

# Biofilm Formation Experiment

## **Background**

Naphthenic acids (NA's) are the major contaminant in oil sands production. It is reported that naphtheinic acids (NA's) are best degraded in a biofilm. We want to enhance the biofilm formation ability of bacteria that are capable of degrading NA's in tailing waters. To give the bacteria the ability to form biofilms we plan on introducing a plasmid with antigen 43 that will make the cells "frizzy".

For this experiment we are testing the native ability of bacteria to form biofilms (without the antigen 43 plasmid). Three strains will be tested for biofilm formation: *Pseudomonas putida* (oil sands), *Pseudomonas fluorescens* (oil sands) and *E. coli* K12. The two *Pseudomonas* strains were given to us and shown to grow in oil sands tailing water. We will use these *Pseudomonas* strains as model organisms that can degrade NAs. These *Pseudomonas* strains are known to form biofilms and we want to characterize the biofilm formation ability. The *E. coli* K12 strain will be used as a reference sample.

We will be modeling the adhesion assay from the following paper:

A rapid screening procedure to identify mini-Tn10 insertion mutants of *Escherichia coli* K-12 with altered adhesion properties. FEMS microbiology letters [0378-1097] Genevoux yr:1996 vol:142 iss:1 pg:27

and we will also be adapting our protocol from Alex's lab procedure

## **Materials**

### **Strains**

- *E. coli* K12
- *P. putida* (oil sands)
- *P. fluorescens* (oil sands)

### **Containers**

- 96 well microplate
- 3- 15 mL falcon tubes
- 3-50 mL falcon tubes

### **Media**

- 25 mL of LB media
- 2 mL of 0.1% crystal violet staining solution
- 2 mL of 200 proof ethanol
- 10 mL sterile DI water for washing

**Media Recipes**

Crystal violet staining solution

- 100 ug of crystal violet
- 100 mL of sterile DI water

**Procedure**

1. Start an overnight culture of the following strains in 2 mL of LB media and grow up at 30C in the shaker
  - a. *E. coli* K12
  - b. *P. putida* (oil sands)
  - c. *P. fluorescens* (oil sands)
2. Two hours before the experiment add 1 mL of the saturated overnight culture to 4 mL of fresh LB media for each strain
3. Allow the cultures to grow out to an OD600 of approximately 0.600 (exponential growth phase). This step will take about 2 hours
4. Add 20 uL of to each microplate well according the the layout below (a polystyrene microplate should be used)

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>												
<b>B</b>		LB <i>E. coli</i> K12				LB <i>P. putida</i>				LB <i>P. fluorescens</i>		
<b>C</b>												
<b>D</b>		LB <i>E. coli</i> K12				LB <i>P. putida</i>				LB <i>P. fluorescens</i>		
<b>E</b>												
<b>F</b>		LB <i>E. coli</i> K12				LB <i>P. putida</i>				LB <i>P. fluorescens</i>		
<b>G</b>			LB Blank	LB Blank				EtOH Blank	EtOH BLank			
<b>H</b>												

5. Add 200 uL of LB media to each well with culture and make LB blanks
6. Allow the plate to incubate (WITHOUT SHAKING OR ANYTYPE OF MOTION) for 2 hours at 30C
7. Measure the OD600 on the plate reader after the 2 hour period
  - a. This step will be used to normalize the faster growing cultures against the slower growing ones
8. Pipet out liquid culture
  - a. Place pipet angled against bottom corner to get all the media
9. Bake microplate reader for 30minutes at 80C to adhere cells from biofilm to surface
10. Stain cells with 220uL of 0.1% crystal violet stain for 1 minute
11. The remaining stain was removed by washing extensively with DI water
  - a. Rinse 3 times with 250 uL of sterile DI water, pipetting water out
12. Let the plates dry completely

13. Add 220  $\mu$ L of de-coloring solution to each well and let it sit for 15 minutes
  - a. Might be better to change to 1 minute to prevent ethanol evaporation...
14. Briefly mix the contents of each well by pipeting up and down
15. Measure the adsorption at 600 to quantify the biofilm formation

## ***Results***

Comparing the adsorption readings of the OD600 of the cultures after biofilm growth (OD) vs the OD 600 after the crystal violet staining CV-OD600 should give us the biofilm formation comparison between the three strains. A plot of CV-OD600/OD600 vs the different strains will be made.

Need to find a control of no biofilm formation or excess biofilm formation