in the regions of increased amplitude, the extrapolymer substances resulted in enhanced coupling between grains, whereas areas of decreased amplitude may be explained by viscous losses or physiochemical alterations at grain contacts due to the nature of the biofilms. We note that during ESEM imaging of the sand samples from regions with increased amplitude (Figure 1a, location A), individual bacterial cells were not clearly distinguishable until the operating temperature of the ESEM was raised from 5 to 20°C, and the relative humidity was decreased from 89% to 14%, which effectively dried out the sample/biomaterial. However, individual cells and attached biomass on sand samples collected from location B (Figure 1a), were evident immediately upon viewing with the ESEM (at 5°C and 89%) and remained virtually the same in appearance when the temperature was increased to 20°C (images not shown).

[14] Wave scattering or interference from spatial heterogeneity of the medium is another mechanism that affects wave attenuation. The biostimulated sample did not exhibit biogenic gas formation, which suggests that air bubbles are not a source of scattering. The density contrast between water-saturated sediment and the biofilm-microbially altered sediment is not large. Thus, a potential source of scattering is a spatial variation in elastic or viscoelastic moduli from microbial alteration of the grain contact, pore-filling material and/or biofilm connecting grains.

[15] Our investigation shows that acoustic imaging techniques are sensitive to spatiotemporal changes in porous media caused by enhanced microbial growth of a biofilm

forming bacteria culture. While the exact microbial-induced mechanisms for the variations in amplitude are yet unclear, we speculate that the differences in amplitude arise from a non-uniform distribution of microbial activity or possible heterogeneity in the biomass distribution and biofilm morphology. The applicability of our laboratory measurements to the field scale will depend on spatial and temporal dispersion. Temporal dispersion connects frequency-dependent attenuation and velocity with elastic moduli, i.e., changes at grain contacts or pore-filling. Spatial dispersion connects wavelength with the size of the scatterer, i.e., the size and/or spatial correlation length of microbially altered regions. Increasing or decreasing attenuation with frequency will provide information on the size of the altered region and on the spatiotemporal distribution of biomass/bioclogging development.

[16] **Acknowledgments.** This material is based in part on work supported by the National Science Foundation under grants OCE-0729642, EAR 0722410 (MRI), EAR 0525316, and REU Award 0552918 and EPA Student Services Contract EP07D000660, Office of Basic Energy Sciences, US Department of Energy (DEFG02-97ER14785 08). S. Pamp and T. Tolker-Nielsen provided the *Pseudomonas* PAO1 Tn7-Gm-gfp strain to S. Rossbach, and we thank S. Rossbach for helpful discussions on culturing the bacteria. Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

References

- Acosta-Colon, A., L. J. Pyrak-Nolte, and D. D. Nolte (2009), Laboratory-scale study of field of view and the seismic interpretation of fracture specific stiffness, *Geophys. Prospect.*, *57*, 209–224, doi:10.1111/j.1365-2478.2008.00771.x.
- Atekwana, E. A., D. D. Werkema, and E. A. Atekwana (2006), Biogeophysics: The effects of microbial processes on geophysical properties of the shallow subsurface, in *Applied Hydrogeophysics*, NATO Sci. Ser., Ser. IV, edited by H. Vereecken et al., pp. 161–193, Springer, New York.
- Baveye, P., P. Vandevivere, B. L. Hoyle, and P. C. DeLeo (1998), Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials, *Critical Rev. Environ. Sci. Technol.*, *28*, 123–191, doi:10.1080/10643389891254197.
- Brovelli, A., F. Malaguerra, and D. A. Barry (2009), Bioclogging in porous media: Model development and sensitivity to initial conditions, *Environ. Model. Software*, *24*, 611–626, doi:10.1016/j.envsoft.2008.10.001.
- Clark, V. A., B. R. Tittman, and T. W. Spencer (1980), Effect of volatiles on attenuation (Q-1) and velocity in sedimentary rocks, *J. Geophys. Res.*, *85*, 5190–5198, doi:10.1029/JB085iB10p05190.
- Davey, M. E., N. C. Caiazza, and O'Toole G. A. (2003), Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1, *J. Bacteriol.*, *185*, 1027–1036, doi:10.1128/JB.185.3.1027-1036.2003.
- DeJong, J. T., M. B. Fritzges, and K. Nusslein (2006), Microbially induced cementation to control sand response to undrained shear, *J. Geotech. Geoenviron. Eng.*, *132*, 1381–1392, doi:10.1061/(ASCE)1090-0241(2006)132:11(1381).
- Dupin, H. J., and P. L. McCarty (2000), Impact of colony morphologies and disinfection on biological clogging in porous media, *Environ. Sci. Technol.*, *34*, 1513–1520, doi:10.1021/es990452f.
- Ecker, C., J. Dvorkin, and A. Nur (1998), Sediments with gas hydrates: Internal structure from seismic AVO, *Geophysics*, *63*, 1659–1669, doi:10.1190/1.1444462.
- Friedman, L., and R. Kolter (2004), Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms, *Mol. Microbiol.*, *51*, 675–690, doi:10.1046/j.1365-2958.2003.03877.x.
- Janknecht, P., and L. F. Melo (2003), Online biofilm monitoring, *Rev. Environ. Sci. Biotechnol.*, *2*, 269–283.
- Knight, R., and R. Nolen-Hoeksema (1990), A laboratory study of the dependence of elastic wave velocities on pore scale fluid distribution, *Geophys. Res. Lett.*, *17*, 1529–1532, doi:10.1029/GL017i010p01529.
- Li, X., L. R. Zhong, and L. J. Pyrak-Nolte (2001), Physics of partially saturated porous media: Residual saturation and seismic-wave propagation, *Annu. Rev. Earth Planet. Sci.*, *29*, 419–460, doi:10.1146/annurev.earth.29.1.419.

- Murphy, W. F., III, K. W. Winkler, and R. L. Kleinberg (1984), Frame modulus reduction in sedimentary rocks: The effect of adsorption on grain contacts, *Geophys. Res. Lett.*, *11*, 805–808, doi:10.1029/GL011i009p00805.
- Nolte, D. D., L. J. Pyrak-Nolte, J. Beachy, and C. Ziegler (2000), Transition from the displacement discontinuity limit to the resonant scattering regime for fracture interface waves, *Int. J. Rock Mech. Min. Sci.*, *37*, 219–230, doi:10.1016/S1365-1609(99)00102-1.
- Pamp, S. J., and T. Tolker-Nielsen (2007), Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*, *J. Bacteriol.*, *189*, 2531–2539, doi:10.1128/JB.01515-06.
- Silverman, M. P., and E. F. Munoz (1974), Microbial metabolism and dynamic changes in the electrical conductivity of soil solutions: A method for detecting extraterrestrial life, *Appl. Microbiol.*, *28*, 960–967.
- Stoodley, P., Z. Lewandowski, J. D. Boyle, and H. M. Lappin-Scott (1999), Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: An in situ investigation of biofilm rheology, *Biotechnol. Bioeng.*, *65*, 83–92, doi:10.1002/(SICI)1097-0290(19991005)65:1<83::AID-BIT10>3.0.CO;2-B.
- Williams, K. H., D. Ntarlagiannis, L. D. Slater, A. Dohnalkova, S. S. Hubbard, and J. F. Banfield (2005), Geophysical imaging of stimulated microbial biomineralization, *Environ. Sci. Technol.*, *39*, 7592–7600, doi:10.1021/es0504035.
- E. A. Atekwana (corresponding author), Boone Pickens School of Geology, Oklahoma State University, Stillwater, OK 74078, USA. (estella.atekwana@okstate.edu)
- C. A. Davis, Geological Sciences and Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA.
- M. E. Haugen and L. J. Pyrak-Nolte, Department of Physics, Purdue University, West Lafayette, IN 47907, USA.
- D. D. Werkema Jr., U.S. Environmental Protection Agency, 944 E. Harmon Ave., Las Vegas, NV 89119, USA.

Acoustic and electrical property changes due to microbial growth and biofilm formation in porous media

Caroline A. Davis,¹ Laura J. Pyrak-Nolte,² Estella A. Atekwana,³
Douglas D. Werkema Jr.,⁴ and Marisa E. Haugen²

Received 31 August 2009; revised 31 January 2010; accepted 19 February 2010; published 16 July 2010.

[1] A laboratory study was conducted to investigate the effect of microbial growth and biofilm formation on compressional waves, and complex conductivity during stimulated microbial growth. Over the 29 day duration of the experiment, compressional wave amplitudes and arrival times for the control (nonbiostimulated) sample were observed to be relatively uniform over the scanned 2-D region. However, the biostimulated sample exhibited a high degree of spatial variability in both the amplitude and arrival times, with portions of the sample exhibiting increased attenuation (~80%) concurrent with an increase in the arrival times, while other portions exhibited decreased attenuation (~45%) and decreased arrival times. The acoustic amplitude and arrival times changed significantly in the biostimulated column between days 5 and 7 of the experiment, consistent with a peak in the imaginary conductivity (σ'') values. The σ'' response is interpreted as recording the different stages of biofilm development with peak σ'' representing maximum biofilm thickness and decreasing σ'' representing cell death or detachment. Environmental scanning electron microscope imaging confirmed microbial cell attachment to sand surfaces and showed apparent differences in the morphology of attached biomass between regions of increased and decreased attenuation. 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.

Citation: Davis, C. A., L. J. Pyrak-Nolte, E. A. Atekwana, D. D. Werkema Jr., and M. E. Haugen (2010), Acoustic and electrical property changes due to microbial growth and biofilm formation in porous media, *J. Geophys. Res.*, 115, G00G06, doi:10.1029/2009JG001143.

1. Introduction

[2] Microorganisms have the ability to create micro-environments and niches in subsurface sediment environments by forming biofilms. Biofilms are created by the attachment, growth, and proliferation of microorganisms at mineral grain surfaces. These highly organized microbial systems consist of microbial cells, microbial byproducts, nutrients, substrates, and solid surfaces [Cunningham *et al.*, 1991; Marshall, 1992]. An important aspect of microbial attachment and growth on mineral surfaces is the production of a “pseudoglu” material consisting of exopolymeric substances (EPS), which helps to bind the microbes to surfaces.

The EPS that connects microbes to mineral particles is a key factor in clogging of sediment pore spaces and fluid flow pathways [Baveye *et al.*, 1998]. Studies have shown that biofilm development significantly reduces the porosity (by 50–90%) and permeability (by 95–99%) of porous media [e.g., Bouwer *et al.*, 2000; Dunsmore *et al.*, 2004]. Hence, microbial colonization of mineral surfaces and the proliferation of biofilms can have a profound effect on the physico-chemical properties of subsurface environments, influencing fluid flow and transport properties [e.g., Cunningham *et al.*, 1991; Baveye *et al.*, 1998; Brovelli *et al.*, 2009].

[3] Such microbial transformations of subsurface media are often coupled; occurring over a wide range of spatial and temporal scales, thus making it difficult to assess the extent of these dynamic microbial induced alterations. In addition, while biofilms are more readily studied in bioreactors, flasks, tanks, etc., they are more difficult to study in porous media [e.g., Cunningham *et al.*, 1991]. For example, the thickness and morphology of biofilms can be quantified using a variety of microscopy techniques such as confocal scanning laser or atomic force microscopy [e.g., Ahimou *et al.*, 2007; Yerly *et al.*, 2008]. Unfortunately, due to the surface roughness of

¹Department of Geological Sciences and Engineering, Missouri University of Science and Technology, Rolla, Missouri, USA.

²Department of Physics and Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana, USA.

³Boone Pickens School of Geology, Oklahoma State University, Stillwater, Oklahoma, USA.

⁴U.S. Environmental Protection Agency, Las Vegas, Nevada, USA.

sediment grains and the inherent difficulties of making such measurements when biofilms are found in geologic material, use of the above listed microscopy techniques is often limited. It is thus essential to develop techniques and proxy indicators that can provide spatiotemporal information on biofilm forming processes especially in field settings.

[4] Geophysical methodologies, such as seismic and electrical methods have the potential to provide a minimally invasive option to remotely detect, characterize and/or quantify the spatial distribution of biogeochemical processes [e.g., Williams *et al.*, 2005; Atekwana *et al.*, 2006; DeJong *et al.*, 2006; Atekwana and Slater, 2009; Slater *et al.*, 2009] and the induced changes in the physical properties of geologic media. The effects of biofilm formation on geophysical properties are currently not well understood, and require further study to determine how the mechanical properties of the geologic media are directly or indirectly affected by microbial activities.

[5] Understanding the mechanical properties of biofilms, such as attachment and detachment, is important for assessing biofilm processes and behavior, as well as for the control of biofilms in industrial and medical environments [Stoodley *et al.*, 1999; Ahimou *et al.*, 2007]. Rheological measurements have shown that biofilms exhibit linear viscoelastic behavior [Stoodley *et al.*, 1999; Klapper *et al.*, 2002; Ahimou *et al.*, 2007]. Acoustic and seismic measurements depend on the rheological properties of the porous medium and fluids that are contained in the pore spaces [Li *et al.*, 2001]. Thus, variations in the temporal viscoelastic properties of biofilms as they develop and evolve suggests that seismic geophysical techniques may be used to assess biofilm distribution and processes in field situations. Although several studies have investigated the rheological properties of biofilms in laboratory settings [e.g., Stoodley *et al.*, 1999], it is not known how such soft gelatinous material affects seismic wave propagation in porous media in the absence of mineral precipitation. Such an understanding is critical for assessing the utility of seismic geophysical techniques for imaging biofilm spatial distribution and the effects of bioclogging in porous media at field scales.

[6] Most biogeophysical investigations have focused on geoelectrical techniques (see, for example, reviews by Atekwana *et al.* [2006] and Atekwana and Slater [2009]) for the investigation of temporal microbial-induced phenomena. Recent studies have focused on the effect of biogenic gases or microbial mediated mineral precipitation on wave propagation in sands and sediments [e.g., Williams, 2002; Williams *et al.*, 2005; DeJong *et al.*, 2006, 2010]. The present study advances the work of Williams *et al.* [2005] and DeJong *et al.* [2006] by investigating the effect of biofilm formation on the spatiotemporal seismic properties of porous media using an acoustic two-dimensional scanning method [e.g., Li and Pyrak-Nolte, 1998; Pyrak-Nolte *et al.*, 1999] in the absence of enhanced precipitation. The primary objective of this research was to investigate the acoustic and electrical property changes due to microbial growth and biofilm formation in porous media. We observed that changes in the acoustic wave properties coincided with peaks in the imaginary component of complex conductivity. Based, in part, on our previous microbial growth study [Davis *et al.*, 2006] which showed that peaks in imaginary conductivity were coincident with peaks in microbial cell and biofilm attachment and

scanning electron microscope (SEM) images, we suggest that changes in the acoustic wave properties may be the direct result of the presence of biofilms. Hence we suggest that variations in biofilm structure and morphology in our samples caused heterogeneity in the elastic properties of the porous media, and thus variations in acoustic wave attenuation and velocity. Acoustic and electrical methods have the potential to (1) provide diagnostic semiquantitative data for testing and validation of bioclogging models and numerical simulations used for assessing microbial induced changes in flow and transport properties, and (2) be used for assessing spatial or temporal variations in biomass distribution in subsurface environments (i.e., microbial enhanced oil recovery (MEOR), and engineered biobarriers).

2. Materials and Methods

2.1. Experimental Column Setup

[7] Rectangular experimental columns were fabricated from 3.2 mm thick clear acrylic, and measured 102 mm by 51 mm by 254 mm (width $\times$ depth $\times$ height). Two sets of experimental columns were constructed: two columns for acoustic wave measurements (e.g., Figure 1a) and two columns for complex conductivity (electrical) measurements (e.g., Figure 1b). The electrical columns were constructed with two Ag-AgCl current injection electrode coils (160 mm apart), and two Ag-AgCl potential electrodes (90 mm apart), which were installed between the current electrodes (Figure 1b). In addition, a plastic divider was installed in the center of the electrical columns (Figures 1b and 1c), constructed from a 190 mm long section of 32 mm inner diameter (1 mm thick) polyvinyl chloride pipe cut lengthwise. Emplacement of the divider was deemed necessary to reduce noise in the electrical data, after calibration measurements using NaCl solutions of known fluid conductivity without the divider showed that the phase shift (ϕ) error exceeded 1 mrad at 10 Hz. This ϕ error is in excess of what has previously been reported by other studies using similar complex conductivity equipment (<0.5 mrad below 10 Hz) [e.g., Abdel Aal *et al.*, 2006], and well beyond the relative ϕ accuracy (~ 0.2 mrad below 100 Hz) reported for this instrumentation by Slater and Lesmes [2002]. After placing the divider in the electrical columns the ϕ error decreased to <0.5 mrad, which was deemed to be an acceptable level.

[8] All columns were initially prepared similarly. All columns were wet-packed with silica sand saturated with sterile 25% BH (Bushnell Haas; Becton Dickinson) nutrient broth, and manually agitated to release any trapped air bubbles. The coarse grain (0.6–1.18 mm) ASTM 20/30 silica sand (Ottawa, IL) consisted of 99.8% silicon dioxide, 0.020% iron oxide, 0.06% aluminum oxide, 0.01% titanium oxide, $<0.01\%$ calcium oxide, $<0.01\%$ magnesium oxide, $<0.01\%$ sodium oxide, $<0.01\%$ potassium oxide. The sands were washed with deionized water (DI), air-dried, and disinfected by autoclaving prior to being packed in the columns. Accessory equipment (e.g., tubing, valves) and columns were disinfected with a 70% ethanol solution before the experiment. Fresh, sterile BH broth was circulated through the sand-packed columns using a peristaltic pump, and baseline acoustic and electrical measurements were collected on all columns prior to commencing the column experimental treatments. Then the experiment began with the following experimental treat-

Figure 1. Schematic drawing showing the column setup for the (a) acoustic and (b and c) electrical columns. The 2-D acoustic scan region measures 60 mm $\times$ 70 mm (width $\times$ height). The drawing is not to scale.

ments. One set of columns (one electrical and one acoustic) was inoculated (herein called the biostimulated column) by saturating with 25% BH broth, 30 mM glucose, *Pseudomonas aeruginosa* PAO1 wild type bacteria culture, and 30 μ g/mL Gentamicin antibiotic. The *P. aeruginosa* bacteria strain (PAO1 Tn7-Gm-gfp) used in this experiment was obtained from the University of Denmark (Lyngby, Denmark) [e.g., Pamp and Tolker-Nielsen, 2007]. The PAO1 bacteria are a Gram-negative, rod-shaped, biofilm (slime) forming bacteria culture [e.g., Klausen *et al.*, 2003] commonly found in soil and water. The antibiotic was added to the biostimulated columns to inhibit growth of microorganisms besides that of the *P. aeruginosa*. The other set of columns had no bacteria inocula (herein called the control column), were saturated with 25% BH and 30 μ g/mL Gentamicin antibiotic and were used to collect background measurements. As this was a static experiment, the fluid in the columns remained stagnant during this experiment and the columns were not fed with additional nutrients.

2.2. Acoustic Wave Measurements

[9] An acoustic wave imaging system [e.g., Li and Pyrak-Nolte, 1998; Pyrak-Nolte *et al.*, 1999; Acosta-Colon *et al.*, 2009] was used to monitor the acoustic response of the experimental columns. The full-waveform imaging system consisted of two water-coupled plane wave transducers as source and receiver, computer-controlled linear actuators (Newport 850-B4 and Motion Master 2000), a high-voltage pulse generator (Panametrics PR1500), and an oscilloscope (Lecroy 9314L). Water-coupled transducers (12.7 mm diameter, 1 MHz central frequency) were used to ensure the same coupling between the transducers and the columns at all locations and over time. The columns were secured to a

platform placed in a tank of water and remained in place at laboratory conditions (22°–24°C) for the duration of the experiment. To perform an acoustic scan, the water-coupled transducers were positioned on opposite sides of the column (Figure 2) at a fixed distance from the column. The distance from the column was determined from the arrival time of the first reflection, i.e., the reflection from the water column interface. Over the 29 day duration of the experiments, the source and receiver path lengths were on average 29.69 mm and 29.37 mm, respectively, with a standard deviation of 680 microns. For the biostimulated column over the same time period, the source and receiver path lengths were on average 30.61 mm and 28.36 mm with standard deviations of 940 microns and 870 microns, respectively. After alignment of the source and receiver, full waveform measurements were recorded over a two-dimensional region of the column. Linear actuators were used to move the source and receiver in increments of 5 mm over the 60 mm by 70 mm acoustic scan region. An oscilloscope recorded and digitized the transmitted signals at each point in the scan region. Acoustic scans were collected over the 2-D scan region every 2–3 days for the 29 day experiment duration.

2.3. Complex Conductivity Measurement

[10] Complex conductivity (σ^*) measurements were collected at 40 frequency intervals between 0.1 and 1000 Hz using instrumentation based around a two-channel National Instruments (NI) dynamic signal analyzer [e.g., Slater and Lesmes, 2002]. Current (typically a swept sine wave) was injected through Ag-AgCl current electrode coils (Figure 1b) and the impedance magnitude ($|\sigma|$) and phase shift ϕ of the sample are recorded relative to a reference resistor [e.g., Slater and Lesmes, 2002]. The imaginary ($\sigma'' = |\sigma| \sin \phi$) part of the

Figure 2. Drawing showing the acoustic imaging system setup and column placement in the water tank. The drawing is not to scale.

complex conductivity was then calculated from the measured $|\sigma|$ and ϕ , and the imaginary conductivity (σ'') is related to the polarization that occurs at interfaces [e.g., *Lesmes and Frye, 2001*]. Prior to introducing the experimental treatments and starting the experiments, the electrical measurement experimental uncertainty was determined from calibration measurements using NaCl solutions of known fluid conductivity (30–3000 $\mu\text{S}/\text{cm}$). The columns were then flushed with DI water prior to injection of the background and experimental solutions. Electrical measurements were collected 2–3 times per week for the 29 day duration of the experiment.

2.4. Geochemical and Sand Sampling

[11] Fluid temperature and pH were measured 1–2 times per week from fluid samples collected from the bottom valve of the columns. At the end of the experiment, the columns were destructively sampled by withdrawing fluid from the bottom valve and retrieving cores of sand. Cores of the wet sand were retrieved on a grid from different areas in the column based on the acoustic scan images. The sands were used for imaging with an environmental scanning electron microscope (ESEM) to investigate the sand surface characteristics and to confirm the presence of any biofilms. An FEI Quanta 600 ESEM operating at 25 kV, 14–89%, 5°–20°C, for accelerating voltage, relative humidity, and temperature, respectively, was used to image the sand grains.

3. Results

3.1. Acoustic Wave Measurement

[12] A full-waveform imaging system was used to monitor the effect of biofilm formation on transmitted compressional wave amplitude and arrival time. Figures 3 and 4 show a

20 microsecond window of compressional waves measured on the control and biostimulated samples from days 1, 5, and 29 of the experiment. The signals are chosen from two locations on the column referred to as position A and position E (see Figure 9 for the location on the column). For the two positions, the signals from the control column (Figure 3) did not change significantly in amplitude, arrival time or in phase over the duration of the experiment or between the two positions. However, the signals from the biostimulated column for the equivalent two locations differed in phase and amplitude and temporally over the duration of the experiment (Figure 4). On day 1, the signals from the biostimulated column at positions A and E are nearly identical (red curves in Figures 4a and 4b). On day 5, for both positions in the biostimulated column, the signal decreased in amplitude and a shift in phase is observed. By day 29, the compressional wave signal at position E in the biostimulated column (Figure 4b) had been strongly attenuated while the change in amplitude for position A (Figure 4a) depends on the phase, for example later arriving phases exhibit amplitudes slightly larger than those from day 1.

[13] Different methods can be used to quantify the change in amplitude of a signal, for example peak-to-peak amplitude of the first arrival or the maximum amplitude from a Fourier Spectrum. Because the signals in the biostimulated column exhibited phase shifts and amplitudes that vary with arrival time, we performed a time-frequency analysis, i.e., wavelet analysis to determine the amplitude and arrival time of the signals as a function of frequency. We used the Nolte-Hilbert wavelet transform [Nolte *et al.*, 2000] that balances time-frequency localization without violating the wavelet admissibility condition that impedes the use of the Morlet wavelet transform.

Figure 3. Transmitted compressional signals from the control column for days 1, 5, and 29 of the experiment for (a) position A and (b) position E. See Figure 9 for location of positions in the two-dimensional acoustic scans.

[14] Figures 5a and 5d show the wavelet transforms for the signals from the control column for days 1 and 29 of the experiment. The amplitude varies with frequency and between day 1 and day 29 the maximum amplitude decreased by 14%. For the biostimulated column position A (Figures 5b and 5e) from day 1 to day 29, the amplitude of the signal is observed to increase for several frequencies. However, a dramatic decrease in amplitude is observed for the signals collected at position E of the biostimulated column (Figures 5c and 5f). The maximum amplitude increased 7% for position A and decreased 80% for position B.

[15] The wavelet transforms also show that the transmitted compressional wave signals are dispersive, i.e., the arrival time varies as a function of frequency and, in particular, the low-frequency components of the signal arrive first. We extracted time-frequency dispersion curves from the wavelets by determining the arrival time of the maximum amplitude at each frequency. Figure 6a shows the time-frequency relationship for the control column for positions A and E for day 1 and day 29. The low-frequency components arrive first and the time dispersion is the same for both locations and over

time except for frequencies greater than 0.8 MHz. For the biostimulated column (Figure 6b), the time-dispersion relationship is the same at positions A and E. However, by the end of the experiment (day 29 in Figure 6b), the dispersion relationship is neither the same for the two positions nor the same as day 1 for the biostimulated column. For position A on day 29, the low-frequency components (<0.5 MHz) arrive later than those on day 1 but the high-frequency (>0.5 MHz) components arrive earlier (Figure 6b). The converse is observed for position E in the biostimulated column. The observed effect of biofilm development and microbial growth on arrival times and hence velocity is frequency dependent for the range of frequencies used in these laboratory experiments.

[16] From the data presented in Figures 4 and 6 for the biostimulated column, the acoustic response is observed to vary temporally and with frequency. To quantify the variations caused by biostimulation, we performed our analysis at a frequency of 0.5 MHz. For each scan, 168 signals were collected and a wavelet analysis was performed on each signal. From each wavelet, the maximum amplitude at a

Figure 4. Transmitted compressional signals from the biostimulated column for days 1, 5, and 29 of the experiment for (a) position A and (b) position E. See Figure 9 for location of positions in the two-dimensional acoustic scans.

Figure 5. Wavelet transforms of the compressional wave signals from the (a and d) control column position A, (b and e) biostimulated column position A, and (c and f) biostimulated column position E for days 1 and 29 of the experiment.

frequency of 0.5 MHz was determined and the arrival time of the maximum amplitude was also recorded. These results were used to create two-dimensional acoustic maps of amplitude and arrival time (Figures 7 and 8).

[17] The time-progression 2-D acoustic maps of the transmitted acoustic amplitude and the group arrival time acquired from the control column are shown in Figures 7a and 7b, respectively. The baseline 2-D image from day 1 of the control column was relatively uniform in both the transmitted amplitude (Figure 7a) and time of arrival (Figure 7b) values,

except for the lower edges of the amplitude scan (Figure 7a) where slightly lower amplitudes were measured. Although the amplitude decreases slightly over the duration of the experiments, the measured amplitudes and arrival times became more spatially uniform over time.

[18] The time-progression 2-D acoustic maps of the transmitted amplitude and the group arrival time acquired from the biostimulated column are shown in Figures 8a and 8b, respectively. At the start of the experiment (day 1), the transmitted amplitude (Figure 8a) and time of arrival (Figure 8b)

Figure 6. Time-frequency relationship extracted from the wavelet transforms for the (a) control column positions A and E for day 1 and day 29, and (b) biostimulated column positions A and E for day 1 and day 29.

Figure 7. Two-dimensional map of the (a) compressional wave amplitude and (b) arrival time data for the control column for days 1, 2, 5, 8, and 29.

values are spatially relatively uniform over the scan region, as was observed in the time-frequency relationship (Figure 6b). By day 5, spatial variations are observed in the acoustic response over the scanned region in the biostimulated column, and a relatively consistent spatial trend in the amplitude response is noted on day 6 (image not shown), which persists through the end of the experiment. While a spatial trend is also observed in the arrival time response on day 6, the response is spatially variable through day 29

as compared to the control column (Figure 7b). The acoustic response measured on day 29 (Figure 8) exhibits both increases in amplitude and time of arrival in some regions, while other regions show a decrease. Overall, 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%.

[19] The temporal percent change in acoustic amplitude and arrival time relative to day 1 for both the biostimulated

Figure 8. Two-dimensional map of the (a) compressional wave amplitude and (b) arrival time data for the biostimulated column for days 1, 2, 5, 8, and 29.

Figure 9. Two-dimensional amplitude scans obtained from day 29 for the (a) biostimulated and (b) control columns, with the select scan region (positions A–E) plotted temporally to the right. Similarly, the 2-D time of arrival scans obtained from day 29 for the (c) biostimulated and (d) control columns, with the select scan region (positions A–E) plotted to the right.

and control columns are shown in Figure 9. By day 5, the transmitted amplitude and arrival time measured on the biostimulated column (Figures 9a and 9c) deviate sharply from the baseline (day 1), whereas the acoustic response from the control column remains relatively steady compared to baseline (Figures 9b and 9d). Acoustic wave amplitudes observed from the biostimulated column are highly variable by day 6, both increasing and decreasing in amplitude in the selected region (Figure 9a; locations A–E). The arrival times for the biostimulated column (Figure 9c) by day 6 also vary in the selected region, though to a lesser degree. After day 6, the trend in amplitude and arrival times generally continued through the end of the experiment with the exception of one data location from the selected region. In contrast, the

amplitude and arrival time results from the control column remain relatively steady over time, and are consistent for all of the selected data points shown (Figures 9b and 9d; locations A–E) and do not exhibit the same heterogeneous behavior as observed for the biostimulated column (Figures 9a and 9c).

[20] We quantified the spatial variability in the control and biostimulated columns by examining the probability distribution of arrival times for each scan. The probability distribution from the control column narrowed over the duration of the experiment (Figure 10a) while the probability distribution from the biostimulated column broadened (Figure 10b). The average group arrival time (based on all measurement locations from a scan) does not change significantly (Figure 11a)

Figure 10. Histogram of the arrival time data for the (a) control and (b) biostimulated sample for days 1, 2, and 29.

for either column. However, the probability distribution (Figure 10b) gets broader with time for the biostimulated column. This is supported by the standard deviation of the probability distributions shown in Figure 11b. Over the duration of the experiment, the width of the probability distribution for the control column is relatively constant and narrow indicating a fairly homogenized distribution of acoustic properties (e.g., elastic moduli, porosity, etc.). On the other hand, the probability distribution for the biostimulated column broadens and the standard deviation increases with experimental time up until about 15 days. The acoustic properties of the biostimulated became more heterogeneous with time.

3.2. Complex Conductivity and Geochemical Measurement

[21] The results of the calibration tests conducted prior to the experiment indicate that experimental uncertainty associated with the ϕ measurements were generally less than 0.5 mrad at 10 Hz. The experimental σ'' results are reported at 10 Hz, as this is the frequency where the lowest phase shift error was observed during calibration measurements, and this

frequency is close to typical frequencies used in field electrical measurements.

[22] The results of the temporal σ'' and pH are shown in Figure 12. The σ'' measured from the biostimulated column (Figure 12a) increased by $\sim 220\%$ to peak values (2.29×10^{-5} S/m) on day 5 relative to preinjection (day -1) values (7.16×10^{-6} S/m), before steadily decreasing to near baseline values by day 14, and remained relatively consistent through day 23. The magnitude of the σ'' response measured from the control column was relatively small compared to the biostimulated column, varying by $\sim 1.09 \times 10^{-6}$ S/m over the duration of the experiment compared to 1.57×10^{-5} S/m for the biostimulated column. The pH values measured for the control column remained steady near a pH of 7 throughout the experiment (Figure 12b). The pH values measured from the biostimulated column, however, steadily decreased from a baseline pH value of 7 to near 4.4 on day 8, and remained steady at a pH of 4.4 to the end of the experiment.

3.3. Grain Surface Characteristics

[23] ESEM images obtained from destructive sampling of the biostimulated and control columns are shown in

Figure 11. A comparison of the variation in (a) average arrival time and (b) standard deviation for the control and biostimulated samples as a function of experimental time.

Figure 12. Plots showing the (a) temporal imaginary conductivity (at 10 Hz) and (b) pH results.

Figure 13. We observe differences in the biofilm structure between areas of increased and decreased amplitude in the biostimulated columns. ESEM images that are representative of the increased acoustic amplitude columns are shown in

Figures 13a and 13b. In these images, the surface texture is rough with a patchy covering of “biomaterial” or EPS covering portions of the sand grain. However, other portions of the grain are not covered and the surface of the grain is clearly visible. Although not clearly distinguishable in these images, rod-shaped bacterial cells are present in this biomaterial (Figures 13a and 13b). We note that during ESEM imaging of the increased amplitude sand samples, individual bacterial cells were not visible until the operating temperature of the ESEM was raised from 5° to 20°C, and the relative humidity was decreased from 89% to 14%, which effectively dried out the sample/biomaterial.

[24] ESEM images that are representative of the decreased acoustic amplitude samples from the biostimulated column are shown in Figures 13c and 13d. In contrast to the previous ESEM images (Figures 13a and 13b), the images obtained from the area of decreased acoustic amplitude (Figures 13c and 13d) indicate that a smooth biomaterial appears to completely cover the surface of the sand grain. Several holes and voids in the biomaterial covering the grain are also observed. Rod-shaped bacteria are also seen in these images on top of the biomaterial as well as along the inner sides of the void spaces. Upon viewing with the ESEM (at 5°C and 89%), the individual cells and attached biomass were clearly visible and appeared to remain the same even when the temperature was increased to 20°C (images not shown). No enhanced precipitation was observed on the ESEM images.

[25] The ESEM images of sand samples obtained from the control column (Figures 13e and 13f) were imaged with

Figure 13. Environmental scanning electron microscope (ESEM) images from sand samples collected after column destruction. (a and b) Images from the biostimulated column collected from an area of increased amplitude on the 2-D acoustic scan, and (c and d) images from an area of decreased amplitude. (e and f) Images from the control column.

ESEM operating conditions of 25 kV, 89%, 5°C, for accelerating voltage, relative humidity, and temperature, respectively. No bacterial cells or biomass are apparent in these images. The surface of the silica sand grain is irregular but relatively smooth.

4. Discussion and Conclusions

4.1. Spatial Variability of Acoustic Properties

[26] Previous seismic studies have demonstrated that seismic methods are sensitive to the products of microbial activity in porous media, such as the production of biogenic gas [Williams, 2002] and enhanced biomineralization [Williams *et al.*, 2005; DeJong *et al.*, 2006]. Based on the fact that mineral precipitation was not induced or enhanced in our current experiment, and no large gas bubble formation was observed on the sides of the biostimulated columns (unlike that of Williams [2002]), we attribute the observed amplitude variation in the biostimulated sample in these experiments to biofilm development. This attribution is based on the observed microbes and biofilms in the ESEM images of samples at locations with enhanced/reduced acoustic attenuation.

[27] The results of our laboratory study indicate that biostimulation of a porous media alters the acoustic properties of the sediment both spatially and temporally. In physical models of sediments, wave propagation depends on the physical properties of the individual constituents (density, elastic moduli, etc., of both the fluid and solid components) of the granular medium and on the structural characteristics of the matrix. The major mechanisms of energy loss are scattering (when the wavelength of the signal approaches the size of the scatterer), frictional losses at grain-grain contacts, and viscous losses caused by relative motion between the pore fluid and the sediment frame. Bio-alteration can potentially alter the pore fluid properties (e.g., viscosity) as well as the mechanical properties of the sediment.

[28] The ESEM images confirm both the presence of bacteria and biomaterial in the biostimulated column, and confirm that the amount and structure (or texture) of the biomaterial varied at different locations in the sample (Figure 13). Furthermore, the ESEM images do not show mineral precipitation. The acoustic measurements taken at the locations of the ESEM images exhibited different trends in attenuation and group arrival times as observed in the acoustic maps and arrival time dispersion curves (Figures 6 and 8). From our data sets, an increase in the transmitted wave amplitude is observed to occur when grains had a patchy or nonuniform covering of biomaterial. A possible hypothesis is that small amounts of biomaterial enhance coupling between grains either at grain contacts or by coupling grains together. Nihei [1992] observed from numerical modeling and laboratory experiments that grain contacts were the primary sites of attenuation in dry and fluid-saturated granular media and can result in frequency-dependent attenuation coefficients caused by scattering at grain contacts. Work on granular media [Nihei, 1992; Liu and Nagel, 1992] has recognized that granular packings are complex structural systems composed of particles that have a finite number of contacts with neighboring particles and form a three-dimensional force-chain network. In such systems, small-amplitude vibrations are sensitive to the degree of contact

between particles. Thus, biofilm networks (hyphae) that link grains may alter the force-chain network of the sediment and potentially strengthen the contacts.

[29] Most locations in the biostimulated sand column exhibited a decrease in amplitude. ESEM images from these locations showed that the biomaterial coating the grains had a smooth texture and contained numerous void spaces/pores or channels (Figures 13c and 13d). Decreases in acoustic amplitude often result from biogenic gas production, weakening of grain contact/coupling, as well as dissipation caused by viscous losses as a bio-altered pore fluid moves relative to the frame, or by losses associated with the skeletal frame characterized by a complex moduli.

[30] The variability in the acoustic wave measurements presented here from the biostimulated column (e.g., Figures 8 and 9) suggests that more than one mechanism may be responsible for the variability in the spatiotemporal acoustic amplitude. Biofilm structures have been shown to be very heterogeneous, consisting of cell clusters, pores, channels, and EPS [Zhan *et al.*, 2006]. It is also documented that thicker biofilms exhibit higher EPS yields, higher porosity, lower density, and higher water content [Zhan *et al.*, 2006], whereas, thinner biofilms tend to have lower porosity, higher density, and lower water content. We infer from our results that spatial variations in acoustic amplitude and travel times most likely result from the nonuniform 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. Additional research is needed to determine the attenuation mechanism or competing mechanisms that result from biofilm evolution.

4.2. Temporal Variability of the Complex Conductivity

[31] In addition to the spatial variability in the acoustic wave amplitude, there is also significant temporal variation measured in the biostimulated column, as shown in the time-progression 2-D acoustic maps (Figure 8) and temporal plots (Figure 9a). Significant changes in the acoustic wave properties occurred in the biostimulated column between day 5 and day 7 of the experiment (Figure 8). These observed acoustic changes are consistent with a peak in the imaginary conductivity values on day 5 (Figure 12). In our earlier work [Davis *et al.*, 2006], we demonstrated that imaginary conductivity measurements are uniquely sensitive to the physicochemical properties at grain-fluid interfaces, and showed that imaginary conductivity measurements may be used as a proxy indicator of microbial growth, attachment, and biofilm formation in porous media. In this earlier work, we observed peak increases in imaginary conductivity that generally paralleled peak increases in attached microbial cell concentrations and biofilms. While we did not measure the concentration of attached biomass or the thickness of biomass in the current experiment, our complex conductivity results are identical to the study by Davis *et al.* [2006]. Thus, we suggest that the peak changes observed in the imaginary conductivity response may reflect a peak in the concentration of attached biomass or biofilm thickness. Based on this assumption, we infer that the variations in the acoustic amplitude that occurred between days 5 and 7 are associated with the change in physical properties of the medium caused

Figure 14. Drawing showing the different stages of biofilm development, including (1) reversible attachment of microbial cells, (2) irreversible attachment, (3) maturation and layering of biofilm, (4) maturation and maximum thickness, and (5) dispersion/detachment of microbial cells and biofilm (modified from <http://www2.binghamton.edu/biology/faculty/davies/research.htm-8/31/09>).

by peak microbial attachment and maximum biofilm thickness. We also note that there is a remarkable similarity in the temporal acoustic and electrical data presented here, and the relative time frame for biofilm development presented by Davey *et al.* [2003] as discussed below in section 4.3. This may be further evidence that biofilm development is a primary contributor to the observed geophysical response.

[32] Similar to Davis *et al.* [2006], we observed a consistent decrease in the imaginary conductivity after peak values were observed and for the remaining duration of the experiment. This decrease in the imaginary conductivity may be attributed to increased rates of detachment, dispersion, or cell death potentially due to nutrient limitations [Davis *et al.*, 2006; Abdel Aal, 2008]. A possible reason for the suggested increase in cell death or detachment may be the result of the accumulation of waste products (e.g., organic acids) and the low pH conditions that developed over time in the biostimulated column contributed to the death of the cells.

[33] Given the above discussion we suggest that the imaginary conductivity was responsive to the biofilm development (Figure 14). Initial attachment of cells to the surfaces (both reversible and irreversible) resulted in a small change in the imaginary conductivity (days 1–2, Figure 12). Studies by Abdel Aal *et al.* [2009] have suggested that attachment of cells to clean quartz sands (such as was used in this experiment) result in an enhancement of the imaginary conductivity of ~15%. Since we measured increases in the imaginary conductivity of >200%, we hypothesize that attachment, rapid colonization, and the production of biofilms may explain the peak in the imaginary conductivity values observed on day 5. Consequent death of cells resulting from possible accumulation of acids (evidenced by low pH) resulted in a rapid decrease in the imaginary conductivity as observed after day 5 (Figure 12). We also note that pH values remained constant after day 8 to the end of the experiment. In contrast to the imaginary conductivity, the acoustic wave amplitude after day 8 remained relatively constant through the end of the experiment (Figure 9). While dead cells have no electrical properties and hence the decrease in imaginary conductivity after day 5 [e.g., Abdel Aal, 2008], it is possible that a steady biofilm or EPS thickness remained in the pore geometries of

the porous media, hence no change was observed in the acoustic properties after day 8.

4.3. Effect of Biofilm Structure and Development on Acoustic Properties

[34] 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 (Figure 14). In addition to the sequence of biofilm development described by Davey *et al.* [2003], the authors also provided a relative time frame for this development, where the third stage (or physiology) occurred at ~3 days of growth, the fourth stage after ~6 days of growth, and the fifth stage after ~9–12 days. Klausen *et al.* [2003] observed that the basic *P. aeruginosa* biofilm structure did not change appreciably after day 7, although the accumulation of biomass within the biofilm on day 7 had not yet reached a plateau. Therefore, the authors referred to the 7 day old biofilms as being mature biofilms. Further, Davey *et al.* [2003] stated that all stages of the sequential development may be present at the same time during the maturation of the biofilm. We note that no significant changes in the acoustic properties were observed in our columns after day 8.

[35] Physically, the presence of biofilms may alter grain contact coupling, decrease the volume and size of pores which alters the pore throat geometry, provide additional coupling among grains, or alter relative motion between the bio-alter pore fluid and the solid grains (i.e., viscous losses). All of the above will affect the sediment matrix stiffness and the elastic and/or visco-elastic moduli of the fluid-saturated media. In addition, if the microbial-induced alterations are not uniformly distributed throughout the sediment, then the sediment textural and mechanical properties may vary spatially leading to spatial heterogeneity in the seismic signatures (i.e., scattering).

[36] Wave scattering or interference from spatial heterogeneity of the medium is another mechanism that affects wave attenuation. In our experiments, air bubbles were not a

source of scattering because no gas formation was observed in the biostimulated sample. Scattering caused by a density contrast between the water-saturated sediment and the microbially altered sediment is not large and is therefore ruled out as a source of scattering. Thus, if scattering is the source of the observed spatial heterogeneity in the acoustic properties of the bio-stimulated sample, the cause of the scattering could be attributed to the spatial variation in elastic or viscoelastic moduli from microbial alteration of the grain contact, pore-filling material and/or biofilm connecting grains.

[37] Variations in pore geometries and elastic properties between regions of highly colonized, dense areas of biofilm (e.g., Figures 13c and 13d) and those with a patchy distribution of biomass (e.g., Figures 13a and 13b) may affect local permeability and pore pressure. When the porous media is excited by the passing acoustic wave, grain-scale heterogeneities (i.e., pore shape, saturation) will induce pressure gradients and cause pore fluid to be squeezed from compliant to less compliant regions. This movement of fluid results in viscous dissipation or “squirt-flow” mechanisms [e.g., Palmer and Traviolia, 1980; Mavko and Jizba, 1991]. Thus, we hypothesize that spatially nonuniform biofilm structure alters the pore geometry and elastic moduli which results in heterogeneous attenuation and travel times. The relatively high spatial variation observed in the acoustic data from our current study suggests that enhanced microbial growth has a variable as well as frequency-dependent effect on the elastic properties of porous media. The effect of the microbial growth on the elastic properties depends on the amount and stages of biofilm development. Future work should focus on delineating the competing attenuation mechanisms that arise from enhanced microbial growth and biofilms. In addition, further work is needed to quantify the relationship between the amount and type of biofilm present in a porous medium and the degree to which the acoustic signal is attenuated by the presence of biomass. Experiments that examine the effect of biofilm properties (such as thickness, density, porosity, viscosity, etc.) on seismic wave attenuation are needed.

[38] The geophysical results presented here are relevant to field applications that require information on the spatial distribution of subsurface biofilms. For example, microbial enhanced oil recovery activities use the in situ growth of microorganisms and biofilm formation to selectively plug highly permeable zones. However, up scaling our seismic laboratory measurements to the field scale will depend on spatial and temporal dispersion, as well as consideration of field site conditions, gas production, and precipitation effects, as quite probably these mechanisms are all involved in complex heterogeneous field settings. Furthermore, temporal dispersion connects frequency-dependent attenuation and velocity with elastic moduli, i.e., changes at grain contacts or pore filling. While, spatial dispersion connects wavelength with the size of the scatterer, that is, the size or spatial correlation length of microbially altered regions. Thus, increasing or decreasing seismic attenuation with frequency will provide information on the size of the altered region and on the spatiotemporal distribution of the biomass or bioclogging development. This potential surface mapping capability would yield new additional information on subsurface sites where microbial activity is of interest.

[39] **Acknowledgments.** This material is based in part on work supported by the National Science Foundation under grants OCE-0729642, EAR 0722410 (MRI), EAR 0525316, and REU award 0552918, and EPA Student Services contract EP07D000660. L.J.P.N. would like to acknowledge support from Geosciences Research Program, Office of Basic Energy Sciences, U.S. Department of Energy (DEFG02-97ER14785 08). S. Pamp and T. Tolker-Nielsen provided the *Pseudomonas* PAO1 Tn7-Gm-gfp strain to S. Rossbach, and we thank S. Rossbach for helpful discussions on growing the bacteria. Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

References

- Abdel Aal, G. Z. (2008), Electrical properties of bacteria in sand columns: Live vs. dead cells, paper presented at Chapman Conference on Biogeophysics, AGU, Portland, Maine.
- Abdel Aal, G. Z., L. D. Slater, and E. A. Atekwana (2006), Induced-polarization measurements on unconsolidated sediments from a site of active hydrocarbon biodegradation, *Geophysics*, 71(2), H13–H24, doi:10.1190/1.2187760.
- 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.
- Acosta-Colon, A., L. J. Pyrak-Nolte, and D. D. Nolte (2009), Laboratory-scale study of field of view and the seismic interpretation of fracture specific stiffness, *Geophys. Prospect.*, 57(2), 209–224, doi:10.1111/j.1365-2478.2008.00771.x.
- Ahimou, F., M. J. Semmens, P. J. Novak, and G. Haugstad (2007), Biofilm cohesiveness measurement using a novel atomic force microscopy methodology, *Appl. Environ. Microbiol.*, 73(9), 2897–2904, doi:10.1128/AEM.02388-06.
- Atekwana, E. A., and L. D. Slater (2009), Biogeophysics: A new frontier in Earth science research, *Rev. Geophys.*, 47, RG4004, doi:10.1029/2009RG000285.
- Atekwana, E. A., E. A. Atekwana, and D. D. Werkema (2006), Biogeophysics: The effects of microbial processes on geophysical properties of the shallow subsurface, in *Applied Hydrogeophysics*, NATO Sci. Ser., vol. 4, edited by H. Vereecken et al., pp. 161–193, Springer, New York.
- Baveye, P., P. Vandevivere, B. L. Hoyle, P. C. DeLeo, and D. Sanchez de Lozada (1998), Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials, *Crit. Rev. Environ. Sci. Technol.*, 28(2), 123–191, doi:10.1080/10643389891254197.
- Bouwer, E. J., H. H. M. Rijnaarts, A. B. Cunningham, and R. Gerlach (2000), Biofilms in porous media, in *Biofilms II: Process Analysis and Applications*, edited by J. D. Bryers, pp. 123–158, Wiley-Liss, Wilmington, Del.
- Brovelli, A., F. Malaguerra, and D. A. Barry (2009), Bioclogging in porous media: Model development and sensitivity to initial conditions, *Environ. Model. Softw.*, 24, 611–626, doi:10.1016/j.envsoft.2008.10.001.
- Cunningham, A. B., W. G. Characklis, F. Abedeen, and D. Crawford (1991), Influence of biofilm accumulation on porous media hydrodynamics, *Environ. Sci. Technol.*, 25, 1305–1310, doi:10.1021/es00019a013.
- Davey, M. E., N. C. Caiazza, and G. A. O'Toole (2003), Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1, *J. Bacteriol.*, 185(3), 1027–1036, doi:10.1128/JB.185.3.1027-1036.2003.
- Davis, C. A., E. A. Atekwana, E. A. Atekwana, L. D. Slater, S. Rossbach, and M. R. Mormile (2006), Microbial growth and biofilm formation in geologic media is detected with complex conductivity measurements, *Geophys. Res. Lett.*, 33, L18403, doi:10.1029/2006GL027312.
- DeJong, J. T., M. B. Fritzges, and K. Nusslein (2006), Microbially induced cementation to control sand response to undrained shear, *J. Geotech. Geoenviron. Eng.*, 132(11), 1381–1392, doi:10.1061/(ASCE)1090-0241(2006)132:11(1381).
- DeJong, J. T., B. M. Mortensen, B. C. Martinez, and D. C. Nelson (2010), Bio-mediated soil improvement, *Ecol. Eng.*, 36, doi:10.1016/j.ecoleng.2008.12.029.
- Dunsmore, B. C., C. J. Bass, and H. M. Lappin-Scott (2004), A novel approach to investigate biofilm accumulation and bacterial transport in porous matrices, *Environ. Microbiol.*, 6(2), 183–187, doi:10.1046/j.1462-2920.2003.00546.x.
- Klapper, I., C. J. Rupp, R. Cargo, B. Purvedorj, and P. Stoodley (2002), Viscoelastic fluid description of bacterial biofilm material properties, *Biotechnol. Bioeng.*, 80(3), 289–296, doi:10.1002/bit.10376.

- Klausen, M., A. Heydorn, P. Ragas, L. Lambertsen, A. Aes-Jørgensen, S. Molin, and T. Tølker-Nielsen (2003), Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants, *Mol. Microbiol.*, 48(6), 1511–1524, doi:10.1046/j.1365-2958.2003.03525.x.
- Lesmes, D. P., and K. M. Frye (2001), Influence of pore fluid chemistry on the complex conductivity and induced polarization responses of Berea sandstone, *J. Geophys. Res.*, 106, 4079–4090, doi:10.1029/2000JB900392.
- Li, X., and L. J. Pyrak-Nolte (1998), Acoustic monitoring of water–sediment interaction, *Geophys. Res. Lett.*, 25(20), 3899–3902, doi:10.1029/1998GL900028.
- Li, X., L. R. Zhong, and L. J. Pyrak-Nolte (2001), Physics of partially saturated porous media: Residual saturation and seismic-wave propagation, *Annu. Rev. Earth Planet. Sci.*, 29, 419–460, doi:10.1146/annurev.earth.29.1.419.
- Liu, C. H., and S. R. Nagel (1992), Sound in sand, *Phys. Rev. Lett.*, 68(15), 2301–2304, doi:10.1103/PhysRevLett.68.2301.
- Marshall, K. C. (1992), Biofilms: An overview of bacterial adhesion, activity, and control at surfaces, *ASM News*, 58(4), 202–207.
- Mavko, G., and D. Jizba (1991), Estimating grain-scale fluid effects on velocity dispersion in rocks, *Geophysics*, 56, 1940–1949, doi:10.1190/1.1443005.
- Nihei, K. T. (1992), Micromechanics of seismic wave propagation in granular rocks, Ph.D. thesis, 167 pp., Univ. of Calif., Berkeley.
- Nolte, D. D., L. J. Pyrak-Nolte, J. Beachy, and C. Ziegler (2000), Transition from the displacement discontinuity limit to the resonant scattering regime for fracture interface waves, *Int. J. Rock Mech. Min. Sci.*, 37(1–2), 219–230, doi:10.1016/S1365-1609(99)00102-1.
- Palmer, I. D., and M. L. Traviolia (1980), Attenuation by squirt flow in undersaturated gas sands, *Geophysics*, 45, 1780–1792, doi:10.1190/1.1441065.
- Pamp, S. J., and T. Tølker-Nielsen (2007), Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*, *J. Bacteriol.*, 189(6), 2531–2539, doi:10.1128/JB.01515-06.
- Pyrak-Nolte, L. J., B. L. Mullenbach, X. Li, D. D. Nolte, and A. S. Grader (1999), Detecting sub-wavelength layers and interfaces in synthetic sediments using seismic wave transmission, *Geophys. Res. Lett.*, 26, 127–130, doi:10.1029/1998GL900237.
- Slater, L., and D. P. Lesmes (2002), IP interpretation in environmental investigations, *Geophysics*, 67, 77–88, doi:10.1190/1.1451353.
- Slater, L., F. D. Day-Lewis, D. Ntarlagiannis, M. O'Brien, and N. Yee (2009), Geoelectrical measurement and modeling of biogeochemical breakthrough behavior during microbial activity, *Geophys. Res. Lett.*, 36, L14402, doi:10.1029/2009GL038695.
- Stoodley, P., Z. Lewandowski, J. D. Boyle, and H. M. Lappin-Scott (1999), Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: An in situ investigation of biofilm rheology, *Biotechnol. Bioeng.*, 65(1), 83–92, doi:10.1002/(SICI)1097-0290(19991005)65:1<83::AID-BIT10>3.0.CO;2-B.
- Williams, K. H. (2002), Monitoring microbe-induced physical property changes using high-frequency acoustic waveform data: Toward the development of a microbial megascope, M.S. thesis, Univ. of Calif., Berkeley.
- Williams, K. H., D. Ntarlagiannis, L. D. Slater, A. Dohnalkova, S. S. Hubbard, and J. F. Banfield (2005), Geophysical imaging of stimulated microbial biomineralization, *Environ. Sci. Technol.*, 39(19), 7592–7600, doi:10.1021/es0504035.
- Yerly, J., Y. Hu, and R. J. Martinuzzi (2008), Biofilm structure differentiation based on multi-resolution analysis, *Biofouling*, 24(5), 323–337, doi:10.1080/08927010802209892.
- Zhan, X. M., M. Rodgers, and E. O'Reilly (2006), Biofilm growth and characteristics in an alternating pumped sequencing batch biofilm reactor (APSBRR), *Water Res.*, 40, 817–825, doi:10.1016/j.watres.2005.12.003.

E. A. Atekwana, Boone Pickens School of Geology, Oklahoma State University, Stillwater, OK 74078, USA. (estella.atekwana@okstate.edu)
 C. A. Davis, Department of Geological Sciences and Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA.
 M. E. Haugen and L. J. Pyrak-Nolte, Department of Physics, Purdue University, West Lafayette, IN 47907, USA.
 D. D. Werkema Jr., U.S. Environmental Protection Agency, Las Vegas, NV 89119, USA.