Ultrasonic Membranes for Biofouling Control

Prepared for the One Water 2018 Fresh Ideas Contest
Anton Rosi, M.S.
What’s Biofouling?

Source: www.global-membrane.com
Biofilms Are Organized, Protective Structures

Extracellular polymeric substances (EPS) give biofilms their structure:

- Stratification & Niches (not blobs)
- Protection from chemical and physical stress

Membrane location

Image: a biofilm viewed in cross-section
How is Biofouling Control Currently Practiced?

Status Quo Cleaning options
- Control of operating parameters
- Chemical treatment
- Physical treatment

Issues facing fouling management options:
- Incomplete flux recovery
- Down time
- Membrane damage by cleaning
- Membrane death
Ultrasound for Fouling Control

- Effective removal and deterrence by cavitation
  - Medical, industrial use

- Synergy with other cleaning methods

- Degradation of pharmaceuticals, other recalcitrant compounds
Piezoelectric membranes have built-in ultrasonic defouling

• Piezoelectric membrane?
• Overcome problems with membrane life & scale-up
• Successful preliminary studies using model foulants
Problem: Not Much Known About Ultrasound-Biofoulant Interaction

- Little known about how ultrasound acts on biological materials
- Difficult to study, both processes complex & heterogeneous
- Result: not much design guidance for US defouling system, no baseline to evaluate ultrasonic membrane performance

An experiment was therefore conducted...
This Study: Interrogating the Effects of Ultrasound on Biofilm Removal

Aim: identify how ultrasound changes structure, EPS composition, and taxonomic makeup

Biopolymer structure and composition
• Does ultrasound create a desirable result?

Do biofilms behave like model foulants?
• Time series shows removal progresses
Experimental Setup for Biofouling Control

- Experimental variable: ultrasound duration
  - 0, 20, or 60 s
- Bioreactor
- Custom filtration vessel with Whatman ceramic membranes
- Ultrasound bath
  - 6.83 W cm\(^{-2}\), 205.5 kHz, 10.5 cm from transducer
- Confocal microscopy & 16S rRNA microbial community analysis
Example of Data from a 3D Image

- Microscope detects stains above membrane surface

Above and below: unsonicated thick and thin biofilms.
Signal Attenuation Prevented Imaging Of Biofilm Interiors

• Biomass obstructs emission of fluorescent probes, preventing detection by microscope

• Result: collected images capture outer biofilm only

vs.
Quantitative Image Analysis

- Depth profiles calculated for each image
  - Plots biofilm probe intensity vs. Height above membrane surface
- Enabled quantitative analysis of biofilm thickness and EPS concentration
All Biofilm Images were Represented as Depth Profiles

- $\text{CH}_{\text{PN,DNA}} \rightarrow$ EPS proteins and nucleic acids (green)
- $\text{CH}_{\text{PS}} \rightarrow$ EPS polysaccharides (red)
Basal Biofilm Not Removed

- Non-zero detection
- Others also show incomplete removal
  - Latex particles – Lamminen dissertation
  - Biofoulants – Xu et al. (2013), Yu et al. (2012)
- Potential for synergy with chemical cleaners
- May be better to avoid exposing this layer?
Biofilms that Survived Ultrasound Exhibited Altered EPS Composition

- Sonicated biofilms retaining over 60 µm of thickness
  - Statistically significant reduction in protein and nucleic acid quantity at biofilm surface ($p = 0.01$)
  - Reduction by 50%
    - Result is unaffected by variance in biofilm surface thickness

- No significant reduction in polysaccharide content ($p=0.20$)
Biofilm Removal Starts With Upper Strata

[Graph showing peak altitude (µm) vs. sonication duration (s) with data points and error bars for different conditions (a, b, c, d, e, f).]
Progressive Biofilm Erosion Dissimilar to Some Studies of Model Foulants

- Properties of biofilms are different
  - Stratified removal, elastic

- Resemblance to removal by high fluidic shear (Walter et al. 2013), not pinpoint obliterations
  - Hypertonic biofilm raises cavitation threshold?
  - Lack of cavitation nuclei in biofilm?

Image adapted from Lamminen, Walker, and Weavers (2004)
Ultrasound Affects Biopolymer Content of Remnant Biofilms

• Depleted middle strata

• Alteration of biofilm physiology by ultrasound

• Better to leave intact?
  • Derlon et al. (2016)
Changes In Community After Sonication

- **Sediminibacterium spp.**
  - Enriched, but US greatly reduced its relative abundance
- **Chryseobacterium spp.**
  - Sonication increased relative abundance
  - Extremely low detection in sonicate
Implication of Community Shifts

- Ultrasonic MBR changed community
  - Yu et al. (2012)

- Differentiation starts after 1st treatment

- Select against species in nutrient-rich biofilm canopy, an example of which may have been *Sediminibacterium* spp.
Inferred Selection of Basal Biofilm Dwellers

- Per imaging results, basal strata were difficult to remove.

- *Chryseobacterium* spp. increased in relative abundance following ultrasound
  - Indicates preferential removal of other taxa
  - Protected from ultrasound by overlying biomass?

- *Chryseobacterium* spp. is chlorine resistant, which poses a problem if physical and chemical cleaning are not options
Summary of *ex-situ* ultrasound study

- Thin biofilms remained following sonication
  - Recolonization
  - Hydraulic resistance
  - Optimized processes will need to ensure enough power transfer

- Ultrasound appears to work differently on biofilms than on model foulants
  - Stratified removal, community shifts

- Ultrasound drives community differentiation, likely as a result of preferential removal of outermost strata

- Baseline for evaluating ultrasonic membranes (next)
Ultrasonic Membrane vs. Biofouling: Experimental Setup

• Short-term bioreactor

• Same module design plus:
  • Electrodes
  • Pressurization
  • Hydrophone

• Experimental conditions
  • Fouling overnight
  • 5 psi driving pressure
  • Operated at resonance
A Promising Result (that needs to be confirmed)

- Time crunch! I graduated – there was only time for a practice trial
  - One trial means no statistical power
  - Someone needs to run this experiment again
  - Electrode corrosion?

Non-poled membrane  poled, inactive membrane  Ultrasonic membrane
Where Does This Leave Us?

• Promising (but yet to be verified) results for PZT membranes

• Simplified framework for experimenting on biological foulants with ultrasound, rather than clays or latex

• Insight into the process of removal of biofilm by ultrasound

• Some guidance for others on differences & considerations for model and biological foulants
  • Biofilm structure, microbial community


Reactor Conditions Over Time

- DO (mg/L)
- pH
- TOC (mg C/L)
- TN (mg N/L)
- VSS (mg/L)
- TSS (mg/L)
- Temperature (Celsius)
Reactor Conditions During Fouling

- **DO (mg/L)**
- **pH**
- **Temperature (°C)**

**Conductivity (µS/cm)**

**Total organic carbon (mg/L)**

**Total nitrogen (mg N/L)**
Crossflow Conditions

TMP = 576+/-8 mbar

18.0+/-0.4 C

TMP = 572+/-10 mbar

19.0+/-0.2 C

Pump Q = 2.00+/-0.06 min/L
## Wilcoxon Test Results

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Property tested</th>
<th>p</th>
<th>ratio of means</th>
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</thead>
<tbody>
<tr>
<td>Thick-NoUS</td>
<td>Thick-US</td>
<td>Intensity of signal at biofilm surface</td>
<td>0.01</td>
<td>0.20 2.0507</td>
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<tr>
<td></td>
<td></td>
<td>Biofilm surface height</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biofilm surface depth range**</td>
<td>1.00</td>
<td>0.14 1.0818</td>
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<tr>
<td>Thin-NoUS</td>
<td>Thin-US</td>
<td>Intensity of signal at biofilm surface</td>
<td>0.34</td>
<td>0.47 1.2185</td>
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<td></td>
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<td>Biofilm surface height</td>
<td>0.43</td>
<td>0.26</td>
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<td></td>
<td>Biofilm surface depth range**</td>
<td>0.24</td>
<td>0.96 0.5913</td>
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<tr>
<td>All thin</td>
<td>All thick</td>
<td>Intensity of signal at biofilm surface</td>
<td>0.01</td>
<td>0.00 0.5552</td>
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<td></td>
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<td>Biofilm surface height</td>
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<td>0.00</td>
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<tr>
<td></td>
<td></td>
<td>Biofilm surface depth range**</td>
<td>0.00</td>
<td>0.00 3.4842</td>
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</table>
### Relative Abundances - Tabulated

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Mixed Liquor</th>
<th>Unsonicated</th>
<th>Sonicated</th>
<th>Sonicate</th>
<th>Unfouled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassified Sphingobacteriaceae</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>0.00</td>
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<tr>
<td>Sediminibacterium spp.</td>
<td>0.03</td>
<td>0.11</td>
<td>0.06</td>
<td>0.03</td>
<td>0.00</td>
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<tr>
<td>Luteimonas spp.</td>
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<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
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<tr>
<td>Chryseobacterium spp.</td>
<td>0.08</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Unclassified Comamonadaceae</td>
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<td>0.17</td>
<td>0.16</td>
<td>0.20</td>
<td>0.02</td>
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<tr>
<td>Unclassified Cytophagaceae</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.03</td>
<td>0.00</td>
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<tr>
<td>Dokdonella spp.</td>
<td>0.17</td>
<td>0.10</td>
<td>0.12</td>
<td>0.14</td>
<td>0.01</td>
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<tr>
<td>Other</td>
<td>0.48</td>
<td>0.45</td>
<td>0.49</td>
<td>0.53</td>
<td>0.35</td>
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</tbody>
</table>
## Nucleic Acid Yields

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nucleic acid yield (ng/µL)</th>
<th>Standard deviation (ng/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Liquor</td>
<td>35</td>
<td>--</td>
</tr>
<tr>
<td>Unsonicated Biofilm</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>Sonicated Biofilm</td>
<td>27</td>
<td>3</td>
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<tr>
<td>Sonicate</td>
<td>29</td>
<td>--</td>
</tr>
<tr>
<td>Unfouled membrane</td>
<td>3</td>
<td>--</td>
</tr>
</tbody>
</table>
Crossflow Schematic
**when can we expect sonic membranes?**

<table>
<thead>
<tr>
<th>The Fourth Quarter of Next Year</th>
<th>The Project Will Be Canceled in Six Months.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five Years</td>
<td>I've solved the interesting research problems. The rest is just business, which is easy, right?</td>
</tr>
<tr>
<td>Ten Years</td>
<td>We haven't finished inventing it yet, but when we do, it'll be awesome.</td>
</tr>
<tr>
<td>25+ Years</td>
<td>It has not been conclusively proven impossible.</td>
</tr>
<tr>
<td>We're not really looking at market applications right now.</td>
<td>I like being the only one with a hovercar.</td>
</tr>
</tbody>
</table>

Source: xkcd.com/678