Abstract: SIMB-RAMC

Antimicrobial Materials for Advanced Microbial Control in Spacecraft Water Systems

Microbial detection, identification, and control are essential for the maintenance and preservation of spacecraft water systems. Requirements set by NASA puts limitations on the energy, mass, materials, noise, cost, and crew time that can be devoted to microbial control. Efforts are being made to attain real-time detection and identification of microbial contamination in microgravity environments. Research for evaluating technologies for capability enhancement on-orbit is currently focused on the use of adenosine triphosphate (ATP) analysis for detection purposes and polymerase chain reaction (PCR) for microbial identification. Additional research is being conducted on how to control for microbial contamination on a continual basis. Existing microbial control methods in spacecraft utilize iodine or ionic silver biocides, physical disinfection, and point-of-use sterilization filters. Although these methods are effective, they require re-dosing due to loss of efficacy, have low human toxicity thresholds, produce poor taste, and consume valuable mass and crew time. Thus, alternative methods for microbial control are needed. This project also explores ultraviolet light-emitting diodes (UV-LEDs), surface passivation methods for maintaining residual biocide levels, and several antimicrobial materials aimed at improving current microbial control techniques, as well as addressing other materials presently under analysis and future directions to be pursued.
ANTIMICROBIAL MATERIALS FOR ADVANCED MICROBIAL CONTROL IN SPACECRAFT WATER SYSTEMS

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Minimize energy requirements
  - Must not exceed 32 V without hazard controls for crew safety

Reduce crew time devoted to maintenance and operation

Minimize hardware and equipment mass
  - Reduce volume and weight
  - Eliminate resupply or dosing for maintaining microbial control

Limitations on materials, biological and chemical components
  - Avoid hazardous by-products, contamination, or bodily injury

Limitations on noise, based on duration time
  - Must not exceed 49-80 dBA

Have rapid turn-around with experimental results
  - Doesn't require return to ground for analysis

Reduce cost

MICROBIAL DETECTION

- Applicable on orbit in ISS
  - Needs to function in microgravity
  - Must meet NASA requirements
- Capable of detecting *How Much* is present
  - Not necessarily what is there

- Currently, culturing methods are used with broad-specificity media
  - Limited to microbes that can be cultivated
  - Requires an incubation period before analysis
- Necessity to move towards an online, real-time assay for quantification
  - Not limited by cultivation
Adenosine Triphosphate (ATP) analysis

- Can be used to monitor levels of metabolically active organisms
  - Healthy cells produce ATP continuously
  - Dying cells rapidly decrease in ATP production
- Detects ATP from active cells in samples
  - Aerobic and anaerobic
  - No bias from growth medium
- Fast
  - Does not require dilutions, plate counts, incubation time, microscopes, or additional molecular methods
  - Results in minutes, rather than days

**ATP ASSAY**

- Mix a sample with a reagent that reacts with ATP to produce light

- Measure amount of light produced with a luminometer

\[
\text{Luciferin + ATP + O}_2 \xrightarrow{\text{Enzyme}} \text{Oxyluciferin} + \text{AMP} + \text{PP}_i + \text{Light}
\]

### Bulk fluid fg ATP/cell results

<table>
<thead>
<tr>
<th>Bulk fluid samples</th>
<th>Standard</th>
<th>Formalin (1%)</th>
<th>Silver (400 μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microgravity (n = 51)</td>
<td>2.02E-03 ± 5.75E-04</td>
<td>N/A</td>
<td>2.30E-05 ± 9.07E-06</td>
</tr>
<tr>
<td>Lunar gravity (n = 51)</td>
<td>2.70E-03 ± 6.05E-04</td>
<td>2.82E-05 ± 5.57E-06</td>
<td>N/A</td>
</tr>
<tr>
<td>Terrestrial gravity (n = 21)</td>
<td>1.10E-03 ± 3.81E-04</td>
<td>1.38E-04 ± 3.31E-05</td>
<td>2.81E-04 ± 8.83E-05</td>
</tr>
</tbody>
</table>

- Analyzed samples on ground, from lunar gravity, and microgravity
- Ground testing resembled metabolic state of benchmark samples
- More ATP per cell in sample exposed to short-term microgravity

FUTURE WORK WITH ATP

- A more extensive flight experiment with bacteria and ATP analysis on ISS
  - Single vs. mixed communities
  - Short-term vs. long-duration exposure to microgravity
  - Total ATP vs. cellular ATP
  - Different media
    - water, food, surfaces

- Interested in studying the ATP to AMP ratio as an alternate microbial community evaluation method
Applicable on orbit in ISS

- Needs to function in microgravity
- Must meet NASA requirements

Interested in What's There

- Example: E. coli
  - presence = significant
- Less concerned with viability

"A geneticist laid bare my genetic blueprint, and now I feel violated."
Polymerase Chain Reaction (PCR)
- Versatile in microbe detection
- Can detect DNA from complex matrices
- Relatively easy to obtain and interpret results
- Low energy requirement
- Minimal creation of biohazardous waste
CURRENTLY EVALUATING
What's There or How Much?
- Identification or Detection

NASA is currently predominantly pursuing *What's There* approach using molecular-based methods
- Presence is significant, not focused on viability

NASA is still evaluating the *How Much* approach however with the ATP assays
- Combining methods may be future direction
Current disinfection on ISS for drinking water
- Chemical-iodine or ionic silver biocides
- Physical-point of use (POU) sterilization filters

Drawbacks
- Low in efficacy requires re-dosing
- Low human toxicity thresholds
- Reportedly poor taste

Goals for next generation water systems
- Minimize power and volume for long-duration mission
- Collect, store, recycle, and disinfect water for use/reuse
- Strive to eliminate need of re-dosing and POU filters
WHAT WE’VE DONE
UV-C LED STATIC TESTING

- Goal: Use germicidal UV-C LEDs as a point-of-use sterilization device
  - Maintain drinking water safety

- Challenge bacteria used to inoculate water samples in 96-well plate

- Samples analyzed at several time points to evaluate amount of exposure required for cellular inactivation

UV-C LEDs were able to produce a $>3 \log_{10}$ CFU/mL reduction within an hour of contact time in the static system.
Goal: Use UV-C LEDs for microbial disinfection in a recirculating potable water system

- Water was inoculated with either one challenge organism or a mixed community of 5 organisms
- Recirculating tests were run for 24 hours
UV-C LED Recirculation Test

- UV-C LEDs were able to produce a $>1.5 \log_{10}$ CFU/mL reduction within 24 hours of contact time in the recirculating system
  - UV-C LEDs degraded quickly
Goal: Test UV-A LEDs for efficacy in disinfecting potable water systems

- Use in conjunction with a photocatalyst: titanium dioxide

- Challenge bacteria used separately to inoculate water samples in 96-well plates

- Samples taken every 15-30 minutes for up to 3 hours

WHAT WE’VE DONE
UV-A LED STATIC TESTING

- UV-A or UV-A plus titanium dioxide were effective
- UV-A with anatase titanium dioxide more effective against *S. paucimobilis* and *M. fujisawaense*

**Table 1. AODC and HPC Results (n=6).**

<table>
<thead>
<tr>
<th></th>
<th>Before exposure</th>
<th>After exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log (10)</td>
<td>AODC (cells/mL)</td>
<td>HPC (CFU/mL)</td>
</tr>
<tr>
<td><strong>Burkholderia cepacia</strong></td>
<td>6.83 ± 0.02</td>
<td>6.92 ± 0.82</td>
</tr>
<tr>
<td><strong>Cupriavidus metallidurans</strong></td>
<td>7.13 ± 0.06</td>
<td>6.97 ± 1.31</td>
</tr>
<tr>
<td><strong>Methylobacterium fujisawaense</strong></td>
<td>6.84 ± 0.04</td>
<td>6.78 ± 0.71</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>7.16 ± 0.02</td>
<td>6.96 ± 0.06</td>
</tr>
<tr>
<td><strong>Sphingomonas paucimobilis</strong></td>
<td>7.42 ± 0.01</td>
<td>7.58 ± 1.12</td>
</tr>
<tr>
<td><strong>Wautersia basilensis</strong></td>
<td>7.27 ± 0.02</td>
<td>7.14 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>AODC (cells/mL)</td>
<td>HPC (CFU/mL)</td>
</tr>
<tr>
<td><strong>Burkholderia cepacia</strong></td>
<td>6.79 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Cupriavidus metallidurans</strong></td>
<td>7.12 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Methylobacterium fujisawaense</strong></td>
<td>6.74 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>7.15 ± 0.02</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Sphingomonas paucimobilis</strong></td>
<td>7.43 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Wautersia basilensis</strong></td>
<td>7.26 ± 0.02</td>
<td>ND</td>
</tr>
</tbody>
</table>
WHAT WE’VE DONE
UV-A LED RECIRCULATING TESTING

- Goal: Test efficacy of UV-A LEDs for disinfection in a recirculating system
  - Use UV-A in conjunction with photocatalyst (titanium dioxide) or silver
- Reactors inoculated with *P. aeruginosa* or *E. coli*
- Reactor run up to 144 hours
  - Samples taken at multiple time points

## DFR Results

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Organism</th>
<th>Experimental Conditions</th>
<th>HPC (log(10) CFU/mL) (n=3)</th>
<th>AODC (log(10) cells/mL) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>DFR 1</td>
<td><em>E. coli</em></td>
<td>UV-A alone</td>
<td>7.32 ± 0.07</td>
<td>5.17 ± 0.67</td>
</tr>
<tr>
<td>DFR 2</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A alone</td>
<td>6.74 ± 0.03</td>
<td>7.35 ± 0.07</td>
</tr>
<tr>
<td>DFR 3</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A alone</td>
<td>7.61 ± 0.04</td>
<td>8.23 ± 0.11</td>
</tr>
<tr>
<td>DFR 4</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A alone</td>
<td>7.60 ± 0.04</td>
<td>8.03 ± 0.07</td>
</tr>
<tr>
<td>DFR 5</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A + titanium dioxide</td>
<td>7.76 ± 0.03</td>
<td>8.10 ± 0.48</td>
</tr>
<tr>
<td>DFR 6</td>
<td><em>E. coli</em></td>
<td>UV-A + titanium dioxide</td>
<td>7.01 ± 0.06</td>
<td>5.82 ± 1.17</td>
</tr>
<tr>
<td>DFR 7</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A + small silver coupon (12 mm)</td>
<td>7.03 ± 0.01</td>
<td>8.00 ± 0.17</td>
</tr>
<tr>
<td>DFR 8</td>
<td><em>P. aeruginosa</em></td>
<td>UV-A + large silver coupon (3 x 1 inch)</td>
<td>7.74 ± 0.05</td>
<td>3.10 ± 1.77</td>
</tr>
<tr>
<td>DFR 9</td>
<td><em>E. coli</em></td>
<td>UV-A + small silver coupon (12 mm)</td>
<td>7.02 ± 0.06</td>
<td>6.45 ± 0.09</td>
</tr>
<tr>
<td>DFR 10</td>
<td><em>E. coli</em></td>
<td>UV-A + large silver coupon (3 x 1 inch)</td>
<td>7.20 ± 0.03</td>
<td>4.53 ± 2.78</td>
</tr>
</tbody>
</table>

**Notes:** Red highlight indicate discussed results. +/- represents the standard deviation between replicates.

- Largest sustained decrease with samples containing ionic silver
- Lack of inactivation by UV-A LEDs is attributed to degradation
WHAT WE’VE DONE
ANTIMICROBIAL MATERIALS

- Goal: Test a variety of antimicrobial materials for efficacy of disinfecting water systems and preventing biofilm formation
  - 4 polymer materials
  - Coated with 14 different antimicrobial coatings
  - Impregnated with 3 different surface topographies
- Reactors inoculated with one challenge organism or 5 challenge organisms
- Coupons evaluated for biofilm prevention
- Bulk fluid evaluated for content

## WHAT WE’VE DONE
### ANTIMICROBIAL MATERIALS

### CBR Bulk Fluid Results

<table>
<thead>
<tr>
<th>CBR</th>
<th>HPC (log(10) CFU/mL)</th>
<th>AODC (log(10) cells/mL) (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>31</td>
<td>5.58</td>
<td>7.46</td>
</tr>
<tr>
<td>32</td>
<td>5.46</td>
<td>ND</td>
</tr>
<tr>
<td>33</td>
<td>5.39</td>
<td>ND</td>
</tr>
<tr>
<td>34</td>
<td>5.67</td>
<td>ND</td>
</tr>
<tr>
<td>35</td>
<td>5.54</td>
<td>5.18</td>
</tr>
<tr>
<td>39</td>
<td>5.61</td>
<td>0.48</td>
</tr>
</tbody>
</table>

+- represents the standard deviation between replicates.

### CBR Coupon Results

<table>
<thead>
<tr>
<th>CBR</th>
<th>HPC (log(10) CFU/coupon)</th>
<th>AODC (log(10) cells/coupon)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td>2</td>
<td>(n=15)</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>(n=12)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treatment</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.82 ± 0.22</td>
<td>7.57 ± 0.20</td>
<td>7.71 ± 0.21</td>
<td>7.50 ± 0.21</td>
</tr>
<tr>
<td>33</td>
<td>7.49 ± 0.15</td>
<td>ND</td>
<td>7.70 ± 0.14</td>
<td>5.37 ± 0.19</td>
</tr>
<tr>
<td>35</td>
<td>7.70 ± 0.19</td>
<td>3.38 ± 0.31</td>
<td>7.60 ± 0.22</td>
<td>4.56 ± 0.17</td>
</tr>
<tr>
<td>41</td>
<td>8.33 ± 0.05</td>
<td>8.10 ± 0.03</td>
<td>8.58 ± 0.30</td>
<td>8.35 ± 0.17</td>
</tr>
</tbody>
</table>

ND= none detected. The control samples did not have the application of the treatment with anticipated antimicrobial efficacy. +/- represents the standard deviation between replicates.

- CBR 31 and CBR 32 contained AM 5
  - CBR 32 also contained Ag
  - AM 5 did not contribute to antimicrobial effect, only Ag did
- CBR 33 and CBR 34 contained AM 6
  - CBR 34 also contained Ag
  - AM 6 contributed to reduction in microbes
  - The compound leached so ND was found in the bulk fluid
- CBR 35 and CBR 39 contained AM 7
  - CBR 39 also contained Ag
  - AM 7 did not contribute to antimicrobial effect, only Ag did

- Surface topography 1 delayed biofilm formation during static testing (CBR 2)
- AM 6, a ceragenin, showed >7 log reduction (CBR 33)
  - Compound leached into bulk fluid
- AM 7, membrane disruption, showed a >4 log reduction (CBR 35)
- AM 11, hydrophobic material, showed a slight decrease (CBR 41)
WHAT WE’VE DONE
PASSIVATION WITH IONIC SILVER

- Goal: Prolong silver efficacy by maintaining solubility
  - Safe limits for human consumption
  - Limit bacterial growth
- Metal coupons used for potable water systems in space exposed to silver fluoride treatments
- Performed in a CDC biofilm reactor to test efficacy of coupons

0.4 ppm silver was an effective biocide
Pre-passivation with 50 ppm provided additional microbial control
Slow degradation of silver by adherence on coupon surfaces
Loss of residual antimicrobial activity minimized
Goals
- Evaluate microbial transfer through the membrane
- Evaluate biofouling of the membrane
- Evaluate materials properties of the membrane
Omniphobic Materials

- Surface treatments for metal and polymer materials that yield contact angles greater than 150° for both polar and non-polar liquids
- May reduce microbial attachment and biofilm formation on wetted surfaces

Omniphobic surfaces developed in Tuteja Group

Omniphobic surface morphologies
## ACKNOWLEDGEMENTS

<table>
<thead>
<tr>
<th>Microbial ID</th>
<th>Microbial Detection</th>
<th>Microbial Control</th>
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<tbody>
<tr>
<td>Monsi Roman (MSFC)</td>
<td>David Smith (KSC)</td>
<td>Lawrence Koss (KSC)</td>
</tr>
<tr>
<td>Victoria Castro (JSC)</td>
<td>Leticia Vega (JSC)</td>
<td>Richard Meshberger (KSC)</td>
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<td>Cherrie Oubre (JSC)</td>
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<td>John Catechis (KSC)</td>
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<td>Kasthuri Venkateswaran(JPL)</td>
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<td>Griffin Lunn (KSC)</td>
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<td>Parag Vaishampayan (JPL)</td>
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<td>LaShelle McCoy (KSC)</td>
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<tr>
<td>Mary Hummerick (KSC)</td>
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<td>Jeremy O’Neal (KSC)</td>
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<td><strong>LuminUltra:</strong></td>
<td>Mike Lee (KSC)</td>
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<td>Lou Banks</td>
<td>Dave Tracey</td>
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<tr>
<td><strong>University of Michigan:</strong></td>
<td></td>
<td></td>
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<tr>
<td>Anish Tuteja</td>
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