Three-dimensional Characterization of Microbial Biomass in Porous Media

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Introduction:

Current understanding of subsurface microbial processes and how they impact fluid hydrodynamics is limited by our ability to observe microbial colonies in their natural spatial arrangement. Biomass in porous media has only been observed in two dimensions or at the nanoscale; scientists are therefore lacking significant information about biofilm form. Using synchrotron-based x-ray microtomography we plan to image biofilms without disturbing their natural spatial arrangement. Data collected will provide information about physical structures of biomass and how they alter the physical properties of soil, e.g. porosity and hydraulic conductivity. Significant changes in physical parameters may impact the rate microbes degrade contaminates at and therefore alter the effectiveness of bioremediation.

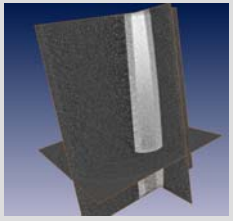
Some issues to overcome:

Diffusion

- Since biofilms behave as a gel-like substance, diffusion of doped water into biomass is a significant issue. X-ray attenuation of water is similar to that of biomass, in order to clearly image biofilm structure we need to determine a method to minimize movement of the dopant from fluid to biomass phase or visa versa. A potential solution is using dopants that are too large to penetrate the biomass, such as liposomes and nanoparticles

NaI doped biomass in nylon tube

Water in nylon tube



NaI doped biofilm diffusion into water in nylon tube

Keeping the microbes alive

- Due to the high energy of beamline most organisms will not survive the imaging process. Eventually we would like to obtain images over an extended period of time, capturing biofilm structure at different stages of development. Therefore we will need to determine if microbes such as Deinococcus radiodurans can withstand and grow in continuous radiation.

The Microorganisms

Deinococcus radiodurans

TGY growth media

- 1.0% Bactotryptone
- 0.1% Glucose
- 0.5% Yeast Extract

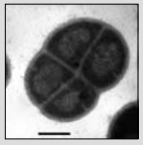


Image obtained from: http://www.snabs.null.jp/deinococcus/index_20.htm

One of the most radioresistant microorganisms yet discovered

Shewanella oneidensis

TSB growth media

- 3 g/L TSB media

Well known metal reducing bacteria

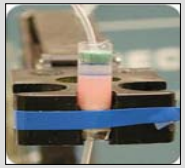
Forms tenacious biofilms

Image obtained from: www.emsl.pnl.gov/capabs/images/10334-2large.jpg

Learning to Grow Microbes

Before attempting to grow biofilms I needed to learn some basic lab skills, including:

- Growth media preparation
- Aseptic lab technique
- Mixing of stock solutions
- Inoculation from frozen stock
- Continuous culture
- Harvesting cells
- Preparation of frozen stock
- Inoculation of flow cell
- Slide preparation



Porous Media

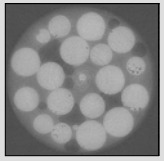
Glass spheres

- 0.8-1.2 mm
- 0.47 mL pore volume

Treated with HF to increase the surface area of the glass beads

Autoclaved at 120˚C and 15 atmospheres

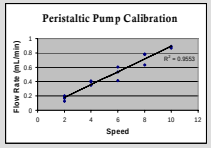
1.5 mm diameter / 2.7 micron



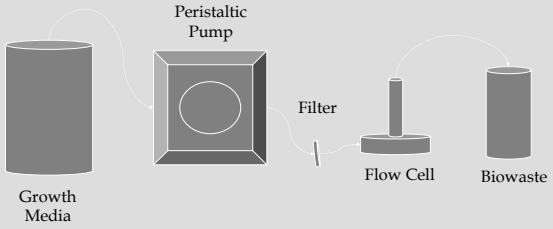
Glass beads with biomass

Pump Calibration

Pump calibration was performed by weight using an analytical scale and timer



Experiment Set Up



Imaging Solutions

- **Using Absorption X-ray CT with Synchrotron Radiation**

Fenestra

- Commercially available iodine encapsulated in lipid emulsions
- Image at the Iodine photoelectric edge, 33 KeV

Iodine encapsulated liposomes

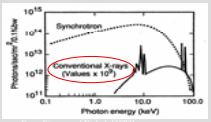
- Purchased through Texas Health Sciences Center at Houston
- Image at the Iodine photoelectric edge, 33 KeV

Colloidal Iron

-Image at 7 KeV

Silver nanoparticles

-Image at 25.5 KeV



From Kinney et al. 2005

- **Using Phase-Contrast X-ray CT with Synchrotron Radiation**

Some valuable lessons I learned:

- Always check for leaks
- O-rings are very important
- Acrylic is not autoclaveable
- Ethanol dissolves acrylic
- Ask questions early to avoid problems later
- Double check information sources and question assumptions

What has been accomplished?

NSF preproposal due Sept. 22.

Future Work:

Future work will focus on solving fundamental flow cell issues that prevented me from collecting images this summer. This may require refabricating the cells out of a more solid material to avoid pressure build up and leaks.

During Fall 2006 we plan to attempt to image biomass at Argonne National Lab. Beam-time Nov. 12th

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