

Status of ISS Biofilm Management Testing for the Water Processor Assembly

Yo-Ann Velez Justiniano[1]
NASA Marshall Space Flight Center, Huntsville, AL, 35808, USA

Connor Murphy[2] and Eric Beitle[3]
Jacobs Space Exploration Group, Huntsville, AL 35808, USA

and

Iulian Cioanta [4], John Jackson[5], and Cary McGhin[6]
SANUWAVE Health, Inc., Alpharetta, GA, 30005, USA

Biofilm growth is a significant concern for NASA's current and future water systems. The International Space Station (ISS) Water Processor Assembly (WPA), which produces potable water from a combination of humidity condensate and urine distillate, has suffered from biofouling incidents. In recent years multiple technologies have been tested to combat this biofouling. Temperature treatments, coated surfaces, and nutrient filters have been tested in-line with bioreactors simulating similar conditions to the wastewater tank. Results from testing indicate that microbial growth persists through multiple conditions, and thus other tests were required to further understand treatment performance. Other plans to tackle biofilm include prototyping a shockwave tank. Future testing of these technologies is being discussed to determine a final biofilm treatment regimen for water systems.

Nomenclature

ASTM	=	American Society for Testing and Materials
CFU	=	Colony Forming Unit
CDC	=	Center for Disease Control
ISS	=	International Space Station
MSFC	=	Marshall Space Flight Center
PWB	=	Potable Water Bus
R2A	=	Reasoner's 2A Agar
RFI	=	Request for Information
WPA	=	Water Processor Assembly

I. Introduction

THE Water Processor Assembly (WPA) on the International Space Station (ISS) is a complex system that is used to recycle urine and condensate into potable water. The current WPA includes adsorption, ion exchange, and catalytic oxidation[1], a process that uses a catalyst to convert volatile organic compounds into carbon dioxide and water. It also includes the multifiltration beds which can reduce organic contaminants. The WPA system is continuously monitored, and samples are regularly taken for microbiological and chemical analysis to ensure the water quality is safe for consumption. However, in the WPA, biofilms can form on surfaces that are in contact with water, such as plumbing and filters[2]. To this day, there are no sensors to detect biofilm and early prevention of hardware failure. All biofilms can harbor a variety of microorganisms, including bacteria, fungi, algae, and protozoa, and can be composed of a mixture of different species. In the case of ISS, biofilms previously studied have been found to

---

[1]ECLSS Engineer, MSFC ES62, B-4755 Tiros St. Redstone Arsenal, AL 35808
[2]Test Engineer, MSFC ES62, B-4755 Tiros St. Redstone Arsenal, AL 35808
[3]Test Engineer, MSFC ES62, B-4755 Tiros St. Redstone Arsenal, AL 35808
[4]Senior Research Engineer, SANUWAVE Health, Inc.3, Alpharetta, GA, 30005, USA
[5]Research Engineer, SANUWAVE Health, Inc.3, Alpharetta, GA, 30005, USA
[6]Research Engineer, SANUWAVE Health, Inc.3, Alpharetta, GA, 30005, USA

harbor mostly bacteria and some fungi. However, analysis of these built-environment biofilms has only been obtained after recovery of
system hardware and ground experiments. The system's geometry and narrow spaces make *in situ* sampling difficult and thus has not been attempted. Biofilms in the WPA could cause various problems, including, but not limited to:

1. Clogging filters and reducing flow rate through the system.
2. Creating potential health hazards by harboring pathogenic microorganisms or producing harmful by-products. However, it is worth noting that the Potable Water Dispenser (PWB) already receives iodinated water from the Potable Water Bus (PWB) and thus engages in robust water disinfection[3]. It has not presented biofilm issues to date.
3. Reducing water quality and reducing the efficacy of disinfectants. Although this has not been observed in ISS, it is an issue that arises with the presence of biofilm[4].

To prevent or control biofilm formation in the WPA, various strategies have been proposed and tested. In this update paper we summarize some of the results that pertain to on-going tests at the NASA George C. Marshall Space Flight Center (MSFC) and by external partners at SANUWAVE.

I. Coated Coupons, Heat Mitigation, and Filtration Systems

Previously described plans in 2021 encompass using a series of bioreactors to test temperature mitigation, coated coupons and nutrient filters[5]. These options were selected for a biofilm mitigation trade study, from a list of design options suggested in a Request for Information (RFI) that took place in 2019[6]. By utilizing the MSFC Biofilm Mitigation Test stand (Fig 1), the effectiveness of several coating and filtration techniques are explored in the design conditions. The two coatings are described as halogenated coatings, the nutrient filters are mixed multifiltration resin beds, and finally, there are also temperature treatments in the form of heat cycles. The purpose of this research is to continue to compare robustness and readiness of suggested technologies.

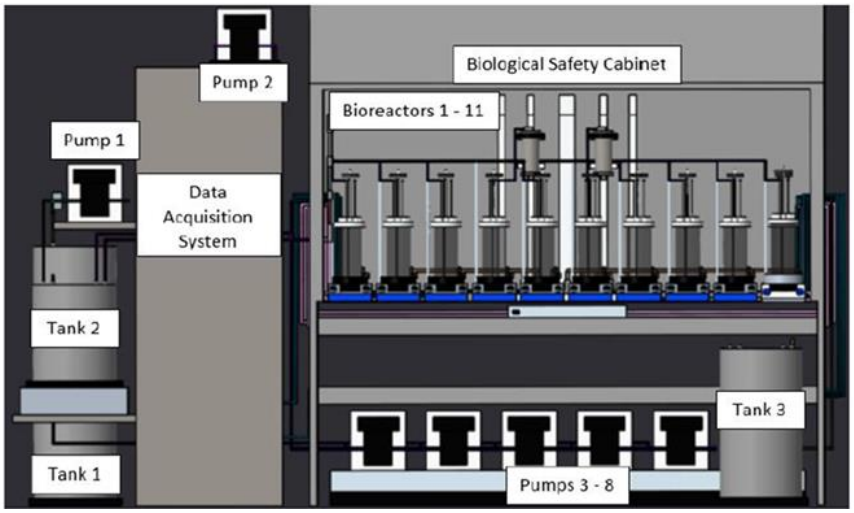

Figure 1. Test stand architecture in BSL2 facility and Class II Biosafety Cabinet. *Tank 1 is used as a reservoir for the wastewater simulant, also called ersatz. Tank 2 is a reservoir for mixed ersatz and microbial inoculants. Pump 1 transfers fresh ersatz into the inoculation tank. Pumps 3-8 pump inoculated ersatz into the reactors. Tank 3 serves to collect waste.*

A. Bioreactor Set Up and Methods

Utilizing an eleven CDC bioreactor test stand setup, each mitigation technique was challenged with a biofilm formation test, consisting of a bacterial and fungal mixture containing *Burkholderia contaminans, Exophiala spinifera, Lecythophora mutabilis, Aureobasidium pullulans, Cupriavidus metallidurans, Ralstonia insidiosa,* and *Pseudomonas aeruginosa.* The fungal organisms were used at lower amounts as previously studied biofilms have found lower CFU of the fungal species[7] Additionally, growth and spore recovery by centrifugation is more difficult in the fungal samples[8] and post-inoculation recovery and CFU counts may vary. The bacterial and fungal inoculation mixture was added into an ersatz solution simulating the wastewater in the ISS WPA. Figure 2 shows the bacterial and fungal amounts seeded into the Tank 2 reservoir to complete the 5L inoculated ersatz and result in a 10^6CFU/mL concentration. Growth, wash and eventual inoculation was performed as described in a previous conference[1]. The

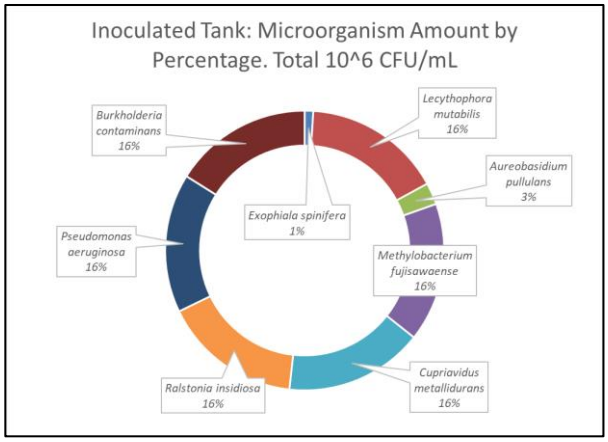

Figure 2. Bacterial and fungal amounts seeded into the Tank 2.

ersatz used for this experiment and nutrient conditions have been described before[9] and this currently used ersatz is a modified recipe from one previously used in an ionic challenge experiment pertaining bed resins[10]. It was modified to better simulate the wastewater conditions, instead of the conditions for the previous targeted chemical test. The inoculated ersatz solution was pumped into each test stand bioreactor at a rate of 0.1 mL/min to allow for continuous influx of nutrients and a low shear environment. The combination stirring and heating plates also yielded weight feedback, and this was utilized to maintain a consistent level above the coupons. Bioreactor 11 was used as an uninoculated control, while Bioreactor 8 is used as an inoculated control without any mitigation strategy. All coupons were made of Inconel 718 and they were obtained for sampling by pulling out a rod from the baffle side. The ASTM E256-22 procedures (part 10) were used for coupon sampling, in triplicate[11]. The main change to the method was the use of Reasoner's 2A Agar (R2A). The temperature used for the plates was 25°C.

B. Coated Coupon Methods

Brominated coating and chlorinated coating applied to Inconel 718 sample coupons, acquired by Auburn University, and halogenated as by Demir and colleagues[12] were loaded into a BioSurface CDC bioreactor setup, occupying bioreactors number 6 and 7. These two types of coatings were challenged in the methods mentioned in the previous section. Both halogenated coatings are intended to prevent any biofilm formations growing or attaching on their surfaces.

C. Heat Mitigation Methods

Temperature application was also explored in the biofilm test stand run. The conditions used were 65, 72.5, 79.5, 86.4, and 93.4 °C, sustained for 15 minutes every 48 hours. These results will not be used in the final assessment due to a now known bioreactor design flaw where a poor thermocouple position brought into question the validity of the temperature treatment. A separate manuscript will focus on results and lessons learned from this experiment.

D. Filtration Methods

The final technology examined by the recent run of the Biofilm Mitigation Test stand was the nutrient filter canister. Intended as a barrier meant to absorb upstream nutrients that biofilm growth could use within the bioreactor, the nutrient filter was placed upstream of the bioreactor (bioreactors 9 and 10). An additional 0.2µm groundwater filter was placed upstream of the nutrient filter canister. Amberlite IRN 150 and Ambersorb 4652 resins were utilized for the filter media within the nutrient filters. Although housed in the same canister, the resins were not mixed, with a separating mesh between the resin sections. Each canister had approximately 325 grams of Amberlite IRN 150, and 116 grams of Ambersorb 4652. The canister flow path brought the inlet ersatz stream through the bottom bed of Ambersorb 4652 and up through the Amberlite IRN 150 resin bed. These resins have been used in space station research in the past as part of a series of upgrades to the WPA[1].

E. Results

Testing ran for five days, and the resulting data was collected immediately after. This was shorter than the planned time and was caused by a blockage of filters from Tank 1 to Tank 2. Based on these results, it was concluded that the brominated coated coupons were inadequate to mitigate biofilm growth. Chlorinated coupons however showed potential as a biofilm mitigation technology (Fig. 3). The difference between the brominated coupon CFU/mL and inoculated, untreated bioreactor was $<10^2$, which means a low effectiveness was achieved when compared to the chlorinated coupons. The nutrient filters varied in total CFU/mL, however, there was a present leakage in the canister detected after the test was stopped and the pressure in the canister indicated potential overpacking of the resin.

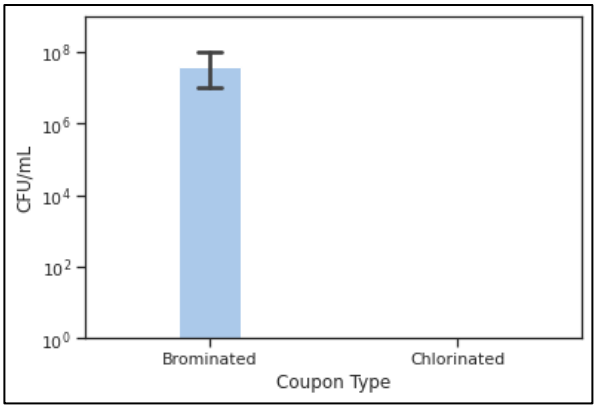

Figure 3. Coated coupons microbial results.

Table 1. Summarized status of testing for the initial test run.

Mitigation Method	Status
Brominated Coupons	Ineffective
Chlorinated Coupons	Effective in Short Term Study
Thermal Mitigation	Inconclusive/Future Testing Required
Nutrient Filtration	Inconclusive/Future Testing Required

F. Discussion

Based on the conditions in which each biocide and filtration system was challenged, determinations can be made for several new functional mitigation technologies. Design modifications are being planned for a future test (Phase II) such as sterility checks for Tank 1. Current diagnostics have already allowed for continuous flow without Tank 1 to Tank 2 blockages for 35 days. Chemical tests are also a planned addition to the current test model. The results from the Phase II of the MSFC biofilm test stand will be described in a future manuscript. future.

II. Shockwave Technology

Shockwaves are asymmetric bursts of pressure that last 5 to 8 microseconds. Each burst is denoted by a single and fast acoustic pressure pulse that increases in tens to hundreds of nanoseconds to a maximum positive compressive pressure of 50 to 110 MPa (500 to 1100 bar), followed by an exponential decrease to negative pressures of -5 to -15 MPa (-50 to -150 bar)[13]. The positive compressive pressures generate "acoustic streaming" that moves the fluid in the direction of shockwave propagation, which produces high tensile and shear forces[13] against bacterial biofilms. The negative pressures create cavitation bubbles or growth of existing air bubbles from a fluid for 300 to 800 microseconds followed by their collapse through the bubble medial portion. This implosion generates secondary shockwaves and microjets, with speeds in excess of 100 meter/second (328 foot/second)[13]. These microjets create "acoustic microstreaming" in fluids and produce additionally localized tensile and shear forces, which can produce cracking, dislodging, and fragmentation of bacterial biofilms. The cavitation bubbles' collapse also induces localized transient high temperatures and sonoluminescence[13-15] that could contribute to sterilization of the surrounding fluid medium. Tests performed by the Center for Biofilm Engineering (CBE) at Montana State University, have shown that SANUWAVE focused shockwave technology is effective in eliminating medical[16], marine[17], and monument[18] biofilms containing both Gram-positive or Gram-negative bacterium species, when biofilms were present in the focal region of the focused shockwaves. Furthermore, experiments performed at University of Georgia, have shown that shockwaves have bactericidal effects against planktonic bacteria[19], by destroying bacterial wall integrity via either tensile forces produced by shockwaves[20] or by creation of opened sensitive channels in bacterial shell via mechanotransduction. This bloats and bursts the bacteria[20]. Based on the shockwave effects on bacterial biofilms and planktonic bacteria, NASA awarded a contract to SANUWAVE to explore the feasibility of using shockwaves to remove patchy biofilms, prevent the biofilm formation, and possibly reduce the overall free bacteria from the Water Tank included in the Water Reclamation System (WRS) aboard the ISS.

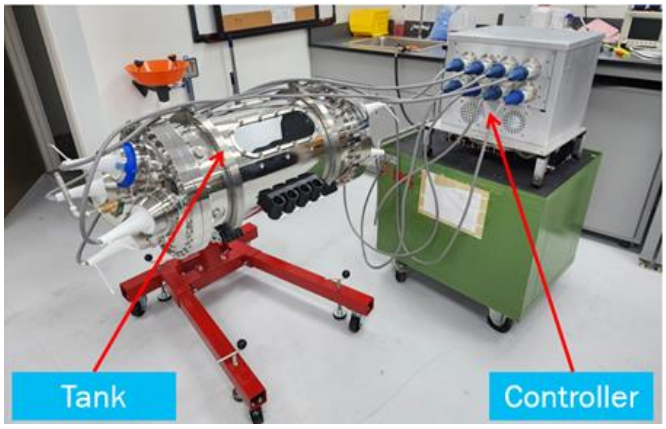

Figure 4. SANUWAVE testing system.

A. Shockwave Test Design

SANUWAVE designed and manufactured a 1/1-dimensional replica of the ISS tank that has modified end caps to accommodate eight (8) focused shockwave medical applicators, as seen in Figure 4. These commercial medical focused applicators have a high efficiency in their focal zone, where the highest forces/pressures are produced. The focal zone and its location relative to the applicators are designed for typical human body dimensions. Thus, the applicators used in the tests were not optimized for the use in a large tank that significantly exceeds their focal region dimensions. This translates to lower forces/pressures produced by the shockwaves at the tank wall, when compared to the forces/pressures from the focal region. To understand the activation sequence for the shockwave applicators, the Top and Bottom Caps were rotated from their parallel position with plus 45 degrees and minus 45 degrees for visualization purpose, as seen in Figure 5a. On each Cap, the shockwave applicators were positioned at 90 degrees apart with the bottom applicators being also shifted with 45 degrees relatively to the top ones. This design feature was necessary to have a better coverage with shockwaves of the water tank internal surface. Two different activation sequences were used for testing. For the first test run, identified as "12345678 applicators' activation sequence", the 1, 2, 3, and 4 applicators were fired sequentially from the Top Cap and then applicators 5, 6, 7, and 8 were fired sequentially from the Bottom Cap. Using this activation sequence, in a first subset of experiments, there was a 1-second delay between the firing of the four (4) applicators from the Top Cap and the four (4) applicators from Bottom Cap and vice versa, to avoid any possible interference in between subsequent shockwaves. Since the 1 second delays were determined to not produce an advantage, a second subset of experiments were then conducted without the 1-second delays. Additionally, a second activation sequence was explored where one applicator from the Top Cap was fired, followed by a relatively opposite applicator from Bottom Cap, and then the same logic was repeated until all 8 applicators were fired. That was identified as the "18362745 applicators' activation sequence". Another important aspect for testing was the applicators' positioning, as seen in Figure 5b. On both end caps, the applicators were held in dedicated slots inside semi-spherical holders, which allowed their reorientation. The first positioning tested had the applicators oriented towards the tank's center. This positioning was identified as "Applicators oriented towards the tank central point" (see Table 1). Another configuration used during testing was orienting the applicators towards the tank wall, using the shortest distance, which allowed more shockwave reflections inside the tank. This positioning was identified as "Applicators oriented towards the tank wall" (see also Table 1). For visualization inside the tank, there are two windows at 90 degrees angle on circumferential direction. Access ports present on the end caps and on the tank's cylindrical wall were used or could be used for water "In" and "Out", aeration, pressure gauges, UV lights, sensors, borescope, or other required sensors and equipment. The semi-ellipsoidal reflectors, incorporated in the 8 applicators, produced focused shockwaves via electrohydraulic principle that uses the discharge of high voltages in between two electrodes of a spark gap. A heavy stand was utilized to support the water tank that allowed the rotation of the tank in the vertical plane (see Figure 4).

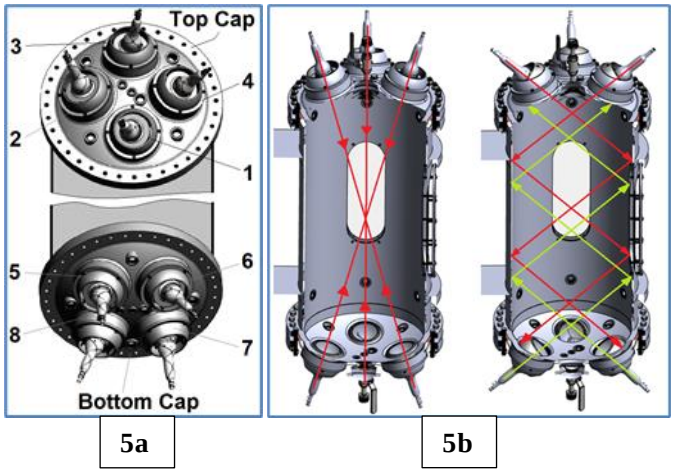

Figure 5a. Applicator activation sequences 5b. Applicator positioning.

Figure 4 also shows the controller for the 8 applicators, capable of reaching high voltages of 27 to 30kV, which to produced 7.3 to 9 Joules. The controller was WiFi remotely actuated from a laptop via LabVIEW software. A biofilm mimicking material was used instead of "live" bacterial biofilm. The mimicking material was a mixture of a heavy glue and blue food color. This mixture did not dissolve or slough off, when painted on a vertical stainless-steel plate and left in a water container for 72 hours. Additionally, the inclusion of the heavy glue mimicked the stickiness characteristic of real bacterial biofilms. It was deemed impractical to paint all the interior surface of the tank with the biofilm mimicking material for each test, and thus targeted portions of the tank were coated with patches of 20.0x20.0x0.2mm[11] for each test. (see Figure 6). The

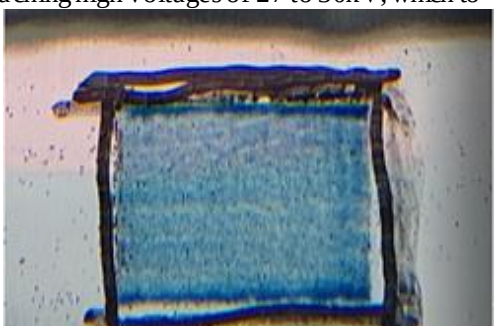

Figure 6. Biofilm mimicking material.

targeted patches were primarily in the middle portion of the water tank's cylindrical wall and in front of one of the two visualization windows. The tests were performed with the tank inclined at 20 degrees from horizontal position, in a gravitational environment, and with tap water that contained a significant amount of air bubbles. Both the gravitation and the amount of air bubbles from the tank played a significant role in the cleaning speed of the biofilm mimicking material.

B. Results

The reflected shockwaves on the tank wall have their mechanism of action given by the shear forces created in between biofilm layers and the compression tensile forces generated at the biofilm surface. Additionally, when air bubbles were present in the tank, shockwaves caused the existing air bubbles to expand and then collapse, producing additional shear forces and high-speed jets near the tank wall, increasing both the overall shear and tensile forces.

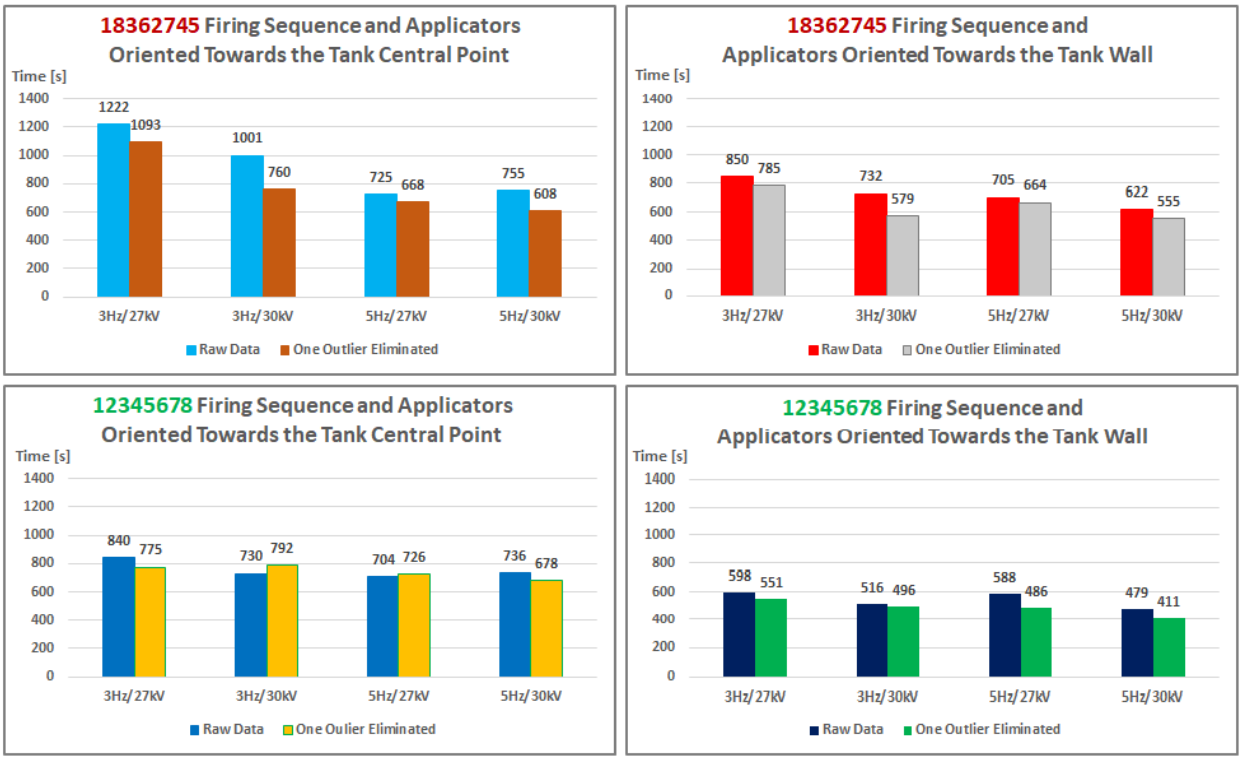

Figure 7. Cleaning time variation function of applicators' activation sequence and orientation.

The tests were performed at SANUWAVE laboratory from Alpharetta, Georgia. For each testing situation, 4 tests were performed, with the exception of four instances where 6 tests and one instance where 8 tests were performed, to verify consistency of the results beyond 4 tests. During testing, different settings were varied including energy input for shockwaves (27kV or 30kV), number of shockwaves per second/shockwave frequency (3Hz or 5Hz), applicators' activation sequence (12345678 or 18362745), and applicators positioning (orientation towards the tank central point or orientation towards the tank wall, as defined in Figure 5b). Time to clean the biofilm mimicking patch was determined, for each of the different test settings, using video recordings. A summary of testing results is presented in Table 1, as mean times and their standard deviations for all test points or with one outlier eliminated. In addition, the test results presented in Table 1 and Figure 7 were performed with air bubbles present in the fluid and around the testing patch area. The first set of tests were done with the applicators oriented towards the tank central point, activated with 12345678-activaton sequence, and with 1-second delay between the firing of the four (4) applicators from Top Cap and the four (4) applicators from Bottom Cap and vice versa. This gave the longest cleaning times for the biofilm mimicking material. All following tests were done without 1-second delays. Then, knowing the time necessary to fire sequentially all 8 applicators, the number of seconds added to the cleaning time due to 1-second delays could be

Table 2. Times for cleaning in seconds for all tested points

APPLICATORS ORIENTED TOWARDS THE TANK CENTRAL POINT / 12345678 SEQUENCE OF APPLICATORS' ACTIVATION					
ALL MEASUREMENTS REGARDLESS OF DELAY OR NOT	**3Hz/27kV**	**3Hz/30kV**		**5Hz/27kV**	**5Hz/30kV**
RAW DATA	1118 ± 383	948 ± 400		930 ± 596	959 ± 319
ONE OUTLIER ELIMINATED	1035 ± 326	842 ± 286		721 ± 79	895 ± 283
ADJUSTED DATA TO ELIMINATE 1 SECOND DELAYS					
RAW DATA	861 ± 169	733 ± 219		641 ± 282	664 ± 162
ONE OUTLIER ELIMINATED	830 ± 157	686 ± 187		562 ± 183	629 ± 139
DATA WITHOUT 1 SECOND DELAYS					
RAW DATA	840 ± 135	730 ± 154		704 ± 47	736 ± 169
ONE OUTLIER ELIMINATED	775 ± 38	792 ± 110		726 ± 16	678 ± 152
APPLICATORS ORIENTED TOWARDS THE TANK WALL / 12345678 SEQUENCE OF APPLICATORS' ACTIVATION					
DATA WITHOUT 1 SECOND DELAYS	**3Hz/27kV**	**3Hz/30kV**		**5Hz/27kV**	**5Hz/30kV**
RAW DATA	598 ± 144	516 ± 48		588 ± 288	479 ± 186
ONE OUTLIER ELIMINATED	551 ± 134	496 ± 30		486 ± 249	411 ± 156
APPLICATORS ORIENTED TOWARDS THE TANK CENTRAL POINT / 18362745 SEQUENCE OF APPLICATORS' ACTIVATION					
	3Hz/27kV	**3Hz/30kV**		**5Hz/27kV**	**5Hz/30kV**
RAW DATA	1222 ± 419	1001 ± 595		725 ± 188	755 ± 368
ONE OUTLIER ELIMINATED	1093 ± 306	760 ± 96		668 ± 142	608 ± 90
APPLICATORS ORIENTED TOWARDS THE TANK WALL / 18362745 SEQUENCE OF APPLICATORS' ACTIVATION					
	3Hz/27kV	**3Hz/30kV**		**5Hz/27kV**	**5Hz/30kV**
RAW DATA	850 ± 177	732 ± 344		705 ± 100	622 ± 226
ONE OUTLIER ELIMINATED	785 ± 212	579 ± 192		664 ± 71	555 ± 133

calculated and the overall time adjusted to a non-delay situation. When the overall/combined data, the adjusted data, and the data without 1-second delays were compared, the shortest times for cleaning were obtained by the activation of the applicators without 1-second delays. Therefore, 1-second delays did not help with a more efficient cleaning of the biofilm mimicking material. Subsequently, the applicators' orientation was changed towards the tank wall and no delays were set in between the top and bottom applicators. In the next set of testing, the 18362745-activation sequence for the applicators was used, with no delays between the activation of different applicators too. The results for all these test points are presented in Table 2.

The data without 1-second delays, which is a sub-set of the results presented in Table 2, is presented in a graph form in Figure 7. These graphs show that high input energy (30kV) and high frequency (5Hz) reduced the cleaning times, regardless of applicators' activation sequence or orientation. More important, note that applicators' orientation has a significant role in reducing the cleaning time for both activation sequences, with the applicators pointing towards the tank wall being the most efficient way to clean the biofilm mimicking material. That can be possible explained by the

more reflections of the shockwaves inside the tank per one firing cycle (see Figure 5b). When the activation sequences for applicators were compared, the results shown that using the 12345678-activation sequence is better than 18362745-activation sequence. Firing sequentially the shockwave applicators from opposite directions (18362745-activation sequence) might partially cancel the shockwaves reflections and thus reducing their effects, which may be a possible explanation for these results. Overall, the best results were obtained by the 12345678-activition sequence and when the applicators were oriented towards the tank wall. Additional tests illustrated that when no bubbles were present in the fluid, biofilm cleaning happened layer by layer mostly due to shear forces and it required more than 3.6 times longer times for a complete removal of biofilm mimicking material. Furthermore, the results indicated the thickness of the biofilm mimicking material is important too, with thicker biofilms (20.0x20.0x0.3mm[11]) requiring more time to clean (> 2.2 times longer). Finally, the assessment of the tank wall surface did not show any damage after the large amount of shockwaves fired during testing.

C. Discussion.

Tests with SANUWAVE medical focused applicators proved that focused shockwaves used in their non-focal region are capable to dislodge the adhesive material, which was the purpose of this feasibility study. However, a better use for shockwave technology is for biofilm prevention for both dormant (no crew on board) and non-dormant periods. For that, the shockwaves systems can be activated for minutes to few hours at set time intervals. Using the shockwave technology can prevent bacteria to aggregate or incipient biofilms can be dislodged. Most important, there is no resistance and adaptability of the bacteria induced by shockwaves, as it happens with chemical methods. In separate experiments at University of Georgia [19], it was shown that shockwaves can kill individual bacterium, but not all the bacterial planktonic cells. However, shockwaves can be combined, without any interference and with additive effects, with other antibacterial options such as biocides, light technologies, biofilm starving, etc., which can accomplish the complete killing of the dislodge bacterial cells.

As observed in our preliminary tests, the technology can work both with bubbles or no bubbles being present. The cavitation bubbles generated by the shockwaves in a gravitational environment are in the dimensional range of millimeters and dissipate fast, especially when frequency of shockwave pulses per second is low. The same should happen in low-gravity or no gravity environments, since the bubbles are small in dimension and the pressure from the fluid is enough to collapse them. However, if residual bubbles are present in a fluid, the effect of shockwaves is amplified.

In general, the shockwaves systems produce noise, although the levels can be different when comparing different technologies used for shockwave generation. The electrohydraulic shockwave applicators are the noisiest and the electromagnetic or piezoelectric shockwave applicators produce less noise. Furthermore, the noise can be reduced significantly by employing noise dampening devices, as SANUWAVE uses in its medical electrohydraulic focused applicators. The applicators have also a limited life and need adjustments or maintenance periodically, which is depending on the technology employed for shockwave generation, with electrohydraulic applicators requiring frequent maintenance work, with electromagnetic applicators requiring significantly less maintenance and the piezoelectric applicators even less maintenance. However, for the electrohydraulic applicators an automated electrode gap adjustment system can be created, which can meaningfully increase the life of the applicators.

To improve biofilm cleaning and prevention, the shockwave applicators and tank designs must be optimized, which can be done via acoustic propagation simulations using the COMSOL Multiphysics software. It has been observed that the shockwave technology can dislodge films from the walls of a cylindrical tank. If a bellow tank is used, the biofilms can easily form in the folds of the bellows. To properly use shockwave for cleaning biofilms in bellow tanks, the bellows will need to be extended to their maximum height and then apply the shockwaves to reach efficiently the bottom of the folds. To increase the productivity of shockwaves for removal or prevention of biofilms, other tank designs may be used as ellipsoidal tanks, spherical tanks, etc.

Regarding the energy consumption, the shockwave system from Figure 4 when running at the upper limits for the settings (5Hz and 30kV) requires a total 100 Watts x hours (Wh), which is the average energy consumed in one hour. However, the amount of energy is depending on the frequency of the shockwaves, with lower frequencies as 1Hz consuming only 20 Wh in one hour. Further work is needed to examine the integration of such shockwave system with existing ISS WRS hardware. The design of the shockwave system would require easy access to the shockwave applicators, to reduce crew time dedicated for system maintenance or recovery from dormant periods and have only non-movable parts that increases their longevity.

Acknowledgments

The authors would like to acknowledge the contributions and funding support from the Exploration Capabilities Life Support Systems, as well as its project management leadership Walt Schneider and Imelda Stambaugh. We would like to thank the NASA MSFC Space Systems Department for their management and support. Funding was provided to the SANUWAVE project via 80NSSC21P2234, to cover IC. Auburn University work was performed under contract 80NSSC19K0368. We would also like to acknowledge the work of Buket Demir and other students and graduates from Auburn University.

References

[1] Kayatin, M. J., Carter, D.L., Schunk, R.G., Pruitt, J. M. Upgrades to the ISS Water Recovery System. Presented at the 46th International Conference on Environmental Systems, Vienna, Austria, 2016.

[2] Zea L. et al. "Design of a spaceflight biofilm experiment," Acta Astronautica, Vol. 148, July 2018, pp. 294-300. https://doi.org/10.1016%2Fj.actaastro.2018.04.039

[3] Maryatt, B.W. and Smith, M.J. Microbial Growth Control in the International Space Station Potable Water Dispenser. Presented at the 47th International Conference on Environmental Systems, Charleston, South Carolina, 2017.

[4] Clayton, G.E., Thorn, R.M.S. and Reynolds, D.M. "The efficacy of chlorine-based disinfectants against planktonic and biofilm bacteria for decentralised point-of-use drinking water," npj Clean Water 4, 2021, 48. https://doi.org/10.1038/s41545-021-00139-w

[5] Justiniano, Y.-A. V., and Johnson, A. Developing a Multi-Bioreactor Test Stand for the Evaluation of Biofilm Mitigation Technologies. Presented at the 50th International Conference on Environmental Systems, Online, 2021.

[6] Justiniano, Y.-A.V., Carter, L., Nur, M. And Angle, G. Developing Methods for Biofilm Control in Microgravity for a Water Recovery System. ICES 2020.

[7] Weir, N., Wilson, M., Yoets, A., Molina, T., Bruce, R., and Carter, L. Microbial Characterization of the International Space Station Water Processor Assembly External Filter Assembly S/N 01. Presented at the 42nd International Conference on Environmental Systems, San Diego, California, 2012.

[8] Mbareche, H. et al. "Recovery of Fungal Cells from Air Samples: a Tale of Loss and Gain" Applied and Environmental Microbiology, Vol. 85(9), 2019, e02941-18. https://doi.org/10.1128%2FAEM.02941-18

[9] Justiniano, Y.-A.V. et al. Biofilm Management in a Microgravity Water Recovery System. Presented at the 50th International Conference on Environmental Systems, Online, 2021.

[10] Bowman, E.M., Carter, L., Wilson, M., Cole, H., Orozco, N. and Snowdon, D. Performance Evaluation of the ISS Water Processor Multifiltration Beds. Presented at the 42nd International Conference on Environmental Systems, San Diego, California, 2012.

[11] ASTM Standard E2562-22, 2022," Standard Test Method for Quantification of Pseudomonas aeruginosa Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor" ASTM International, West Conshohocken, PA, 2022, DOI: 10.1520/E2562-22,www.astm.org.

[12] Demir, B., Taylor, A., Broughton, R. M., Huang, T.-S., Bozack, M. J., and Worley, S. D. "N-Halamine Surface Coating for Mitigation of Biofilm and Microbial Contamination in Water Systems for Space Travel." Biofilm, Vol. 4, 2022, p. 100076. https://doi.org/10.1016/j.bioflm.2022.100076.

[13] Cleveland R.O. and McAteer J.A. "The Physics of Shock Wave Lithotripsy," Smith's Textbook of Urology, edited by Smith A.D., Preminger G.M., Kavoussi L.R., Gopal H., 2007, pp. 317-332

[14] Brenner M.P, Hilgenfeldt S, Lohse D. "Single bubble Sonoluminescence," Reviews of Modern Physics, Vol. 74. April 2002, pp. 425-484.

[15] Putterman S., "Sonoluminescence: Sound into Light," Scientific American, February 1995, pp. 33-37.

[16] James G.A, Cioanta I., Jackson J., Fisher S.T., "Shockwave Disruption of Biofilms," 7th ASM Conference on Biofilms, Chicago IL, October 24-29, 2015.

[17] Sturman P, "Assessment of SANUWAVE PACE Technology for the Removal of Marine Biofilms," Center for Biofilm Engineering, 2015.

[18] Sturman P, "Assessment of SANUWAVE PACE Technology for the Removal of Biofilm from Marble," Center for Biofilm Engineering, 2017.

[19] Cioanta I., "Acoustic Pressure Shock Wave Technology Successfully Disrupts Medical and Non-Medical Biofilms," Montana Science and Technology Meeting, Bozeman, MT, July 19-21, 2016.

[20] Willis M.J, Ahrens T.J., Bertani L.E., Nash C.Z., "Bugbuster – Survivability of Living Bacteria Upon Shock Compression" Earth and Planetary Science Letters, Vol. 247, 2006, pp. 185-196.

[21] Booth I.R, Edwards M.D., Black S., Schumann U, Miller S., "Mechanosensitive Channels in Bacteria: Signs of Closure?,", Nature Reviews – Microbiology, Vol. 5, June 2007, pp. 431-440.