Antimicrobial Susceptibility of Multispecies Biofilms

Ryan Carpenter[1], Jinzi Deng[2], Leslie M. Shor[2]
[1]University at Buffalo, Department of Chemical and Biological Engineering
[2]University of Connecticut, Department of Chemical and Biomolecular Engineering
BASF

The Technology:

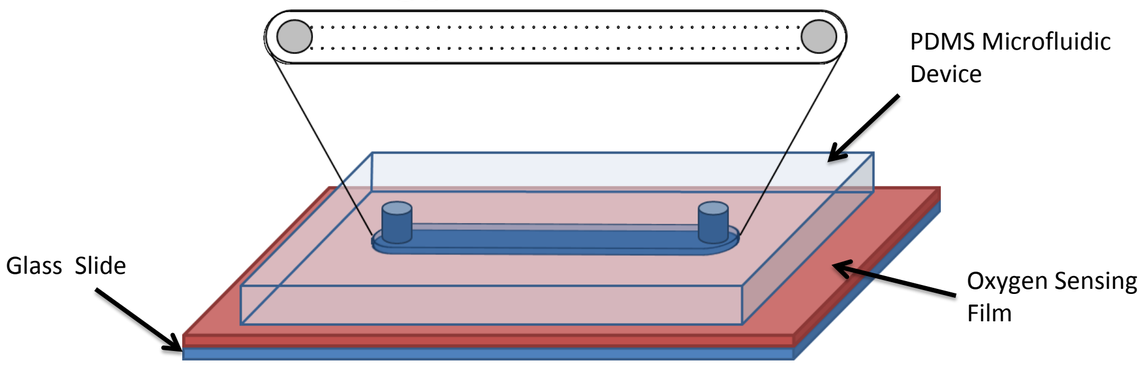


Figure 1: Cross sectional schematic of microfluidic flow cell

PDMS Microfluidic Device

- Microenvironment control
  - Flow rates, flow shape, shear stress
- Small scale, Low fluid volume
- Biologically inert

Oxygen Sensing Film

- Pt(II) meso-Tetra(pentafluorophenyl) porphine
- Real time fluorescent monitoring of cellular respiration

The Market:

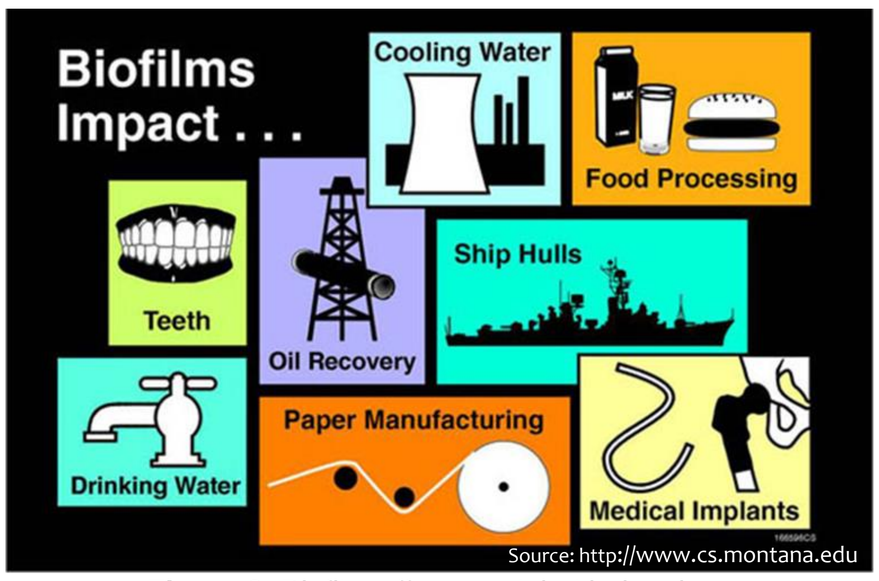


Figure 2: Biofilm affect on global industries

- Costs industries **over $500 billion annually** worldwide
- Biofilms generally exist as complex communities of multiple bacterial species
  - Current research focuses on single species biofilms

Objective: To develop a method to observe the antimicrobial susceptibility of multispecies biofilms grown in a microfluidic device

Methods:

Inject Cell Culture → **2 Hr. Incubation (No Flow)** → **Rinse with Media** → **Apply Antimicrobial**

- Images taken at 4 positions perpendicular to flow at center of device
- Pure species liquid culture grown overnight
- **Control** - *P. aeruginosa* : TSB media 1:1 (v/v)
- **Coculture** - *P. aeruginosa* : *S. aureus* 1:1 (v/v)

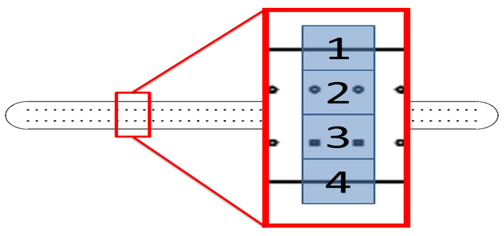
Strains

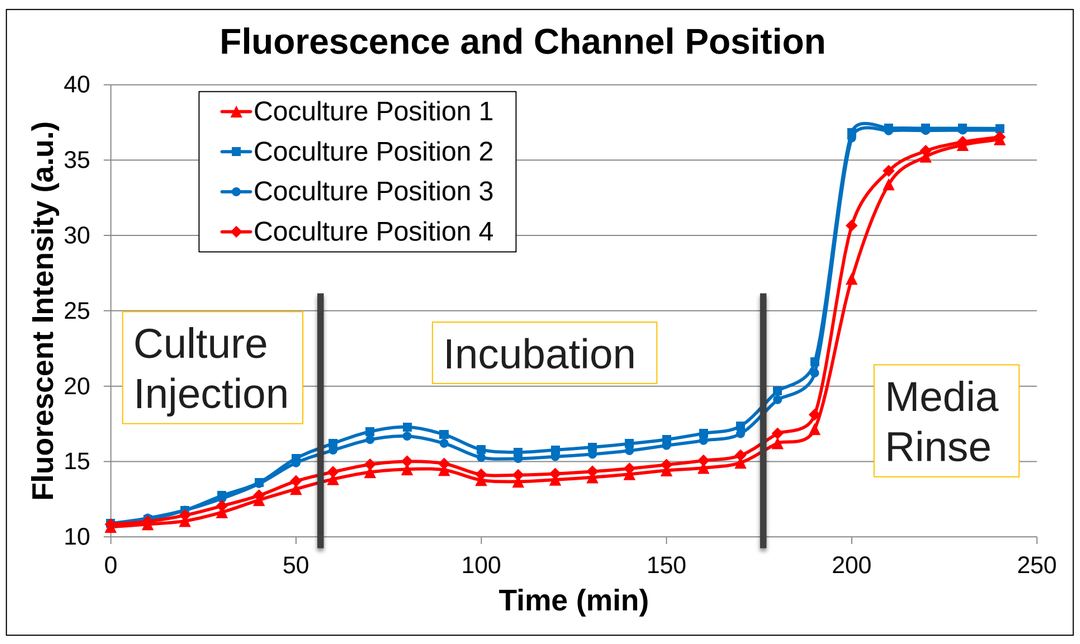
- GFP *Pseudomonas aeruginosa*
- wt *Staphylococcus aureus*

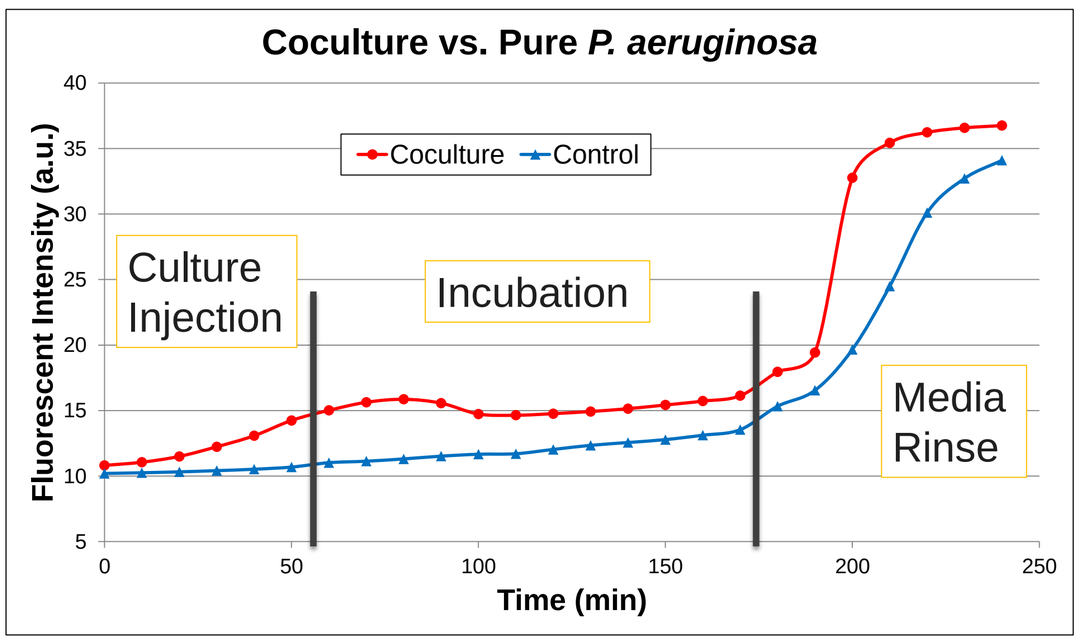
Figure 3: Experimental set up over a Carl Zeiss AXIO-observer Z1 automated inverted microscope

Results:

Figure 4: (Right) Location of four microscope image positions on microfluidic channel (Below) Fluorescent intensity with respect to channel position







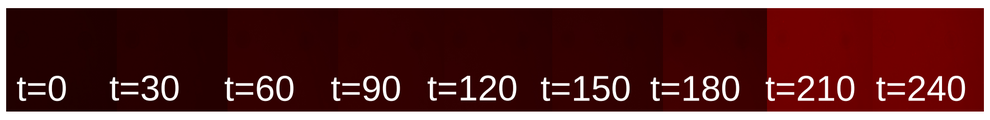


Figure 5: (Top) Average fluorescent intensity from sensing film (Bottom) Time lapse fluorescent images of coculture position 2

Conclusions:

- Successfully observed biofilm respiration and abundance in real time
- Center positions of channel have higher rates of oxygen consumption due to laminar flow
- Larger respiration rates of coculture biofilms may be due to presence of *S. aureus*
- Media rinsing causes large increase in respiration

Student Contact: ryancarp@buffalo.edu
Advisor Contact: leslie@engr.uconn.edu

Future Direction:

- Expose biofilms to antimicrobials
- Measure cell densities of “eradicated” biofilm
- Introduce YFP/CFP *S. aureus* to better characterize biofilm

Acknowledgements:

- National Science Foundation Research Experience for Undergraduates Program
- The Shor Lab