

Evaluation of Electrochemically Generated Potable Water Disinfectants for Use on the International Space Station

Branelle Rodriguez,¹ Molly Anderson,² and Niklas Adam³
NASA Johnson Space Center, Houston, Texas, 77058

Leticia Vega⁴
Jacobs Technologies, Houston, Texas, 77058

and

Douglas Botkin⁵
Enterprise Advisory Services, Inc., Houston, Texas, 77058

Microbial contamination and subsequent growth in spacecraft water systems are constant concerns for missions involving human crews. The current potable water disinfectant for the International Space Station (ISS) is iodine; however, with the end of the Space Shuttle Program, there is a need to develop redundant biocide systems that do not require regular up-mass dependencies. Throughout the course of a year, four different electrochemical systems were investigated as a possible biocide for potable water on the ISS. Research has indicated that a wide variability exists with regards to efficacy in both concentration and exposure time of these disinfectants; therefore, baseline efficacy values were established. This paper describes a series of tests performed to establish optimal concentrations and exposure times for four disinfectants against single and mixed species planktonic and biofilm bacteria. Results of the testing determined whether these electrochemical disinfection systems are able to produce a sufficient amount of chemical in both concentration and volume to act as a biocide for potable water on the ISS.

Nomenclature

<i>cfu</i>	= colony-forming units
<i>DI</i>	= deionized water
<i>ECD</i>	= Electrochemical Disinfection
<i>H₂O₂</i>	= hydrogen peroxide
<i>ISS</i>	= International Space Station
<i>JSC</i>	= Johnson Space Center
<i>L</i>	= liter
<i>MBECTM</i>	= Minimum Biofilm Eradication Concentration
<i>min</i>	= minutes
<i>mg</i>	= milligram

¹ Aerospace Engineer, Crew & Thermal Systems Division, 2101 NASA Parkway, Houston, Texas, 77058, Mail Stop: EC3, not AIAA affiliated.

² Chemical Engineer, Crew & Thermal Systems Division, 2101 NASA Parkway, Houston, Texas, 77058, Mail Stop: EC2, not AIAA affiliated.

³ Chemist, Crew & Thermal Systems Division, 2101 NASA Parkway, Houston, Texas, 77058, Mail Stop: EC3, not AIAA affiliated.

⁴ Microbiologist, Crew & Thermal Systems Division, 2101 NASA Parkway, Houston, Texas, 77058, Mail Stop: EC3, not AIAA affiliated.

⁵ Senior Scientist, NASA-JSC Microbiology Laboratory, Wyle IS&E/Enterprise Advisory Services, Houston, Texas, 77058 and AIAA Member.

<i>mL</i>	=	milliliter
<i>NaOCl</i>	=	sodium hypochlorite
<i>NASA</i>	=	National Aeronautics and Space Administration
<i>PAA</i>	=	peracetic acid
<i>PBS</i>	=	phosphate buffered saline
<i>R2A</i>	=	Reasoner's 2A
μL	=	microliter

I. Introduction

The Electrochemical Disinfection (ECD) feasibility assessment investigated the capability of four disinfectants for possible use on the International Space Station (ISS) as a secondary means of disinfection. The four biocides selected for this study were hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), peracetic acid (PAA), and ozone. All four biocides are considered strong oxidizers; however, they have different mechanisms of reacting with bacteria. Each biocide was tested to understand its biocidal efficacy on planktonic and biofilm bacteria. Planktonic testing determines whether the disinfectant can kill cells that are already free-floating in water. Biofilm testing is designed to evaluate whether the disinfectant agent can disrupt or penetrate established cell communities located on a surface.

Testing was first conducted using commercially obtained biocides with *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) to optimize test procedures and methods. Electrochemically generated biocides were then tested against bacteria that have been identified, on the ISS, as iodine resistant. These bacteria are defined as the ISS consortium and consist of the following five bacteria: *Methylobacterium fujisawaense*, *Burkholderia cepacia*, *Sphingomonas paucimobilis*, *Cupriavidus metallidurans*, and *Cupriavidus basilensis*.

During test methodology development, the team measured the range of performance by determining how many orders of magnitude of cell reduction that occurred in viable populations of bacteria. A reduction criteria of 5 logs was set as a performance goal, but not as a pass-fail criteria. A 1×10^7 colony-forming units (cfu)/milliliter (mL) starting concentration of bacteria was used to ensure that the difference between starting concentrations and final concentrations could be clearly identified. Planktonic and biofilm testing were conducted in three distinct phases. The first phase was to determine the approximate concentration and exposure time necessary to reduce the number of bacteria by 5 logs. The second phase of testing was conducted with commercially available versions of the four biocides against the ISS consortium. This testing provided a baseline comparison for the evaluation of the electrochemically generated biocides. The final phase of testing was conducted with the four electrochemically generated biocides against the ISS consortium.

Iodine is the current disinfectant for the United States (U.S.) potable water system on the ISS. Chemical characteristics of each biocide were investigated to characterize the shelf life both with and without bacteria, as well as to characterize the compatibility of each biocide with iodine. These tests were conducted to provide an understanding of any chemical compatibility issues prior to integrating future electrochemically generated biocides on the ISS.

Metallic and non-metallic materials testing was also conducted with the four commercially available biocides. Details of the testing and results are discussed in a separate paper.¹

II. Test Methodology Development

The team examined the effect of each of the four biocides on the concentrated planktonic and biofilm bacteria. The first phase was to determine the approximate concentration and exposure time necessary to reduce the number of bacteria by 5 logs. This was conducted using commercially available versions of the four biocides. The bacterium selected was *E. coli* and *P. aeruginosa*, which provided the means to optimize test procedures for both planktonic and biofilm methodology before testing against the ISS consortium. *E. coli* (American Type Culture Collection #8739) was selected for use as the planktonic bacteria since it is considered a standard test organism for a number of different methods, including evaluation of antimicrobial hand washing soaps, disinfection efficacy testing, and quality control testing. *P. aeruginosa* was selected for use as the biofilm bacteria since it is known to efficiently form biofilms, as well as being recommended by the American Society for Testing and Materials.

Ozone test procedures were modified, relative to the other three biocides, due to the unique characteristics of ozone. The lifetime of ozone in solution was determined to be approximately 15 minutes (min); therefore, a modified test plan was required to conduct testing. An overview of the testing is discussed below.

A. Planktonic Testing

The first phase of planktonic tests was performed to determine the approximate concentration and exposure time necessary to reduce the number of planktonic bacteria in phosphate buffered saline (PBS) by 5 logs, as well as to determine the best methodology for testing the ISS consortium. This first series was conducted using commercially available versions of the four biocides with standard *E. coli* test bacterium. With the exception of ozone, which will be discussed below, the test method was as follows: a bacterial concentrate was added to sterile solution of PBS, mixed well, and divided into three to four beakers, depending on the number of test concentrations per biocide. A baseline sample was collected from each beaker prior to the addition of the biocide to confirm that each test solution contained the required concentration of bacteria for testing and to ensure there was nothing in the test solution that would potentially interfere with the biocide measurements. The biocide was then added to the solution and allowed to mix thoroughly. Samples were gently stirred and collected, after the addition of the bacteria, at the following time points: 0, 10, 30, 60, and 120 (H₂O₂ only) min. All test points were collected in triplicate. Samples collected for microbial enumeration were immediately added to a sample tube containing quench solution to inactivate the biocide, thereby mitigating any effects of biocide carryover on cell viability.

Since ozone had to be produced in PBS, the test method was modified. The PBS was ozonated to the targeted concentration, the concentration was verified, and a bacterial inoculum was added to the ozonated PBS. Ozone testing then followed the same test procedures as the other three biocides. The solution was allowed to mix thoroughly, and samples were collected at time points 0, 10, 30 and 60 min for analysis.

All samples were directly plated using an Autoplate[®] 4000. A 0.1 mL aliquot of quenched sample was added to 9.9 mL buffer solution. A total of 50 microliter (μL) was dispensed onto a Reasoner's 2A (R2A) nutrient agar plate, an additional 50 μL from the same tube was spiral plated onto a second R2A plate, and finally a 250 μL sample was dispensed from the sample tube onto a third R2A plate. The objective was to determine the concentration of bacteria using a wide range of sample dilutions while minimizing the number of agar plates used. All plates were incubated at 30°C ± 2°C for 48 hours.

B. Biofilm Testing

The first phase of biofilm tests was performed to determine the approximate concentration and exposure time necessary to reduce the number of biofilm bacteria, as well as to determine the best methodology for testing against the ISS consortium. *P. aeruginosa* was grown on nutrient agar plates and enumerated. A standardized inoculum of 1 x 10⁷ cfu/mL was used to inoculate Minimum Biofilm Eradication Concentration (MBEC[™]) lids and trough devices. Biofilm growth was present on individual pegs attached to the MBEC[™] lid after 24 hours at 35°C. The growth control pegs remaining on each challenge plate throughout the testing were used to help determine the log reduction in biofilm concentration after biocide exposure. This setup permitted examination of test conditions and controls (both assayed in triplicate) using a 96 well plate during biocide challenge.

Three controls were used on each MBEC[™] plate. One control was PBS in the plate wells with the corresponding pegs removed (negative control). The second control was biofilm-laden pegs treated with biocide quench solution. The final control was biofilm-laden pegs treated with PBS. One MBEC[™] lid was inoculated per time point tested, and the efficacy of one biocide was evaluated per test day. Working concentrations of H₂O₂, NaOCl, and PAA were made using PBS.

Ozone was generated in sterile PBS. Aliquots of each stock and working solution were removed for determination of biocide concentration and pH prior to addition to the challenge plates. No further measurements were possible due to the very small (< 200 μL) volume remaining in the challenge plates.

P. aeruginosa was exposed to different concentrations of each of four potable water biocides at contact times up to 2 hours. At each time point, the MBEC[™] lid was rinsed and sonicated to remove the remaining attached biofilm. Samples from each well were spot plated for bacterial enumeration. Spread plating was also employed to permit a more accurate count of each of the remaining strains in the biofilm population.

III. Establishing an Efficacy Baseline

The objective of the second phase of testing was to establish an efficacy baseline using commercially available versions of the four biocides. Additionally, optimal concentrations and exposure times were determined for each biocide. The five bacteria identified as part of the ISS consortium were used for both planktonic and biofilm testing. As stated previously, the ISS potable water system disinfectant is iodine. The bacterial species in the ISS consortium are considered to be iodine resistant.^{2,3}

A. Planktonic Bacteria Testing and Results

ISS consortium bacteria were exposed to different concentrations of each of the four biocides over the course of 60 min (120 min for H₂O₂). Test methodology was defined during previous testing and implemented in this phase of testing. Samples were removed at different time points to determine bacterial concentration, biocide concentration, and pH of the mixture. Concentrations of the commercially available versions of the four biocides, maximum log reductions, and the associated exposure times are listed in Table 1.

Three concentrations of H₂O₂ were selected for testing (Table 1): 500 milligram (mg)/liter (L), 1,000 mg/L, and 30,000 mg/L. Previous experiments revealed low levels of biocide activity (data not shown) for H₂O₂. Therefore, a 30,000 mg/L concentration of H₂O₂ was tested as a positive control for the antimicrobial activity of this biocide. The H₂O₂ exhibited minimal effectiveness at test concentrations of 500 mg/L and 1,000 mg/L, with a less than 1-log reduction in the concentration of bacteria. The 30,000 mg/L concentration demonstrated more than a 5-log reduction of bacteria after relatively long exposure periods (60 min and 120 min). One notable observation was made during planktonic testing. The bacteria that were viable after exposure to the 30,000 mg/L H₂O₂ were primarily *Sphingomonas paucimobilis*. Based on the results of the commercial H₂O₂ testing, higher concentrations were investigated for breadboard-generated H₂O₂.

Three concentrations of NaOCl were selected for testing (Table 1): 0.01 mg/L, 0.1 mg/L and 1 mg/L. It was challenging to determine an estimate of relative species abundance due to relatively high concentrations of bacteria remaining after being exposed to either 0.01 mg/L or 0.1 mg/L of NaOCl. Although *Methylobacterium fujisawaense*, *Sphingomonas paucimobilis*, *Burkholderia cepacia*, and *Cupriavidus* species (the two strains are indistinguishable on agar plates) were present after being exposed to 1 mg/L NaOCl, *Methylobacterium fujisawaense* was the most abundant species present. The best observed performance was a moderate 2.9-log reduction in the bacterial concentration after treatment with 1 mg/L NaOCl after 60 min of exposure, which remained below the 5-log reduction goal.

Three concentrations of PAA were selected for testing (Table 1): 1 mg/L, 5 mg/L, and 10 mg/L. Peracetic acid exhibited limited effectiveness with a 1 mg/L biocide concentration achieving only a 0.2-log reduction, 5 mg/L achieving a 1.2-log reduction, and 10 mg/L achieving a 1.4-log reduction in overall bacterial concentration after 60 min of exposure. A high survival rate of all of the ISS consortium members occurred in the final cultured samples. As a result, higher concentrations were selected to be evaluated during the breadboard-generated NaOCl testing.

Three concentrations of ozone were selected for testing (Table 1): 0.1 mg/L, 0.5 mg/L, and 1 mg/L. The 1 mg/L ozone treatment of bacteria demonstrated a very high biocidal activity upon contact with the bacteria. A greater than 4-log reduction in viable bacteria was observed in the first sample taken (time = 0 min). Treatment with either 0.1 mg/L or 0.5 mg/L ozone resulted in similar log reductions of bacteria, but the exposure times were longer.

Two lessons learned from this portion of the study were applied to subsequent planktonic bacteria testing. First, excluding the ozone testing results, only the higher concentrations of each biocide demonstrated log reductions close to the performance goal of 5 logs, and only at the longer exposure times. Since biofilms are known to be more resistant than planktonic bacteria to disinfectants, the concentration of biocide was increased for breadboard-generated biocide testing. In addition, since the results from the samples at 10 min were similar to those samples collected at 30 min, the 10-min time point was removed from the third test phase.

Table 1. Commercial planktonic evaluation biocide concentrations and results

Biocide	Planktonic Commercial Concentration (mg/L)		
	Low	Mid	High
	Log Reduction @ Time Point (min.)		
H ₂ O ₂	500	1,000	30,000
	0.6 @ 120	0.6 @ 120	5.8 @ 60
Ozone	0.1	0.5	1
	4.0 @ 30	4.1 @ 30	4.3 @ 0
PAA	1	5	10
	0.2 @ 60	1.2 @ 60	1.4 @ 60
NaOCl	0.01	0.1	1
	0.1 @ 10	0.2 @ 60	2.9 @ 60

B. Biofilm Testing and Results

The second phase of the testing evaluated the effect of commercially available versions of the four biocides on the concentration of ISS consortium biofilms. MBEC™ plates were inoculated with 1×10^8 cells/mL of the ISS consortium for 24 hours at 35°C. A spread plating step following spot plating was included during testing of the ISS consortium. This was a process improvement that increased the sensitivity of bacterial enumeration to 10 cfu/mL, and permitted a more straightforward method for identification of ISS consortium members. Concentrations of the commercially available versions of the four biocides, maximum log reductions, and the associated exposure times are listed in Table 2.

Three concentrations of H₂O₂ were selected for biofilm testing (Table 2): 500 mg/L, 1,000 mg/L, and 30,000 mg/L. The 500 mg/L and 1,000 mg/L were unable to reduce the concentration of bacteria by more than approximately 2 logs. The 30,000 mg/L concentration was able to reduce the biofilm concentration by 3.8 logs, with the predominant remaining bacteria being *Burkholderia cepacia*. The other members of the ISS consortium, if detected on the particular spot plate counted, were in approximately equal ratios per well. The highest H₂O₂ concentration tested (30,000 mg/L) is impractical for breadboard production. Therefore, future test concentrations of H₂O₂ were substantially increased due to marginal biocide activity of the 500 mg/L and 1,000 mg/L solutions against the ISS consortium biofilms.

Two concentrations of NaOCl were selected for biofilm testing (Table 2): 5 mg/L and 10 mg/L. The 5 mg/L test solution yielded a maximum 0.4-log reduction at 10 min. After 10 min in the 5 mg/L of NaOCl, two of three MBEC™ wells exhibited a shift in relative position between *Sphingomonas paucimobilis* and the *Cupriavidus* species. This result is not unexpected as low concentrations (<1 mg/L) of NaOCl are known to change the microbial composition of a multispecies proteobacterial biofilm.⁴ The 10 mg/L test solution yielded a maximum log reduction of only 0.8 at 30 min. One well yielded no detectable organisms on the spread plate. The remaining two wells exhibited an ISS consortium distribution similar to that described for the 10-min treatment with 5 mg/L NaOCl. This biocide exhibited the lowest biocidal activity of any commercially available biocide or concentration tested in this biofilm study. Future NaOCl test concentrations were increased due to the very low biocidal efficacy against ISS consortium biofilms.

Two concentrations of PAA were selected for biofilm testing (Table 2): 30 mg/L and 180 mg/L. After a 30-min exposure to both 30 mg/L and 180 mg/L concentrations, an approximate 5-log reduction in the biofilm concentration was achieved. At the 30-min timepoint, no organisms were detected in samples from two of three MBEC™ wells treated with 30 mg/L of biocide and three of three wells treated with 180 mg/L of the biocide. The biocidal effect was present within 10 min of exposure, where approximately 3.3- and 2.6-log reductions were observed for 30 mg/L and 180 mg/L PAA concentrations, respectively.

Two concentrations of ozone were selected for biofilm testing (Table 2): 0.5 mg/L and 1 mg/L. Treatment with a 0.5 mg/L solution resulted in a maximum reduction of 1.3 logs at 30 min. The most abundant species was *Sphingomonas paucimobilis*, followed by *Burkholderia cepacia*, *Cupriavidus* species, and *Methylobacterium fujisawaense*. A maximum 1.9-log reduction in biofilm concentration was observed at 10 min after exposure to a 1 mg/L ozone solution. After a 10-min exposure to 1 mg/L ozone, the relative distribution (most to least abundant) of bacteria in the biofilm was *Burkholderia cepacia*, *Sphingomonas paucimobilis*, *Cupriavidus* species, and *Methylobacterium fujisawaense*. Future ozone test concentrations were increased due to their marginal effectiveness against the ISS consortium bacteria.

The spread plate analysis provided allowed the capability of easily distinguishing the majority of the ISS consortium (the *Cupriavidus* species were indistinguishable on agar plates), leading to the opportunity to gain a general understanding of the specific effects each biocide has on biofilm composition. Based on the test results with the commercially available versions of the four biocides, the test concentrations for the breadboard-generated biocides were increased, taking into account the practical limitations of the maximum output from the breadboard modules.

Table 2. Commerical biofilm evaluation biocide concentrations and results.

Biocide	Biofilm Commercial Concentration (mg/L)		
	Low	Mid	High
	Log Reduction @ Time Point (min.)		
H ₂ O ₂	500	1,000	30,000
	1.7 @ 10	1.8 @ 10	3.8 @ 10
Ozone	0.5	1	-
	1.3 @ 30	1.9 @ 10	-
PAA	30	180	-
	4.8 @ 30	5.3 @ 30	-
NaOCl	5	10	-
	0.4 @ 10	0.8 @ 30	-

IV. Electrochemically Produced Biocide Testing

The objective of the third and final phase of testing was to test the breadboard-generated biocides against the ISS consortium and compare the results to the baseline established in the second phase of this project. Biocide concentrations were determined based on the previous testing, and the capability of the biocide breadboard production. The H₂O₂ breadboard unit that was developed during this phase of the project successfully generated 10 mL of H₂O₂ at a concentration of 30,000 mg/L in 1 hour. The NaOCl breadboard unit that was developed during this phase of the project successfully generated 0.5 L of NaOCl at a concentration of 10,000 mg/L in 1 hour. The PAA breadboard unit developed during this phase of the project successfully generated 1 L of PAA at a concentration of 2,000 mg/L in 1 hour. The ozone breadboard unit developed during this project successfully generated 0.5 L of ozone at a concentration of 1 mg/L in 15 min.

A. Planktonic Testing and Results

The results from the commercially available biocide testing illustrated the need to increase the test concentrations for the breadboard evaluation. The concentrations that were selected were based on the anticipated likelihood of biocidal activity and materials compatibility, and the ability of the breadboard units to produce the test concentrations.

Planktonic bacteria were tested using the breadboard-generated H₂O₂ concentrations of 1,000 mg/L and 10,000 mg/L (Table 3). The 1,000 mg/L concentration was retained to ensure that a minimum of one test point would be available for comparison to the results of the commercial H₂O₂. The 30,000 mg/L concentration was not replicated due to the impractical amount of time required to produce the volume of biocide required for planktonic bacteria testing. The 10,000 mg/L concentration was selected for evaluation based on commercial biocide results. This concentration was determined to be within an acceptable breadboard production timeframe, and it was considered a concentration that may be effective in meeting the 5-log reduction of bacteria. The 1,000 mg/L concentration had minimal effect with a maximum 0.5-log reduction (Fig. 1).

Table 3. Breadboard-generated planktonic evaluation concentrations.

Biocide	Planktonic Breadboard Concentration (mg/L)		
	Low	Mid	High
H ₂ O ₂	1,000	10,000	-
Ozone	0.1	0.5	1
PAA	10	20	30
NaOCl	1	5	10

The 10,000 mg/L concentration was very effective, with an overall 5.3-log reduction in viable bacteria at 60 min. Observations made during testing of breadboard-generated H₂O₂ were similar to those made during testing of the commercial H₂O₂. Bubbles were visually present in all test vessels, with more bubbles present in the higher concentrated solutions. Additionally, in the 1,000 mg/L H₂O₂-treated samples, the predominant bacteria appeared to be yellow, indicating the presence of *Sphingomonas paucimobilis*.

Based on the poor performance of the commercial NaOCl, the concentrations of NaOCl tested were increased for breadboard-generated planktonic testing. A 1 mg/L concentration was tested to compare the results against the established baseline. The 5 mg/L and 10 mg/L concentrations were added in an attempt to achieve greater biocidal efficacy from the NaOCl (Table 3). Interestingly, the breadboard-generated NaOCl did appear to be more effective than the commercial NaOCl. The 1 mg/L concentration achieved a calculated 6.9-log overall reduction in bacteria after 60 min (Fig. 1). Most of the surviving bacteria appeared to be *Methylobacterium fujisawaense*. Immediately after exposure, no viable bacteria were detected in test solutions treated with NaOCl concentrations of 5 mg/L and 10 mg/L. At timepoint zero, bacteria concentrations were reduced greater than 5 logs. In these tests, the concentration of NaOCl is measured as free chlorine in the concentrate produced by the breadboard generator and then diluted to the proper concentration for testing. It is likely that different active species may be present in the breadboard-generated biocide, which could contribute to the increased reduction of bacteria. Another possible reason for increased effectiveness is that the concentration of biocide was increased up to 1 log-fold higher.

Based on the effectiveness of the commercial PAA testing, a change in concentrations was required to see improvement in biocidal activity. PAA concentrations of 10 mg/L, 20 mg/L, and 30 mg/L generated by the breadboard were selected for testing (Table 3). Of note, a greater biocidal efficacy was observed with the breadboard-generated solution when the same concentration of PAA was examined. It is assumed that intermediates or residual reactants from the process of making PAA also reduce bacterial viability. The 10 mg/L concentration achieved a 4.7-log reduction, nearly reaching the 5-log target, whereas testing of the 20 mg/L and 30 mg/L concentrations revealed bacterial counts below the level of detection, suggesting a biocidal efficacy at or near the 5-log reduction goal (Fig. 1).

Ozone solutions of 0.1 mg/L, 0.5 mg/L, and 1 mg/L were generated by the breadboard unit for planktonic testing (Table 3). The 1 mg/L concentration reduced the bacterial concentration by more than the 5 logs, exceeding the target goal. The 0.5 mg/L and 0.1 mg/L concentrations did not reach the same level of effectiveness as the commercial ozone solutions after 60 min, as the breadboard-generated ozone reached maximum reductions of 3.3 logs (at 30 min) and 4.2 logs (at 30 and 60 min) (Fig. 1).

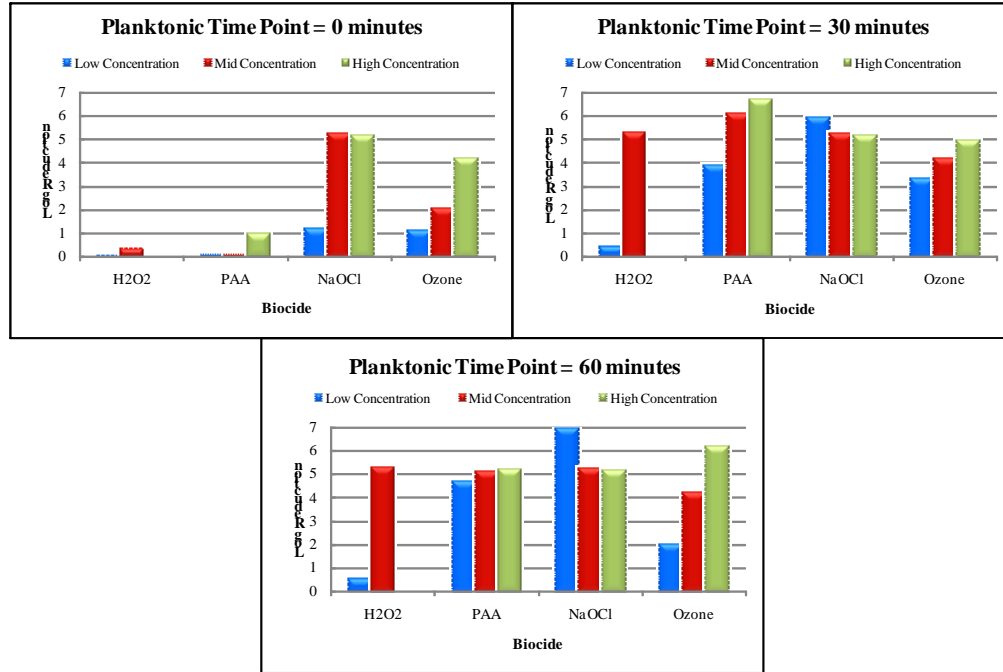


Figure 1. Breadboard-generated planktonic evaluation results.

B. Biofilm Testing and Results

ISS consortium species were used to inoculate MBEC™ devices for biofilm production. These ISS consortium biofilms were exposed to different concentrations of each of the four electrochemically produced potable water biocides at contact times up to 60 min. Testing was performed as described when evaluating the antimicrobial efficacy of commercially produced biocides on ISS consortium biofilms.

Concentrations selected to determine the effectiveness of the H₂O₂ generated by the breadboard units against biofilms were 1,000 mg/L, 10,000 mg/L, and 30,000 mg/L (Table 4). The 1,000 mg/L concentration was selected to provide a comparison against the commercial biocide results. The 10,000 mg/L concentration was used to identify a higher concentration that would potentially be more effective. The 30,000 mg/L concentration was intended as a control to demonstrate that H₂O₂ could eventually be effective at high enough concentrations. Contrary to the planktonic bacteria evaluation, the 30,000 mg/L concentration was tested against biofilms due to the smaller biocide volumes required for biofilm testing. The maximum log reduction in bacterial concentration observed while testing 1,000 mg/L and 10,000 mg/L solutions was 1.6 and 1.0, respectively, each observed at 30 min after biocide exposure (Fig. 2). The greatest log reduction of the positive control concentration of 30,000 mg/L was 5.8, observed 60 min after biocide exposure. In fact, no colonies were detected on the spread plates, indicating a high level of killing since the limit of detection is just 10 cfu/mL.

Breadboard-generated PAA concentrations were increased to 30 mg/L and 180 mg/L due to the minimal effectiveness of commercial PAA at the lower concentrations (Table 4). The greatest reduction in biofilm concentration (3.9 logs) was observed following a 30-min contact time with 180 mg/L of the biocide. A maximum log reduction of 3.2 was noted for 30 mg/L of PAA at 60 min after exposure (Fig. 2). This is higher than the test with the

Table 4. Breadboard-generated biofilm evaluation concentrations and results.

Biocide	Biofilm Breadboard Concentration (mg/L)		
	Low	Mid	High
H ₂ O ₂	1,000	10,000	30,000
Ozone	0.5	1	-
PAA	30	180	-
NaOCl	5	10	-

commercially available PAA, but still below the 5-log reduction goal that was used as the performance target.

Despite the limited biocidal efficacy of the commercial NaOCl solution, concentrations were not increased for the breadboard testing. Initial metals immersion data indicated that the 10 mg/L concentration had compatibility concerns¹; therefore, it was determined to keep the maximum concentration of NaOCl at 10 mg/L. The NaOCl concentrations tested were 5 mg/L and 10 mg/L (Table 4). The 5 mg/L solution yielded a maximum log reduction in the biofilm concentration of 4.3 at 60 min, whereas the 10 mg/L solution produced a 5.6-log reduction in 60 min (Fig. 2). The biofilm results also demonstrated that the biocide generated by the breadboard was more effective than the commercial disinfectant when equivalent concentrations of the NaOCl were present. In these tests, the concentration of NaOCl is measured as free chlorine in the concentrate produced by the breadboard generator and then diluted to the proper concentration for testing. It is likely that different active species may be present in the breadboard-generated biocide, which could contribute to the increased reduction of bacteria.

Concentrations of breadboard-generated ozone were not increased due to production limitations. Biofilm testing of breadboard-generated ozone was conducted with concentrations of 0.5 mg/L and 1 mg/L (Table 4). The largest log reduction for 0.5 mg/L ozone was 0.6, observed after a 60-min contact time (Fig. 2). The 1 mg/L solution yielded a maximum log reduction in biofilm concentration of 0.1 immediately upon contact with the biofilm. Again, the ozone was minimally effective against the established biofilms of bacteria with less than a 1-log reduction in viable bacteria.

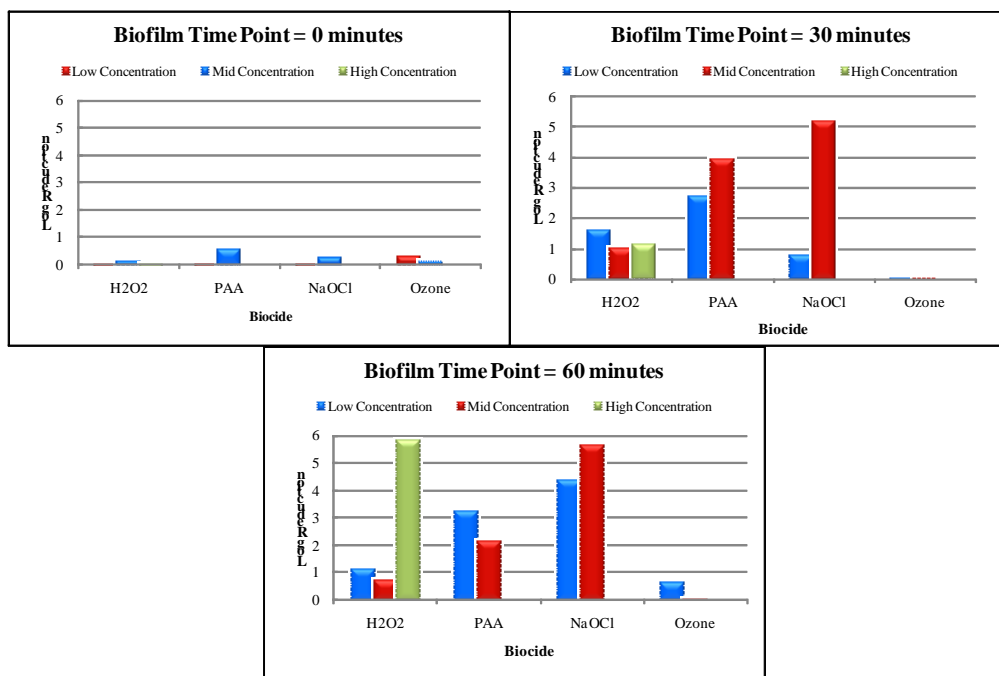


Figure 2. Breadboard-generated biofilm evaluation results.

V. Chemical Characteristics of Biocides

Shelf life tests examined the stability of the biocides without including the presence of bacteria. A test was conducted to identify the stability of biocide solutions from both commercially available solutions and the breadboard-generated biocides. Each solution was stored for a 35-day duration with periodic data collection (approximately every 7 days). Stability testing was initially performed with commercially available biocides to obtain a baseline to compare to the generated biocides' stability. Only the breadboard-generated biocide data will be discussed in this paper. Additionally, during planktonic testing, concentration measurements were taken over the test duration to characterize the stability of the biocides once introduced to a contaminated solution.

As stated previously, the current disinfectant for the U.S. potable water system on the ISS is iodine. Therefore, a study of the compatibility of each biocide to iodine/iodide was completed to investigate the interaction and assess whether any initial compatibility issues exist. Since the electrochemically generated biocide may be used on the ISS, it is important to test the compatibility of ozone with iodine and iodide. Iodine and iodide were mixed together, and concentrations were measured over the course of a 48-hour period. Testing was conducted with both commercially

available and breadboard-generated biocides; however, only the breadboard-generated biocide data is discussed in this paper.

A. Chemical Stability Testing

The concentration of H_2O_2 at $1,000 \text{ mg/L} \pm 100 \text{ mg/L}$ was selected for stability testing. Hydrogen peroxide was stored in a Teflon[®] bottle, sealed, wrapped in foil, and placed in a dark environment that maintained a constant temperature of 25°C . A total of seven test points were measured (days 0, 1, 7, 14, 21, 28, and 35). Over the course of the test, the H_2O_2 maintained its concentration. The measured starting concentration of generated H_2O_2 was 949 mg/L with a pH of 4.81 (Fig. 3). Over the course of the test, the H_2O_2 decreased in concentration to 805 mg/L with a pH of 5.07 by day 35. This implies that there was approximately a 15% decrease in breadboard-generated H_2O_2 over 35 days. During planktonic testing, stability testing was also conducted to characterize how breadboard-generated H_2O_2 would behave when exposed to a contaminated solution. Stability testing was conducted with breadboard-generated H_2O_2 at $1,000 \text{ mg/L}$ over the course of 60 min. The measured starting concentration was 915 mg/L with a pH of 7.45. At the 30-min test point, the H_2O_2 decreased to 802 mg/L . The final concentration at the 60-min test point was 769 mg/L with a pH of 7.35. This indicates that there was approximately a 16% decrease in breadboard-generated H_2O_2 over the course of 60 min during planktonic testing.

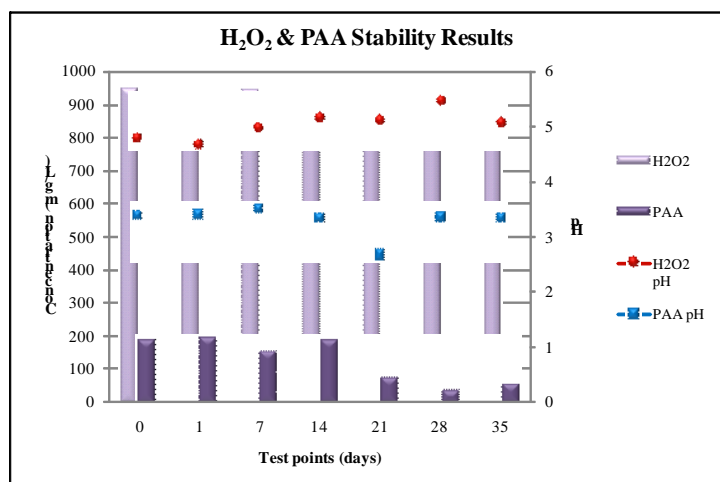


Figure 3. Stability results of H_2O_2 and PAA

Testing was conducted to measure the concentration of PAA over the course of 35 days. PAA was stored in a Teflon[®] bottle, sealed, wrapped in foil, and placed in a dark environment that maintained a constant temperature of 25°C . Stability tests were conducted with breadboard-generated PAA at 180 mg/L . The measured starting concentration of generated PAA was 185 mg/L with a pH of 3.37. Over the course of the test, the PAA decreased in concentration to 56 mg/L with a pH of 3.33 by day 35 (Fig. 3). This implies that there was approximately a 70% decrease from initial concentration in breadboard-generated PAA over 35 days. During planktonic testing, measurements were made to characterize how breadboard-generated PAA would behave with a contaminated solution.

Stability testing was conducted with breadboard-generated PAA at 30 mg/L over the course of 60 min. The measured starting concentration was 17 mg/L with a pH of 7.10. The final concentration at the 60-min test point was 15 mg/L with a pH of 7.04. This indicates that there was approximately a 50% decrease in breadboard-generated PAA over the course of 60 min during planktonic testing.

The stability of the NaOCl concentration when stored is an important variable when identifying possible on-orbit applications. Sodium hypochlorite was stored in a Teflon[®] bottle, sealed, wrapped in foil, and placed in a dark environment that maintained a constant temperature of 25°C . Due to the free chlorine in hypochlorite, the chlorine demand needed to be taken into account when selecting the bottles. Bottles (or containers) needed to be treated with chlorine compounds to destroy any organic compounds that would react with hypochlorite and eliminate the chlorine demand of the container. Once the bottle was treated, chlorine concentrations remained more stable. Testing was conducted with an untreated as well as a conditioned or treated bottle. Stability tests were conducted with breadboard-generated NaOCl at $10 \text{ mg/L} \pm 1 \text{ mg/L}$. A total of two tests were conducted with breadboard-generated hypochlorite: one test with untreated Teflon[®] bottles, and another with conditioned (or treated) Teflon[®] bottles. For the untreated Teflon[®] bottles, the measured starting concentration was 10 mg/L with a pH of 7.95. Over the course of the test, the NaOCl significantly reduced by the 14-day test point with a concentration of 2.4 mg/L , and had been nearly consumed by the 35-day test point with a concentration of 0.40 mg/L with a pH of 5.68 (Fig. 4). This implies that NaOCl decreased by approximately 94% over the course of the 35 days with the untreated Teflon[®] bottles. For the conditioned (or treated) Teflon[®] bottles, the measured starting concentration was 10 mg/L with a pH of 7.99. Over the course of the test, the NaOCl maintained its concentration. On day 35, the generated NaOCl was 8.2 mg/L with a pH of 7.05 (Fig. 4). This implies that the NaOCl decreased approximately 18% over the course of the 35-day study with conditioned Teflon[®] bottles. Future testing is recommended to establish a methodology of

conditioning NaOCl storage bottles. During planktonic testing, measurements were made to characterize how the biocide would behave with a contaminated solution. Stability testing was conducted with a breadboard-generated NaOCl at 10 mg/L over the course of 60 min. The equipment used during planktonic testing had been previously used in testing with NaOCl and, therefore, was considered conditioned. The measured starting concentration was 6.7 mg/L with a pH of 7.40. At the 30-min test point, the concentration reduced to 5.0 mg/L, and at the 60-min test point, it had decreased to 4.2 mg/L with a pH of 7.41. This implies that over the course of 60 min, breadboard-generated NaOCl mixed with a contaminated solution is decreased by approximately 58%.

Testing was conducted to measure the concentration of ozone over the course of 35 days. However, due to the short half-life of ozone, testing was stopped after 7 days. Once ozone was generated, it was stored in a Teflon[®] bottle that was sealed, wrapped in foil, and placed in a dark environment that maintained a constant temperature of 25°C. Test points were measured on days 0, 1, and 7. Stability tests were conducted with the breadboard-generated ozone concentration of 1 mg/L. The ozone concentration was measured using a spectrophotometer. The measured starting concentration was 1 mg/L with a pH of 5.93. Over the course of the test, the ozone decreased in concentration. On day 7, the breadboard-generated ozone was 0 mg/L. The decrease in ozone is expected since it has a very short half-life (approximately 15 min) (Fig. 4). During planktonic testing, measurements were made to characterize how breadboard-generated ozone would behave with a contaminated solution. Stability testing was conducted with the breadboard-generated ozone at 1 mg/L. The measured starting concentration was 0 mg/L. The concentration of ozone remained at 0 mg/L for the entirety of the test. This implies that over the course of 60 min, breadboard-generated ozone mixed with a contaminated solution is completely consumed.

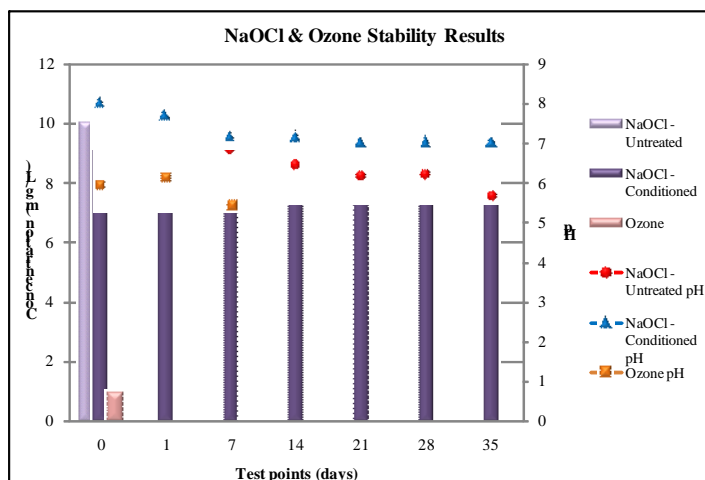


Figure 4. Stability results for NaOCl and ozone.

B. Compatibility with Iodine Testing

Compatibility testing was conducted to characterize the effects that each biocide would have on iodine and iodide. A nominal concentration of iodine (3 mg/L) and iodide (1 mg/L) was mixed with each solution, and concentrations were measured over the course of 48 hours. The objective was to determine at what rate the concentrations of the biocide, iodine, and iodide change when the solutions mix.

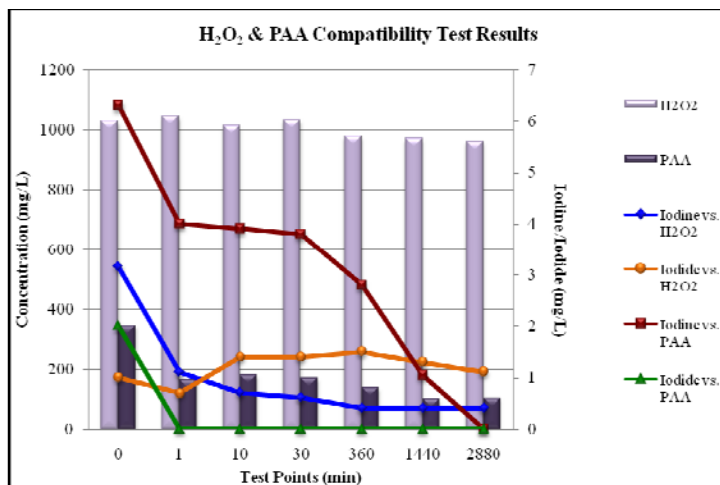


Figure 5. Compatibility results for H₂O₂ and PAA.

For H₂O₂, NaOCl, and PAA, the biocide and iodine-iodide solutions were made separately at half the final volume and double the target concentration, then mixed together so that they diluted each other down to the target biocidal concentrations. However, ozone solutions were made differently. Ozone was bubbled into deionized (DI) water until the required concentration was produced. A small volume of concentrated iodine-iodide was added to the ozonated solution. Measurements of all species were taken frequently over the first hour, then again at 24 and 48 hours.

Breadboard-generated H₂O₂ underwent compatibility testing at a concentration of 1,000 mg/L. Over the 48-hour test, breadboard-generated H₂O₂ decreased in

concentration to 961 mg/L. Iodine decreased to 0.7 mg/L within 10 min of the test, and reduced to 0.4 mg/L at the completion of the test. Iodide increased to 1.4 mg/L within 10 min of the test, and completed the test with a concentration of 1.11 mg/L (Fig. 5). Breadboard-generated H_2O_2 decreased by approximately 6.5% when introduced to iodine and iodide. Iodine levels reduced significantly, and it is likely the disinfecting capability is also diminished. Iodide increased, which indicates that iodine is breaking down into iodide.

Breadboard-generated PAA underwent compatibility testing at a concentration of 180 mg/L. Over the 48-hour test, breadboard-generated PAA decreased in concentration to 105 mg/L. Iodine increased to 3.9 mg/L within 10 min of the test and maintained the higher concentration until the 24-hour test point. The final concentration for iodine was 0 mg/L at 48 hours (Fig. 5). Iodide decreased to 0.0 mg/L within 10 min of the test and maintained that concentration until the test completed. The increase in iodine and the decrease in iodide may indicate an oxidation of the iodide into iodine when reacted with PAA. During the breadboard-generated PAA testing, the iodine decreased rapidly after the 30-min test point. The rapid decrease is not understood at this point in time; however, it could be attributed to the added constituents (due to generation process) in the breadboard-generated PAA.

Breadboard-generated NaOCl underwent compatibility testing at a concentration of 10 mg/L. Over the 48-hour test, breadboard-generated NaOCl decreased in concentration to 3.4 mg/L, approximately 66%. Iodine initially increased to 5.6 mg/L within 1 min of the test; however, iodine proceeded to decrease to 0 mg/L by the 48-hour test point (Fig. 6). Iodide decreased to 0 mg/L within 1 min of the test, and maintained that concentration until the test completed. The decrease of NaOCl may be a result of the reaction with the iodide; however, it should be noted that untreated containers were used during this testing. For future testing, it is recommended that testing be conducted with conditioned containers.

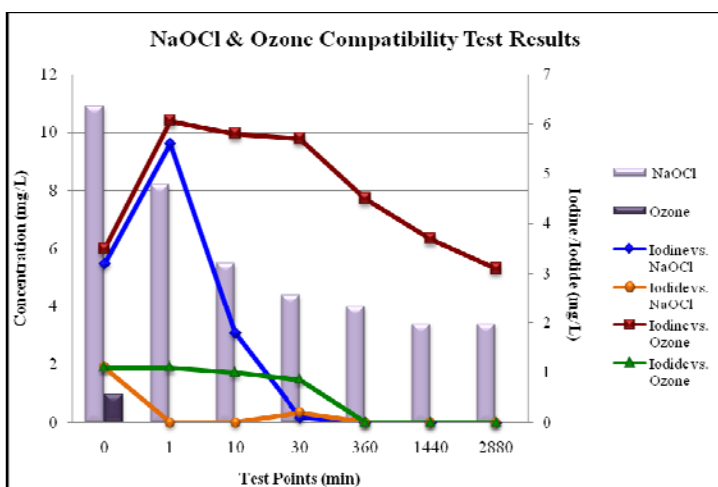


Figure 6. Compatibility results for NaOCl and ozone.

Breadboard-generated ozone underwent compatibility testing at a concentration of 1 mg/L. Over the 48-hour period, ozone decreased in concentration to 0 mg/L within 1 min (Fig. 6). The iodine concentration increased to 5.8 mg/L within 10 min of the test and maintained the higher concentration until the 24-hour test point. The final concentration of iodine was 3.1 mg/L. Iodide decreased to 1 mg/L within 10 min, and decreased to 0 mg/L by the 48-hour time point. The increase in iodine and decrease in iodide could be due to oxidation of iodide over the course of the test.

VI. Conclusion

The ECD feasibility assessment investigated four biocides identified for their disinfectant capabilities. Testing was conducted in three phases: test methodology development, ISS consortium against commercial biocides, and ISS consortium against breadboard-generated biocides. Based on the test results outlined in this report, it is recommended to conduct additional testing on breadboard-generated biocides to establish confidence and repeatability in the data for future ISS applications or exploration applications. Sodium hypochlorite possessed the capability to reduce both planktonic and biofilm populations by at least 5-fold, and is recommended for possible stand-alone ISS applications. PAA also performed well in both studies, but increases to contact time and concentration are recommended to achieve a greater degree of biofilm reduction. Whereas, 10,000 mg/L of H_2O_2 appeared sufficient to effectively reduce levels of planktonic bacteria, higher concentrations should be investigated to obtain a similar level of efficacy against biofilm bacteria. Ozone demonstrated moderate-to-high activity against planktonic bacteria, but very weak activity against biofilm bacteria. Increasing the concentration of ozone is favorable to increasing the contact time, since ozone is unstable in solution and no trend of increased biocidal activity was observed during testing.

All testing to date has evaluated the use of a single dosing model to determine biocide efficacy. Future testing should consider testing within a defined on-orbit application. It is assumed that all potential modifications to testing

will depend upon breadboard outputs (production volume and concentration), materials compatibility, operational constraints, and other considerations.

Acknowledgments

This paper summarizes the hard work that was performed by numerous NASA Johnson Space Center (JSC), Engineering Support Contract, and Bioastronautics Contract engineers, microbiologists, chemists, and technicians. The ECD feasibility assessment was funded by NASA JSC ISS Vehicle Office.

References

- ¹Rodriguez, B. R., Shindo, D., Montgomery, E. "Material Testing in Support of the ISS Electrochemical Disinfection Feasibility Assessment," *AIAA International Conference on Environmental Systems*, Vail, CO, 2013.
- ²Koenig, D. W. and Pierson, D. L. Microbiology of the space shuttle water systems. *Water Science and Technology*. 35:59-64, 1997..
- ³Pyle, B.H and McFeters, G. A. "Population dynamics of pseudomonads after iodination." *Canadian Journal of Microbiology*. 36(11): 801-803, 1990.
- ⁴Mathieu, L., Bouteleux, C., Fass, S., Angel, E., Block, J. C. Reversible shift in the alpha-, beta- and gamma-proteobacteria populations of drinking water biofilms during discontinuous chlorination. *Water Res.* 43(14): 3375-86, 2009.