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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NASA REQUIREMENTS FOR ISS

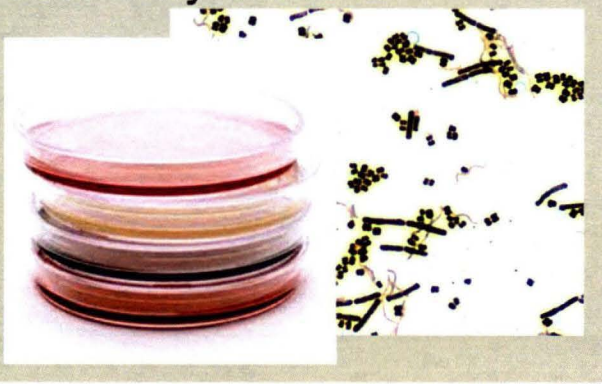
HUMAN SYSTEM STANDARDS

- 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

NASA Space Flight Human-System Standard, *NASA Technical Standard*, 2011, NASA-STD-3001, vol. 2.

MICROBIAL DETECTION

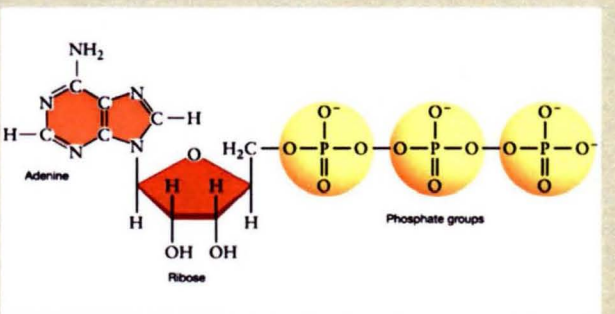
- Applicable on orbit in ISS
  - Needs to function in microgravity
  - Must meet NASA requirements
- Capable of detecting ***How Much*** is present
  - Not necessarily what is there



- 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

METHOD IN PURSUIT

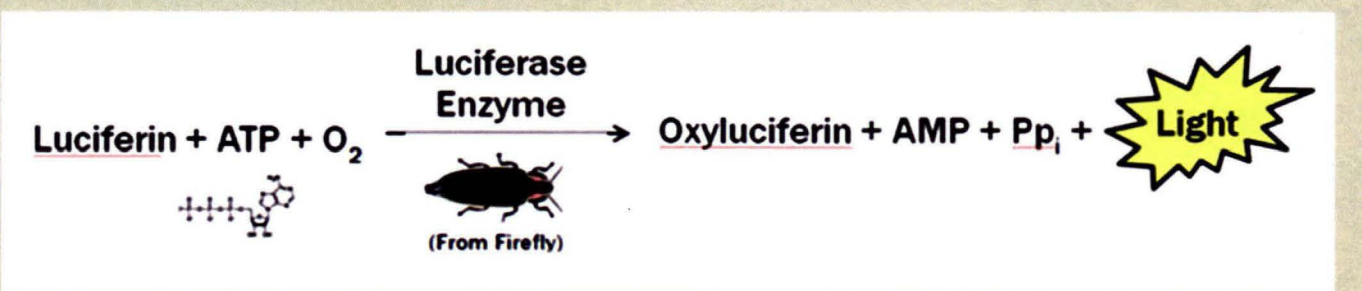
- Adenosine Triphosphate (ATP) analysis
  - Can be used to monitor levels of metabolically active organisms
    - Healthy cells produce ATP continuously
    - Dying cells rapidly decrease in ATP production
  - Detects ATP from active cells in samples
    - Aerobic and anaerobic
    - No bias from growth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



Crouch, S.P., Kozlowski, M.R., Slater, K.J., and Fletcher, J., "The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity," *Journal of Immunology Methods*, Vol. 160, 1993, pp. 81–88.

ATP ASSAY

- Mix a sample with a reagent that reacts with ATP to produce light
- Measure amount of light produced with a luminometer



Chappelle, E.W., and Levin, G. V., "Use of the firefly bioluminescence reaction for rapid detection and counting of bacteria," *Biochemistry Medical*, Vol. 2, 1968, pp. 41–52.

PARABOLIC FLIGHT-ATP ANALYSIS

Bulk fluid fg ATP/cell results			
Bulk fluid samples	Standard	Formalin (1%)	Silver (400 µg/L)
Microgravity (n = 51)	2.02E-03 ± 5.75E-04	N/A	2.30E-05 ± 9.07E-06
Lunar gravity (n = 51)	2.70E-03 ± 6.05E-04	2.82E-05 ± 5.57E-06	N/A
Terrestrial gravity (n = 21)	1.10E-03 ± 3.81E-04	1.38E-04 ± 3.31E-05	2.81E-04 ± 8.83E-05

- 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

Birmele, M., Smith, D., Morford, M., Roberson, L., Roberts, M., "Evaluation of an ATP Assay to Quantify Bacterial Attachment to Wetted Surfaces in Variable Gravity Conditions," ICES 2012, AIAA-2012-3508.

Crombrugge, J., and Waes, G., "ATP method. In Methods for assessing the bacteriological quality of raw milk from the farm ed. Heeschen, W," *Brussels: Intl Dairy Fed*, 1991, pp. 53–60.

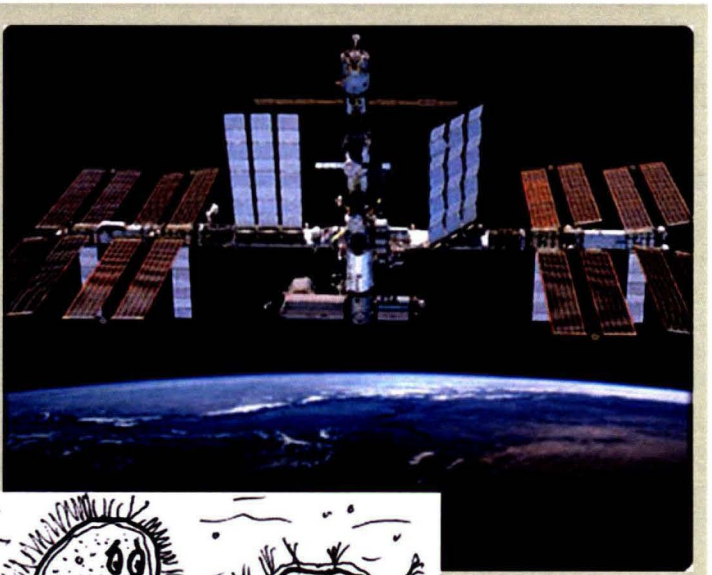
Hampp, R., Hoffmann, E., Schonherr, K., Johann, P., and Filippis, L., "Fusion and Metabolism of Plant Cells as Affected by Microgravity," *Planta*, Vol. 203, 1997, pp. S42–S53.

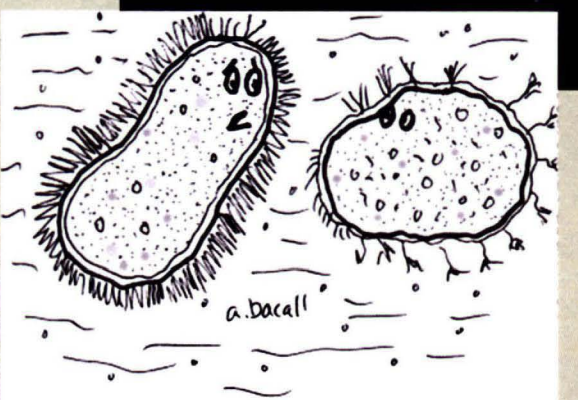
FUTURE WORK WITH ATP

- 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

MICROBIAL IDENTIFICATION

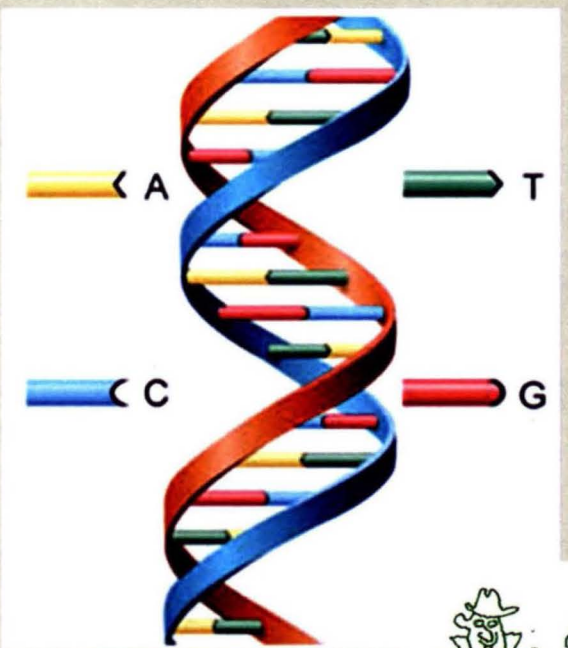
- Applicable on orbit in ISS
  - Needs to function in microgravity
  - Must meet NASA requirements
- Interested in ***<u>What's There</u>***
  - Example: *E.coli*
    - presence = significant
  - Less concerned with viability

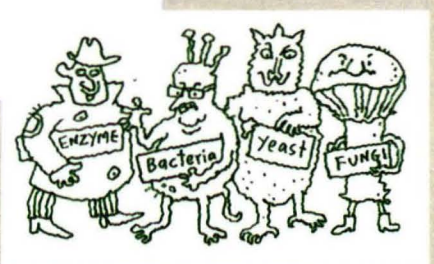




"A geneticist laid bare my genetic blueprint, and now I feel violated."

METHOD IN PURSUIT





- 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 MORE IMPORTANT?

- ***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

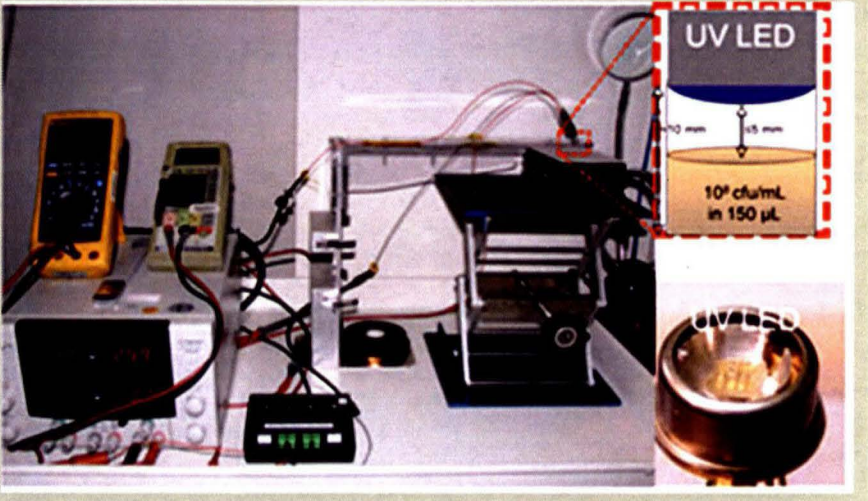
MICROBIAL CONTROL

- 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

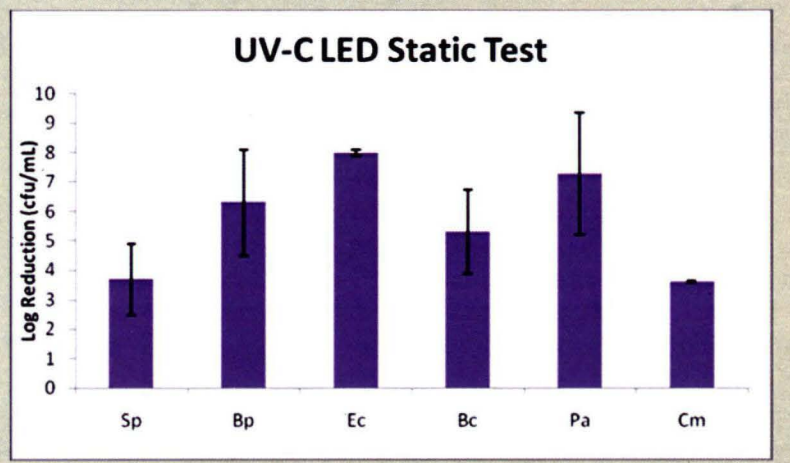
- Goal: Use germicidal UV-C LEDs as a point-of-use sterilization device
  - Maintain drinking water safety
- Challenge bacteria used to inoculate water samples in 96-well plate
- Samples analyzed at several time points to evaluate amount of exposure required for cellular inactivation



Birmele, M., McCoy, L., Soler, R., and Roberts, M.S., Ultraviolet Light Emitting Diodes for Disinfection of Spacecraft Potable Water Systems, 09ICES-0083 / 2009-01-2508.

WHAT WE'VE DONE

UV-C LED STATIC TESTING

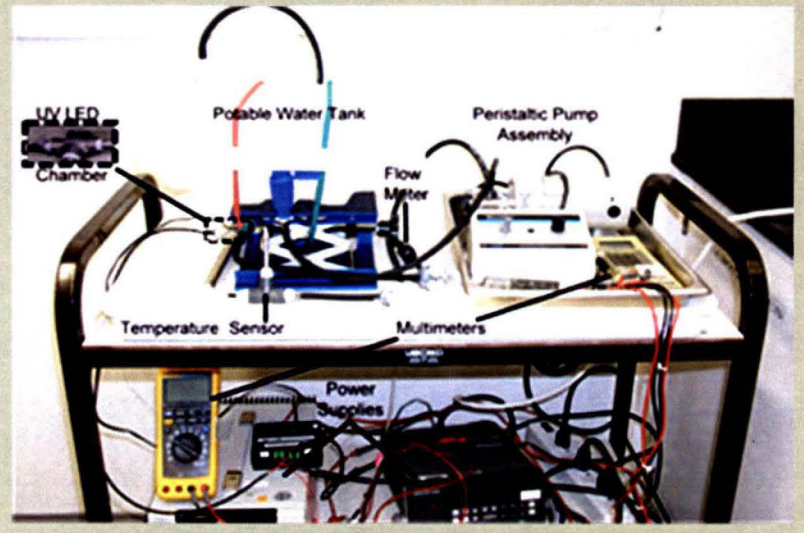


AODCs		
bacteria	log cells/mL at t=0 hour	log cells/mL at t=1 hour
Bc	8.25±0.18	8.75±0.32
Pa	8.45±0.17	8.93±0.32
Cm	7.48±0.72	8.40±0.21
Ec	7.62±0.69	8.52±0.21
Bp	8.08±0.31	8.65±0.26
Sp	7.93±0.30	8.52±0.28

- UV-C LEDs were able to produce a >3 $\log_{10}$ CFU/mL reduction within an hour of contact time in the static system

WHAT WE'VE DONE

UV-C LED RECIRCULATION TESTING

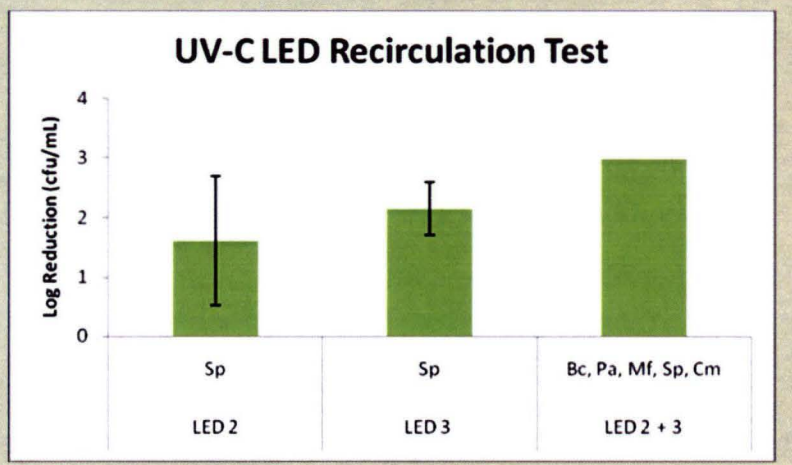


- Goal: Use UV-C LEDs for microbial disinfection in a recirculating potable water system
  - Water was inoculated with either one challenge organism or a mixed community of 5 organisms
- Recirculating tests were run for 24 hours

Birmele, M., McCoy, L., Soler, R., and Roberts, M.S., Ultraviolet Light Emitting Diodes for Disinfection of Spacecraft Potable Water Systems, 09ICES-0083 / 2009-01-2508.

WHAT WE'VE DONE

UV-C LED RECIRCULATION TESTING



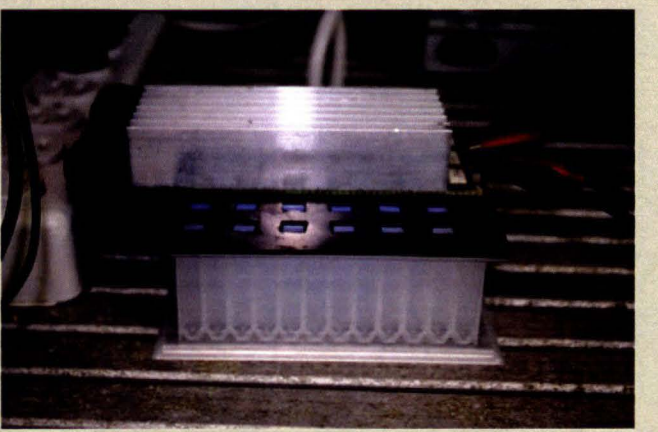
AODCs			
	$\log_{(10)}$ cells/mL for specific LED source treatment		
exposure (hours)	LED 2	LED 3	Both
0:00:00	7.80±0.39	7.44±0.58	7.23
4:00:00	7.74±0.22	7.43±0.38	7.19
16:00:00	7.52±0.17	7.28±0.18	7.17
20:00:00	7.83±0.57	7.03±0.16	7.11
24:00:00	7.62±0.29	7.21±0.02	7.04

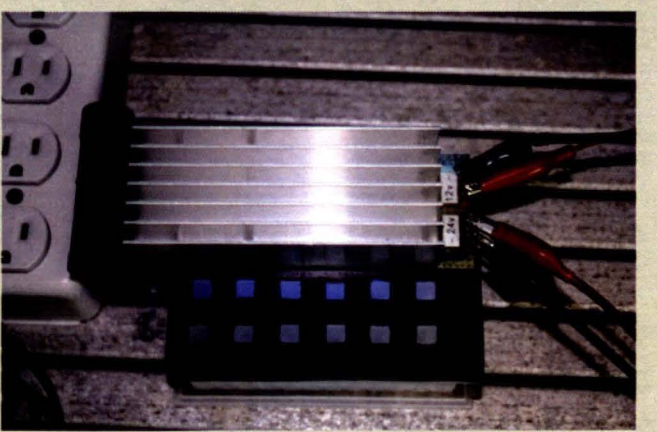
- UV-C LEDs were able to produce a >1.5 $\log_{10}$ CFU/mL reduction within 24 hours of contact time in the recirculating system
  - UV-C LEDs degraded quickly

WHAT WE'VE DONE

UV-A LED STATIC TESTING

- 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





Birmele, M., O'Neil, J., and Roberts, M. Disinfection of Spacecraft Potable Water Systems by Photocatalytic Oxidation Using UV-A Light Emitting Diodes. ICES 2011. AIAA Technical Paper 2011-5276.

WHAT WE'VE DONE

UV-A LED STATIC TESTING

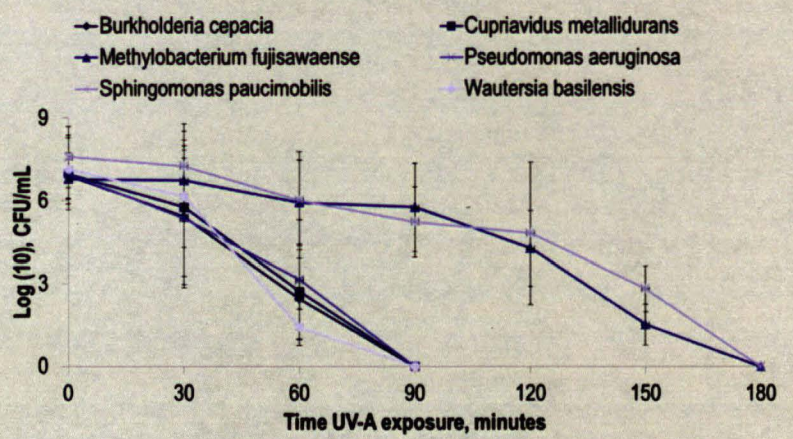


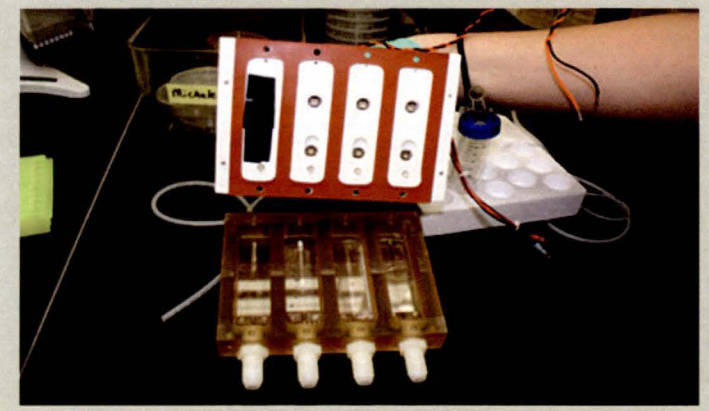
Table 1. AODC and HPC Results (n=6).

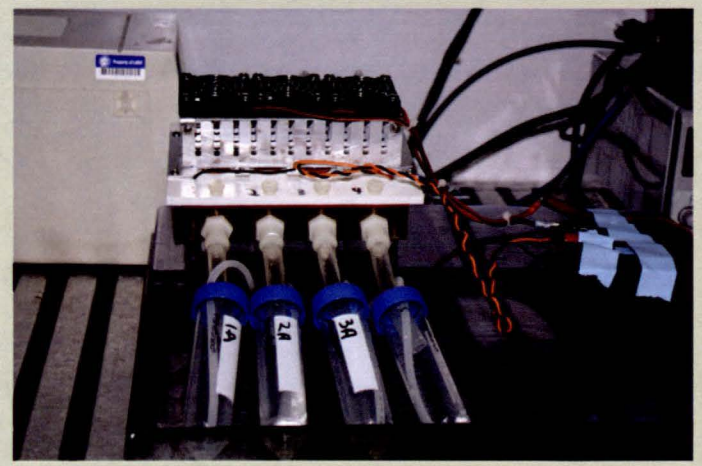
	Before exposure		After exposure	
Log (10)	AODC (cells/mL)	HPC (CFU/mL)	AODC (cells/mL)	HPC (CFU/mL)
Burkholderia cepacia	6.83 ± 0.02	6.92 ± 0.82	6.79 ± 0.04	ND
Cupriavidus metallidurans	7.13 ± 0.06	6.97 ± 1.31	7.12 ± 0.01	ND
Methylobacterium fujisawaense	6.84 ± 0.04	6.78 ± 0.71	6.74 ± 006	ND
Pseudomonas aeruginosa	7.16 ± 0.02	6.96 ± 0.06	7.15 ± 0.02	ND
Sphingomonas paucimobilis	7.42 ± 0.01	7.58 ± 1.12	7.43 ± 0.04	ND
Wautersia basilensis	7.27 ± 0.02	7.14 ± 1.25	7.26 ± 0.02	ND

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

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

Birmele, M., Morford, M., and Roberts, M., "Antimicrobial Resources for Disinfection of Potable Water Systems for Future Spacecraft," ICES 2012, AIAA-2012-3507.

WHAT WE'VE DONE

UV-A LED RECIRCULATING TESTING

DFR Results

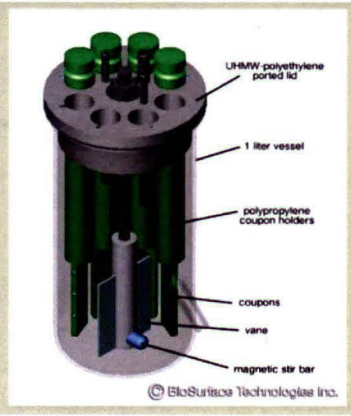
			HPC (log(10) CFU/mL) (n=3)		AODC (log(10) cells/mL) (n=3)	
Expt. #	**Organism**	**Experimental Conditions**	**Initial**	**Final**	**Initial**	**Final**
DFR 1	*E. coli*	UV-A alone	7.32 ± 0.07	**5.17 ± 0.67**	7.09 ± 0.12	7.24 ± 0.18
DFR 2	*P. aeruginosa*	UV-A alone	6.74 ± 0.03	7.35 ± 0.07	6.25 ± 0.12	6.81 ± 0.07
DFR 3	*P. aeruginosa*	UV-A alone	7.61 ± 0.04	8.23 ± 0.11	7.43 ± 0.06	7.90 ± 0.08
DFR 4	*P. aeruginosa*	UV-A alone	7.60 ± 0.04	8.03 ± 0.07	7.48 ± 0.04	8.11 ± 0.52
DFR 5	*P. aeruginosa*	UV-A + titanium dioxide	7.76 ± 0.03	8.10 ± 0.48	7.49 ± 0.02	7.54 ± 0.20
DFR 6	*E. coli*	UV-A + titanium dioxide	7.01 ± 0.06	**5.82 ± 1.17**	6.96 ± 0.18	7.00 ± 0.11
DFR 7	*P. aeruginosa*	UV-A + small silver coupon (12 mm)	7.03 ± 0.01	8.00 ± 0.17	7.33 ± 0.20	8.51 ± 0.06
DFR 8	*P. aeruginosa*	UV-A + large silver coupon (3 x 1 inch)	7.74 ± 0.05	**3.10 ± 1.77**	7.58 ± 0.05	7.60 ± 0.07
DFR 9	*E. coli*	UV-A + small silver coupon (12 mm)	7.02 ± 0.06	6.45 ± 0.09	6.97 ± 0.01	6.84 ± 0.09
DFR 10	*E. coli*	UV-A + large silver coupon (3 x 1 inch)	7.20 ± 0.03	**4.53 ± 2.78**	7.03 ± 0.02	7.05 ± 0.08

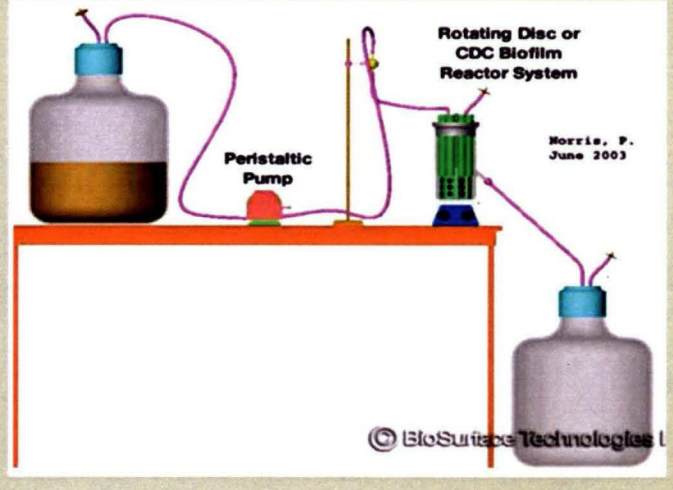
Notes- Red highlight indicate discussed results. +/- represents the standard deviation between replicates.

- 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





Birmele, M., Morford, M., and Roberts, M., "Antimicrobial Resources for Disinfection of Potable Water Systems for Future Spacecraft," ICES 2012, AIAA-2012-3507.

WHAT WE'VE DONE

ANTIMICROBIAL MATERIALS

CBR Bulk Fluid Results

	HPC (log(10) CFU/mL)		AODC (log(10) cells/mL) (n=5)	
CBR	Initial	Final	Initial	Final
31	5.58	7.46	6.34 ± 0.01	67.17 ± 0.04
32	5.46	ND	6.31 ± 0.06	5.87 ± 0.06
33	5.39	ND	6.45 ± 0.05	4.61 ± 0.29
34	5.67	ND	6.43 ± 0.04	ND
35	5.54	5.18	6.45 ± 0.04	5.36 ± 0.08
39	5.61	0.48	5.62 ± 0.03	4.74 ± 0.20

+/- represents the standard deviation between replicates.

CBR Coupon Results

	HPC (log(10) CFU/coupon)		AODC (log(10) cells/coupon)	
CBR	Control	Treatment	Control	Treatment
2 (n=15)	7.82 ± 0.22	7.57 ± 0.20	7.71 ± 0.21	7.50 ± 0.21
33 (n=10)	7.49 ± 0.15	ND	7.70 ± 0.14	5.37 ± 0.19
35 (n=10)	7.70 ± 0.19	3.38 ± 0.31	7.60 ± 0.22	4.56 ± 0.17
41 (n=12)	8.33 ± 0.05	8.10 ± 0.03	8.58 ± 0.30	8.35 ± 0.17

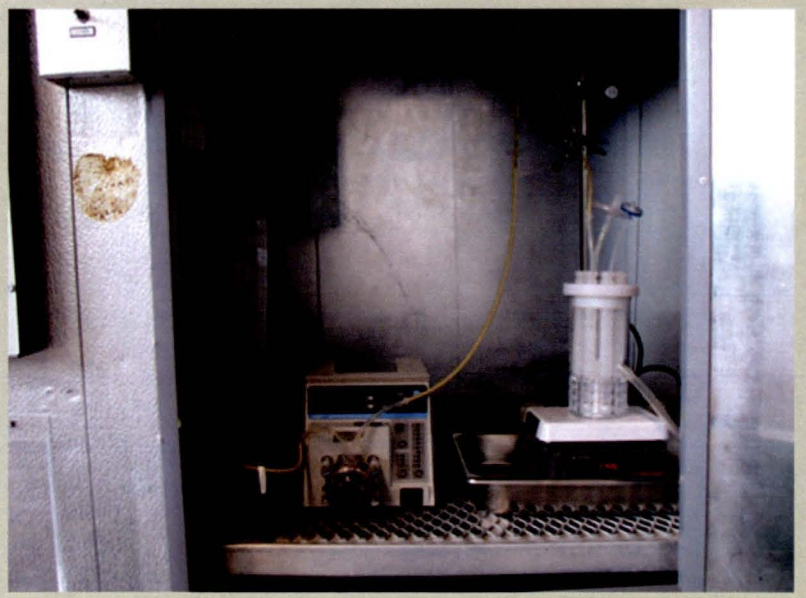
ND= none detected. The control samples did not have the application of the treatment with anticipated antimicrobial efficacy. +/- represents the standard deviation between replicates.

- 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 leeched 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

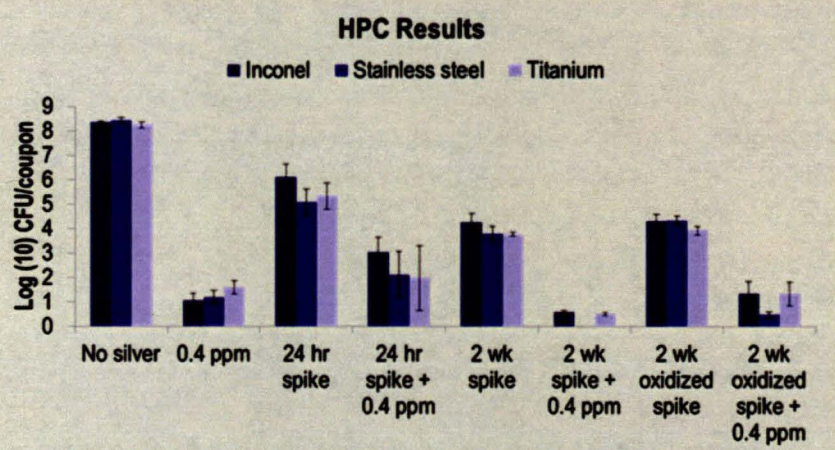


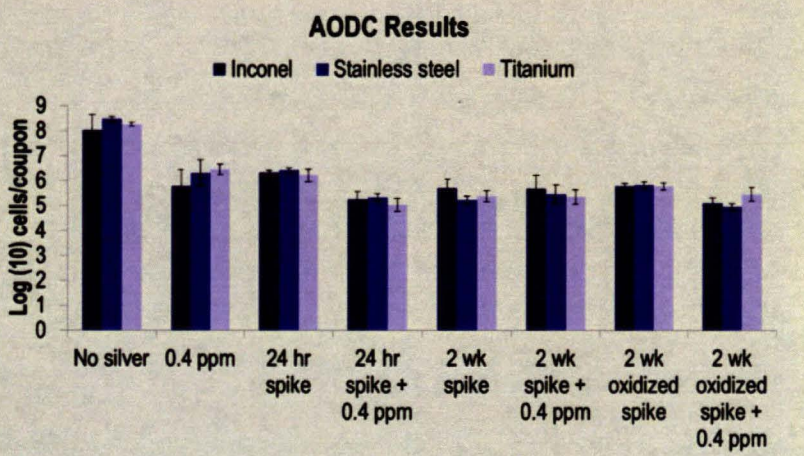
- Goal: Prolong silver efficacy by maintaining solubility
  - Safe limits for human consumption
  - Limit bacterial growth
- Metal coupons used for potable water systems in space exposed to silver fluoride treatments
- Performed in a CDC biofilm reactor to test efficacy of coupons

Birmele, M., McCoy, L., and Roberts, M.S., Disinfection of Spacecraft Potable Water Systems by Passivation with Ionic Silver, ICES 2011, AIAA Technical Paper 2011-5278.

WHAT WE'VE DONE

PASSIVATION WITH IONIC SILVER



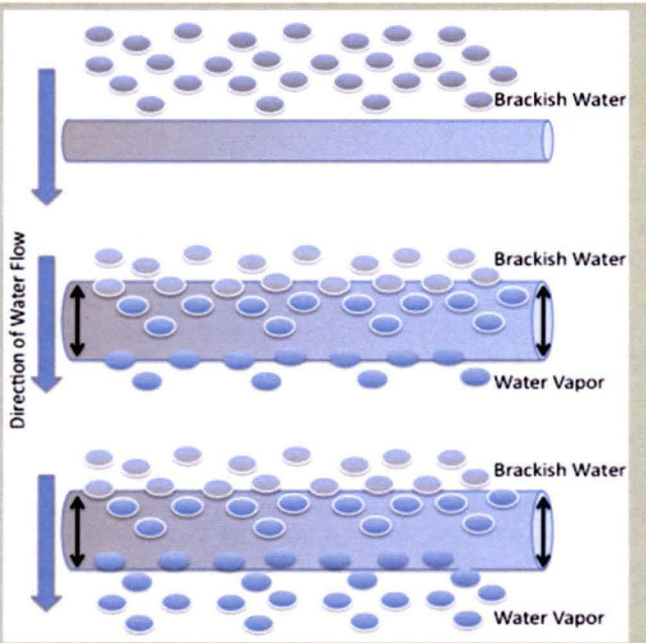


- 0.4 ppm silver was an effective biocide
- Pre-passivation with 50 ppm provided additional microbial control
- Slow degradation of silver by adherence on coupon surfaces
- Loss of residual antimicrobial activity minimized

WHAT WE'VE BEEN DOING

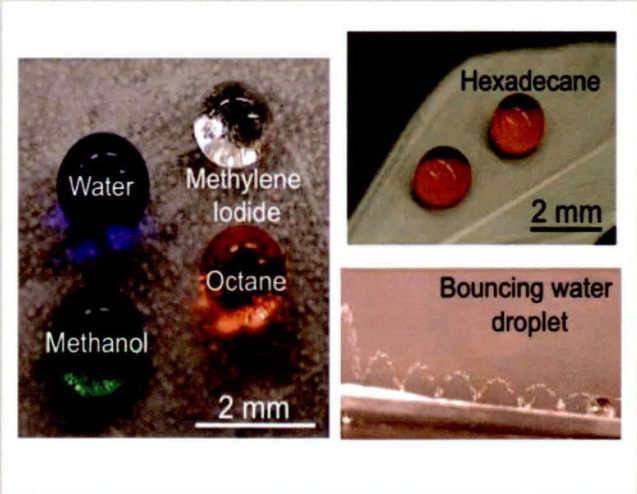
PERVAPORATION MEMBRANE STUDIES

- Goals
  - Evaluate microbial transfer through the membrane
  - Evaluate biofouling of the membrane
  - Evaluate materials properties of the membrane

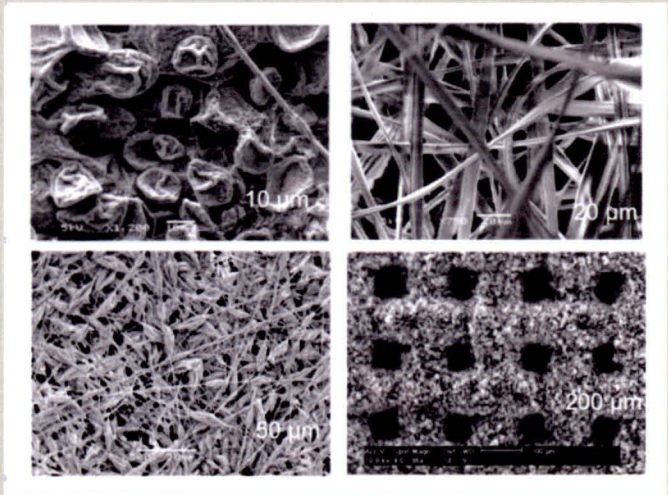


WHAT WE'D LIKE TO EXPLORE

- Omniphobic Materials
  - Surface treatments for metal and polymer materials that yield contact angles great 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

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