Detection, Characterization and Control of Bacteria in UPW Systems

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NSF/SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing
International TIE Project

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NSF/SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

NSF I/UCRC Center for Microcontamination Control

NSF I/UCRC Hazardous Substance Management Research Center

I/UCRC Queen’s University Environmental Science and Technology Research Centre

NSF I/UCRC Center for Biosurfaces
Objectives

• Characterize Bacteria in UPW Systems
• Explore the interactions of biofilm bacteria, their metabolic products and growth substrates, and advanced oxidation reaction species
  • Develop a system for evaluating the adhesion characteristics of representative strains of bacteria within an UPW system
  • Examine the adhesion of bacteria when subjected to simple irradiation vs. AOP with and without the presence of potential energy sources
• Develop an efficient biofilm destruction approach
  • Formulate a biofilm control scheme with an emphasis on conditions for minimal adhesion
Biofilm Bacteria and Semiconductor Manufacturing

*Oligotrophs*

- Cells are often <0.2 μm in size
- May produce extracellular polysaccharides (EPS)
- Ability to scavenge a broad substrate range
- Capable of growth on <1mg carbon/liter
- Often the first bacteria to become established within biofilms

*Advantages for Microorganisms in Biofilms*

- Protection from shearing
- Constant flow of nutrients
- Genetic exchange between bacteria
- Increased resistance to bacteriocidal agents

*Current Industry Trends*

- One bacterium is large enough to cause a short circuit given the current standard of 1 μm transistor linewidth.
- There is currently a general lack of knowledge regarding biofilm formation and behavior under ultrapure water and recycling conditions.
Bacterial Adhesion

Conditioning

Transport to Surface

Initial Adhesion

Irreversible attachment

Mature Biofilm
Standard Biocidal Techniques

_Ultrapure Water Production_

**Ultraviolet irradiation**
- UV, 254 nm
- UV, 185 nm

**Chemical oxidation**
- Chlorination
- Peroxide
- Ozone
- Proprietary chemical blends
- Advanced oxidation
Advanced Oxidation Processes

• Reactions that involve the generation of free radical intermediates, particularly •OH

• Capable of breaking down organic contaminants as far as CO₂ and H₂O

• Relatively non-invasive treatment: chemicals used decompose into “harmless” species

• Efficiency is often contaminant-specific and treatment environment-specific
Isolation of Bacteria from UPWS

**Incoming Water Strains**
- 46 bacterial strains isolated (40 Gram −, 6 Gram +)
- Above strains generally not detected beyond port 3
- Growth on nutrient rich media (poor growth on OM media)

**UPW Strains**
- 58 bacterial strains isolated (54 Gram −, 4 Gram +)
- UPW bacteria may have adapted to a unique environmental niche
- Poor growth on nutrient rich media, positive growth on 1:10,000 R2A
- Potential heat-tolerant strains isolated (growth at ≤ 70°C)
## Basic Characterization of Main Nuisance Bacteria

<table>
<thead>
<tr>
<th>Area of Isolation</th>
<th>MF254A After $\mathrm{UV}_{254}$</th>
<th>5E After 2nd $\mathrm{UV}_{254}$</th>
<th>5F3 After 0.1µm filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>25-42°C (37°C)</td>
<td>25-50°C (37°C)</td>
<td>25-37°C (30°C)</td>
</tr>
<tr>
<td>pH range</td>
<td>5.0-9.0 (6.5)</td>
<td>5.0-9.0 (6.0)</td>
<td>5.0-9.0 (7.0)</td>
</tr>
<tr>
<td>Growth under anaerobic conditions</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Identification (16s rRNA Sequencing)</td>
<td>Pseudomonas syzygii</td>
<td>Pseudomonas syzygii</td>
<td>Bradyrhizobium sp.</td>
</tr>
<tr>
<td>% Homology</td>
<td>99%</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>

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Characteristics of Nuisance Bacteria

- Plate counts require incubation for one month at 25°C
  - 5x fold increase after one month compared to two weeks

- Many microorganisms are less than 0.2µm in size

- Viable bacteria represent approximately 10-28% of the total bacterial population

- MF254A / 5E - Growth on 33 out of 44 carbon sources tested (mainly amino acids and carbohydrates)

- 5F3 - Growth on 29 out of 44 carbon sources tested (mainly carbohydrates)

- Broad substrate range typical of oligotrophic bacteria

- Currently examining humic acid/ethanol utilization
FISH Probes

- Rapid Detection of Bacteria
- Generic Probes
- Species-specific Probes
Relevance to Industrial Systems

• *Psuedomonas syzygii*
  – Isolated after UV254
  – Found throughout our UPW system
  – Found in samples obtained from industrial partner system
    • No continuous ozonation
    • Confirmed via PCR

• *Bradyrhizobium* sp.
  – Isolate after 0.1 µm filter
  – Not yet detected in industrial partner systems
Materials and Methods

• **Isolation of representative biofilm-forming strains**
  – “MF254A”: *Pseudomonas syzyggi* – rod-shaped, Gram (-) oligotroph

• **Foundation of approach - adhesion characterization**
  – Flow cell apparatus
    • Parallel plate design (Center for Biosurfaces, SUNY at Buffalo)
  – Water source
    • UPW Test-bed (UA)
  – Other environments
    • R2A medium
    • Humic acid
  – Buffer (phosphate, nitrite)
  – Treated humic acid

• **Treatment schemes**
  – Control: no treatment
  – AOP: \( \text{UV}_{185} \) lamp
  – Germicidal irradiation: \( \text{UV}_{254} \) lamp
Flow Cell and Apparatus Design

Flow Cell Cross-Section
- Test Material Coupon
- Spacer
- Test Fluid

Cell Death Treatment
- Lamp Housing
- Source
- Insulating Walls
- Stir Plate
- Treatment

Adhesion Experiments
- Treated Cell Suspension
- Infusion Pump
- Sterile, High Purity Tubing
- Flow Cell
- Waste

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Bacterial Adhesion Analysis

- Epifluorescent Microscopy
- CTC – viable cells
- DAPI – total cells
MF254A Strain attached to Germanium IRE

Analysis performed by Center for Biosurfaces, SUNY at Buffalo

- Large ring-bound –OH fraction at bacteria surface (associated with strong bioadhesive characteristics)

- \( \text{GeO}_2/\text{Ge(OH)}_x \) corrosion with attached microbes in a matrix

- Secondary attachment of additional microbes to the already-present oxide/hydroxide-microbe matrix
Cell Death Kinetics: MF254A in UPW

- $\text{UV}_{254}$ Treatment: $C = C_0 e^{-kt}$, $k_d = 0.3$ s$^{-1}$
- $\text{UV}_{185}$ Treatment: $C = C_0 e^{-kt}$, $k_d = 0.2$ s$^{-1}$
- 100% cell death within 2 min of exposure

$y = 0.2867x + 1.4068$
$R^2 = 0.9611$
Cell Death Kinetics: MF254A in R2A Medium

- **UV\textsubscript{254}** Treatment: \( C = C_0 e^{-kd} \), \( kd = 1.2 \text{ s}^{-1} \)
- **UV\textsubscript{185}** Treatment: \( C = C_0 e^{-kd} \), \( kd = 0.6 \text{ s}^{-1} \)
- Much longer doses needed for cell death compared to UPW
Cell Death Kinetics:
MF254A in 100 mM Sodium Phosphate Buffer

- **UV$_{254}$ Treatment:** $C = C_0 e^{-k_d t}$, $k_d = 0.4$ s$^{-1}$
- **UV$_{185}$ Treatment:** $C = C_0 e^{-k_d t}$, $k_d = 0.2$ s$^{-1}$
- Required dose for cell death similar to UPW
Cell Death Kinetics:
MF254A in 10 ppm Humic Acid/UPW

- UV$_{254}$ Treatment: $C = C_0 e^{-k_d t}$, $k_d = 2.0$ s$^{-1}$
- UV$_{185}$ Treatment: $C = C_0 e^{-k_d t}$, $k_d = 4.6$ s$^{-1}$
- Longest tailing section of any treatment environment tested
AOP vs. Germicidal Irradiation in UPW
Adhesion Comparisons, Short Course

Adhesion to Representative Piping Materials
$10^8$ cfu/ml MF254A in UPW, 4.2 h run time

- **Halar**
- **Kynar**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>UV254</th>
<th>UV185</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Bacteria Coverage (cfu/cm²)</td>
<td>1.50E+05</td>
<td>6.00E+05</td>
<td>0.00E+00</td>
</tr>
</tbody>
</table>

**Notes:**
- **Control UV254 UV185**

**Graph:**
- Bar graph showing adhesion coverage for different treatments.
AOP vs. Germicidal Irradiation in UPW
Adhesion Comparisons, Long Course

Adhesion to Representative Piping Materials
108 cfu/ml MF254A in UPW, 16.7 h run time

Surface Bacteria Coverage (cfu/cm²)

- Halar
- Kynar

Treatment
- Control
- UV254
- UV185

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AOP vs. Germicidal Irradiation in R2A Medium

Adhesion to Representative Piping Surfaces
$10^8$ cfu/ml MF254A in R2A Medium, 4.2 h run time

<table>
<thead>
<tr>
<th>Surface Bacteria Coverage (cfu/cm²)</th>
<th>Control</th>
<th>UV254</th>
<th>UV185</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kynar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
AOP vs. Germicidal Irradiation in Buffer
100 mM Sodium Phosphate Buffer

Adhesion to Representative Piping Surfaces
10^8 cfu/ml MF254A in 100 mM Sodium Phosphate Buffer, 4.2 h run time

Surface Bacteria Coverage (cfu/cm²)

- Control
- UV254
- UV185

Treatments:
- Halar
- Kynar
AOP vs. Germicidal Irradiation in Buffer
100 mM Sodium Nitrite

Adhesion to Representative Piping Surfaces
10^8 cfu/ml MF254A in 100 mM Sodium Nitrite Buffer, 4.2 h run time

Surface Bacteria Coverage (cfu/cm²)
- Control
- UV254
- UV185

- Halar
- Kynar
AOP vs. Germicidal Irradiation w/Nutrient Source
10 ppm Humic Acid and Treated Humic Acid

Adhesion to Representative Piping Surfaces
$10^8$ cfu/ml MF254A in 10 ppm Humic Acid/UPW, 4.2 h run time

Surface Bacteria Coverage (cfu/cm²)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>UV254</th>
<th>UV185</th>
<th>UV254-treated solution</th>
<th>UV185-treated solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Halar</td>
<td>Kynar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Halar
Kynar

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Adhesion Comparisons by Treatment and Environment

\[ 10^8 \text{ cfu/ml Adhesion on Halar}^\text{®} \]

<table>
<thead>
<tr>
<th></th>
<th>Control (x 10^5 cfu/cm^2)</th>
<th>UV\text{254} (x 10^5 cfu/cm^2)</th>
<th>UV\text{185} (x 10^5 cfu/cm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPW, short course</td>
<td>2.17 +/- 1.39</td>
<td>4.98 +/- 1.95</td>
<td>0.55 +/- 0.20</td>
</tr>
<tr>
<td>UPW, long course (avg)</td>
<td>1.18 +/- 0.44</td>
<td>1.53 +/- 0.58</td>
<td>0.77 +/- 0.28</td>
</tr>
<tr>
<td>R2A growth medium</td>
<td>1.12 +/- 0.61</td>
<td>2.26 +/- 0.93</td>
<td>1.97 +/- 0.76</td>
</tr>
<tr>
<td>100 mM phosphate buffer</td>
<td>1.06 +/- 0.41</td>
<td>1.84 +/- 0.39</td>
<td>1.03 +/- 0.27</td>
</tr>
<tr>
<td>100 mM nitrite buffer</td>
<td>0.71 +/- 0.32</td>
<td>0.90 +/- 0.15</td>
<td>0.42 +/- 0.14</td>
</tr>
<tr>
<td>10 ppm humic acid</td>
<td>2.26 +/- 0.71</td>
<td>2.44 +/- 0.60</td>
<td>0.57 +/- 0.13</td>
</tr>
<tr>
<td>10 ppm treated humic acid</td>
<td>2.26 +/- 0.71</td>
<td>13.6 +/- 1.75</td>
<td>&gt; 20.6</td>
</tr>
</tbody>
</table>

- Differences in adhesion by treatment were more pronounced in UPW and humic acid/UPW than in media and buffers
- Adhesion comparisons on Kynar® follow same trends as Halar® with high adhesion in almost every case
Conclusions

- Cell death in UPW and similar environments using ultraviolet light follows conventional bacterial inactivation kinetics (first order exponential with tailing).

- Bacterial adhesion does not result in a uniform coverage of the solid surface, nor does it occur in a stepwise, uniform manner as time increases.

- In most cases, Halar® exhibits minimal adhesion over Kynar®.

- In both UPW and more nutrient-rich environments, germicidal irradiation treatment with UV$_{254}$ results in greater adhesion than no treatment and advanced oxidation.

- Advanced oxidation treatment with UV$_{185}$ varies in adhesion effects with the treatment environment.

- Treatment of potential biofilm nutrients prior to their contact with bacteria causes much greater adhesion than simultaneous treatment.
Further Work

- Perfect FISH Technique for specificity
- Continue detection of bacteria in industrial UPW systems
- Mimic tests on representative piping materials using higher water flow rates
- Further adhesion testing of alternative biofilm-forming bacteria species to piping surfaces
- Time-dependent series adhesion tests to determine rates of adhesion in the presence of various treatments
- Test different sources of advanced oxidation, such as UV/H$_2$O$_2$ or UV/TiO$_2$ catalyst
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