

Mitigation of Biofouling in Plant Watering Systems Using AgXX, a Novel Surface Treatment

Tesia D. Irwin[1], Wenyan Li[2], Angie Diaz[2], and Mary Hummerick[2]
NASA, Kennedy Space Center, FL, 32899

The development of plant growth systems with high yields and low maintenance for food production is a key focus area for NASA. One of the remaining technical challenges is keeping the plant watering systems clean without affecting plant growth, requiring consumables, or demanding crew time. Plant watering systems, such as the one onboard the International Space Station (ISS), provide a nutrient rich environment for biofilm formation. Frequent maintenance is necessary to prevent biofouling, which currently requires crew time and a mechanical means of cleaning. Better solutions are needed. The current ISS practices for biofilm mitigation in the water recovery and distribution system include the use of biocides (silver ion or iodine) along with regular maintenance (e.g. flushing, filter replacement). These biocide-based strategies could be problematic for plant watering systems, such as in Ohalo, the Exploration Garden, and the APH, due to incompatibilities of the biocides with plants. We propose the application of AgXX, a novel antifouling surface treatment that meets the above requirements. This paper will report on an initial study that was completed to determine whether AgXX would be effective in a plant nutrient solution, and whether it would negatively impact plant growth in a hydroponics-type system.

Nomenclature

Ag	=	silver
APH	=	Advanced Plant Habitat
CFU	=	Colony Forming Units
CFU/mL	=	Colony Forming Units per Milliliter
COTS	=	commercial off-the-shelf
g	=	gram
H_2O_2	=	hydrogen peroxide
IMA	=	Inhibitory Mould Agar
ISS	=	International Space Station
mL	=	milliliter
mm	=	millimeter
NASA	=	National Aeronautics and Space Administration
NA	=	nutrient agar
NB	=	nutrient broth
O_2	=	oxygen
ROS	=	Reactive Oxygen Species
RPM	=	rotations per minute
Ru	=	ruthenium
TSA	=	Tryptic Soy Agar

[1] R&D Engineer, The Bionetics Corporation, Mail Code: LASSO-008, Kennedy Space Center, FL, 32899.
[2] Research Scientist, Amentum, Mail Code: LASSO-001, Kennedy Space Center, FL, 32899.

I. Introduction

The development of plant growth systems with high yields and low maintenance for food production in space is a key focus area for NASA. Plant growth systems in micro-gravity present a myriad of challenges, which must be overcome before NASA can reach its goals of human habitation of extra-terrestrial surfaces and long-duration spaceflights. Some of these challenges include reliable and sustainable food production, volume optimization concepts, water and nutrient delivery system technologies, food sanitization, and, last but not least, biofouling and contamination prevention.

The International Space Station (ISS) has experienced problems associated with biofouling of distribution plumbing in the water lines of spaceflight plant growth hardware (e.g., the Advanced Plant Habitat (APH) on the ISS). The Ohalo Project received an action at its principal design review in August 2021 to coordinate its design, its plans for the prevention of biofouling, and payload maintenance requirements with Agency Subject Matter Experts (i.e., KSC Crop Production Team, JSC microbiology, SCLT Food/Nutrition Roadmap Lead, and LSS Water Disinfection Team).

To achieve biofouling mitigation for space plant growth system, a technical solution is needed to keep the plant watering systems clean without affecting plant growth, requiring biocide consumable, or demanding crew time. We have proposed the application of AGXX, a novel antifouling surface treatment that meets the above requirement, to meet the challenge.

Our objective was to investigate the use of a commercial off-the-self (COTS) product (AgXX) to prevent biofouling of spacecraft plant watering systems and to evaluate any detrimental effects to plant growth and health. Here we report the outcome of this investigation following relevant background information and some description of the proposal.

II. Background, State of the art, and Proposal Summary

The plant watering systems, such as the one onboard ISS, provide nutrient rich environments for biofilm formation. These systems need frequent maintenance, which currently requires crew time to scrub biofouling surfaces between plant growth cycles. Better solutions are needed. The current ISS practices for biofilm mitigation in the water recovery and distribution system includes the use of biocides (silver ion or iodine) along with regular maintenance (e.g. flushing, filter replacement).[1] These biocide-based strategies could be problematic for plant watering systems, such as in Ohalo, the Exploration Garden, and the APH, due to incompatibilities of the biocides with plants.[2,3]

Several extensive literature reviews were carried out on the state of art technologies to identify a viable solution for biofilm control.[4,5,6,7] In general, biofilm mitigation approaches include physical removal, chemical treatment, radiation, or biological methods.[4] Each method has its own limitation, such as time requirements (physical removal), potential toxic byproducts (chemical treatment), hardware installation (radiation), and limited applications (biological method). Surface treatments or coatings have also been used for biofouling mitigation, but they often fail to provide long term protection without re-application or regeneration of the coatings.[2] Antifouling coatings generally rely on one of two principles, each having its own inherent failure mode that requires periodic recoat or risk of biofouling: (1) antifouling through the release of a biocidal component from the coating, failure at time of biocide depletion; and (2) varying types of hydrophobic surfaces preventing direct contact of water, failure at time of the inevitable wetting under immersion, which leads to biofilm formation.

AgXX is a novel surface coating that prevents biofouling via the passive generation of peroxide at the coating surface. AgXX has the potential to decrease or eliminate manual system cleaning and reduce consumables (filters, biocides, etc.). This investigation supports plant growth systems by providing a means to manage biofouling concerns while lowering maintenance, down-time, and consumables requirements.

A. AgXX and Technology Advancement

As the result of persistent effort, AgXX was identified as a promising candidate technology for solving the biofouling problem in plant growth water systems. AgXX is a novel antifouling coating technology that uses micro-galvanic cells comprised of silver (anode) and activated ruthenium (cathode) to generate Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2), in the presence of dissolved oxygen and organics.

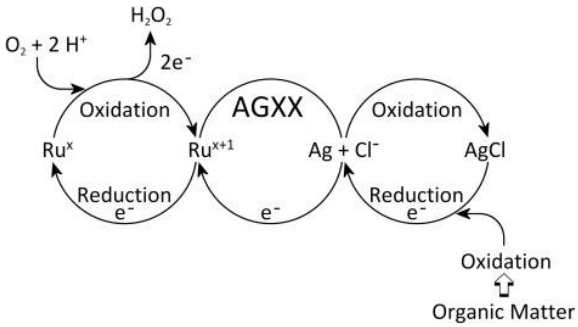

Figure 1: AgXX Mechanism.

AgXX is a commercially available product of a novel technology, as an electroplated coating or microparticles that can be used to coat surfaces including metals, glass, and polymers.[8,9] It also has well-documented success in submersed applications and proven long-term effectiveness. It stands apart from other antifouling coating applications and traditional biofilm prevention strategies for the following reasons.

First, AgXX does not dose biocide, but rather generates H_2O_2 from dissolved oxygen (O_2) at the AgXX surface. Therefore, there are no consumables and H_2O_2 is one of very few disinfectants that does not leave behind a toxic byproduct. This helps to alleviate concerns of plant incompatibility, as it offers localized biofilm inhibition where biofouling is a concern, without impacting beneficial bacteria at the plant root zone.[10, 11]

Second, AgXX does not rely on the releasing of silver ions for its antifouling function, therefore, does not require replenishment. This contributes to its long-term performance.

Third, AgXX has proven to be durable when used in multiple industrial settings as part of a biofilm mitigation strategy for long durations (upwards of 19 months to 5 years).[12,13]

The well documented use and fundamental technological differences between AgXX and other coating approaches make AgXX a good candidate technology in preventing biofilm growth in plant watering systems. However, its performance is yet to be confirmed in plant watering system, and equally importantly, its impact on plant growth must be investigated.

B. Project Goal and Technical Approach:

The main goal of this project was to determine whether AgXX could be used in watering systems for plants to inhibit biofouling and in turn lower the maintenance requirements for such systems. We aimed to test the efficacy of implementing AgXX within the water recirculation system of a plant growth test bed and did so by monitoring for both microbial inhibition and plant health via microbial plate counts, live/dead imaging, root and vegetation fresh and dry weights, and hydrogen peroxide concentrations among other testing.

This project was to determine the effectiveness of AgXX for biofilm control in plant watering systems, from both a plant health and microbial control perspective. To test for biofilm prevention, we used immersion test with both AgXX and control coupons in a nutrient solution which mimics that of a plant watering system. For plant compatibility testing, two hydroponic based testbeds with recirculating water (one with AgXX-coated meshes and one without) were constructed and used to perform initial proof-of-concept testing on the effects of using AgXX in plant water systems. Multiple crop grow-outs, with short harvest periods, were grown in each system under the same environmental conditions. Bacterial counts were performed throughout the experiment, and the growth along with the general health of the plants was monitored. At the conclusion of the experiment, the total crop weight from each system was measured and the water systems were deconstructed and examined for biofilm growth.

III. Experimental Methods and Test Results: Microbiological Tests

A. Experimental

Materials

An AgXX coated stainless steel mesh and a control stainless steel mesh were used for testing. The AgXX product, Ae-aqua C2-100 strainer baskets, contains AgXX coated stainless steel mesh rings. It is sold as a water purification product that is quoted as being able to "be used for two years to protect a 100L water tank against bacterial growth." It contains 60 rings, each made of a 2cm x 6cm mesh sheet rolled into a ring.

For the biofilm coupon test, 1cm x 1cm AgXX coupons of AgXX coated mesh and uncoated stainless steel mesh coupons (control) were used. These meshes have the similar microstructure as in Guridi et.al.,[13] a control sample can be prepared using stainless steel mesh of similar size.

Figure 2. AgXX product as purchased (left) and a single AgXX mesh (right).

Methods

After reviewing some published AgXX test data,[13] it was determined that long term well plate coupon testing was appropriate for the evaluation of the AgXX biofilm resistance; details of the test method can be found in the reference.[14]

Coupons (1 cm^2) were challenged with *Pseudomonas aeruginosa* (ATCC 10145), which has been isolated in ISS water systems and is also known to produce biofilm. Cultures were prepared by inoculating 25 milliliters (mL) of nutrient broth (NB) in a 125 mL sterile flask with a few colonies from a 24-hour culture grown on nutrient agar (NA). The flask was incubated for 18 hours at 27° C and 150 rotations per minute (RPM) in a shaker/incubator. Sterile 12 well plates containing the AGXX mesh test coupons were inoculated with 4 mL of washed (centrifuge at 5000 RPM and resuspend pellet in sterile water or sterile plant nutrient solution, repeat 3 times) bacterial culture adjusted to approximately 1 x 10^6 cells per mL. Plates were sealed with parafilm and placed in a rotary shaker and incubated at 27°C at 110 RPM. Untreated mesh material coupon controls were included in testing. Both sterile water and plant nutrient solution were tested to determine the effect on bacterial growth and biofilm formation. Tests were performed in triplicate and duplicate plates were prepared: one for imaging and one for enumeration plating. The plates were incubated in the rotary shaker/incubator at 27 °C at 110 RPM and sampled after 24 and 48 hours, one week and four weeks. Figure 3 illustrates the plate set-up for one organism per sample time (total of 16 plates).

Sample processing

Coupons were removed from wells using sterile forceps. Coupons were then dipped individually three times in sterile DI water to remove unattached cells. Individual coupons were placed in sterile 15 mL centrifuge tubes containing 4 mL sterile water, vortexed for 30 seconds and placed in a bath sonicator and sonicated for 30 seconds. This process was repeated 3 times to remove attached cells. After sonication, water was diluted and plated in triplicate onto nutrient agar for enumeration of bacteria. Dilution and plating were also performed on the solution containing the coupon in the 12 well plate to enumerate the unattached planktonic bacteria.

An aliquot of 1 mL of each sample was stained with BacLight, live/dead stain and filtered through 25-millimeter (mm) diameter black filters. Filters were placed on a microscope slide fixed with oil and a coverslip and viewed using a Zeiss axioscope fluorescent microscope.

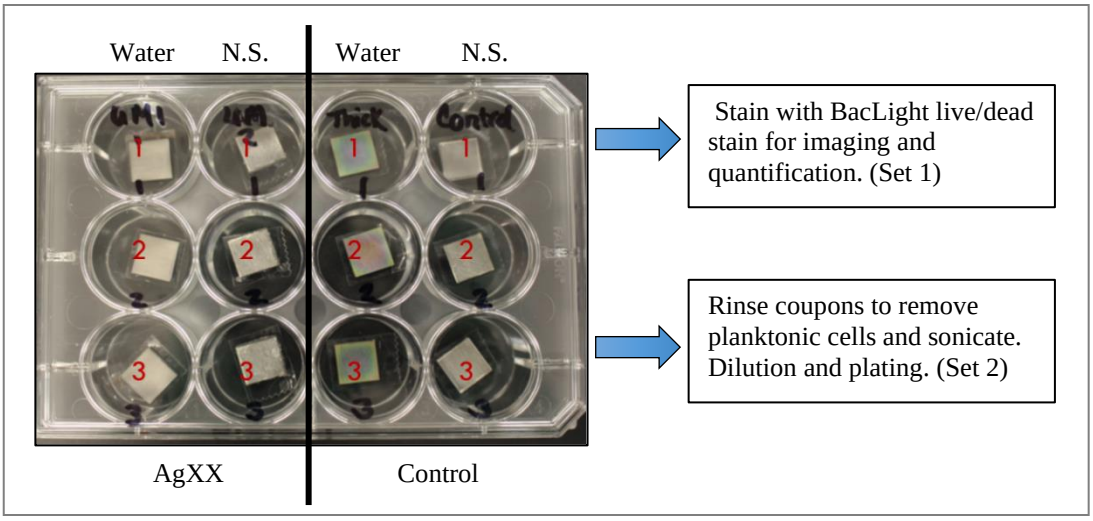

Figure 3. Plate set-up per organism and sample time point. Duplicate plates are prepared to accommodate imaging and bacterial enumeration.

C. Results

CFU plate counts

Figure 4 and Figure 5 show the CFU plate counts of AGXX compared with the control mesh. Under test condition, AgXX showed an impressive ability to prohibit bacterial growth both on the surface and in solution. The AgXX meshes decreased bacterial growth to below the detection level of the plating method consistently for all time points, while the control mesh did allow bacterial growth on the surface and in solution. In all cases, AgXX achieved greater than a 5-log reduction.

The cell count results for the control mesh are shown in Figure 4 for DI water and Figure 5 for the plant nutrient solution and are compared with the live/dead cell counts in the next section.

Live/Dead Images

When we closely examined the live/dead cell images, additional details on the AgXX effect were revealed. The green (live) and red (dead) trendlines in Figure 4 (DI water) and Figure 5 (plant nutrient solution) below show the live/dead stain counts for the control mesh and the AgXX mesh. These figures also break the counts down by location, showing bacteria found on the meshes themselves (top graphs) as well as in the solution (bottom graphs).

Figure 4 shows the live/dead imaging counts along with plate counts in DI water. It was revealed that due to the low nutrient level in DI water, for the control mesh, the plate counts decreased throughout the test. The live cell counts decreased slower than the plate counts, as cells became unculturable before losing membrane integrity. At day 1, the plate count was higher than the live cell count, likely due to the presence of ultrasmall cells that are culturable, but difficult to count. The AgXX mesh greatly affected bacterial growth in DI water and directly impacted cell viability. This is evidenced by the large number of dead cells (with compromised cell membranes) in water.

When combining these observations with the cell count results in Figure 4, we can see that on the control mesh the cell numbers increased over time, indicating the growth of the biofilm. However, the plate counts decreased at the end, likely due to starvation. On the AgXX mesh, the live cell numbers decreased after day 2, indicating the biofilm resistance of the AgXX surface.

Figure 5 shows the live/dead imaging counts and plate counts in plant nutrient solution. The data shows that the nutrient solution with the control mesh supported bacterial growth until day 7. The plate counts were higher than the live cell counts, likely due to the presence of ultrasmall cells that are culturable, but difficult to count. The AgXX was successful in preventing bacteria from growing in the nutrient solution, as evidenced by the live cell count decreasing overtime while the dead cell count increased.

When comparing the control mesh with the AGXX on the mesh surface: on the control mesh, the plate counts decreased more slowly at the end (Day 7 to Day 28), indicating that the biofilm helped to preserve the culturability of

the cells as the nutrients were consumed in the solution. During the test, there were fewer cells on the AgXX mesh than on the control mesh, as expected.

In summary, the AgXX prevented bacteria from growing, achieving greater than 5 log reduction, in DI water and in the plant nutrient solution. The AgXX also directly impacted cell viability as evidenced by (in DI water) the large number of dead cells (with compromised cell membranes) in water and with a lower total cell number on the mesh at day 28; and (in the nutrient solution) a decrease of the live cell number in the solution, and the lower cell number on the mesh.

Live/Dead and Plate Count: comparison

Figure 4 and Figure 5 show the comparison between the CFU plate count and the live/dead cell count for DI water and the plant nutrient solution respectively.

In DI water, the live/dead cell counts showed that AgXX also directly impacts cell viability as evidenced by the large number of dead cells (with compromised cell membranes) in water and a lower total cell number on the mesh at day 28. In the nutrient solution, AgXX similarly impacts cell viability.

It is also evident that the total bacterial growth is much higher (and the growth period longer) in the nutrient solution than in DI water, due to the higher nutrient level.

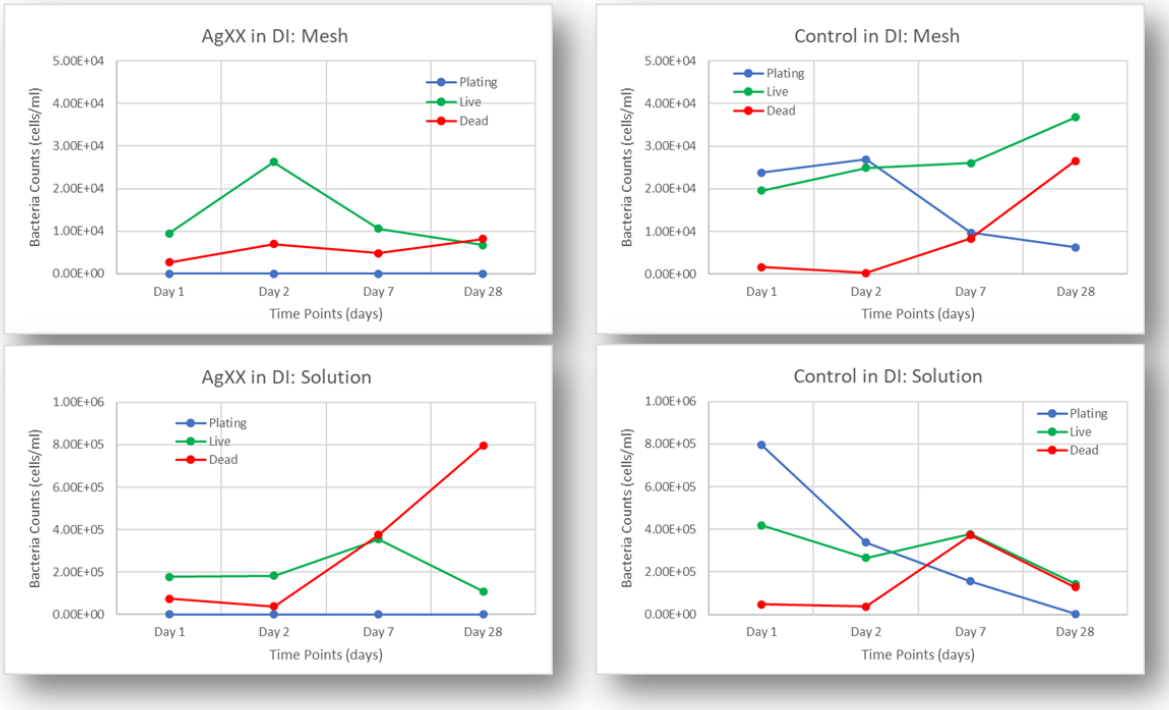

Figure 4. Live/Dead cell count compared to CFU cell counts: DI water.

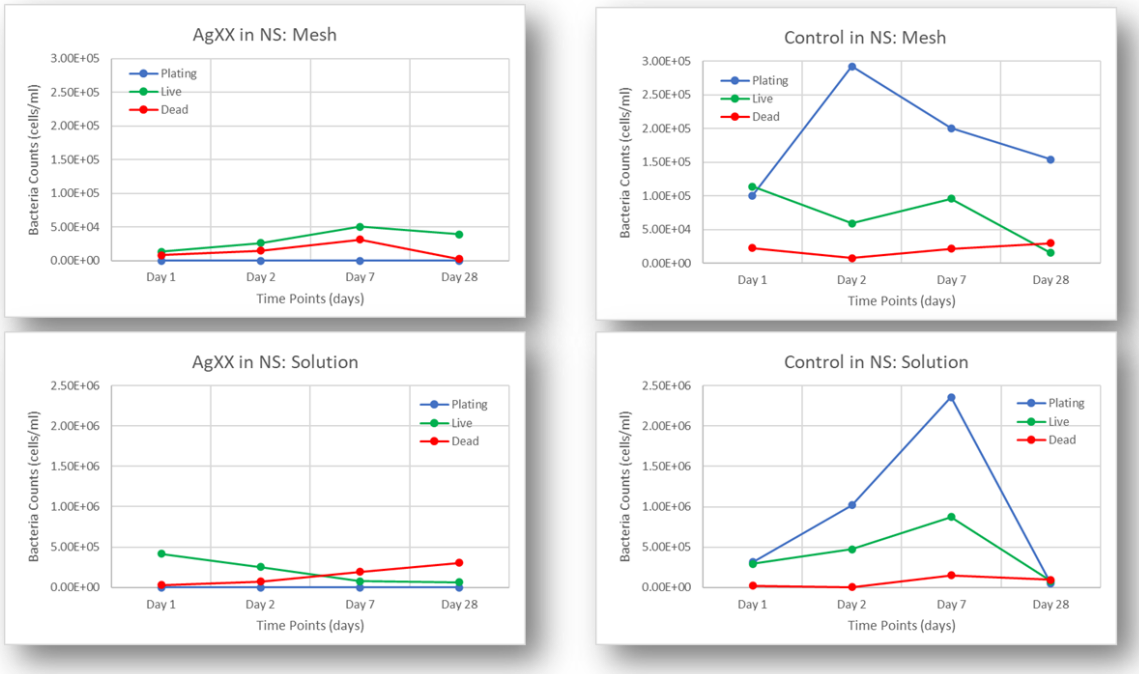

Figure 5. Live/Dead cell count compared to CFU cell counts: Plant Nutrient Solution

IV. Plant Growth Experiments

The purpose of the Plant Growth experiments was to determine whether the use of the AgXX in a plant watering system has detrimental effects on plant health and growth. To do this, we performed five consecutive growth tests and harvests in an Aerogarden Farm 24Plus system.

A. Methods

Crop & Fertilizer Selection

Red romaine lettuce, Lactuca sativa cv 'Outredgeous', was selected for use throughout the five growth experiments for its short growth cycle (~3-4 weeks to harvest) and known sensitivity to environmental stressors. The fertilizer provided by Aerogarden was used along with the recommended dosing recipe and strategy.

System Assembly and Pre-run

Prior to any experiment, the Aerogarden Farm 24Plus system was assembled based on manufacturer instructions and a test batch of lettuce was grown in it as assembled, to assess any potential problems. All standard sanitization, daily checks, and final harvest data collection was performed during this test to provide an opportunity for procedural changes prior to starting experimentation.

Grow-out Procedure

Each of the five growth tests followed the same general procedures which are outlined below. The first growth test was to determine an appropriate amount of AgXX to use, the last four growth tests all used the same planting and harvesting procedure with no changes to system water or test coupons in between growth tests. At the start of test #1, the AgXX trial amount growth test, the Aerogarden system was sanitized by first scrubbing all components with soap and water followed by a 10 min soak in a 5% bleach solution, rinsing with copious amount of DI water, and filling the system with a dilute peroxide solution which was recirculated through all components for 24hrs prior to draining and re-rinsing with sterile DI water. At the start of test #2 the system was sanitized and new sterile DI water was used to fill each water bowl. This sanitization step was also repeated at the end of the 5th harvest cycle (not between harvests).

Within the Aerogarden, there are two separate water bowls and grow decks. One contained AgXX in the form of mesh rings purchased from ae-aqua, the other acted as a control and contained an equivalent amount of stainless-steel mesh rings. For test #1, three 2cm x 6cm AgXX mesh rings were tested and determined to be too harmful to plant health at that loading. For tests 2-5 one mesh ring was used but was cut in half and each half was placed on opposite ends of the water bowl to distribute the product. Ten round plastic coupons were also placed throughout each system at the start of test #1 to be used as biofilm indication coupons. Two of these were pulled from each system at the end of each test, from approximately the same area.

Seeds were planted in the Aerogarden pods upon the start of each experiment using the following method. Prior to planting all of the supplied Aerogarden growth media pods were adjusted by filling it in with extra pod media so that the hole depth for each pod was approximately 1/8th inch. All pods were also autoclaved before used and soaked in sterile DI water for ~1hr prior to planting to ensure they were all fully wetted. Two Outredgeous lettuce seeds were planted in each pod to ensure successful germination. Daily checks were performed, details are outlined in AGXX-SLP-002-Daily Experimental Checks. Temperature, humidity, light height, pest presence, pH, and conductivity were all monitored daily. Water samples were also taken 2-3 times per week to measure hydrogen peroxide levels in both systems. A Hach DR3700 was used to measure peroxide levels, using the Peracetic Acid and Hydrogen Peroxide programs.

Harvests and Sampling

Harvests were broken up into a mid harvest (the four central plant pods) and a final harvest (remaining eight plant pods) so-as to give adequate growing space and minimize overcrowding. Each harvest went through the same procedure, which is described below. For each plant, the following were measured and recorded: height and width dimensions, anthocyanin content, chlorophyl content (using a SPAD meter), and both wet and dry weights of the vegetation and roots. For vegetation weights, each plant was cut at the base just above the roots and plug. For root weights, the roots were carefully trimmed from the outside of each growth plug and patted dry with a paper towel prior to measuring the wet weight. All plants were also photographed after each harvest on both black and white backgrounds.

During each final harvest, water samples were pulled from each system for plate counts and chemical analysis. Plastic biofilm coupons (two/system/harvest) were also taken to perform plate counts.

Microbial Growth Sampling

During each final harvest, water samples were pulled from each system for plate counts and chemical analysis. Autoclaved polycarbonate biofilm coupons (two/system/harvest) were also taken to perform plate counts. The solution was taken and vortexed for 30 seconds in a 50ml falcon tube before dilution of 100 µl in microcentrifuge tubes containing 900 microliters (µL) sterile water in series. The tubes were then vortexed for five seconds before immediately plating 100 µL each onto Tryptic Soy Agar (TSA) or Inhibitory Mold Agar (IMA). While TSA is a non-selective growth medium, IMA inhibits bacterial growth which allows for selectivity of filamentous fungi and yeasts. All plates were inverted and placed in a 30°C incubator for seven days. On day seven, a count of the colony forming units (CFU) was taken to estimate the original sample population density.

Coupons pulled from the Aerogarden were transported individually in a sterile 15 mL centrifuge tube with 10 mL of solution from the immediate vicinity of the coupon in the Aerogarden. The solution was drained and the coupon transferred to a fresh tube of 4 mL sterile water before being vortexed for 30 seconds. This was followed by sonication for 30 seconds, and one more vortexing round for 30 seconds to help release whatever microbial loads might be on the coupon. The solution was then diluted and plated like the previous solution samples. The same process was done with the filters but in 15 mL of solution due to the size of the filters.

B. Plant Growth Test Results – Plant Measurements & Environmental Conditions

A summary of the final harvest data is presented in this report, as it is representative of the same trends that were seen in the mid harvest data. Additional charts will be included in the Appendix section. Figure 6 shows the environmental conditions throughout the entire five month growth test period, including humidity, temperature, lighting adjustments, system pH, and conductivity. There were no appreciable impacts from the environment on either crop. The pH was only adjusted at the end of each growth test or if the system pH dipped below a pH of 4, and was adjusted by adding equal amounts of 1M KOH to both systems. Apart from the first growth test, the pH in both systems was nearly identical. System conductivity did appear to be slowly increasing in both systems over time, which is

understandable and expected considering that the water reservoir was not being turned over or cleaned in between tests.

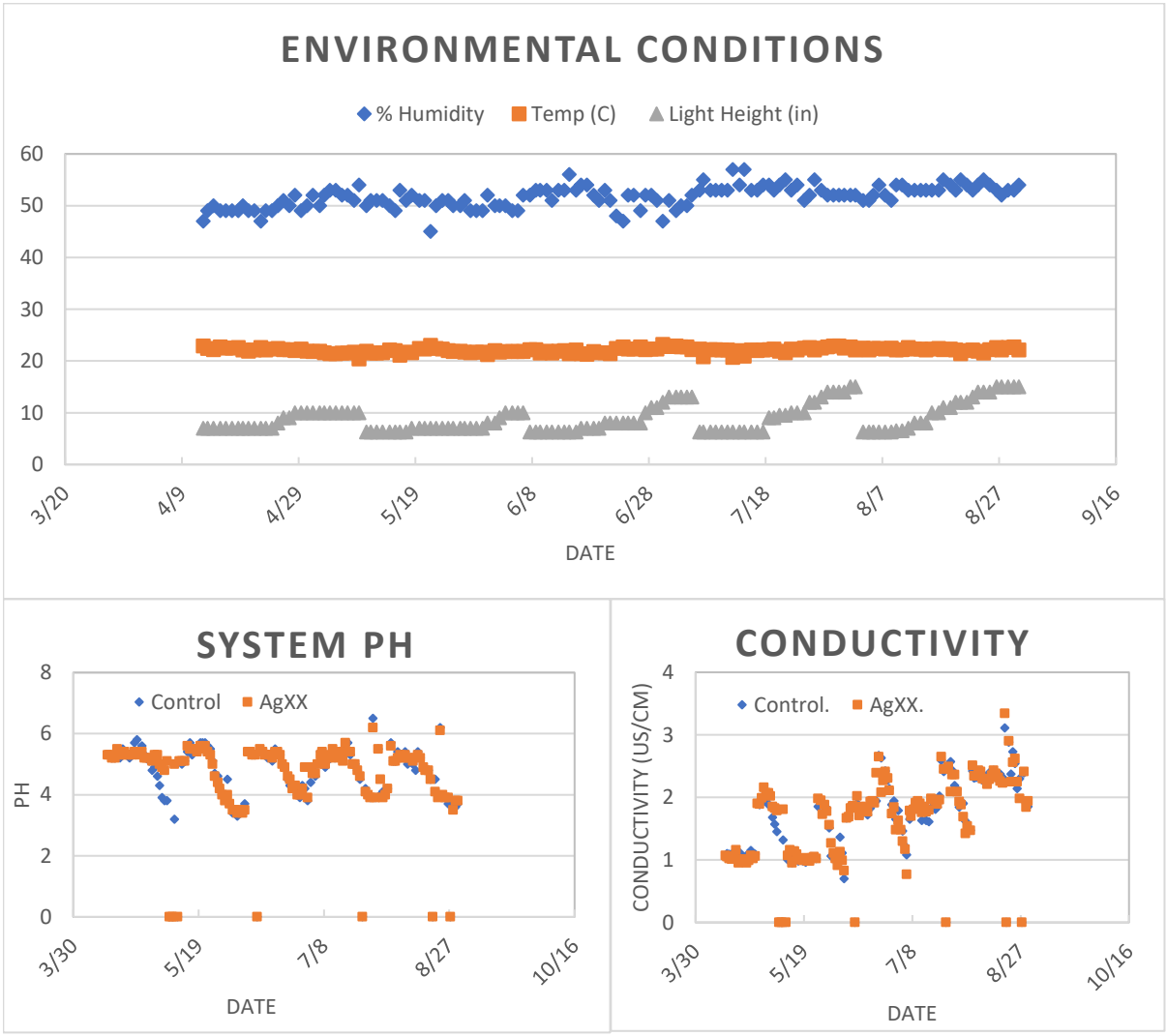

Figure 6: Environmental conditions throughout the 5 month Grow Out period.

Hydrogen peroxide concentrations were monitored throughout the plant growth experiment by collecting water samples 2-3 times per week and measuring the H_2O_2 immediately after sampling. This was done to determine whether the peroxide would accumulate over time in the AgXX system if the water is not fully changed out in between GOs. Figure 7 shows the H_2O_2 data for the entire plant growth experiment, with the AgXX system data shown in blue and the Control shown in orange. Both systems trended together the entire time indicating that there was no significant buildup of H_2O_2 in the bulk water from the AgXX.

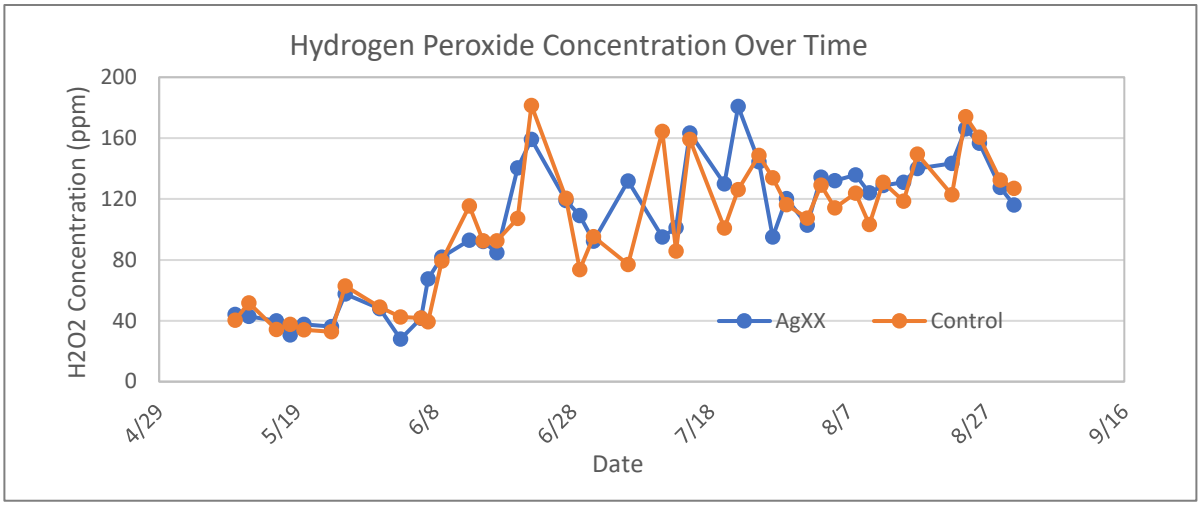

Figure 7: Hydrogen peroxide concentrations in the water bowls of the AgXX and Control systems through the plant growth experiment.

The following plots and figures show a summary of some of the harvest data that was gathered in all five growth tests. Figure 8 shows images of the growth trays at the ends of week 1-4 for test #3, where the control crop is shown in the left tray and the AgXX crop is shown on the right. This growth test was typical of what was seen for tests 2-5 and showed no visible difference between the control crop and the AgXX crop.

Figure 8: Week end images of Control (left) and AgXX (right) crops throughout growth test #3.

Figure 9 shows the final harvest averages (shown as bar plots) and individual plant measurements (shown as points) for both the plant wet and dry mass and root wet and dry mass for the control and AgXX crops. As stated earlier, growth test #1 was a trial to determine an appropriate amount of AgXX to utilize in the water system. The amount used was clearly too much, harming both the vegetation and roots. This first growth test was also harvested early, since it was only a practice run, but is shown for totality in the plots below. Once the AgXX amount was adjusted, tests 2-5 showed no statistical difference between the vegetation or root mass of the AgXX crop vs the Control. It was important to show that this trend would continue over time to show that there is not an accumulation of peroxide or other ROS forms over time that would have greater impacts on crop yields.

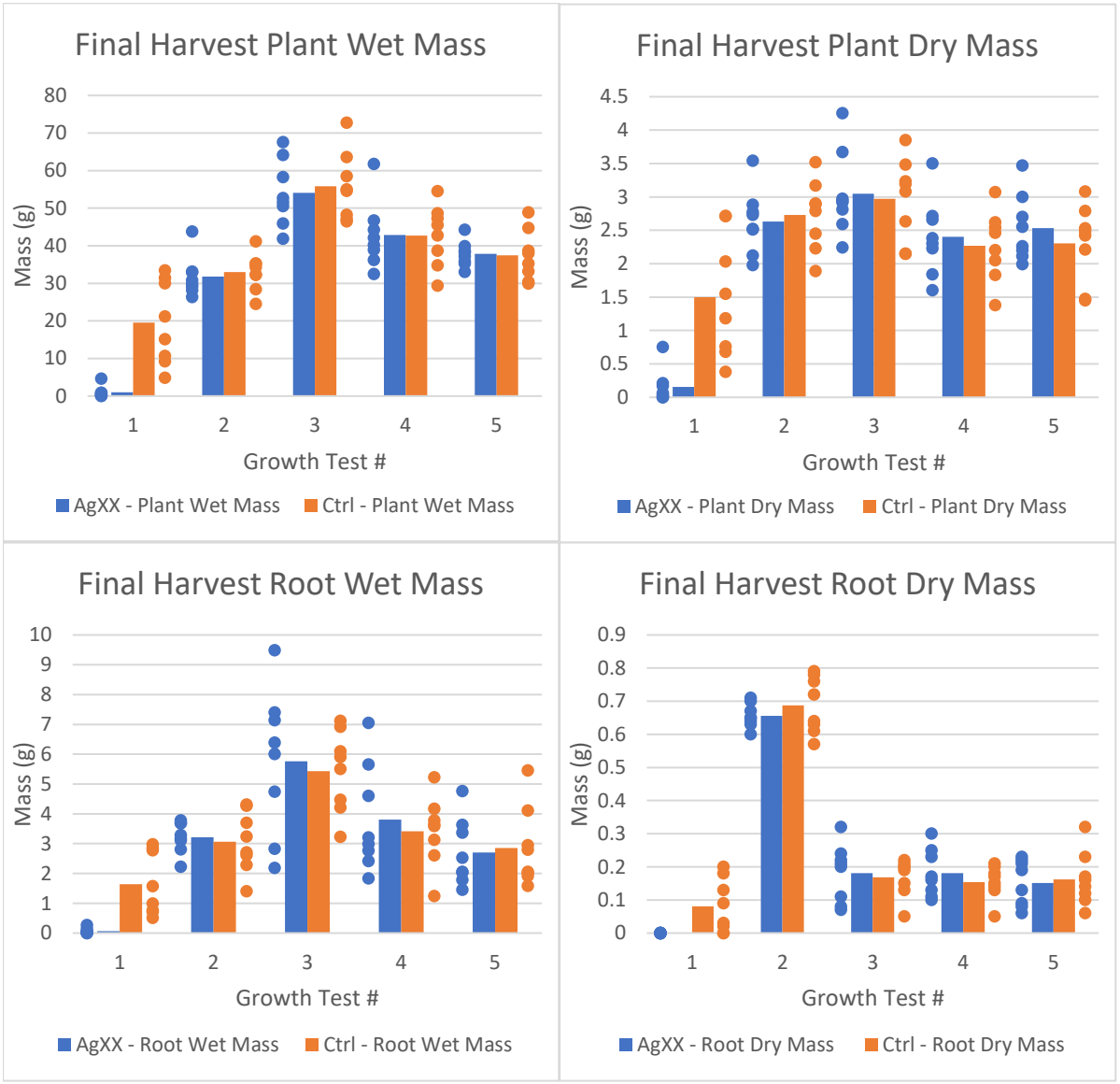

Figure 9: The two charts above show the final harvest wet (left) and dry (right) mass of the plant (top) and roots (bottom) for both the AgXX (blue) and Control (orange) crops for all 5 growth tests. The averages for each harvest are represented as a bar plot, whereas the individual values for each harvest are shown as scatter plots.

Figure 10 shows the anthocyanin and chlorophyl content measurements from all five final harvests. As in Figure 9, the harvest averages are shown as bar plots while the individual measurements are shown as a corresponding scatter plot. In can be seen that for test #1, which had a higher amount of AgXX, that the AgXX crop had much higher levels of anthocyanin on average and lower chlorophyl levels than the control crop. This may be due to an increased stress response in the AgXX crop due to the higher-than-necessary surface area of AgXX in that system for that growth test. While tests 2-5 show no statistically significant differences between the two crops for anthocyanin or chlorophyl content, the last three tests did show a consistently higher average for both values in the AgXX crop. A longer-term study and a more in-depth nutrient analysis of the plants will need to be performed to determine if this trend continues over time and indicates a potential for a healthier crop in the AgXX system.

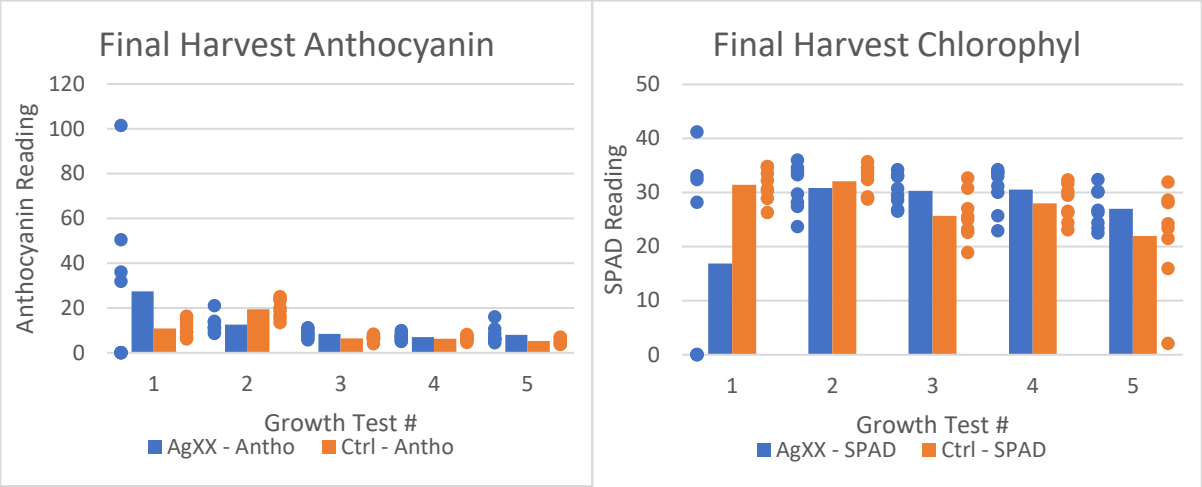

Figure 10: Anthocyanin and chlorophyl content measurements from all 5 final harvests. The harvest averages are shown as bar plots while the individual reading are shown as a corresponding scatter plot.

C. Plant Growth Test Results – Microbial Sampling

At the end of each harvest, several coupons were placed within the Aerogarden bowl during planting sessions, and two were pulled for testing at each harvest. When comparing the solution samples of the four harvests, the control samples had higher microbial counts in comparison to the AgXX circulating solution in TSA, but the difference was less than an order of magnitude (Figure 11A). However, the AgXX maintained a lower count consistently without full cleaning between harvests. IMA growth over the harvests continued to increase in both the AgXX and control environments, but still had a higher population count in the controls by the final sample (Figure 11B). The increase of IMA media population could be due to those species outcompeting of the overall bacterial load in the initial Aerogarden population, although further testing would be required in which the species growing could be isolated and identified.

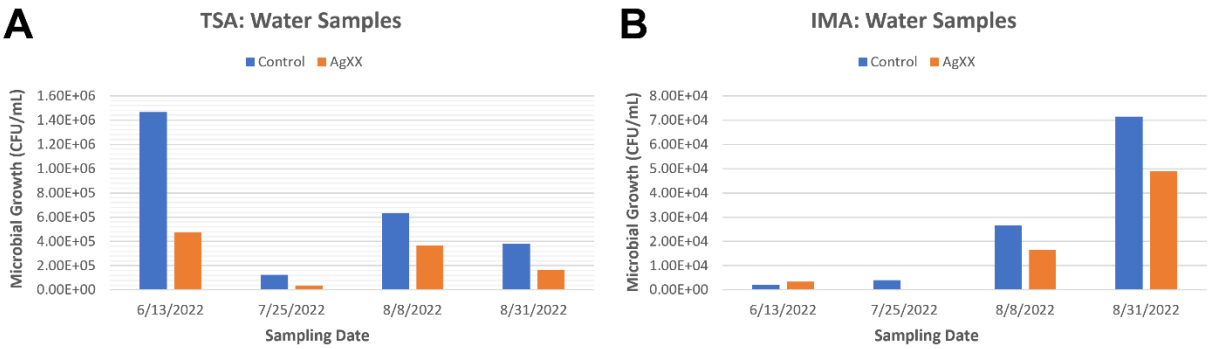

Figure 11. Comparison of microbial count in the control (blue) and AgXX (orange) conditions. Non-selective TSA (A) growth, and selective IMA (B) of the solutions pulled from the Aerogarden during harvests.

When comparing the TSA coupons, the first samples (coupon 1 and 8) for the second harvest had over an order of magnitude of population concentration difference. The rest of the harvests did not show as big of a difference between the control and AgXX conditions as the first set, nor a trend in decrease/increase of populations. The release of peroxides by the AgXX could account for the difference in population in the second harvest samples, though why there would be a population increase in harvest 3 is unclear. Coupon placement could have also affected population comparisons due to the effects of pump flow, which saw some movement of coupons from their original locations.

The fungal counts did not grow past 10^4 CFU/mL but showed no consistently higher count when comparing the AgXX and control coupons in harvests 2 and 3 (Figure 12). Harvest 4 had the highest population for both AgXX and controls, but there was an overall decline at harvest 5. For harvests 4 and 5, control coupons had a higher population. Though there were variations in pH, a clear correlation between the conditions documented in the previous section and the microbial growth could not be determined.

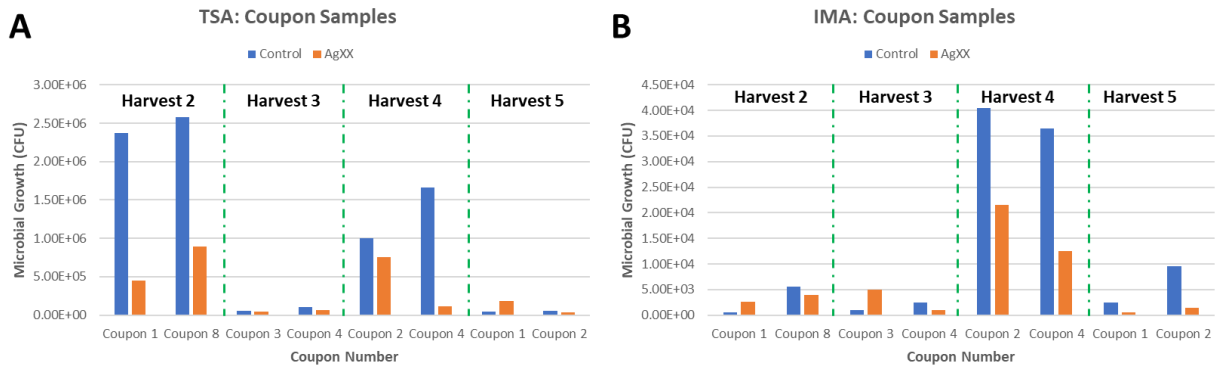

Figure 12. TSA (A) and IMA (B) counts from coupon samples pulled from each harvest.

The filter in front of the pump in each bowl was taken out and tested for growth. The overall microbial growth was in the range of low 10^6 CFU on TSA (Table 1), but the IMA samples were two orders of magnitude lower (Table 2). On TSA, the count was higher in the control, though there was not as much difference as the control versus the AgXX samples on the IMA. At this point, contact with AgXX could be minimized by the circulation of water, therefore lowering the potency of released peroxides. Whereas coupons and the solution itself are more directly in contact with the meshes downstream.

Sampling of the stainless-steel control and the AgXX meshes indicated a higher microbial load by at least an order of magnitude in both the IMA and TSA growth plates. While the control filter showed growth within the range of 10^4 CFU, the AgXX filter was below the detection limit of the plating dilution, indicating if there was a surviving population it would be below 1×10^3 CFU. Apart from the AgXX filter, both left and right mesh samples and the TSA filter sample had higher control condition microbial loads. Overall, no indication of biofilm formation was seen with the naked eye on the coupons (Figure 22A), control mesh (Figure 22B), or AgXX mesh (Figure 22C).

Table 1. TSA microbial counts of the filter, right mesh, and left mesh in either control or AgXX environments.

TSA		
	Control	**AgXX**
Filter	2.17E+06	1.53E+06
Right Mesh	2.96E+05	4.15E+04
Left Mesh	3.07E+05	2.35E+04

Table 2. IMA microbial counts of the filter, right mesh, and left mesh in either control or AgXX environments.

IMA		
	Control	**AgXX**
Filter	3.80E+04	6.80E+04
Right Mesh	1.80E+04	$<1\times10^3$
Left Mesh	1.55E+04	$<1\times10^3$

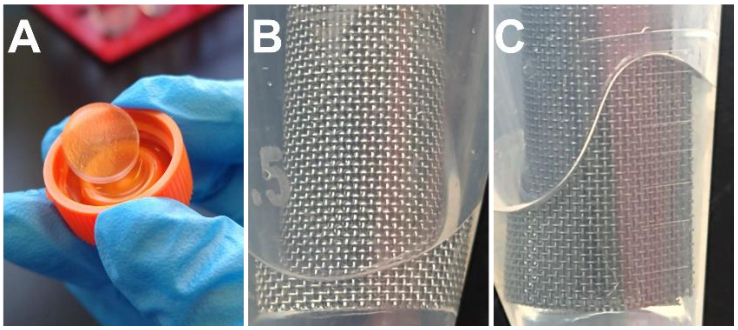

Figure 13. A polycarbonate coupon (A), stainless steel control mesh (B), and AgXX mesh (C) after being pulled from their respective Aerogardens. No biofilm was noted on the samples. The textured effect on the coupon was due to both the coupon itself, as well as a film of Aerogarden solution still on the coupon prior to replacing the solution with sterile water.

V. Conclusion

This study aimed to determine whether the use of a novel antifouling coating, AgXX, would be effective and safe in a plant water environment. To do this the task was broken into two separate sets of experiments: a coupon test to determine AgXX effectiveness in plant nutrient solution; and a plant growth test to determine if AgXX had any negative impacts on plant growth and health. Both tests yielded successful and promising results that indicate that AgXX may be a useful tool in maintaining cleanliness in plant watering systems. Growth test #1 (with three AgXX coupons rather than one) also provided valuable results as it proved that there is still an upper limit of AgXX usage that will have detrimental effects on plant health. This first test emphasizes the importance of targeted AgXX placement, such as for use in tubing and valves that are further away from plant roots but more susceptible to clogging from biofilm.

In the coupon testing, AgXX achieved a 5-log reduction resulting in CFU counts that were below the detection limit for all timepoints both in solution and on the mesh itself, whereas the control coupons showed CFU counts above detection for most timepoints and of upwards of 2.00E+06. In the plant growth testing, growth tests 2-5 showed no statistical difference between the vegetation and root growth of the AgXX crop versus the control crop. Microbial testing of each system throughout the four growth tests also showed an overall decrease (but not elimination) in the microbial population in the AgXX system compared to the control for both bacterial and fungal cultures. Equally important are the microbial results from the meshes themselves at the conclusion of the plant growth testing, which showed CFU counts for the AgXX meshes that were below detection for aerobic bacteria and lower than the control in fungal counts.

These experiments and results show that AgXX is a promising tool to mitigate against biofouling in plant watering systems at a level that is safe for the crops being grown in them. Further investigations into optimizing the specific placement and amounts of AgXX in real spaceflight systems are necessary to understand the full potential of this technology.

Acknowledgments

The authors would like to thank the Ohalo team at the Kennedy Space Center for funding this investigation. Many thanks to the Greenwerks and MicroBio labs at KSC for lending us lab space to perform our experiments. It took a lot of expertise from all backgrounds to bring this project together; we would like to thank Lance Delzit, Raymond Wheeler, Ralph Fritsche, LaShelle Spencer, Oscar Monje, and Jen Gooden for their help and expert advice.

References

[1] Carter, L., Williamson, J., Christopher, A. B., Bazley, J., Gazda, D., Schaezler, R., Thomas, F., and Molina, S., *Status of ISS Water Management and Recovery*, ICES-2019-036, 49th International Conference on Environmental Systems, 7-11 July 2019, Boston, Massachusetts.

[2] Yan, An, and Zhong Chen. "Impacts of Silver Nanoparticles on Plants: A Focus on the Phytotoxicity and Underlying Mechanism." *International journal of molecular sciences* vol. 20,5 1003. 26 Feb. 2019

[3] Mackowiak, C.L., Grossl, P.R. & Cook, K.L. Iodine toxicity in a plant-solution system with and without humic acid. *Plant Soil* 269, 141–150 (2005). https://doi.org/10.1007/s11104-004-0401-6

[4] Azim, N., Diaz, A., Li, W., Calle, L., Irwin, T., and Callahan, M., Literature review of disinfection techniques for water treatment, ICES-2020-132, *50th International Conference on Environmental Systems*.

[5] Diaz, A., Li, W., Irwin, T., Calle, L., and Callahan, M., A literature review of antifouling surfaces and treatments for long-term synergistic biofilm control, ICES-2020-131, *50th International Conference on Environmental Systems*.

[6] Diaz, A., Li, W., Irwin, T., Calle, L., and Callahan, M., Investigation of biofilm formation and control for spacecraft – an early literature review, ICES -2019-271, *49th International Conference on Environmental Systems*, 7-11 July 2019, Boston, Massachusetts.

[7] Li, W., Calle, L., Hanford, A., Stambaugh, I., and Callahan, M., Investigation of silver biocide as a disinfection technology for spacecraft – an early literature review, ICES-2018-82, *48th International Conference on Environmental Systems*, 8-12 July 2018, Albuquerque, New Mexico.

[8] Vaishampayan, A., De Jong, A., Wight, D. J., Kok, J., and Grohmann, E. (2018). A novel antimicrobial coating represses biofilm and virulence-related genes in methicillin-resistant *Staphylococcus aureus*. *Front. Microbiol.* 9:221. doi: 10.3389/fmicb.2018.00221

[9] Wischer, D., Schneider, D., Poehlein, A., Herrmann, F., Oruc, H., Meinhardt, J., Wagner, O., Ahmed, R., Kharin, S., Novikova, N., Haag, R., Daniel, R., Grohmann, E., Novel Antimicrobial Cellulose Fleece Inhibits Growth of Human-Derived Biofilm-Forming Staphylococci During the SIRIUS19 Simulated Space Mission, *Frontiers in Microbiology*, vol. 11 1626. 20.

[10] U. Landau, C. Meyer, E. Grohmann, AGXX: Contribution of surface technology to the avoidance of biofilms (Part 2), *Galvanotechnik*, 6/2017, Eugen G. Leuze Verlag.

[11] The Effects of Hydrogen Peroxide on Plant Growth, Mineral Accumulation, as Well as Biological and Chemical Properties of Ficus deltoidei

[12] Sobisch, Lydia-Yasmin et al. "Biofilm Forming Antibiotic Resistant Gram-Positive Pathogens Isolated From Surfaces on the International Space Station." *Frontiers in microbiology* vol. 10 543. 19 Mar. 2019.

[13] Guridi A, Diederich AK, Aguila-Arcos S, Garcia-Moreno M, Blasi R, Broszat M, Schmieder W, Clauss-Lendzian E, Sakinc-Gueler T, Andrade R, Alkorta I, Meyer C, Landau U, Grohmann E. New antimicrobial contact catalyst killing antibiotic resistant clinical and waterborne pathogens. Mater Sci Eng C Mater Biol Appl. 2015 May;50:1-11. doi: 10.1016/j.msec.2015.01.080. Epub 2015 Jan 26. PMID: 25746238.

[14] W. Li, M. Hummerick, et al, " Biofilm Resistant Coatings for Space Appication," 48th International Conference on Environmental Systems, ICES-2018-83