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Hydrophilic and antimicrobial Ag-exchanged zeolite a coatings: A year-long durability study and preliminary evidence for their general microbiocidal efficacy to bacteria, fungus and yeast

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ABSTRACT

Silver exchanged zeolite A (Ag–ZA) coatings have been proposed as effective materials in providing hydrophilic and antimicrobial surface properties for condensing heat exchangers onboard manned spacecraft. Ag–ZA coatings demonstrated super hydrophilic properties, retention of silver content, and high antimicrobial activity to *Escherichia Coli* after submersion in double de-ionized water over a one-year period. Ag–ZA coatings kill *E. coli* on contact with a small loss of Ag (~0.4%) after each exposure to the bacterium. The coatings remain super hydrophilic even after 24 repeated *E. coli* exposures. The coatings have also shown effective resistance against bacteria *Listeria innocua, Staphylococcus epidermidis* and *Pseudomonas putida,* Fungus *Aureobasidium pullulans,* and marine yeast *Rhodotorula mucilaginosa*, and may provide an enhanced capacity to prevent outbreaks of several microbial species onboard manned spacecraft.

1. Introduction

A key part of the environmental control system onboard manned spacecraft includes a condensing heat exchanger (condenser) used for controlling the temperature and humidity of the spacecraft cabin. Within the condenser, moisture laden air cools in small channels or air passageways and condenses creating water droplets. In the zero or microgravity conditions of space, the condensed water droplets are entrained in the air stream and carried back into the cabin, causing fog or rain. A hydrophilic coating on the air passageway surface will allow the condensate to form a thin spreading film that can be easily removed by vacuum sipping. Antimicrobial properties of the hydrophilic coatings are necessary because damp surfaces tend to grow bacteria and fungi which could adversely affect the health of crew members within spacecraft cabin environments [1,2].

Current sol–gel coatings typically require high-temperature curing (300–600 °C) that is incompatible with the metal alloy construction of condensers. The current method of providing antimicrobial resistance to condensers involves the incorporation of silver oxide into the sol–gel coating [1,2]. Long lasting antimicrobial activity of these coatings requires high concentrations of silver oxide. There is a resulting tradeoff between the hydrophilic property and antimicrobial resistance because higher concentrations of silver oxide within the coating increase hydrophobicity. After repeated exposures, the antimicrobial agent is completely dissolved and the coating must be removed and a fresh coating be applied. To circumvent the problem of restoring the antimicrobial coating, previous attempts involved using thicker coatings (~100 μm) resulting in decreased heat transfer efficiency of the condenser. Thus, it would be beneficial to use a thin, yet very durable coating.

We have proposed using silver exchanged zeolite A (Ag–ZA) as a hydrophilic and antimicrobial coating for condensers used within manned spacecraft [3–5]. A sodium zeolite A (Na–ZA) coatings are easily deposited using a low temperature (e.g., 65 °C) *in situ* crystallization process which can coat the surface of complex shapes and in confined spaces on several metal alloys. Silver ions can be loaded into the ZA coating by a fast and easy ion exchange process. With the incorporation of the silver ions into the zeolite framework, the hydrophilicity of the coating is not sacrificed, as opposed to the use of hydrophobic silver oxide. Furthermore, once depleted, the antimicrobial capability of zeolite coatings can easily be regenerated by another ion-exchange without a potentially expensive recoating process. This Ag–ZA coating is relatively thin with a thickness of about 5 μm and an improved heat transfer of the heat exchanger using hydrophilic zeolite coatings has been

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recently demonstrated [6,7]. We have previously demonstrated that the Ag–ZA coating is super-hydrophilic, extremely antimicrobial toward *Escherichia Coli* and adheres very well to the substrate [3]. Additional durability tests over an eight-week time period showed that the coatings retained their hydrophilic and antimicrobial nature after complete submersion in water [4].

The long-term retention of the hydrophilic and antimicrobial efficiency of these coatings is a primary concern because they will be utilized on coated condensers within spacecraft cabins for a long time period of 10 year or more. In this study, the long-term durability was investigated in two cases: one year submersion in DDI water, and 24 repeated bacterial *E. coli* exposures while evaluating hydrophilicity, silver content, and antimicrobial activity of the coatings. In conjunction with the long term durability of the coatings, their general biocidal efficacy was assessed with several microbial species that included three types of bacteria, yeast and a fungus. Bacteria *Listeria innocua, Staphylococcus epidermidis,* and *Pseudomonas putida* were selected because they are non-pathogenic and genetically similar to bacteria commonly associated with causing various diseases in humans. By studying the biocidal effect on these bacteria, one can anticipate the biocidal effect of Ag–ZA coatings on the corresponding pathogenic bacterial strains. Furthermore, fungus *Aureobasidium pullulans,* and marine yeast *Rhodotorula mucilaginosa*, were also subjected to the microbiocidal coatings to study their general microbiocidal effect.

2. Experimental

2.1. Preparation of zeolite A (ZA) coatings and silver ion exchanged ZA coatings (Ag–ZA)

The procedure for ZA coating preparation and silver ion exchange has been described in detail in our previous studies [4]. In brief, a clear synthesis solution with molar composition of $10Na_2O{:}0.2Al_2O_3{:}SiO_2{:}200H_2O$ was prepared. Stainless steel SS-304 coupons (2 cm by 3 cm) were cleaned in a 1.3 wt.% Alconox detergent solution. Polypropylene balls (2 cm diameter) were slit with a razor blade and the clean coupons were then individually inserted into the slits. The assemblies were then floated in the ZA synthesis solution in a sealed polypropylene bottle, and heated at 65 °C for 11–12 h. After the synthesis, the coated coupon was thoroughly washed with double de-ionized (DDI) water and dried with compressed air. The structure of ZA was verified by X-ray diffraction. The ZA coating obtained its antimicrobial properties by exchanging the sodium ions in the pores of zeolite with silver ions. The ZA coated coupon was inserted into slit polypropylene balls and floated in a 400 mL of 0.01 mol/L $AgNO_3$ solution for 6 h at room temperature. The coupons were then washed thoroughly with de-ionized (DI) water and then soaked in DI water for 1 h.

2.2. SEM and EDS characterizations of ZA coatings

ZA coatings were characterized before and after silver exchange using scanning electron microscopy (SEM, Philips XL30-FEG) at 20 kV and semi quantitative energy dispersive X-ray spectroscopy (EDS) attached to the SEM.

2.3. Year-long durability test by submersion in DDI water

Ag–ZA coated samples were immersed into DDI water for up to 1 yr in order to determine the effect of silver leaching. Every 3 months, 10 samples were removed from the leaching environment and tested for hydrophilicity, silver content and microbiocidal efficacy against bacterium *E. coli*.

2.4. Hydrophilicity testing

The hydrophilicity of the coatings was determined by water contact angle measurement using VCA-Optima XE. A coating is considered to be hydrophilic if the water contact angle is below 30°. Twelve measurements were made on every tested sample and averages were reported.

2.5. Silver content testing

The amount of silver contained within the ZA coating was determined by atomic absorption (AA). The Ag–ZA coating was dissolved in 1.5 mL of 1.0 mol/L HNO_3 and then brought to 10 mL with 0.1 mol/L HNO_3. The solution was then diluted 200-fold with 0.1 mol/L HNO_3, and examined by AA. The data are reported here as the percentage of Ag within the coating by dividing the mass

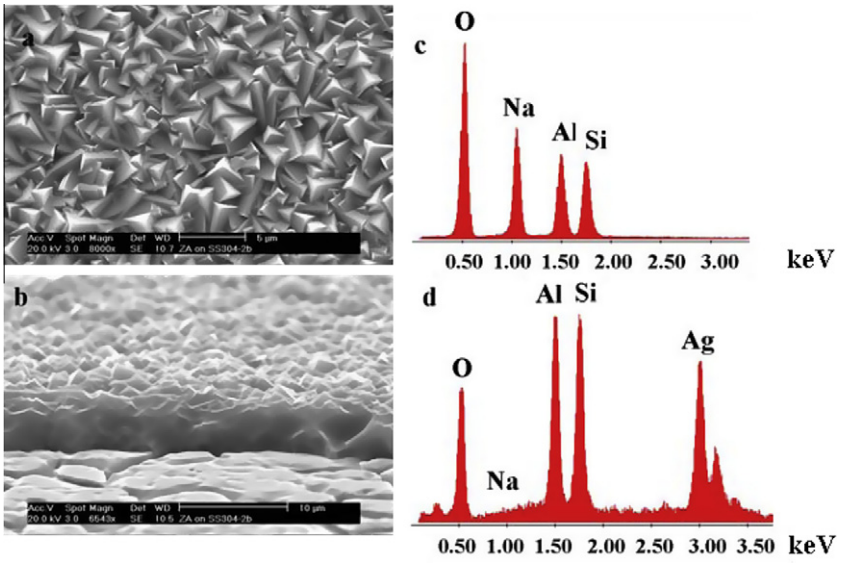

Fig. 1. (a) Surface and (b) cross-sectional SEM images of Ag–ZA coatings on SS304-2 and semiquantitative elemental analysis by EDS of (c) Na–ZA/SS-304-2b and (d) Ag–ZA/SS-304–2b.

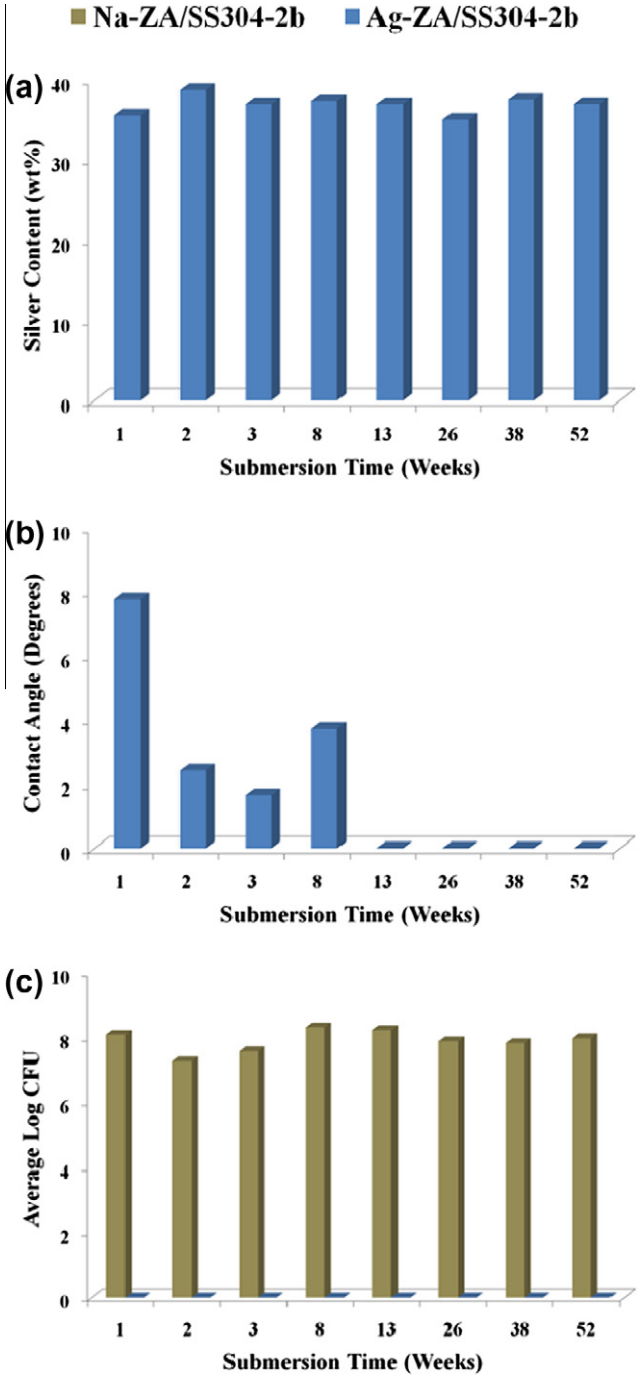

Fig. 2. After submerging Ag–ZA coatings in DDI water up to 52 weeks (a) weight percentage Ag (g/g) within the Ag–ZA coating, (b) water contact angles on Ag–ZA coatings after submersion in DDI water up to 52 weeks, and (c) antimicrobial activity (*E. coli*) at 0 h incubation time of Ag–ZA coatings (blue bars) and Na–ZA coatings (yellow bars). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of silver by the mass of zeolite coating. A more detailed description about silver content testing can be found in our previous paper [4].

2.6. Antimicrobial testing

The antimicrobial function against *E. coli* was investigated following the protocols described by Cowan et al. [8]. *E. coli* (JM 109) was spread on a Luria–Bertani (LB) agar plate and incubated at 37 °C for 24 h such that individual colonies could be found. An individual colony was dabbed with a sterile toothpick, placed into a sterile test tube containing LB medium, and incubated at 37 °C overnight. The bacteria were centrifuged down at 4 °C for 15 min at 3000 rpm, and then resuspended in butterfields buffer (BFB) until an optical density at 540 nm between 0.1 and 1.0 was achieved.

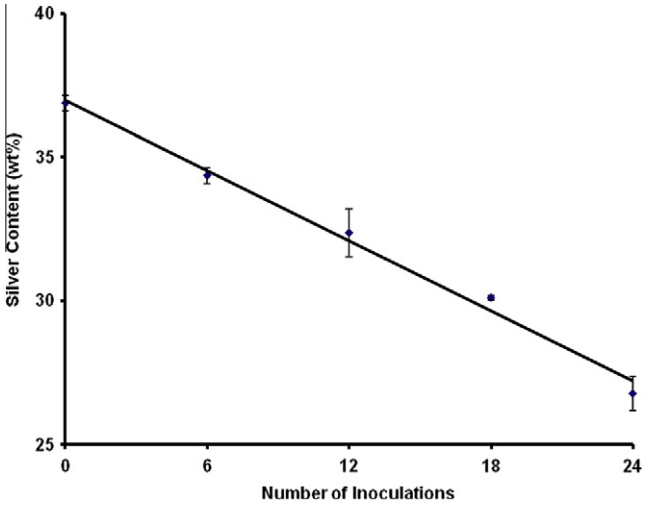

Fig. 3. Percentage Ag (g/g) within the Ag–ZA coating after up to 24 repeated *E. coli* challenges (24 h incubation).

Teflon rings were placed into small Petri dishes containing 6.5 mL BFB. Ag–ZA coupons were placed on the Teflon rings and 0.5 mL of the bacterial suspension was placed on the coupon. They were then incubated at 37 °C for 0, 4, and 24 h. Three samples were used for each incubation period. After incubation, the Teflon stands were removed, and the Petri dish was gently swirled. Each coupon was scraped with a sterile cell spreader three times along the major axis and three times along the minor axis. The cell spreader and the coupon were rinsed with 3 mL BFB, bringing the volume within the Petri dish up to 10 mL. They were then serially diluted to have the appropriate number of colony forming units (CFUs) for bacterial plating. Aliquots of 100 μL were spread across an LB agar plate and then incubated at 37 °C for 20 h. The CFUs were counted visually, and back calculated to find the number of surviving CFUs. The same procedure was applied for the Na–ZA coating controls.

2.7. Repeated bacterial exposures

Total 36 Ag–ZA samples were tested. The 36 samples were equally divided into three groups of 12 samples, one group for 0 h, one group for 4 h, and one group for 24 h incubation time.

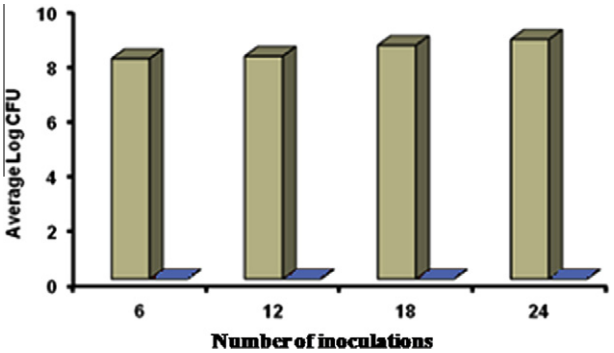

Fig. 4. Antimicrobial activity (*E. coli*) of ZA coatings after up to 24 repeated bacterial challenges (*E. coli*) (0 h incubation) of Ag–ZA (blue bars) and Na–ZA (yellow bars) coatings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

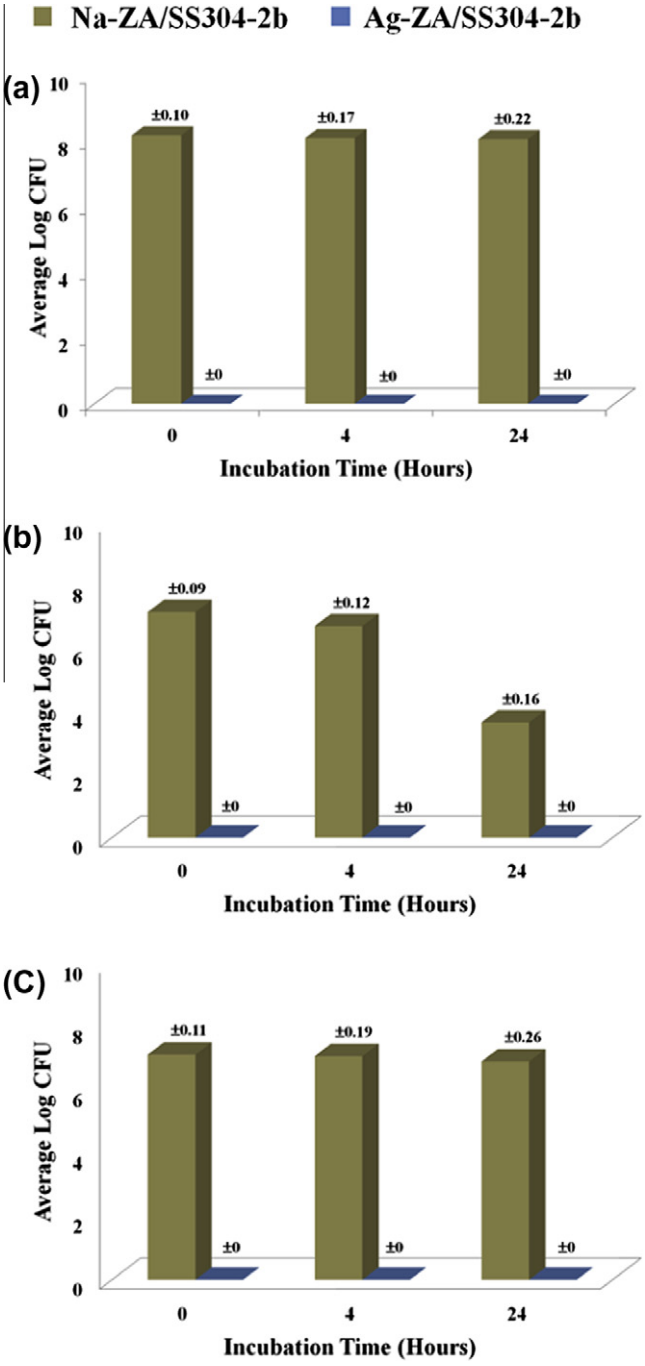

Fig. 5. Surviving colony-forming units on Na–ZA coatings (yellow bars) and Ag–ZA coatings (blue bars) over a 24 h incubation period; (a) *L. innocua*; (b) *S. epidermidis*; (c) *P. putida*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For each group, three samples were taken out for checking the number of surviving cells, as well as the hydrophilicity and silver content after 6th inoculation. Similarly, three sample after 12th inoculation, three samples after 18th inoculation, and final three after 24th inoculation. All ZA coated samples were washed and sterilized in ethanol overnight, and soaked in DDI water for 24 h before inoculation and incubation.

2.8. General antimicrobial testing

L. innocua cultures were grown in BHI broth (Oxoid), with a solid media of 5% (w/v) agar (Difco, Lawrence, KS), at 35 °C while shaking at 200 rpm. All *Listeria* strains were grown aerobically in Brain Heart Infusion (BHI, Difco, Franklin Lakes, NY) at 37 °C on BHI agar plates. Overnight cultures of *L. innocua* in 25 mL brain–heart infusion (BHI) were centrifuged and resuspended in the same volume of minimal medium. After 6 h adaptation to the medium, 400 mL cultures were adjusted to an OD_{600} = 0.05 and incubated for about 17 h at 377 °C with shaking. The cells were harvested by centrifugation (10 min, 3000 6*g*, 47 °C) at late exponential phase (*L. innocua*: OD_{600} = 0.9). *S. epidermidis* and *P. putida* were also cultured similarly in BHI broth at 37 °C and growth was measured turbidimetrically by noting optical density at 600 nm.

Fungus *A. pullulans*, and marine yeast *R. mucilaginosa* were cultured on agar plates and Na–ZA/SS-304-2b and Ag–ZA/SS-304-2b coupons were subsequently placed on the colonies, and incubated for 24 h.

3. Results and discussion

Zeolite A (ZA) coatings on stainless steel (SS-304-2B) substrates displayed complete and even coverage with a thickness of 4–6 μm (Fig. 1a and b). No changes were observed before and after the Ag ion exchange. Before Ag ion exchange, EDS scan confirms the presence of Na and the absence of Ag (Fig. 1c). Once Ag ion exchange is achieved, complete loss of Na from the zeolite A coating is evident, and Ag is detected in the EDS scan (Fig. 1d). The Na ions are exchanged with Ag ions by a single-displacement ion-exchange process.

The Ag ion content of Ag–ZA coating is extremely important for maintaining the coating's antimicrobial activity. Ideally, the best antimicrobial coatings only release silver ions upon bacterial exposure. One year leaching tests were carried out by immersing the Ag–ZA coated coupon in DDI water to verify whether leaching occurs without bacterial presence. Atomic adsorption elemental analyses show that the silver contents of Ag–ZA coatings remain relatively constant during the whole year, always above 35 wt.%, as shown in Fig. 2a. From the chemical formula of zeolite A, $[Na^{+}_{12}(H_2O)_{27}]_8[Al_{12}Si_{12}O_{48}]_8$, if there is a silver ion for every aluminum atom in the thoroughly exchanged Ag–ZA coating, the theoretical maximum percentage of silver in ZA is about 40 wt.%. This means around 90% of Na ion in the as-synthesized coatings was replaced by Ag ion before the immersion or repeated bacteria attach tests. Such high ion exchange ratio is achieved because Ag ions are highly polarizable by the strong electric fields within zeolites and are very tightly bound to the anionic framework [9]. The EDS elemental analysis of the coating after the immersion tests confirmed that the atomic ratio of Ag:Al was roughly 1:1, indicating that very few silver ions had leached out from the coating to DDI water during the whole year submersion.

The hydrophilicity of Ag–ZA coatings during the one year submersion was also tested by water contact angle measurement (Fig. 2b). The data for the first 8 weeks were reported previously [4]. After 8 weeks of immersion, the Ag–ZA coating maintained its super hydrophilic property (<8°) throughout the entire year. The lower hydrophilicity observed for the first 8 weeks was due to the small amount of Ag_2O formed on the surface of the zeolite coating by decomposition of $AgNO_3$ used for the ion exchange experiment. If the samples were washed thoroughly after the Ag ion exchange experiment the coatings do exhibit high hydrophilicity from the beginning of the immersion experiments.

For the rest of the samples which were used for the test period of 13–52 weeks, a more thoroughly stirred solution was used during the ion exchange, avoiding the accumulation of silver oxides on the coating surface. For those samples, the contact angles are 0° after ion exchange, and remain 0° throughout the whole year submersion in DDI water.

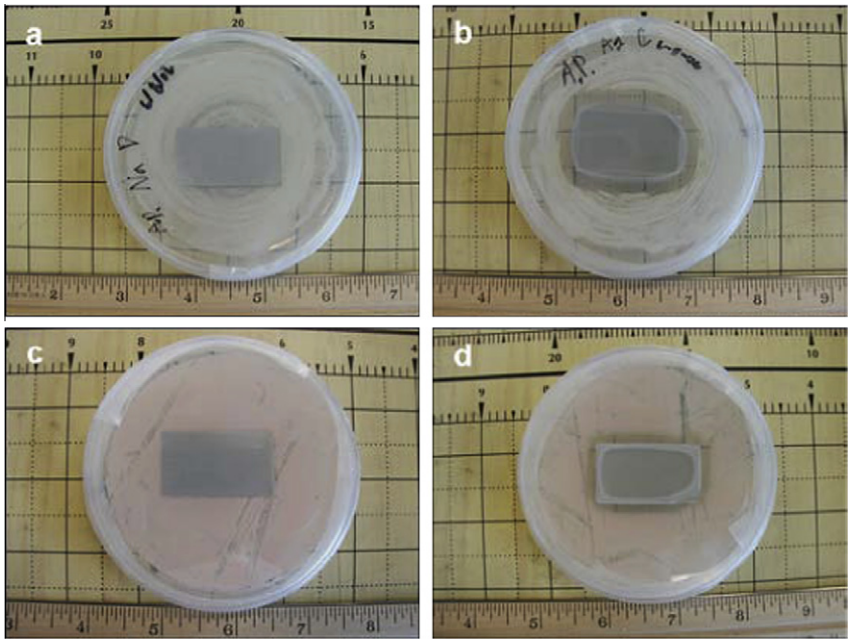

Fig. 6. Optical images of Na–ZA coatings (a and c) and Ag–ZA coatings (b and d) coupons on *A. pullulans* (a and b) and *R. mucilaginosa* (c and d) streaked agar plates after 24 h incubation.

The long term antimicrobial activity of the Ag–ZA coatings after up to 52 weeks of submersion in DDI water is shown in Fig. 2c. It can be seen that these coatings are extremely antimicrobial, killing all *E. coli* bacteria even without incubation, while the Na–ZA controls yield 10^6–10^7 CFU. Similar data was obtained for the 4 and 24 h incubation periods. As no antibacterial activity was observed with the Na–ZA coatings, silver ions released from zeolite A were responsible for the efficacy of killing a large number of bacteria in a very short period of time. The exact mechanisms for of how Ag ion attacks the bacteria are still being debated. Two possible mechanisms are listed. The first one is that the contact of bacterial cells with silver loaded zeolite coating allows the transfer of Ag ion to the cells, which then inhibit the essential cell functions and eventually damage the cells; the second one is that the transfer of Ag ion from zeolite coating to the cell inhibit the respiratory functions of the cells to generate reactive oxygen that damages the cells [11].

All the results show that Ag–ZA coatings are highly durable under wet conditions. They retain their silver content, super hydrophilicity, and antimicrobial activity after one year submersion in water. Since the silver ions are not readily released in water, the only loss of silver is from the act of killing bacteria. The slow and continuous release of silver ions upon bacterial attack is a critical factor needed to ensure the durability of highly antimicrobial activity of Ag–ZA coatings [10].

Fig. 3 shows the steady decrease in the silver content of Ag–ZA coatings upon repeated *E. coli* bacterial challenges (10^6–10^7 CFU) up to 24 times. The silver content lost about 0.4% after each bacterial challenge. This linear decline is expected since the same amount of silver ions was required to be released from the ZA coating to kill the same amount of bacteria, which also indicates that silver ions in Ag–ZA coatings only release upon bacterial attack. The silver content in Ag–ZA coating remains quite high (>25 wt.% from the original max 38–40%) even after 24 repeated large amount of bacterial exposures. The consumption of Ag ion observed during the bacteria exposure tests is consistent with literature that silver-loaded zeolites in their powder form in aqueous media would only release silver ions in the presence of bacteria [11]. When the Ag ion is consumed it is expected that K ion will be exchanged into the zeolite as K ion is the dominant cation in the buffer solution.

It can be seen from Fig. 4 that Ag–ZA coatings have very high antimicrobial activity. They killed all bacteria immediately without incubation, even after 24 total repeat bacterial tests. Similar data was obtained for the 4 and 24 h incubation periods. The hydrophilicity of Ag–ZA coatings after 24 repeated bacterial tests (24 h incubation period) was also tested by water contact angle measurements. These contact angle measurements showed an increase but less than 20° because the silver ions were released from ZA after each bacterial test. It is possible to form silver oxide on the surface of Ag–ZA coating, subsequently increasing the contact angle. With water contact angles below 30°, these surfaces are still classified as super hydrophilic and will be highly effective at performing the wetting and wicking function required in condensing heat exchanger onboard manned spacecraft.

Microbiocidal function of the Ag–ZA coating was carried out to analyze the general biocidal efficacy of the coating on a broad spectrum of microbial species (much beyond *E. coli*): three bacteria, marine yeast and a fungus. For bacterial species *L. innocua*, *S. epidermidis*, and *P. putida*, instant biocidal effect was observed for the Ag–ZA coating, and no growth of these species was observed at 4 and 24 h incubation periods (Fig. 5). On the contrary, non silver-exchanged ZA coatings showed no microbiocidal effect on contact, and bacteria were present even after 4 and 24 h incubation periods. A live-dead stain of *S. epidermis* confirmed the antimicrobial effect of Ag–ZA coatings. Live-dead stain on Na–ZA coating revealed live bacteria with dye uptake, while Ag–ZA coatings showed no dye uptake occurred indicating that bacteria were dead (data not shown).

Fungus *A. pullulans*, and marine yeast *R. mucilaginosa* were also subjugated to the Ag–ZA and Na–ZA coatings to determine the microcidal effect of the coating. Both, yeast and fungus species, were showed to have no growth on Ag–ZA coatings, and a zone of no growth is also seen around the coupon (Fig. 6b and d). As silver ions leached from the coating into the solution, they could have migrated to the agar surface next to the coupons. This could

have prevented any growth of yeast and fungus species in the immediate vicinity of the coating. Non silver ion-exchange ZA coatings show growth of both yeast and fungus species beginning at the edges of the coupons (Fig. 6a and c).

Our results indicate that Ag–ZA coatings possess high degree of biocidal activity over a broad spectrum of microbes. Zeolite coating leaches Ag ions in a controlled fashion independent of the microbial species attaching to the coating surface. This demonstrates that the coating's microbiocidal function has great utility and can be used in widespread applications.

4. Conclusions

Ag–ZA coatings are extremely durable. Their silver content, hydrophilicity, and antimicrobial activity remain the same after one year submersion in DDI water. After 24 repeated bacterial exposures, Ag–ZA coatings still show excellent antimicrobial activity and remain hydrophilic. The demonstrated long life span and the general biocidal effect of Ag–ZA coating makes it a great candidate for application in heat exchangers on board manned spacecraft for preventing cabin condensation and growth of potentially dangerous microbes. Antimicrocidal zeolite coatings may also find applications in many other areas such as foods and building industry.

Acknowledgments

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