EVALUATION OF A SILANE QUATERNARY AMMONIUM SALT AS AN ANTIMICROBIAL SURFACE TREATMENT

Daniel Blustein[1], Nona Hinkle[2] and Ami Smith[2]
[1]*Kalamazoo College, MI;* [2] *The Bionetics Corporation, Kennedy Space Center, FL, 32899 USA*

NASA guidelines for planetary protection aim to preserve the unaltered environments of both our own planet and all other planetary bodies in our solar system. Preventing the spread of Earth-based life forms to other planets and the contamination of our own planet by extraterrestrial life forms is an important consideration when planning space missions. Even with extensive sterilization techniques during the assembly of spacecraft, bacteria, mainly extremophiles, still survive[1,2].

Another pressing concern when discussing the control of microbes in space is the maintenance of astronaut health. Astronauts traveling in spacecraft are subject to close-quarter living for an extended period of time. Ideal conditions for microbe growth often arise as a result of irregularity in temperature and humidity control onboard the spacecraft. An impaired human immune system during spacflight and the lack of available medical care necessitate bacterial growth prevention measures[3].

To prevent the survival and growth of microbes and to decrease the pathogenic stress on crewmembers, we tested whether the ÆGIS Microbe Shield™ could be used as an antimicrobial surface treatment for materials in and on spacecraft using a dynamic contact bacterial solution method.[4]

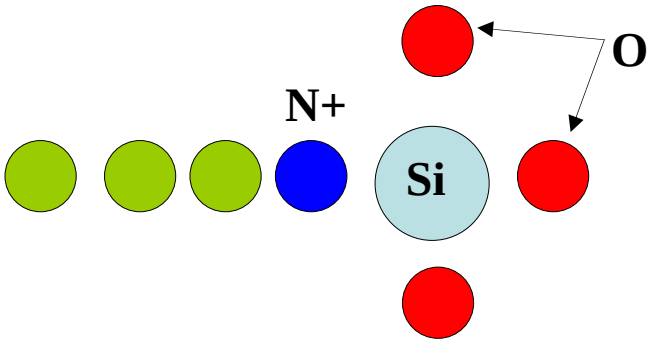


Figure 1. *Chemical makeup of the ÆGIS Microbe Shield™ with silicone base, oxygen atoms, nitrogen ion and hydrocarbon 'spear'*[5].

Surface Preparation: Four aluminum coupons (1.7 cm x 5.5 cm x 0.2 cm) were treated with a 1:100 water dilution of silane QAS (42% in methanol; AEM 5700) to 0.42% (Figure 2). The pieces were flipped after 7.5 minutes and soaked for a total of 15 minutes. The surfacing was performed in a Nuaire™ Biological Safety Cabinet at room temperature. The coupons were hung on a wire in the safety cabinet to dry overnight.

Culture Preparation: Cultures of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus epidermidis* were prepared in Tryptic Soy Broth (TSB) overnight at 35 ºC. The broth was diluted to a transmittance of 72% on a Vitek Colorimeter (1.5-3.0 x 10^8 CFU/mL). This bacterial solution was mixed with 0.003 M KH_2PO_4 buffer to a 1:1000 dilution (1.5-3.0 x 10^5 CFU/mL).

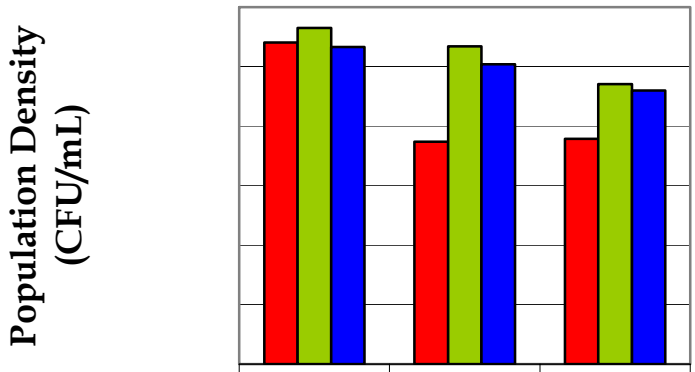
A company protocol was followed for testing the effectiveness of the QAS.[4]

Figure 2. *Aluminum coupon used in surface treatment testing.*

Surface Testing: The bacterial suspension was transferred into 9 flasks in 50 mL portions; 4 flasks contained treated surface, 4 flasks untreated surface, and 1 flask no surface to act as a growth control. A sample was taken from the growth control at time=0 and plates were poured. The nine flasks were placed in a horizontal shaking water bath at 35 ºC and ~120 RPM (Rate 6 on Lindberg/Blue M Agitator). After one hour, samples were taken from every flask, plated, and incubated at 35 ºC overnight. Bacterial colonies were counted on the plates, population densities were calculated across all trials, and t-tests were completed. This information was used to determine the effectiveness of the QAS by examining its toxicity towards bacteria.

Aluminum coupons, treated and untreated, were autoclaved, rinsed with deionized water, and reused for the next trial. A confidence level of 95% was used to test the significance of the logarithmic data.

For each of the species tested, the numbers of live bacteria were significantly reduced on the coupons treated with QAS according to t-tests of the logarithmic data (Figure 3). The exception was the second trial of *B. subtilis* in which a p- value of 0.428 was obtained. The effectiveness of the QAS on *E. coli* was less than that obtained for the other bacterial species.



	E. coli	S. epidermidis	B. subtilis
Treated	2,52E+05	5,46E+03	6,15E+03
Untreated	4,46E+05	2,19E+05	5,06E+04
Control	2,14E+05	1,09E+05	3,94E+04

Figure 3. *Logarithmic comparison of bacterial growth on aluminum coupons treated and untreated with the QAS for each bacteria species.*

According to the claim of the manufacturer (ÆGIS Environments; Midland, MI.) that the QAS is permanent, repeated use of the treated surfaces was not expected to have an effect on the outcome. The microbe shield was also intended to withstand temperatures well over the autoclaving temperature of 121 °C[6]. The reduction in activity of the product after autoclaving and rinsing increased the difficulty of comparing the QAS effectiveness across species of bacteria (Figure 4).

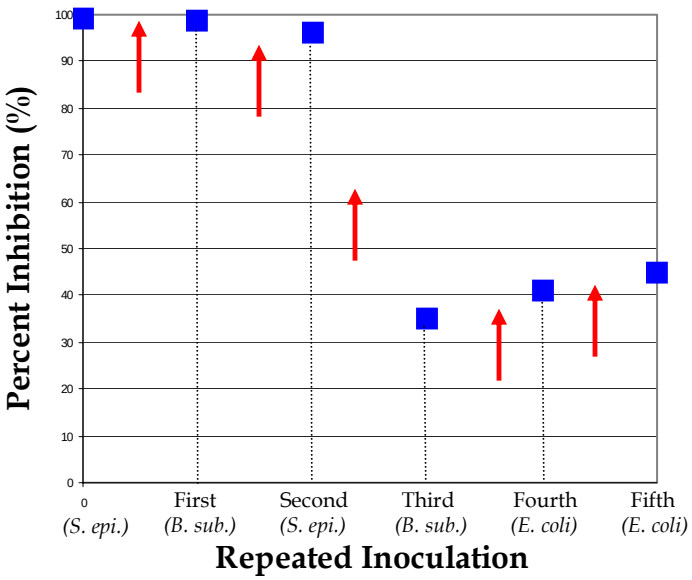


Figure 4. *The effectiveness of the QAS after repeated inoculations of Escherichia coli, Staphylococcus epidermidis, and Bacillus subtilis. The red arrows mark the points at which the treated metal coupons were autoclaved and rinsed.*

The results, except from the fourth use of the metal (second trial of *B. subtilis*), correlated with the expected antimicrobial effects of the QAS. However, company claims of the permanency of the product were not supported by results of the experiments. *E. coli*, expected to be particularly vulnerable to the QAS from previous testing and because it is non-spore-forming, was less affected by the treatment compared to the other species. Since *E. coli* was used in the final two trials, a degrading microbe shield would explain this result possibily attributed to the effect of autoclaving and rinsing the coupons between uses. The surface darkened after autoclaving in the spent media, suggesting that some sort of change occurred to the metal. Autoclaving could have simulated the effect of burning, which the product is not designed to withstand.

Even with the evidence of the loss of effectiveness over time, the results suggested that this product may be of some use in controlling microbes in future space travel. If surfacing techniques could be refined and the scope of the product better understood, this antimicrobial shield could be a very important tool in the prevention of bacterial growth on and in spacecraft of the future.

Since this was a preliminary analysis of the product only demonstrating effectiveness of the QAS in a solution of bacteria under dynamic contact conditions, much work still needs to be completed. While possible applications such as using the shield within water-filled pipes could arise from this testing, the ability of the microbe shield to prevent bacterial colonization in other spacecraft environments needs to be explored. Testing of bacteria using newly treated metal should be performed and the durability of the surfacing agent should be explored. Other investigations should include determining the concentrations of bacteria against which the product is effective and in what concentration the substance must be applied to the surface to retain antimicrobial properties.

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