Hydrophobicity Determination of Bacteria, Colloids and Surfaces

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Introduction and Objectives

Bacteria are essential microbes used in everyday processes, with applications ranging from bioremediation to biofilms to water filtration. Knowledge of bacterial adhesion to surfaces is therefore essential in completely understanding interactions and roles that bacteria play in the natural and synthetic environments. Associated with bacteria adhesion is their cell surface hydrophobicity (CSH). There have been a number of techniques utilized to measure bacteria hydrophobicity, including the Microbial Adhesion to Hydrocarbons (MATH) test and the contact angle measurement (CAM) test. These techniques are mainly used due to their simplicity in experimental protocol and analysis. These techniques can also be used for measuring the hydrophobicity of colloids and prepared surfaces.

The MATH test utilizes partitioning of aqueous and hydrocarbon phases. The method consists of vortexing the bacteria (suspended in 10mM Potassium Chloride) with a hydrocarbon (n-Dodecane) in 4:1 ratio, allowing for phase separation (2 minutes) and measuring the absorbance of aqueous phase. This absorbance is then compared with initial absorbance of the bacterial suspension and the difference is used as the measure of bacteria suspended in the hydrocarbon phase. MATH test result is usually expressed as % cell surface hydrophobicity and is determined by following expression:

$$\% \text{ hydrophobicity} = 100*(A_{control} - A_{MATH})/ (A_{control})$$

where, $A_{control}$ is the absorbance of a control culture not subjected to MATH test and A_{MATH} is the absorbance of aqueous phase of cell culture subjected to MATH test. The addition of Ammonium Sulfate saturation test (MATH-SAT) used 2M Ammonium sulfate in 10mM Potassium chloride as the bacterial suspension medium, to enhance the hydrophobicity of weakly hydrophobic bacteria strains.

The objectives of this internship were to learn the techniques of the MATH and CAM tests, and to utilize them for the determination of the hydrophobic interaction potential of the bacteria as well as hydrophobicized surfaces.

I. Differentiating weakly hydrophobic bacteria

Experimental Details

Gram-negative Bacteria: *Escherichia coli strains:* D21, D21f2 & JM109; *Shewanella oneidensis* MR-1

Gram-Positive Bacteria: *Deinococcus radiodurans*, *Streptococcus salivarius HB*

Parameters monitored: Absorbance, cell size

Salt Concentrations employed:

0M, 0.5M, 1M, 1.5M, 2M of Ammonium sulfate.

Hydrophobicity measurement:

MATH and MATH test amended with addition of Ammonium sulfate (MATH-SAT).

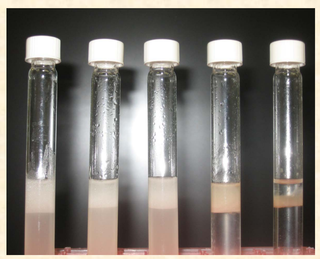

Figure I-1: MATH-SAT results using *Shewanella oneidensis* MR-1. Salt concentrations are 0, 0.5, 1, 1.5 and 2 M Ammonium sulfate (left to right).

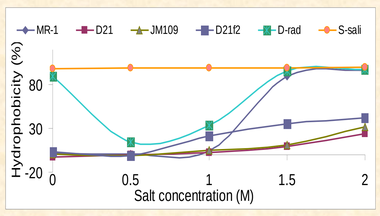

Figure I-2: Hydrophobicity trends of bacteria during the MATH-SAT test of 6 different strains of bacteria.

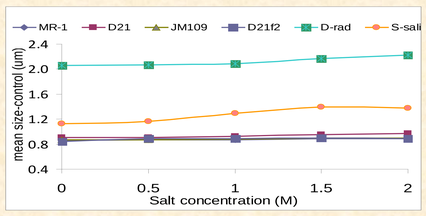

Figure I-3: Mean cell size (um) trends during the MATH-SAT experiment.

Conclusions

The MATH test with the Ammonium sulfate addition showed that weakly hydrophobic bacteria exhibit higher levels of hydrophobicity in the presence of Ammonium sulfate, and thus can be differentiated by modified MATH test. Generally, the addition of Ammonium sulfate in the MATH test influenced the hydrophobicity greatly in gram-negative bacteria and little in gram-positive bacteria, with the greatest hydrophobicity increase in MR-1 after increasing to 1M Ammonium sulfate. We also found that cell size stayed relatively constant throughout the experiment.

II. Growth & Starvation effects on bacterial adhesion

Experimental Details

Gram-negative Bacterial Strains used:

Escherichia coli D21, D21f2 & JM109, *Shewanella oneidensis* MR-1

Parameters monitored:

Absorbance (OD 600),cell size(um), total protein (ppm)

Duration:

Growth:30 hours with measurements taken every 3 hrs for hrs 0-24, then at hr 30.

Starvation: 30 days, measurements everyday for 1-7 days, 10, 20, and 30 days.

Salt Concentration:

2M Ammonium Sulfate

Hydrophobicity measurement:

MATH and MATH-SAT at each sampling.

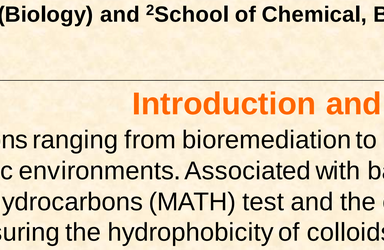

Figure II-1: Hydrophobicity and mean size trends during MATH and SAT experiments under growth conditions of *Shewanella oneidensis* MR-1.

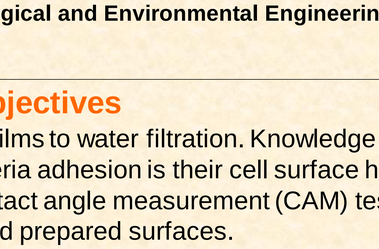

Figure II-2: Biomass and protein concentration during MATH and SAT experiments under growth conditions with *Shewanella oneidensis* MR-1.

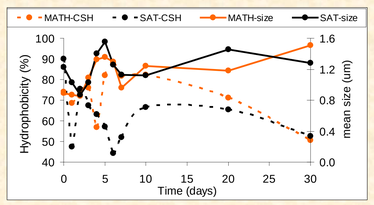

Figure II-3: Hydrophobicity and mean size trends during MATH and SAT experiments under starvation conditions of *Shewanella oneidensis* MR-1.

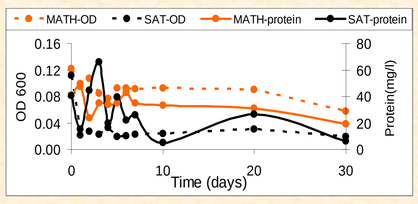

Figure II-4: Cell count and protein concentration during MATH and SAT experiments under starvation conditions with *Shewanella oneidensis* MR-1.

Conclusions:

During growth conditions, there was a general trend of stability in the cell count, cell size, total proteins and hydrophobicity after approximately 9 hours. In the starvation conditions, stability occurred in the cell count (OD600) but not in the cell size or protein concentration.

III. Contact Angle Measurement

Contact angle measurement (CAMs) is another technique used to determine the hydrophobic or hydrophilic properties of bacteria, colloids and natural or synthetic surfaces. In this internship I learned how to set up an apparatus to capture the image of a water and a hydrocarbon (dodecane) droplet on an OTS-coated glass disc, and how to measure the contact angle of the droplet to the surface.

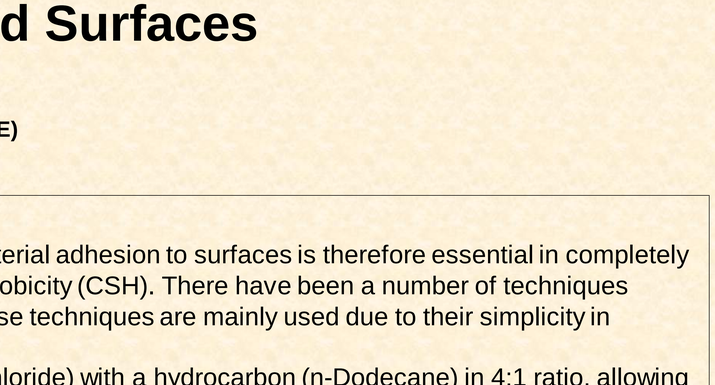

Figure III-1: Water contact angle apparatus.

Using the NIH **ImageJ** software, we were able to measure the contact angle of water or a hydrocarbon to the surface of interest, as a quantitative measure of hydrophobicity of the surface.

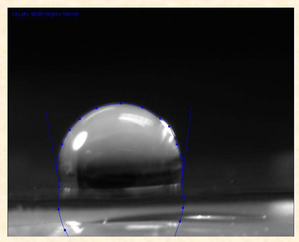

Figure III-2:Analysis of a water droplet on an OTS-coated glass disc using Drop Analysis Package for **ImageJ.** Contact angle: 99.9°.

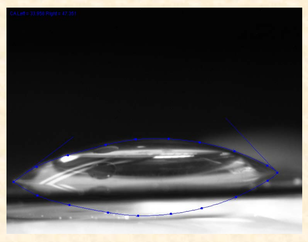

Figure III-3:Analysis of a dodecane droplet on an OTS-coated glass disc using Drop Analysis Package for **ImageJ.** Contact angle: 40.7°.

Subsurface Biosphere Initiative

Research and education focused on life below Earth's surface.

Acknowledgements

I would like to thank the Subsurface Bioshere Initiative at Oregon State University for funding this internship.