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Environmental significance statement

This paper evaluated the long-term performance of different silver-based nanoparticle coatings for biofouling control on stainless steel, in a context of dormancy in water treatment system in deep space mission. The results showed that passivation can achieve similar performance to regular silver nanoparticles coating but with significantly less silver release into the water. This approach represents a more efficient and environmentally friendly of using this biocide for biofouling control, in space system but also on earth, where loss of silver ions is an issue for silver based antimicrobial coatings.

Coating of complex metallic surfaces with passivated silver nanoparticles for long-term biofilm control

Ali Ansari [abc], *Afsana Munni* [abc], *Dianne Carillo* [a], *Matthew Pedersen* [ab], *Rafiqul Islam* [a], *François Perreault* [bcd*]

[a] Cactus Materials, Inc., Tempe, AZ 85282, USA
[b] School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ 85287, USA
[c] Nanosystems Engineering Research Center for Nanotechnology-Enabled Water Treatment, Arizona State University, Tempe, AZ 85287, USA
[d] Department of Chemistry, University of Quebec in Montreal, CP 8888, Succ. Centre-Ville, Montreal, QC H3C 3P8, Canada

[*]Corresponding Author: Email: perreault.francois@uqam.ca

Abstract

Deep space missions will bring new challenges, beyond our experience so far with International Space Station, to life support systems including water supply. The complexity of these missions might leave spacecrafts and facilities uncrewed for several months. In this situation, biofilm growth can deteriorate the quality of stored water and cause water supply system failure during reinitiation, threating the mission success. Antimicrobial coatings have been used for biofilm mitigation in various conditions. A successful coating to control biofilm formation in deep space mission, among other things, must have long lifetime considering the duration of such missions. In this study, a solution was provided to the biggest drawback of silver nanoparticles as antimicrobial coating; short lifetime. Passivating with sulfide was tested to control silver ion release from silver nanoparticles, hence, prolonging antimicrobial activity. Stainless steel bellow pieces, as the most prone parts to biofilm growth, was chosen as the substrate. The pieces were coated with silver and passivated silver with different passivation degree to find the optimum condition. The substrates were exposed to *Pseudomonas aeruginosa* in M9 medium for 12 months for biofilm formation. The bacteria count on the bellow pieces as a representative of biofilm as well as bacteria count and silver ion concentration in M9 medium were measured at 1.5, 3, 6, and 12-month timepoints. Passivation slowed down silver ion release rate from silver nanoparticles, however, biofilm mitigation at the end of the experiment for one passivated coating was the same as silver coating, which means the passivated coating can last longer by releasing less antimicrobial agent, silver ions. Besides performance in biofilm mitigation, we demonstrated that the bellows can be coated homogeneously in a continuous reactor and passivation can enhance the stability of the coating to mechanical stress during expansion/retraction of the bellow, paving the way for application of passivated silver coating for space missions.

1. Introduction

As we turn into space missions beyond low Earth orbit (LEO) and resupplying water from Earth becomes a nonviable option, we must overcome new challenges in providing water in Environmental Control and Life Support Systems (ECLSS), such as ensuring long term water quality and high water recycling efficiency. In June 2023, the National Aeronautics and Space Administration (NASA) announced they had achieved 98% water recovery in a demonstration system in the International Space Station (ISS) thanks to a new Brine Processor Assembly (BPA) (1), an improvement from ~85% obtained through the Water Process Assembly (WPA) unit (2). However, water quality deterioration remains a concern for missions on the Moon and Mars (3). Unlike ISS, deep space missions, considering the complexity of the mission, will include uncrewed habitats and spacecraft (4). For instance, a mission to Mars and back can occur every two years, given the desired locations of the Earth and Mars. During the mission, the water supply system on the spacecraft will remain in partial or total dormancy for ~ 17 months (3). For the Moon, the current plan is to have a Lunar Orbital Platform (Gateway) in which the astronauts will stop before landing on the Moon. Gateway is planned to be crewed ~ one month at least once a year (3). Besides, there might be a gap between one group leaving and the next group arriving on the facilities on Mars and Moon (5). During these times the ECLSS system on the spacecraft or habitat will face dormancy conditions, a situation less experienced and with unique challenges for the aerospace community (4). Learning from ISS experience, the main concerns for water treatment systems in dormancy condition are microbial growth in water and biofilm formation on components (4, 6), the former can threaten crew health and put extra burden on the treatment system, the latter, besides health threat, can cause total system failure by corrosion and filter clogging (3). Problems stemming from biofilm have been observed in multiple space stations such as Soviet Salyut 6 & 7, Mir, and the ISS (3). From the ISS experience, bellows tanks are prone to biofilm growth because of the low liquid velocity (3, 4, 7). Specifically, biofilm tends to grow on bellows probably because their shape provide a suitable place for bacteria attachment and growth (7).

As researchers navigate through the uncharted territory of dormancy in space missions, various methods have been suggested to mitigate microbial growth in water or on the surface of the WPA components (3). One approach suggested draining the wastewater and flushing the system with potable water, which is time-consuming, waste potable water, damage parts, and may take a long time until the system can get to steady condition when it restarts (4). Another method is replacing the parts before running the treatment system again, which is complicated considering the difficulty of supplying parts in space as well as increasing the chance of system failure with multiple assembly/disassembly (4). Literature review on mitigating biofilm in space conditions (3), regardless of dormancy, shows using biocides or applying coating to vulnerable parts can be effective solutions (4). Iodine, ozone, and silver as biocides, lubricants, synthetic polymers, and silicone as coating have been suggested (3, 4, 8). However, the ultimate solution is most probably a combination of using both biocides and coatings.

An ideal biocide should be effective at low concentrations, not cause a health threat to the crew, and be compatible with other components of the water treatment system (3). In the ISS, two biocides are being used to disinfect water: iodine, used by NASA, and silver, which is used by the Russian Space Agency (9). Iodine is an effective disinfectant but it requires an extra treatment step to remove it before consumption, otherwise, it can have a negative effect on the thyroid. This treatment step, adds to the complexity and weight of the whole water treatment system (9). On the other hand, silver is a biocide effective against a broad range of microorganisms at a concentration that is safe for human consumption, making it an interesting candidate to be used across international spacecraft and deep space facilities (9). In addition, silver, in the form of silver nanoparticles, has also been used as a coating material on various surfaces to prevent biofilm formation (3). The nanoparticulate form provides a reservoir of silver ions that can be released near the surface to inactivate microorganisms that comes in contact with it, an approach that has been shown to be effective when applied to materials such as membranes (10, 11), metals (12), or fabrics (13). The use of silver-based antimicrobial coatings is of particular interest for space applications since they will be compatible with the current use of silver ions as a biocide for water disinfection.

A prominent coating for biofilm mitigation, especially for long-term missions, must have good longevity as well as high mechanical and chemical stability (3). Considering these criteria, the fast dissolution of silver nanoparticles when applied as a coating is a concerns for using this material (4, 12). As the silver NPs dissolve from the surface, the antimicrobial action of the coating can be lost. To mitigate this effect, different strategies have been proposed to slow down silver release, such as using amine groups (14), dialdehyde nanofobrillated cellulose (15), or biopolymer beads (16). In our previous studies (10, 12), we have shown that silver ions (Ag^+) release rate from silver NPs can be controlled by passivation, hence, increasing the longevity of antimicrobial properties. Sodium sulfide was found to be the best passivation agent compared to sodium iodide and sodium chloride (12). Because silver sulfide is virtually insoluble in water, Ag NPs with partial shell of Ag_2S have a lower dissolution rate than Ag NPs. The Ag^+ release rate can be adjusted by passivation degree through sodium sulfide concentration and passivation reaction time. This approach provide a simple solution to extend the lifetime of silver NPs coatings used for biofouling mitigation. However, for using this technology in deep space missions several aspects should be considered. The sulfidized silver NPs coatings have not been evaluated for the extended duration representative of dormancy conditions for space applications. In the dormant condition, the longevity of antimicrobial properties is unknown. Another concern is that sulfidation may limit Ag^+ release in such a way that low Ag^+ concentration will exacerbate biofilm formation (17). For real-world applications, the stability of this coating against mechanical stress and its homogeneity when applied to bellows should be investigated.

In this study, the performance of various silver-based coatings for biofilm mitigation in stagnant aqueous conditions was studied over the course of one year. Silver and passivated silver NPs coatings were applied to bellow pieces, as the most prone components to biofilm formation, and the pieces were exposed to a synthetic wastewater supplemented with the model biofilm forming bacterium *Pseudomonas aeruginosa*. Biofilm in terms of culturable bacteria count as well as silver release rate in the wastewater were measured at different time points over one year to compare the performance of different coatings. The stability of different coatings to mechanical stress was also investigated, the assess the applicability of the coating on this stainless steel structure.

2. Material and methods

2.1. Materials

Stainless steel (SS) bellows with 6 cm length and 3 cm diameter as well as sheets made of 316 SS were purchased from Duraflex, Inc (IL, US). Silver nitrate 99% ACS reagent, D-(+)-Glucose ≥99.5% BioReagent, Ammonium hydroxide 27% ACS reagents, Sodium nitrate ≥99% ACS reagent, Sodium sulfide hydrate ≥60%, and nitric acid 70%, ≥99.999% trace metal basis were purchased from Sigma-Aldrich (MO, US). The bacteria strain was *Pseudomonas aeruginosa* ATCC 25668, was obtained from NASA.

2.2. Bellows coating in a flow through system

To determine the homogeneity of nanoparticles coating on bellows in a continuous reactor, a flow through system was used to coat the SS316 bellow with Ag NPs. The system had a feed tank to put the coating solutions, a housing chamber that contained the bellow, and a pump to circulate the coating solutions in the system. To facilitate the characterization of the coating on the bellow after coating, stripes of SS 316 were attached to different parts of the bellow with double sided carbon tape. In total, three stripes were attached to the bellow. Based on the direction of the flow in the membrane housing, the stripes were named front, center, and back.

The coating was done in two steps at room temperature. After washing the bellow with 20% ethanol, in the first step, 2 L of a solution of $AgNO_3$ (10 mM) and NH_4OH (50 mM) was put in the feed tank and circulated through the system for 20 min. The solution was discarded and in the second step, 2 L solution of glucose (100 mM) was circulated for 24 h (18). After that the stripes were cut into small pieces based on their position on the bellow with duplicate stripe pieces for each position. To determine silver loading, stripes were weighed and then the Ag NPs were digested by placing the stripes in 5 mL of 10% HNO_3 for 24 h. The silver containing acid solutions were diluted 5 times and the silver concentration in the nitric acid solution was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Thermo Scientific iCap Qtega ISDS). The weight of the stripes was used to calculate the stripes surface area to report the silver loading on the stripes in ng/cm^2. Scanning Electron Microscopy (SEM, Auriga, Zeiss) was used for imaging at 20 kV and 25,000 magnification.

2.3. Bellow pieces coating

Bellow pieces, as the representative of whole bellows, were prepared by cutting the bellows into different shapes. To test the stability of coating on the bellows under mechanical stress, twelve half circle bellow pieces with five threads width were coated with Ag and Ag/Ag_2S NPs in batch. Each piece was put in a 50 mL conical tube and submerged under 20 mL of $AgNO_3$ (10 mM) and NH_4OH (50 mM) solution for 20 min. After that the solution was discarded, leaving a thin film of silver ions on the pieces. To reduce the activated silver ions to Ag NPs, 20 mL of glucose (100 mM) was poured into the tubes and the samples were shook for 24 h. Six of the pieces were removed from the tubes and washed gently by dipping them into Milli Q water. To passivate the Ag NPs on the other six pieces, after discarding the glucose solution, 20 mL of a solution containing $NaNO_3$ (10 mM) and Na_2S (1 mM) were added to the tubes. The passivation was done for 24 h and at the end the pieces with Ag/Ag_2S NPs coating were washed similarly to pieces Ag NPs coating. SEM images were obtained as described in Section 2.2.

To study the resistance of the silver-based coatings to biofilm formation and the effect of coating on water quality in dormancy condition over a year, bellows were cut into 45° pieces with four threads width. Four different coatings were applied to bellow pieces in batch. The general procedure for coating was similar to what described above; however, the concentration of certain reactants was changed according to **Table 1**. For standard Ag and standard Ag/Ag_2S no changes were made to the coating procedure. To test if coating with higher NPs loading can improve performance, the high loading Ag/Ag_2S (HL) coating was applied by increasing the silver concentration in the first solution by 2.5 times. To test if coating with less passivation i.e. higher release rate, can perform better than standard coating, the high release Ag/Ag_2S (HR) coating was used by reducing Na_2S concentration in the passivation stage. The NPs loading on the bellow pieces was measured by digesting the coatings with 10% nitric acid as described in Section 2.2.

Table 1: Concentration of reactants for different coatings on bellow pieces

Coating	Ag concentration (mM)	NH_4OH concentration (mM)	Glucose concentration (mM)	Na_2S concentration (mM)
Standard Ag	10	50	100	-
Standard Ag/Ag_2S (std.)	10	50	100	1
High loading Ag/Ag_2S (HL)	25	50	100	1
High release Ag/Ag_2S (HR)	10	50	100	0.01

2.4. Compression tests

To test the mechanical stability of the coatings under mechanical stress, the half circle coated bellow pieces with Ag and Ag/Ag_2S were divided into two groups. Three pieces of each coating were subjected to compression tests, an intended deformation for bellows, performed with a Deluxe-Action wedge grip (United Testing Systems) with 2” diameter. The use of this device for mechanical tests has been reported in the literature (19), however, modifications were done to simulate the conditions in a simplified manner (20). The pieces were individually fixed in the wedge grip. Two pieces of soft foam were placed between the sample and wedge grip to avoid any coating removal due to friction. The fixed samples were submerged into 200 mL of MilliQ water and compressed by rotating the T-handle two full circles. For the first two hours the pieces were compressed for 10 min and decompressed by rotating the T-handle in the opposite direction and leaving the samples for 10 min to simulate the expansion and retraction of bellow pieces. Aliquot of 5 mL were taken from water to measure the silver concentration as an indicator of detachment of the coating from the metal surface. After that the compression/decompression cycles were longer at 12 h for 2 days. Similarly to the 2 h point, water samples were taken to measure silver concentration by ICP-MS as described in Section 2.2.

<u>2.5. Twelve-month biofilm formation assay</u>

The performance of the coatings mitigating biofilm formation on 45° bellow pieces was studied by submerging the pieces into 350 mL M9 minimum medium (12) spiked with 2×10^7 CFU/mL of *P. aeruginosa* in a 500 mL sterile Erlenmeyer flask. After sterilizing the pieces with dry heat at 90 °C for 1 h, in each flask 8 pieces with same coatings were put. As a control, flasks with the same number of pieces and same volume of M9 without bacteria were prepared. The flasks were put on a bench top, no shaking, at room temperature. After 1.5, 3, 6, and 12 months, three parameters were measured: 1. Silver concentration in bulk, 2. Bacteria count in bulk, and 3. Bacteria count on bellow pieces. The 12 months period was chosen using current longest dormant operation experienced between Tiangong Expeditions 1 and 2 (4).

To measure silver concentration, an aliquot of 3 mL was taken from each flask under aseptic conditions. The sample was filtered with 0.2 µm sterile polyethersulfone (PES) syringe filter (VWR, US) and 10% nitric acid was added to digest any remaining NPs before analyzing the sample with ICP-MS as described in Section 2.2.

For bacteria count in bulk and on the bellow pieces, colony forming unit (CFU) assay was used. For bulk, an aliquot of 0.1 mL was taken from the flask and serially diluted in sterile NaCl 0.9% solution to the desired concentration. For bellow pieces, they were first rinsed gently with sterile saline solution in three 1" diameter sterile petri dishes to remove any M9 film and loosely attached bacteria. The pieces were then put separately into 50 mL sterile conical tubes with 5 mL sterile saline solutions and sonicated with a bath sonicator for 10 min to detach biofilm from the bellow pieces. An aliquot of 0.1 mL was taken from the tube and serially diluted as described above. For both bacteria in bulk and on bellow pieces, the diluted bacteria samples were plated in triplicates by adding 0.1 mL of the sample onto Tryptic Soy Agar (TSA) plates. The plates were incubated overnight at 37 °C and the colonies were counted to report the number of bacteria in CFU/mL for bulk and CFU/cm^2 for bellow pieces. Use of more accurate techniques for biofilm volume measurement such as Confocal Laser Scanning Microscopy was not possible because of the substrate shape.

3. Results and Discussion

3.1. Nanoparticles coating on bellows distribution and stability

The flow pattern in continuous reactors around parts with complex geometry such as a bellow may result in nonuniform or patchy coating on the part, which may provide vulnerable spots for biofilm formation. The stripes attached to the bellow facilitated testing the homogeneity of the coating. Figure 1 shows the positions of the stripes as well and their associated silver loadings, as well as an SEM imaging of the surface. No statistically significant difference was observed for silver loading among the three stripes located in front, center, and back of the bellow (Fig. 1 b), which shows the coating is homogeneous. The SEM images (Fig. 1 d-f) showed the morphology and size of the NPs are similar.

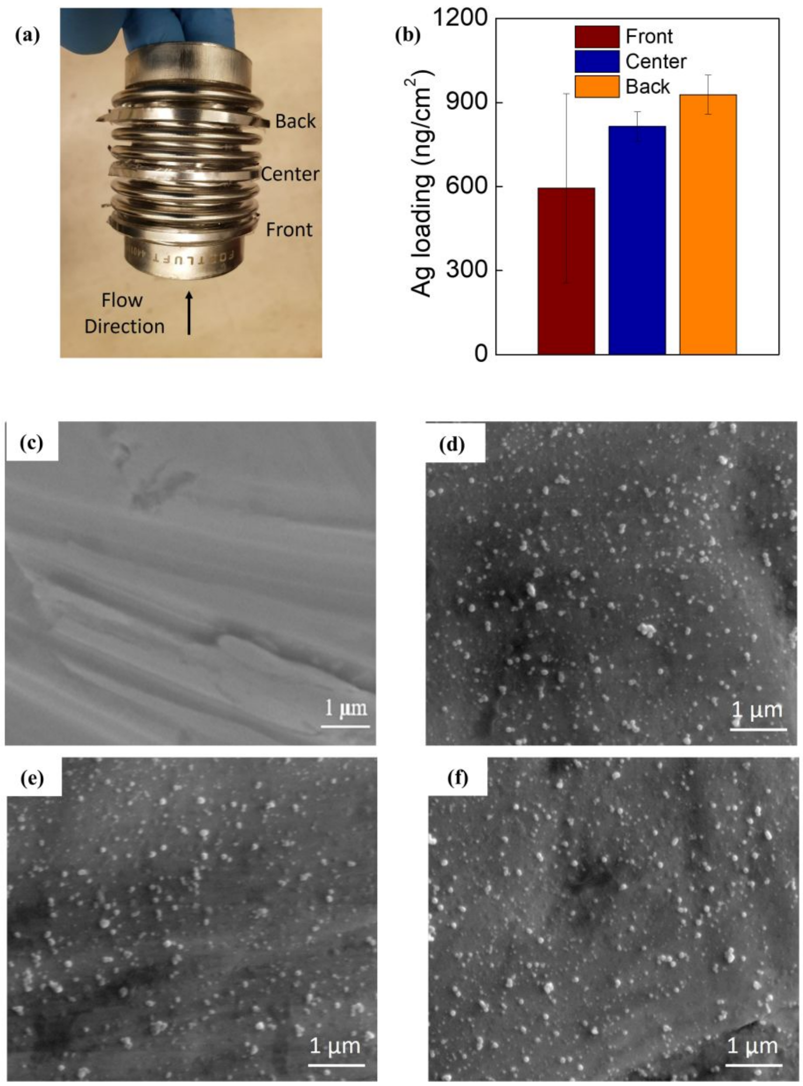

Figure 1: (a) Bellow with three SS316 stripes attached to it, (b) Silver loading on three points of the bellow after coating the bellow with Ag Nanoparticles, SEM images of (c) Pristine stripe, (d)

Coated stripe at front, (e) Coated stripe at center, and (f) Coated stripe at back. The SEM images obtained at 20 kV and 25,000× magnification.

These results show that NPs with this method can be applied to parts with complex geometry in a continuous reactor, which facilitates scale up of the coating procedure for industrial applications. In addition to applying the coating, the stability of the coating under various stress is important for real world applicability of the coating. Bellows in the ISS routinely go through mechanical stress by expansion and retraction (21). A promising coating should be able to tolerate the stress and do not get detached from the surface.

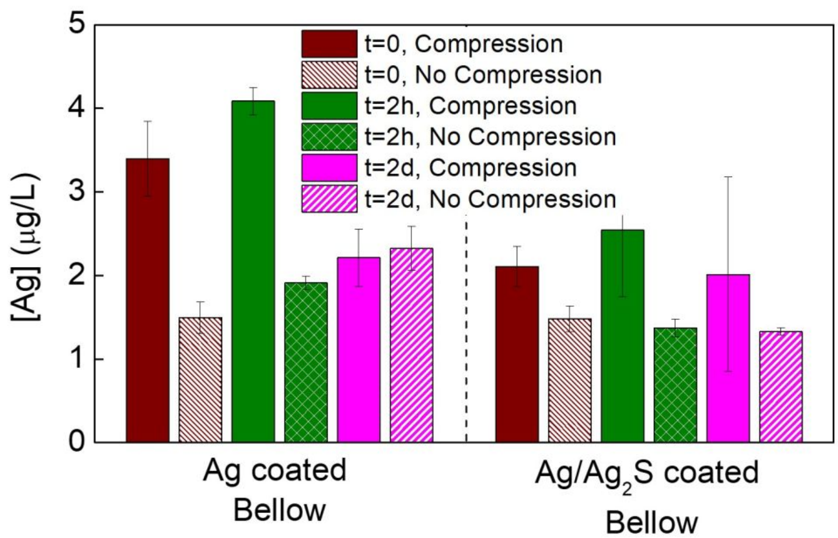

Figure 2: Silver concentration in Milli Q water for containers with bellow pieces coated with Ag or Ag/Ag_2S subjected to compression and decompression cycles.

Silver concentration in water was used as an indicator of coating detachment from the surface because of mechanical stress. The results showed that for both Ag and Ag/Ag_2S coating, compression and decrompression of the bellow pieces increase the coating detachment at time zero and after two hours, as noted by the increase in silver concentration for the tested samples compared to the controls (without compression/decompression cycles). It can be noted that at a longer test time, after 2 days of compression and decompression cycles (see section 2.4 for details), the silver concentration in the water decreased compared to the t=2h time point for the samples that underwent compression/decompression. This decrease may be explained by the gradual deposition of silver ions as metallic silver on the metallic surface of the bellow pieces, a phenomenon known to occur when ionic silver solutions are in contact with stainless steel (22). Because this effect is likely to be more important over time, the comparison of silver concentrations between the Ag and passivated samples was focused on the first two time points, t=0 and t=2h. At both these initial times, the detachment was higher for Ag coating, meaning that passivation makes the coatings more stable. The maximum measured detachment for Ag and Ag/Ag_2S coatings were 1.3 and 0.8% of total loaded silver, respectively. For Ag/Ag_2S after initial silver loss due to mechanical stress, which is hypothesized to be due to the release of loosely attached NPs, no further detachment was observed and the silver concentration remained constant in the water over time. This shows that the passivated NP coating is not significantly affected by the compression mechanical stress as the non-passivated Ag coated sample. It should be noted this experiment covered only one type of mechanical stress for part of the systems that will be used in space missions. To further qualify the coating mechanical stability, expansion tests with a more sophisticated setup (20) should be done. The expansion is particularly important because the surface area of the bellow exposed to water increases, which might cause further detachment of the coating (21). Another typical mechanical stress for space missions is the vibration during shuttle launch (23). Nevertheless, the compression/decompression test results confirm the higher stability of the passivate Ag coatings as compared to the non-passivated one.

3.2. Coated bellow pieces characterization

The 45° bellow pieces that will be used in twelve-month biofilm formation assay first had to be characterized to identify the differences among the four coatings, namely Ag std., Ag/Ag_2S std., Ag/Ag_2S HL, and Ag/Ag_2S HR. Silver loading measurement on pieces (Figure 3 (a)) yielded the expected results that we had observed in our previous study (12) which is the passivation does not decrease silver loading. The NPs number on Ag/Ag_2S HLwas highest, while for the three other coatings there was no significant difference.

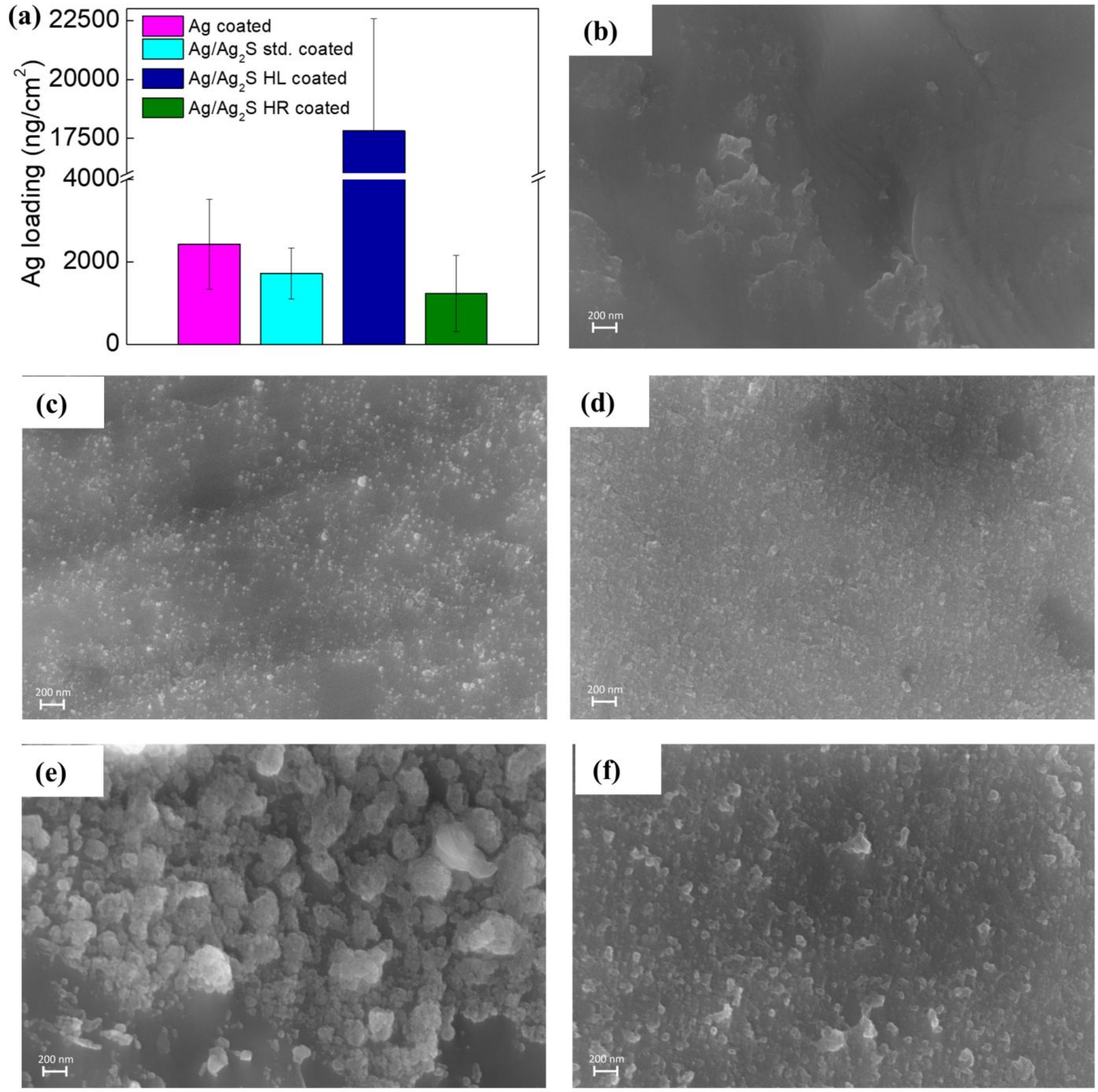

Figure 3: (a) Silver loading on SS316 bellow pieces coated with Ag, Ag/Ag_2S standard (std.), Ag/Ag_2S high loading (HL), and Ag/Ag_2S high release (HR) NPs. The bellow pieces were microwave digested in 70% nitric acid. SEM images of (b) SS316 bellow pieces coated with (c) Ag, (d) Ag/Ag_2S standard (std.), (e) Ag/Ag_2S high loading (HL), and (f) Ag/Ag_2S high release (HR) NPs.

This result was further confirmed by SEM imaging and EDS. The NPs on all the coatings (Figure 3 (c, d, f)) expect for Ag/Ag_2S HL (Figure 3 (e)) had the same morphology and size. No nanoparticles were observed on SS (Figure 3 (b)). For Ag/Ag_2S HL coating, the NPs were bigger and more aggregates were observed, which explains the high silver loading. The elemental composition analysis (Table 2), showed Ag coating has the highest silver percentage. For passivated samples, silver percentages are lower and sulfur percentages are higher because part of silver NPs are covered by sulfur.

Table 2: Elements percentage of bellow pieces with different coatings

Sample	Fe	C	Cr	Ni	O	S	Ag
SS 316	46.4	29.4	13.0	6.4	4.0	0.7	ND
Standard Ag	41.5	36.2	11.9	5.6	3.9	0.7	0.2
Standard Ag/Ag_2S (std.)	53.9	20.1	14.8	7.3	2.8	1	0.1
High loading Ag/Ag_2S (HL)	50.4	24.5	14.1	6.9	3.1	0.8	0.1
High release Ag/Ag_2S (HR)	47.3	28.2	13.2	6.4	4.2	0.7	0.1

3.3. Twelve-month biofilm formation assay

In projected space missions to the Moon and Mars, the water treatment system may remain unused and in dormancy conditions for months. Therefore, it is important to study the performance of the coating in preventing biofilm formation as well as its effect on water quality in bulk in stagnant conditions. Bacteria count on 45° bellow piece was used as biofilm volume indicator and silver and bacteria concentration in water were measured as criteria for water quality.

3.3.1. Biofilm formation on bellow pieces

Over the course of the experiment, SS pieces had the highest biofilm volume compared to the coated samples (Figure 4). The biofilm volume, reported in CFU/cm^2, peaked at 6 months then reduced at 12 months which is consistent with biofilm life cycle (24). For coated samples, Ag and Ag/Ag_2S HR had the lowest biofilm volume and similar to each other for the 3-month sampling point. The two coatings with the highest passivation, Ag/Ag_2S std. and Ag/Ag_2S HL, had similar biofilm volume which was higher than Ag and Ag/Ag_2S std. samples (Figure 4).

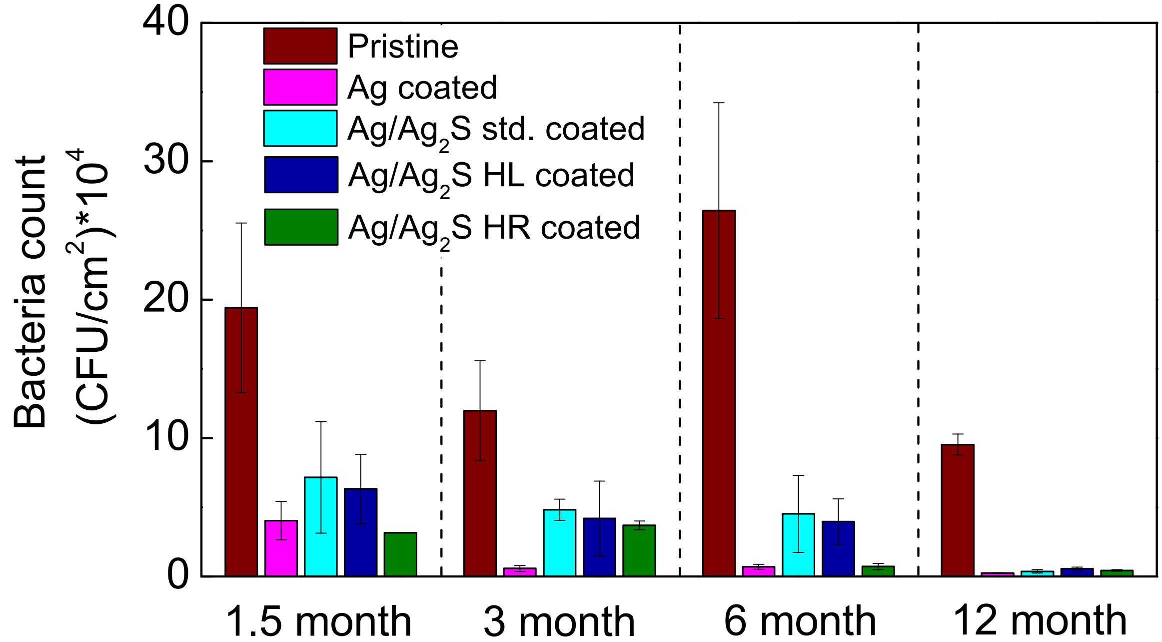

Figure 4: Viable bacteria count on the bellow pieces in the long-term biofilm formation test after 1.5, 3, 6, and 12 months.

The biofilm volume reduction was over 95% for all the coated samples compared to pristine samples after 12 months. This result shows that although high passivation degree weakens antimicrobial property at first because NPs dissolution rate is slowed down, over time the three passivated coatings have the similar effect as Ag coating. One potential concern regarding low concentrations of silver is that it can enhance biofilm formation, as suggested by Yang et al. (17). However, controlling silver NPs' dissolution on coating did not have negative effect on biofilm mitigation.

One explanation for good performance of Ag coating could be that because of high silver ion release rate, the Ag^+ concentration in bulk might have reached biocidal levels, killing bacteria in bulk and reducing biofilm volume on bellow pieces. To test this hypothesis, the bacteria count in bulk was measured in flasks.

3.3.2. Bacteria count in bulk

The number of bacteria in water affects biofilm formation potential on the surfaces. The 12-month experiment started with $2*10^7$ CFU/mL of bacteria. The number of bacteria showed an increase at 1.5-month sampling because bacteria consumed glucose in the M9 medium and grew. However, after that, the bacteria count decreased for all flasks at the 3-month sampling point to initial number (~ $2*10^7$ CFU/mL) and remained almost the same until the end of the experiment, following bacteria natural life cycle (Figure 5). Except for Ag/Ag_2S HR at the 6-month sampling, which seems to be an artefact.

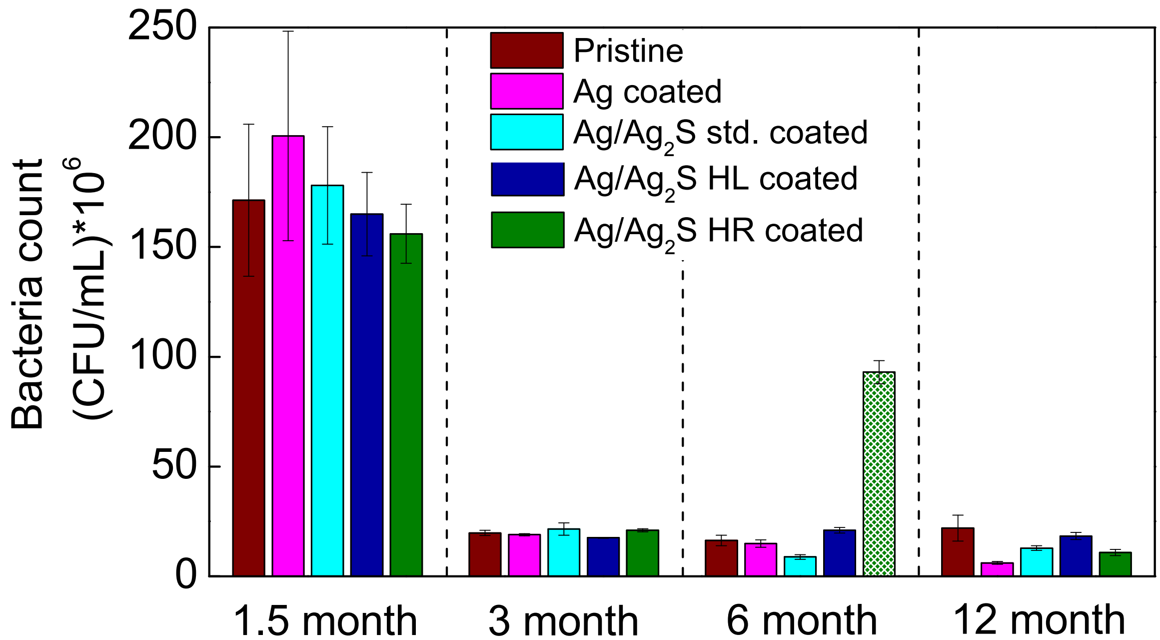

Figure 5: Viable bacteria count in the flasks in the long-term biofilm formation test after 1.5, 3, 6, and 12 months. The data for Ag/Ag_2S HR at 6 month seems to be an outlier.

According to Figure 5, all flasks have a considerable number of bacteria after 12 months which means that silver ions did not completely inactivated bacteria. For flasks containing Ag coated samples the bacteria count is the lowest, possibly due to having the highest silver ions concentration (quantified in the next section). The partial inactivation of bacteria in bulk possibly contributed, to some extent, to reducing biofilm volume on bellow pieces coated with silver while for passivated samples, despite higher bacteria in the bulk, the biofilm volume remains low. The ability to mitigate biofilm formation when the surface is exposed to highly biologically contaminated water is an important feature of the coating when long-term performance is considered. Passivated NPs maintain the surface antimicrobial property over time because the inactivation of bacteria rooted in the surface not bulk, therefore, exposure to continuous flow of water will not increase biofilm formation. However for Ag coated surfaces, when water flows, the water with high silver ions concentration leaves and fresh water with high number of bacteria and no silver ions replaces it. In this situation, the Ag coated surface that lost its ability to kill bacteria in bulk will become more prone to biofilm formation. This was emphasized by the work of Barrios et al. where the non-passivated silver coating, despite high static antimicrobial performance, had low anti-biofouling results when tested in a continuous cross-flow cell (10). Similarly, in space water systems, each cycle of water replacement will remove the silver ions and expose the vulnerability of the surface.

3.3.3. Silver release into bulk

The NPs with silver core will dissolve over time in water and release silver ions. With a silver-based coating, the silver concentration in the bulk should be monitored to study bacteria inactivation in bulk and in case of drinking water to assure the silver concentration is not above the safe limits, which set by WHO and EPA at 100 µg/L (25). Moreover, the silver release rate inform on the longevity of the antimicrobial performance of each coating type, as faster release coating will lose their biocidal potential quicker and therefore will be limited for applications in long dormancy scenarios. In all flasks the silver concentration increasing over time and flasks containing Ag coated bellow pieces had the highest silver concentration in M9 medium throughout the four sampling over one year, followed by Ag/Ag_2S HR, Ag/Ag_2S HL, and Ag/Ag_2S std., respectively (Figure 6).

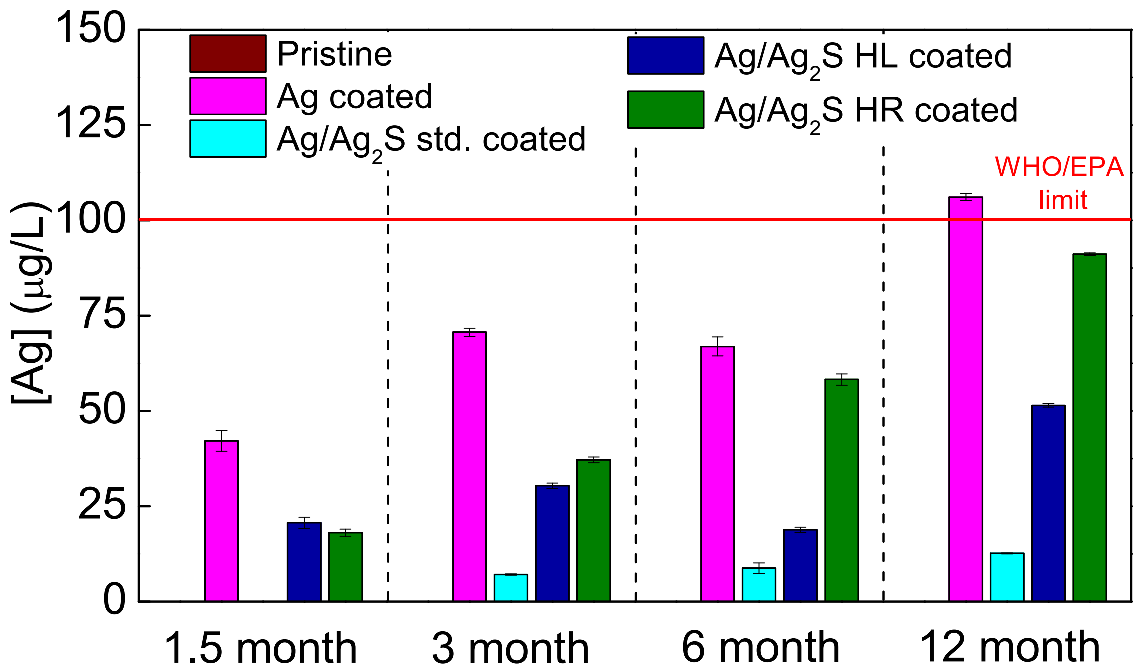

Figure 6: Concentration of Ag released in the flasks by the different coatings after 1.5, 3, 6, and 12 months. The red line indicates the recommended limit of silver in drinking water by WHO and EPA.

The effect of passivation on silver release can be seen in the silver release data. As expected, the non-passivated Ag coated sample had the highest release, followed by the Ag/Ag_2S sample with low passivation level, which was designed to have high release (Ag/Ag_2S HR). The lowest amount of silver release was observed for the Ag/Ag_2S std. pieces, followed by the sample that was treated with the same concentration of passivating agent but that used a highest silver concentration for Ag coating (Ag/Ag_2S HL). These results confirm that the passivation degree is the dominant factor compared to NPs loading in determining the silver release rate. When compared to the microbial abundance data for the surface (Figure 4) and the liquid (Figure 5), it can be seen that the sample with the highest release had the lowest amount of microbial growth, as expected considering the biocidal nature of the silver ion; however, microbial abundance did not correlate with silver release for the other types of coatings. For example, the Ag/Ag_2S std.

had a significantly lower silver release but similar level of biofilm growth inhibition as the Ag/Ag_2S HL condition. Therefore, silver release to the bulk liquid is not the only factor in determining the ability to control biofilm on the bellows surface.

Assuming one year period of dormancy and considering M9 medium volume to bellow pieces surface area of 3.5 cm, the result shows the silver concentration in flasks containing the bellow pieces coated with non-passivated Ag could reach silver concentrations above the recommended health levels for drinking water. However, for bellow pieces coated with any of the three passivated Ag/Ag_2S coatings (std., HR, or HL), the silver concentrations remained below the safe limit. Therefore, passivation not only prolongs antimicrobial property of the surface by reducing the silver release, it also keeps the water safer in case of using this type of coating for drinking water storage tanks. Therefore, passivated silver coatings offer clear benefits over regular silver for long-term biofouling control in dormancy conditions.

4. Conclusion

Passivating silver nanoparticles with sulfide can prolong antimicrobial property of this material without decreasing performance when applied as a coating to stainless steel surfaces. Moreover, passivation enhances the stability of the coating to mechanical stress, as evidenced by the lower silver release upon compression and decompression of the bellow pieces. This coating can be applied by a green chemistry, homogeneously to complex shapes such as bellows in a flow through system. Passivation can overcome one of the main disadvantages of using silver coatings and facilitate use of this well-known antimicrobial material to protect water supply systems in deep space missions. Future work should evaluate other form of mechanical stresses, such as expansion and vibration, to better characterize the applicability of this antimicrobial approach to a range of metallic surfaces and applications.

Conflict of Interest Statement

Cactus Materials, Inc. is developing commercial applications for antimicrobial coating materials.

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Data availability statements

The primary data originating from the research done for this article are presented in tables or figures in the manuscript. No crystallographic data, software, or code were developed or created in this work.