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# Biobarriers in Fractured Bedrock: Effects of Gasohol on Biofilm Structure

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# Outline

## **1. The Biobarrier Concept**

- From Laboratory Testing to Field Demonstration
- Our Goal

## **2. What can the microscale tell us?**

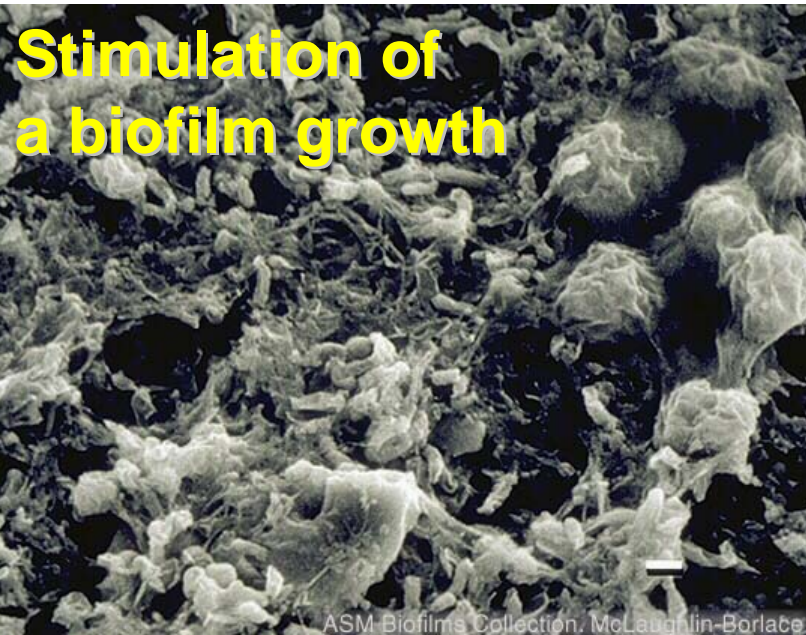
- The Tools
- The Responses

## **3. Future Work and Application**

- @ microscale
- @ macroscale

# The Biobarrier Concept @ Microscopic and Macroscopic Scales

**Stimulation of  
a biofilm growth**



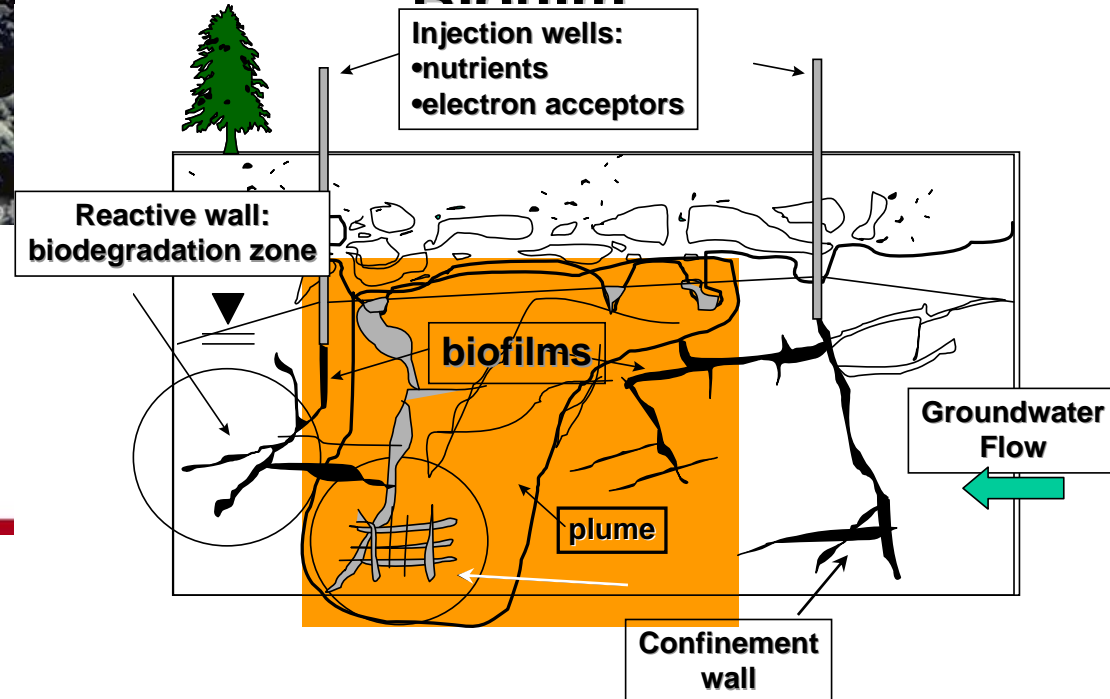
ASM Biofilms Collection, McLaughlin-Borlace

**Development of  
a biobarrier**

**Bacteria  
+  
Exopolymeric substances (EPS)**

**=**

**Biofilm**



# Highlights of the Development of the Biobarrier Concept



## ➤ ***On Bioclogging:***

- ✓ Biofilm thickness of 1100  $\mu\text{m}$
- ✓ Decrease in K of 99.99 %

(Ross et al. 2001. Wat. Res. 35:8)



## ➤ ***On Monitoring Tools:***

- ✓ Eh and planktonic bacteria as indicators
- ✓ Tracer experiments to develop mathematical models

(Ross et al. 2005, JEES, Submitted)



## ➤ ***On Field Demonstrating:***

- ✓ After 15d of biostimulation: up to 20-fold  $\downarrow$  GW velocity and 40% aperture reduction

(Ross, N., Bickerton, G., Remediation.12:5-21, 2002)

# **Fractured Bedrock can be Effectively Clogged. How Does Contamination Affect the Biobarrier?**

## **Our Goal:**

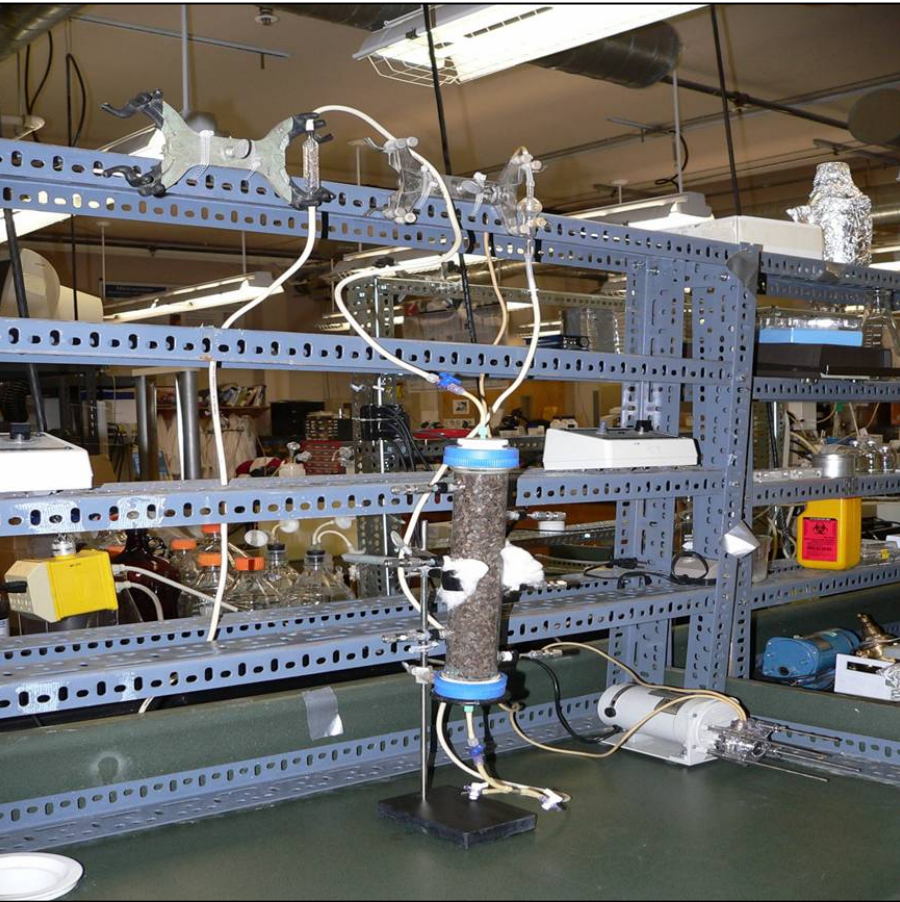
To observe the effects at the microscale of the  
addition of gasohol to a biofilm

## **The Objectives are to Measure the:**

- 1- Effects of Gasohol on Hydrogeological Parameters
- 2- Removal of Gasohol by a Biofilm
- 3- Effects of Gasohol on the Biofilm Structure



# Obj. 1 & 2: Hydrogeological Parameters and Gasohol Removal



Two species culture



4-Day feeding in  
"Recycle mode"



7-Day  
fresh continuous feed



Tracer tests

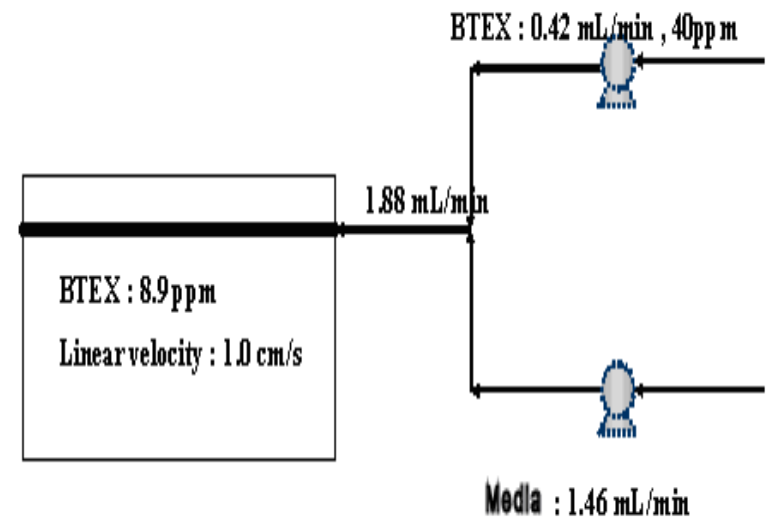
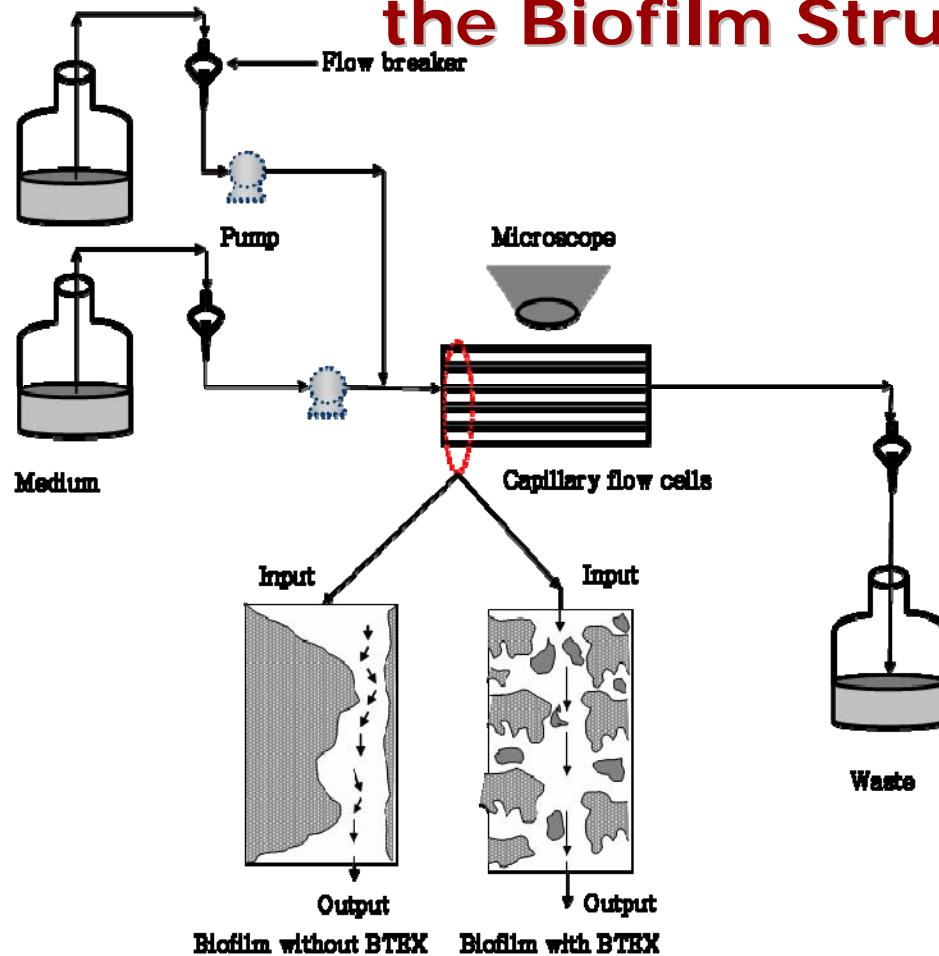


Increasing concentrations  
of gasohol

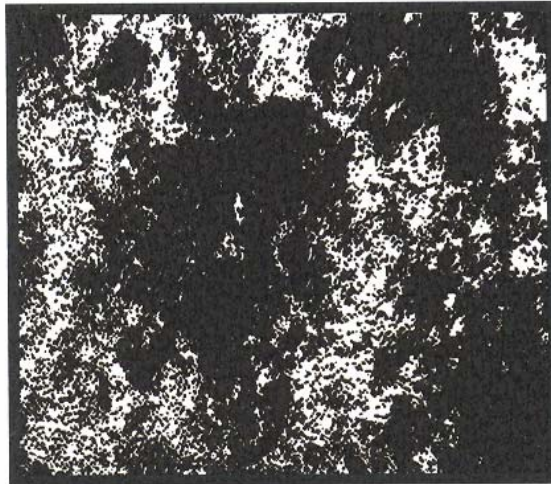
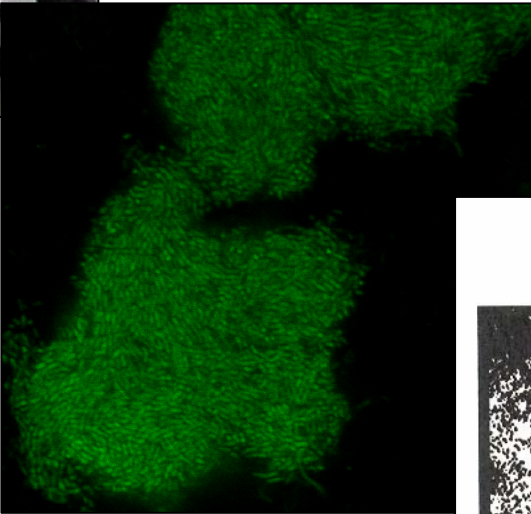


Measurements of  
hydraulic conductivity  
and gasohol removal

# Obj. 3- Effects of Gasohol on the Biofilm Structure



## Obj. 3- Effects of Gasohol on the Biofilm Structure



Horizontal = 640 pixel

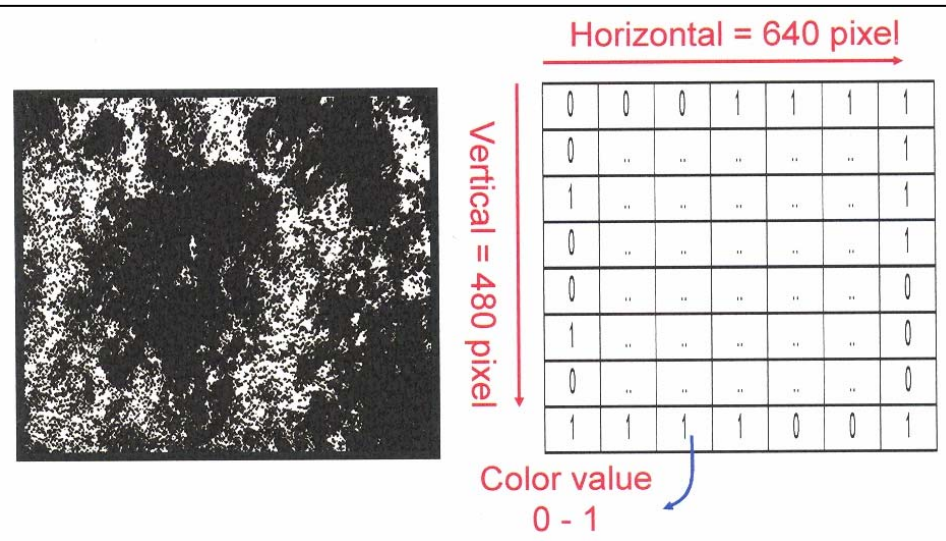
Vertical = 480 pixel

0	0	0	1	1	1	1
0	..	..	..	..	..	1
1	..	..	..	..	..	1
0	..	..	..	..	..	1
0	..	..	..	..	..	0
1	..	..	..	..	..	0
0	..	..	..	..	..	0
1	1	1	1	0	0	1

Color value  
0 - 1

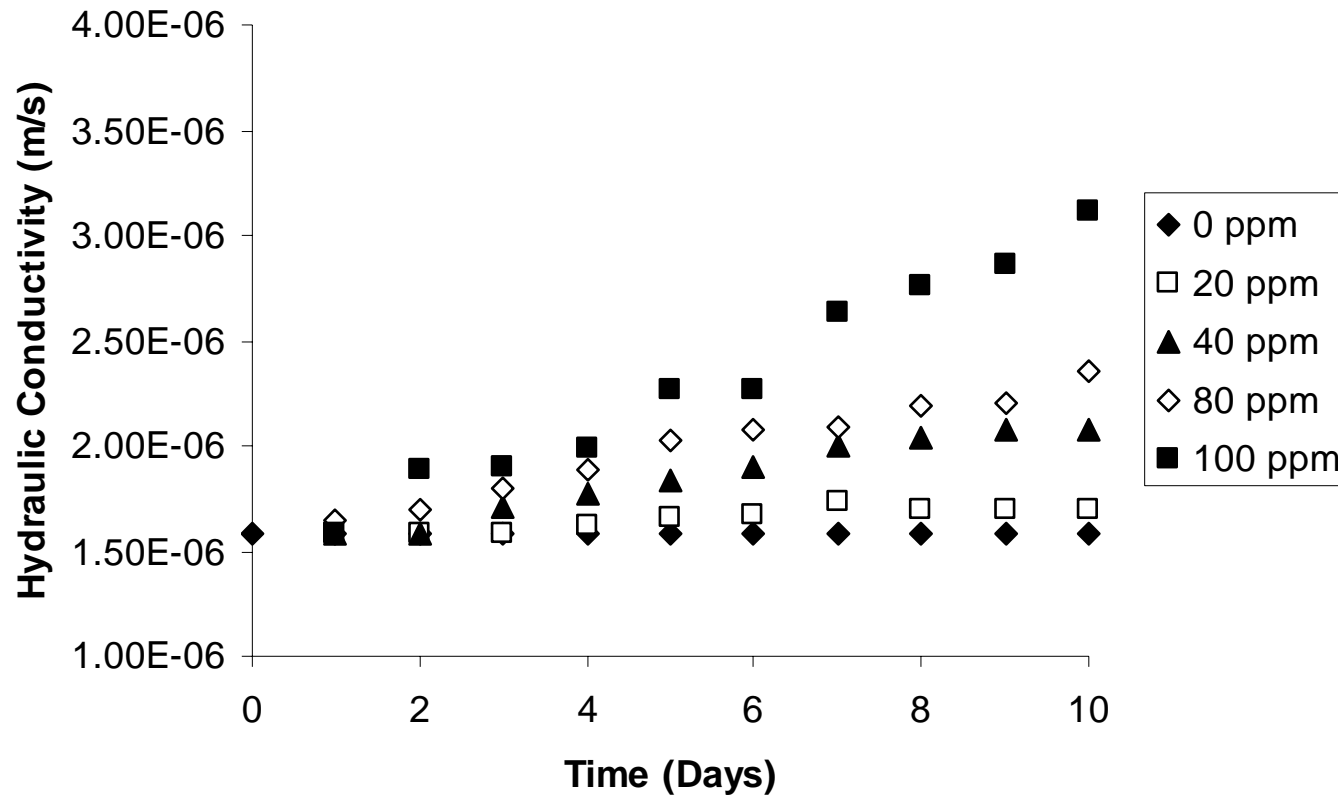


# Obj. 3- Effects of Gasohol on the Biofilm Structure

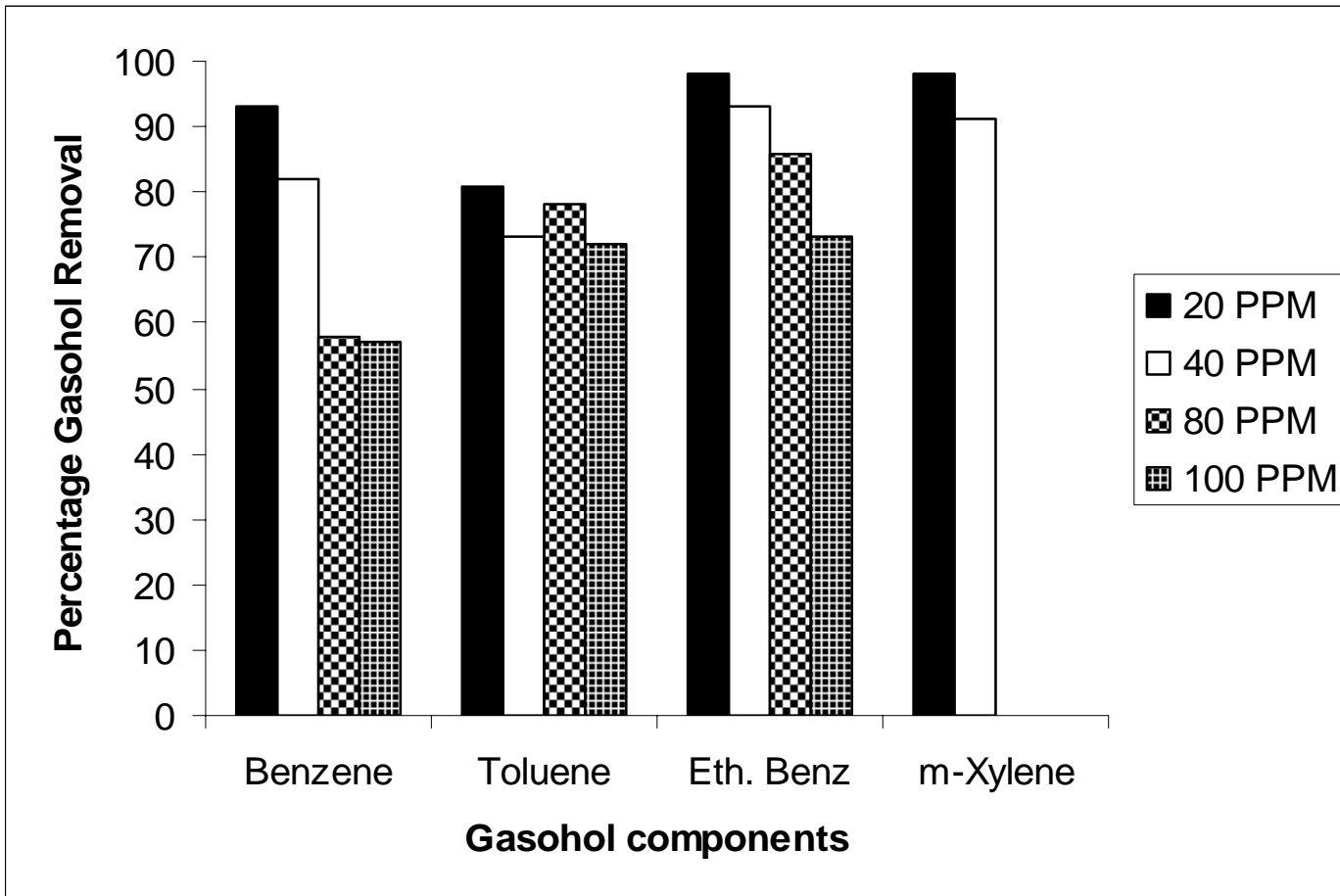


- ✓ **Areal Porosity:** Ratio of void area to total area
- ✓ **Biovolume:** Total biomass volume in the biofilm. It is calculated by summing number of cluster pixels in the image set.
- ✓ **Fractal Dimension:** Rate of change in the perimeter of an object. The rougher the biofilm boundary, the higher the fractal dimension.
- ✓ **Biofilm Roughness:** Describes the coarseness of the biofilm

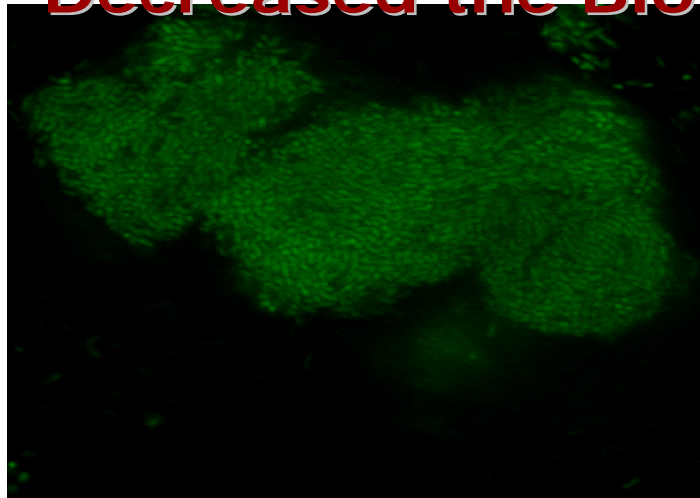
# Exposure to an Increasing Concentration of Gasohol led to a Two-fold Increase in K



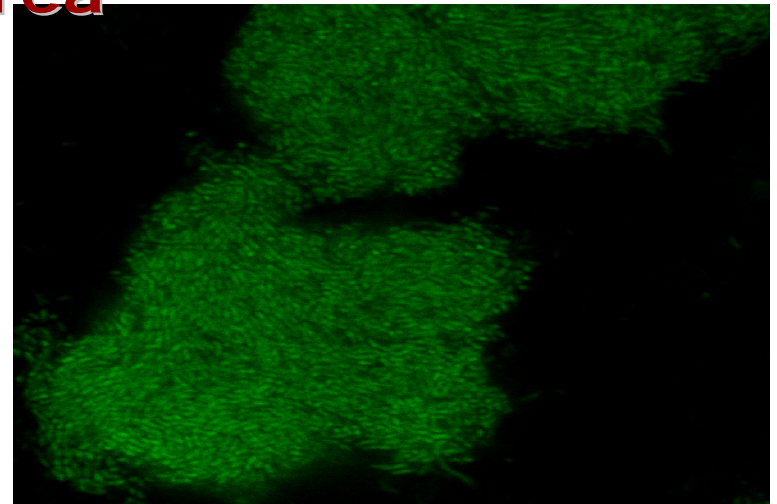
# Biofilm was Effective to Remove BTEX up to 40 ppm



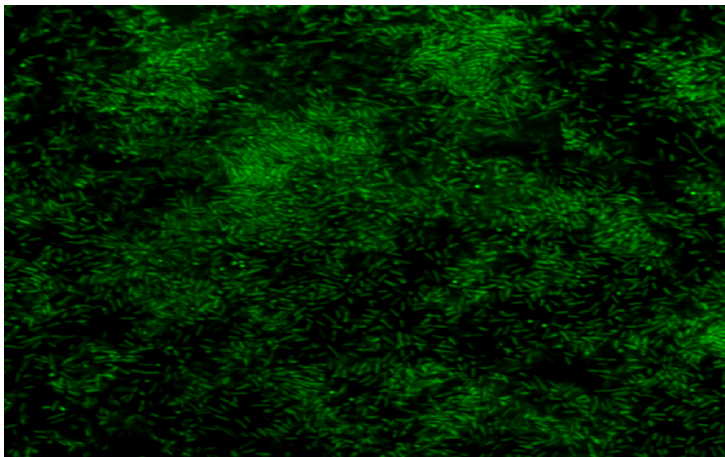
# Increasing Concentration of Gasohol Decreased the Biofilm Area



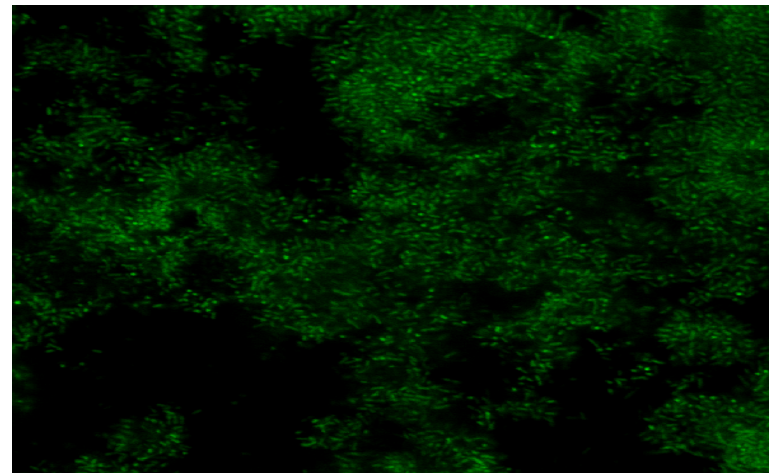
Control



20 ppm



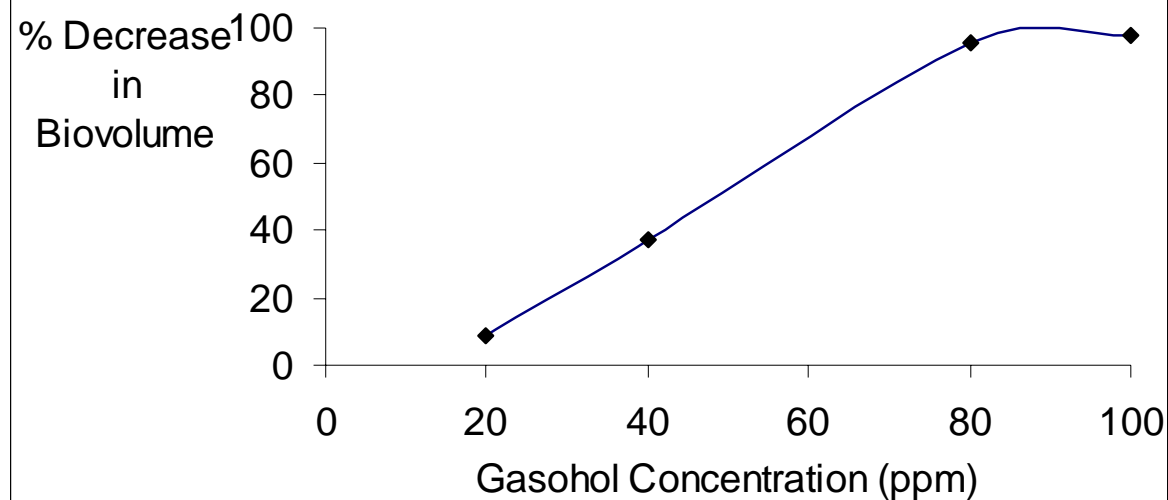
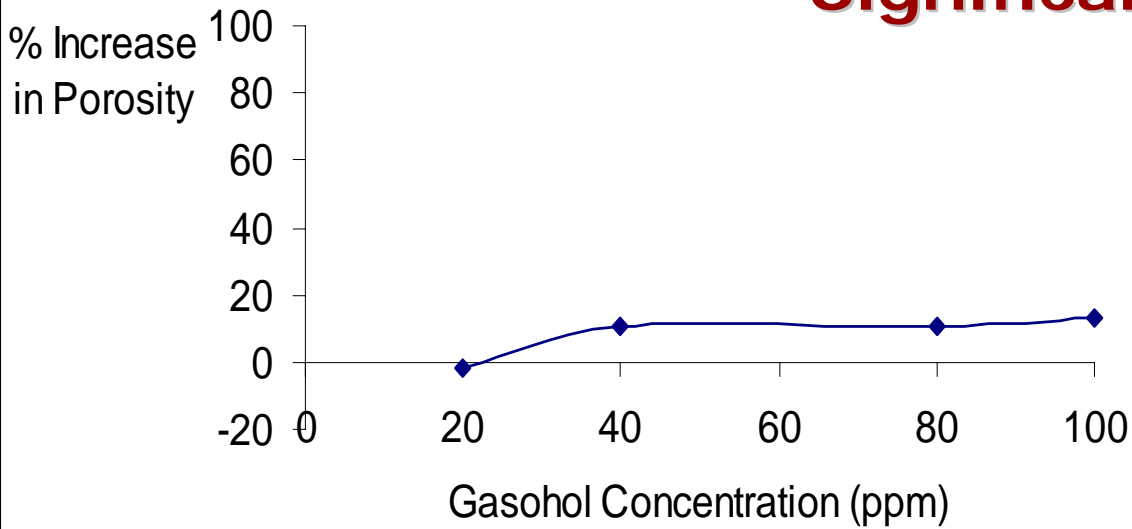
40 ppm



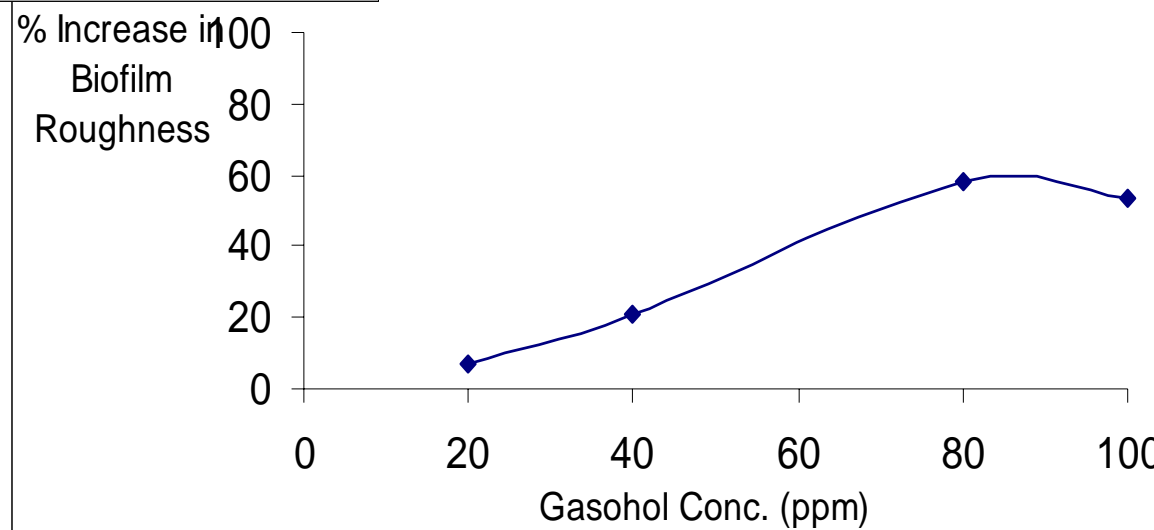
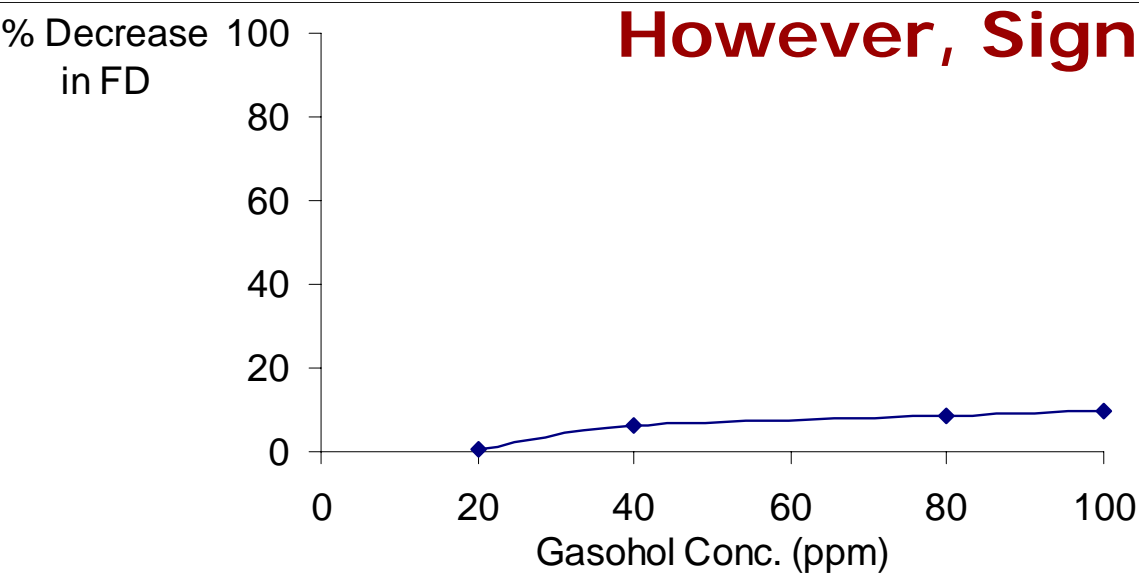
100 ppm

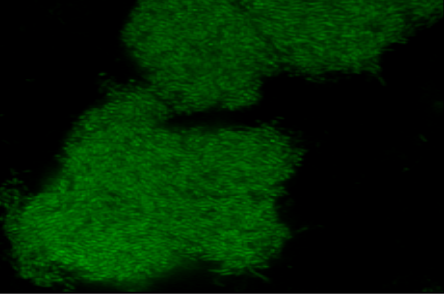


# Biofilm Slightly more Porous; However Significantly less Clustery



# Biofilm Slightly more Heterogeneous; However, Significantly Rougher





# Conclusions

1. At high concentrations, over 80 ppm gasohol in this study, an **increase in  $K$  of a couple of folds** is expected in a bioclogged media;
2. In the conditions tested, **higher removal** were measured at gasohol concentrations around and below 40 ppm;
3. The **microstructure of biofilm is significantly affected** by the presence of gasohol at concentration higher than 40 ppm.

# Future Work

1. Correlate changes in biofilm structure with **bacterial activity and viability** when exposed to gasohol;
2. Explore mathematical models on **contaminant diffusivity** to measure the effect of biofilm on transport in fractured rock;
3. Gain information at macroscale from a bioclogging of a complex fracture network at **field demonstration scale**.



# Acknowledgments

- PERD
- Dr. Zbigniew Lewandowski and Dr. Haluk Beyenal, CBE
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