

Interaction of Bacteria With Hydrophobic and Hydrophilic Interfaces

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Overview

- The free energy of adhesion is calculated based on contact angle measurements between interacting surfaces
- *Pseudomonas putida* were observed using laser confocal scanning microscopy and results were compared to theory
- Hydrophobic and hydrophilic surfaces were achieved using SAMs of dodecanethiol and mercaptoundecanol respectively on gold films

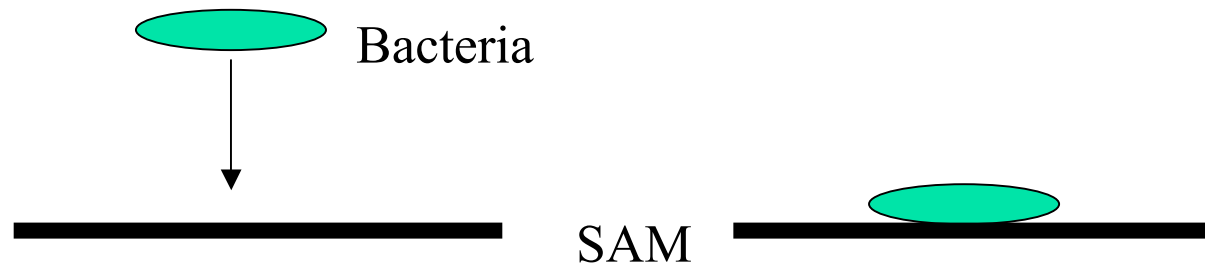
Theory

- Thermodynamics
 - Interaction between the bacteria and substrate is assumed to be a thermodynamic process governed by interfacial free energy
- Free energy of the system is proportional to the work done by the system to bring unit areas of two bodies into contact in a third medium

Thermodynamics

- The system we are considering

Liquid suspension medium



- For dispersion forces

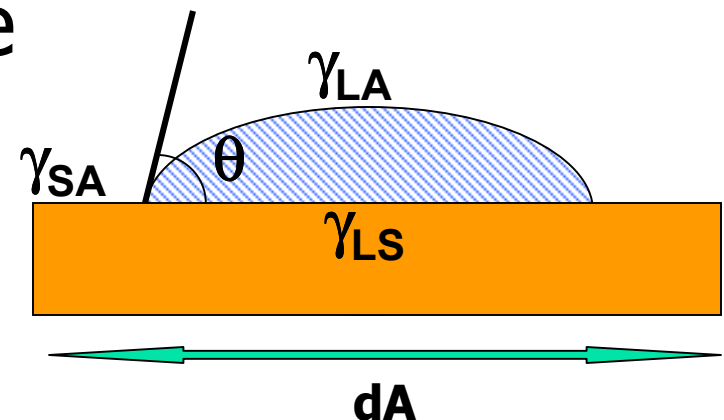
$$W_{AB} = \sqrt{W_{AA}W_{BB}}$$

- Work can be expressed in terms of surface energies and interfacial free energies

$$W_{AB} = \gamma_A + \gamma_B - \gamma_{AB}$$

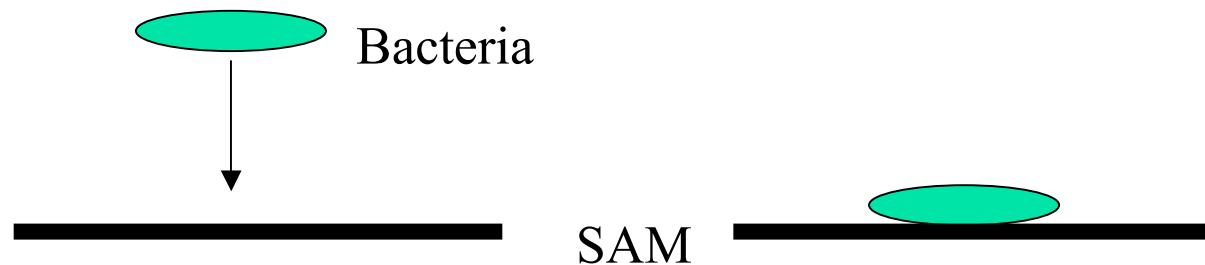
- We use the Young-Dupre equation to calculate the work done

$$W_{sl} = \gamma_l(1 + \cos\theta)$$



- For a bacterium (b) in a liquid medium (l) in the presence of a surface (s)

Liquid suspension medium



$$W_{sbw} = -\gamma_{sb} + \gamma_{sw} + \gamma_{bw}$$

- After some algebra, we obtain

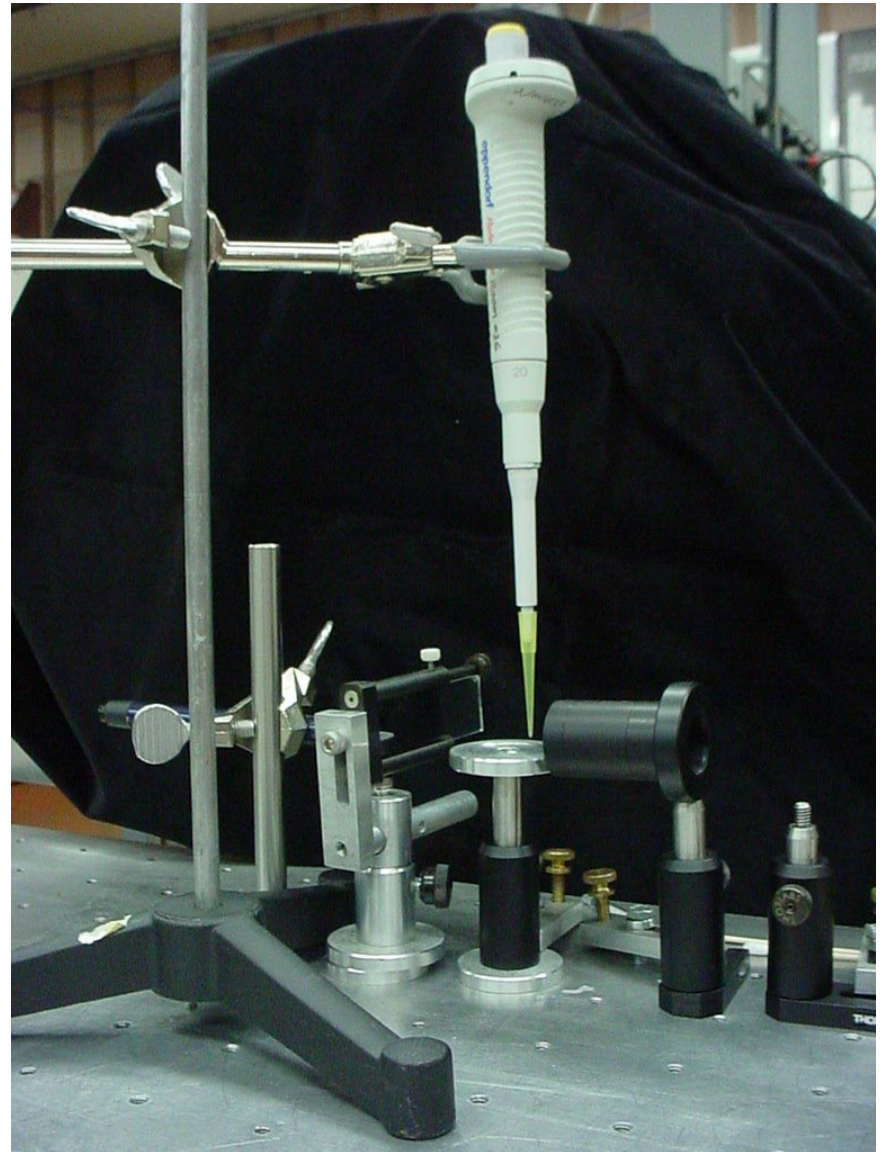
$$W_{sbw} = \frac{1}{2} \gamma_w (1 - \cos \theta_b)(1 - \cos \theta_s)$$

Implications

- According to the equation, as θ_s approaches zero (i.e. hydrophilic surface) the work of adhesion does too
- A bacterium can not distinguish a hydrophilic surface from a watery environment and attaches preferentially to a hydrophobic surface

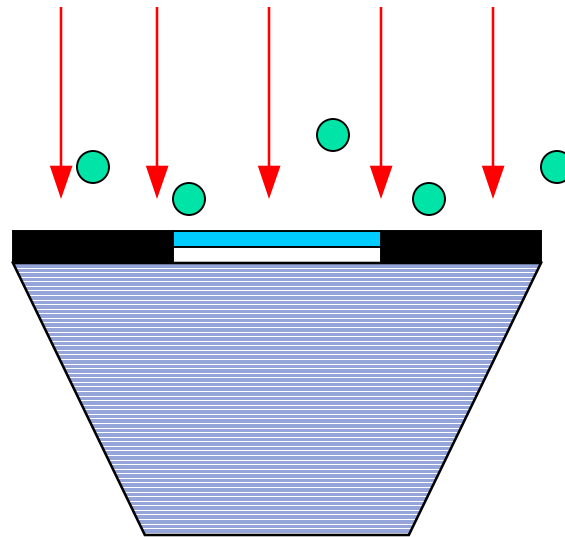
Contact Angle Measurements

- Contact angles were measured on horizontal profiles of sessile drops of water using a 5X eye piece constructed in the lab.
- Water drops were 10 μ L were deposited using a micropipette.
- Image was taken with a Sony DSC-P30/P50 digital camera 10-15 s after the drop was deposited.



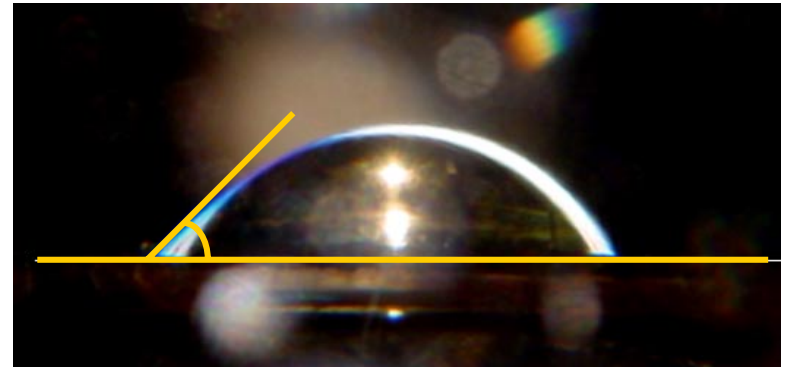
Centrifugation to Obtain Contact Angles on Bacterial Surfaces

- **The bacteria were centrifuged at 3000g for 10 minutes to harvest a uniform layer which was then dried under the biohood**
- **The bacterial layer is uniform in thickness and distribution (average thickness = 22 μm)**



Contact Angle Results

*Angles were measured by ImageJ software, or by hand



Average Contact Angles (degrees)	
Substrate	Average Contact Angle
SAM of mercaptoundecanol	27.8 (1.36)
SAM of dodecanethiol	98.3 (0.41)
Bacteria Centrifuged onto Mercaptoundecanol SAM	36 (6)
Bacteria Centrifuged onto Dodecanethiol SAM	41 (8)

Work of Adhesion Calculations

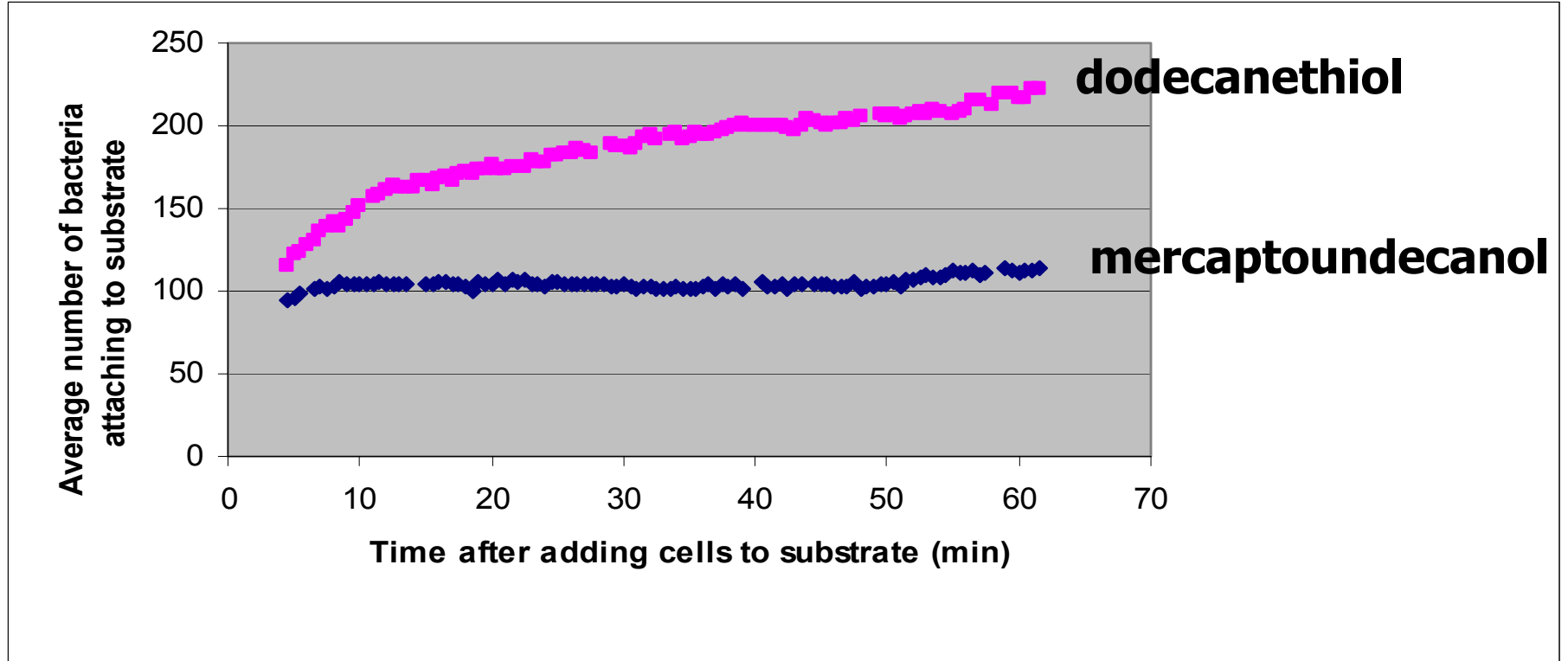
$$W_{sbw} = \frac{1}{2} \gamma_w (1 - \cos\theta_b)(1 - \cos\theta_s)$$

Work of Adhesion (mJ/m²)	
$W_{mbw} = 1.41$	(hydrophilic)
$W_{dbw} = 7.66$	(hydrophobic)

Confocal Microscopy

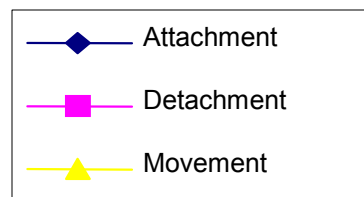
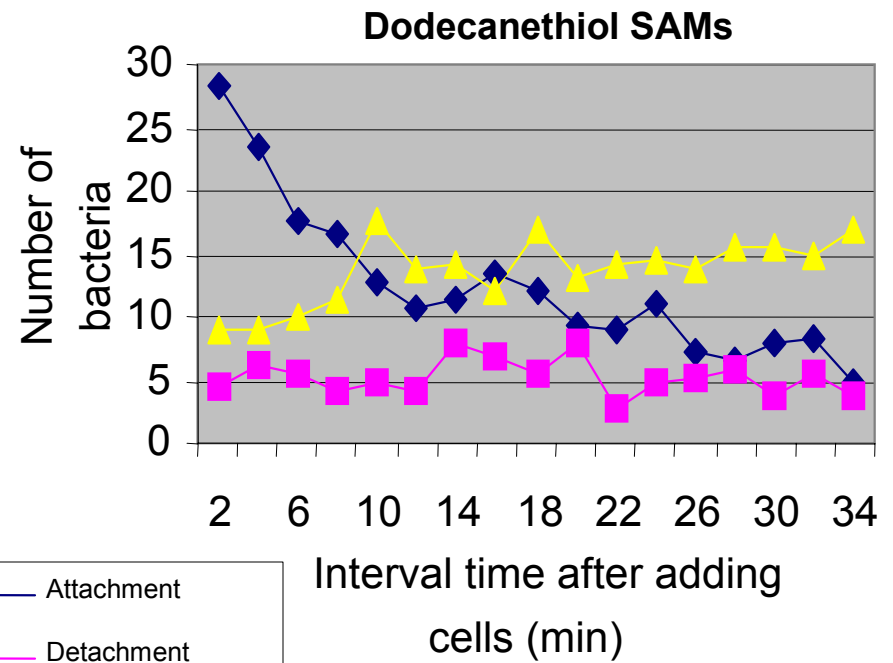
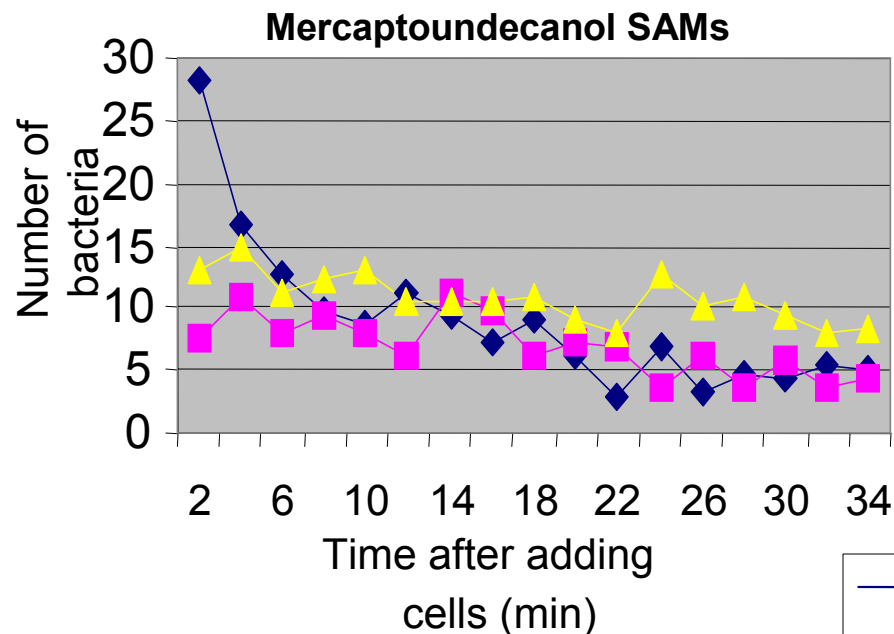
- Confocal analysis of *P. putida* biofilm formation was done to study the “initial” attachment process.
- It was found that adhesion was higher on the dodecanethiol SAM than on the mercaptoundecanol SAM (overall, more bacteria attached to dodecanethiol SAMs over the period of one hour).

Average Number of Bacteria Attaching During One Hour



Movement on Substrate

- Initially, number of bacteria detaching is higher on hydrophilic surface
- After eight minutes, number of bacteria attaching was similar to number detaching on hydrophilic surface
- After ten minutes, biological effects may dominate



Conclusion/further Research

- Our model agrees qualitatively with experimental observations
- Our model ignores more complex interactions and may need refinement
- Further experiments are required