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SHORT COMMUNICATION

Efficacy of various chemical disinfectants on biofilms formed in spacecraft potable water system components

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As the provision of potable water is critical for successful habitation of the International Space Station (ISS), life support systems were installed in December 2008 to recycle both humidity from the atmosphere and urine to conserve available water in the Station. In-flight pre-consumption testing from the dispensing needle at the Potable Water Dispenser (PWD) indicated that bacterial concentrations exceeded the current ISS specifications of 50 colony-forming units (CFU) ml^{-1}. Subsequent investigations revealed that a corrugated stainless steel flex hose upstream of the dispensing needle in the PWD was filled with nonsterile water and left at room temperature for more than 1 month before launch. To simulate biofilm formation that was suspected in the flight system, sterile flex hoses were seeded with a consortium of bacterial isolates previously recovered from other ISS water systems, including *Ralstonia pickettii, Burkholderia multivorans, Caulobacter vibrioides*, and *Cupriavidus pauculus*. After incubation for 5 days, the hoses were challenged with various chemical disinfectants including hydrogen peroxide (H_2O_2), colloidal silver, and buffered pH solutions to determine the ability of the disinfectants to decrease and maintain bacterial concentrations below ISS specifications. The disinfection efficacy over time was measured by collecting daily heterotrophic plate counts after exposure to the disinfectants. A single flush with either 6% H_2O_2 solution or a mixture of 3% H_2O_2 and 400 ppb colloidal silver effectively reduced the bacterial concentrations to <1 CFU ml^{-1} for a period of up to 3 months.

Keywords: international space station; water recovery system; spacecraft potable water dispesnser; hydrogen peroxide; colloidal silver; biofilms

Introduction

In order to accommodate the increased demand for potable water of a six person crew onboard the International Space Station (ISS), the Water Recovery System (WRS) was installed in December 2008. The WRS consists of an urine processor and a Water Processing Assembly (WPA). The WPA is a water purification system that recovers potable water from urine distillate, cabin air humidity condensate, and other hygiene waste water (Carrasquillo 2005).

To certify that water produced from the WPA met ISS potable water quality requirements, microbial testing of the water was performed prior to crew consumption. Samples were collected from the dispensing needle of the galley system called the Potable Water Dispenser (PWD) (Figure 1) and, during the certification process, in-flight water analysis indicated that bacterial concentrations exceeded the current ISS specifications of 50 colony forming units (CFU) ml^{-1}. Subsequent investigations revealed that a corrugated stainless steel (SS) flex hose upstream of the dispensing needle in the PWD was flushed with 30 ppm of iodine for only 5 min, drained, and then refilled with non-sterile water. The refilled hose was left at room temperature for over 1 month before launch. Silver (ionic and colloidal) and iodine are commonly used to disinfect potable water systems onboard the ISS and Space Shuttles (Koenig et al. 1995; Roberts et al. 2007). After failing the ISS specifications, an in-flight treatment of the PWD ambient leg with 40 ppm iodine was attempted, but was not successful in reducing the bacterial concentration.

The purpose of this study was to determine the efficacy of alternative chemical disinfectants on biofilms formed in spacecraft potable water system components. These alternative chemical disinfectants must have low toxicity level for crew safety and be compatible with the materials that are used in ISS water systems. A 6% H_2O_2 solution was chosen because of its known disinfection capability (Lucia et al. 1993). A mixture of 3% H_2O_2 and 400 ppb colloidal silver was selected because of its increasing popularity in the biosafety arena as a disinfectant solution for water systems (Funk G, personal communication). A phosphoric acid buffered solution with a pH of 2.85 was used because of its initial success in

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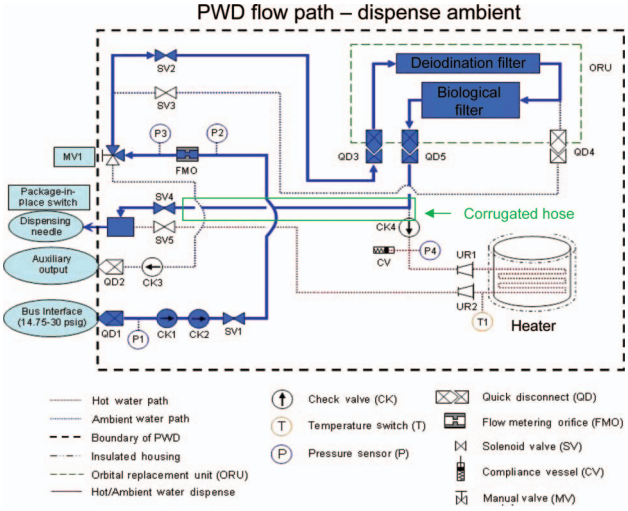

Figure 1. Schematic of potable water dispenser flow path.

eliminating planktonic water-borne bacteria (unpublished data).

Materials and methods

Formation of biofilms

To simulate biofilm formation that was suspected in the flight system, corrugated SS flex system (Swagelok SS-FL4TA6TA6-9) identical to that in the PWD were purchased from Hardware Inc. (Gretna, LA). A total of four hoses was sterilized by autoclaving at 121°C for 15 min in a dry cycle. After the hoses had been cooled for 24 h, they were seeded with a consortium of bacterial isolates previously recovered from ISS water systems, including *Ralstonia pickettii, Burkholderia multivorans, Caulobacter vibrioides*, and *Cupriavidus pauculus*. Identification of the bacteria was performed using MicroSeq 500 16S rDNA Bacterial Identification Kits and a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). One milliter of each bacterial suspension (approximately 10^5 CFU ml^{-1} in sterile de-ionized water) was inoculated into each hose, which held a maximum of 5 ml. Each hose was capped with a sterile stopper at the outlet, whereas the inlet was covered by a sterile 15 ml Falcon conical tube to prevent contamination and to allow for air exchange. The hoses were secured in a plastic test tubes rack and incubated at room temperature (23–26°C) for 5 days under a Class II biosafety cabinet. On Days 2 and 4 of the incubation period, bacterial suspension from each hose was drained and plated on R2A agar plates (Hardy Diagnostics, Santa Maria, CA) by serial dilutions. The hoses were then flushed with 400 ml of sterile de-ionized water. A post-flushing sample of 50 ml was collected from each hose at the end of the flush and filter-plated using the Milliflex filter unit and R2A agar cassette (Millipore, Billerica, MA). The hoses were then refilled with 4 ml of sterile de-ionized water after each drain and flush. The purpose of performing the heterotrophic plate count during incubation was to verify that a biofilm had been established in each hose.

Exposure to disinfectants

Prior to exposure to the disinfectants of interest, a drain sample was collected from each hose and plated on R2A agar plates by serial dilutions. Three of the hoses were then flushed for 20 min (at 20 ml min^{-1}) with either a 6% H_2O_2 solution, a mixture of 3% H_2O_2 and 400 ppb colloidal silver, or a phosphoric acid buffered solution with a pH of 2.85. The remaining hose was not exposed to any chemical disinfectant and was flushed with sterile water to serve as a control. The flushing of the hoses was conducted using a Cole-Parmer MasterFlex 7518-00 peristaltic pump (Vernon Hills, IL) and autoclave-sterilized Cole-Parmer MasterFlex platinum silicone tubing (Vernon Hills, IL). A post-flushing sample of 50 ml was collected from each hose at the end of the flush and filter-plated using the Milliflex filter unit and R2A agar cassette (Millipore, Billerica, MA). After the collection of the post-flush samples, each hose was refilled with its respective disinfectant. The disinfectants remained in the hoses for 18 h to simulate in-flight disinfection protocol. Samples were collected and plated from each hose at the end of 18 h. Each hose was then refilled with 4 ml of sterile de-ionized water. The hoses were then drained and refilled on a daily basis. All drain samples were plated on R2A agar plates and incubated at room temperature for 48 h.

When the phosphoric acid buffered solution treated hose was subjected to an additional exposure of H_2O_2 and colloidal silver solution, the soak, drain and flush procedure described above was repeated.

Results

Pre-exposure

Heterotrophic plate counts collected on Days 2 and 4 during the 5-day biofilm incubation period fluctuated around 100,000 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 5$) whereas all post flush samples collected on Days 2 and 4 between drain and refill during the biofilm incubation period were <1 CFU ml^{-1}.

6% H_2O_2

The bacterial concentration of the hose that was exposed to a 6% H_2O_2 solution decreased from an

initial concentration of 2.3×10^5 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 5.36$) to <1 CFU ml^{-1} after flushing for 20 min. The concentration remained at <1 CFU ml^{-1} for over 90 days after exposure (Figure 2).

3% H_2O_2 + 400 ppb colloidal silver

The bacterial concentration of the hose that was exposed to a mixture of 3% H_2O_2 and 400 ppb colloidal silver solution decreased from an initial concentration of 1.1×10^5 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 5.04$) to <1 CFU ml^{-1} after 20 min of flushing. The concentration remained at <1 CFU ml^{-1} for over 90 days after exposure (Figure 3).

Phosphoric acid buffered solution (pH 2.85)/re-shocked with 3% H_2O_2 + 400 ppb colloidal silver. The bacterial concentration of the hose that was exposed to a phosphoric acid buffered solution (pH 2.85) decreased from an initial concentration of 5.8×10^4 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 4.76$) to 7.9×10^3 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 3.9$) after flushing for 20 min. The concentration dropped further to 80 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 1.9$) after being exposed to the solution for 18 h. However, the concentration increased to 6.0×10^4 CFU ml^{-1} (Log_{10} CFU ml^{-1} 4.78) 5 days after exposure (Figure 4). At the end of Day 5, the hose was re-exposed to a different disinfectant solution (a mixture of 3% H_2O_2 and 400 ppb colloidal silver) for 18 h. The bacterial concentration dropped to and remained at <1 CFU ml^{-1} for a period of 8 day after re-exposure. The bacterial concentration began to increase gradually 8 days after re-exposure. The concentration fluctuated around 300 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 2.5$) for the remainder of the study.

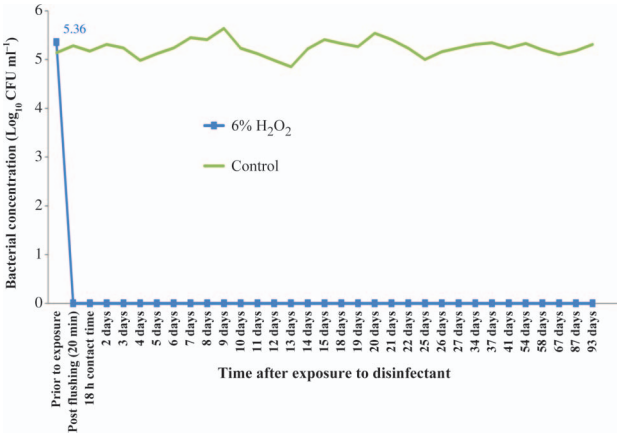

Figure 2. Bacterial concentrations recovered from hose after exposure to a 6% H_2O_2 solution.

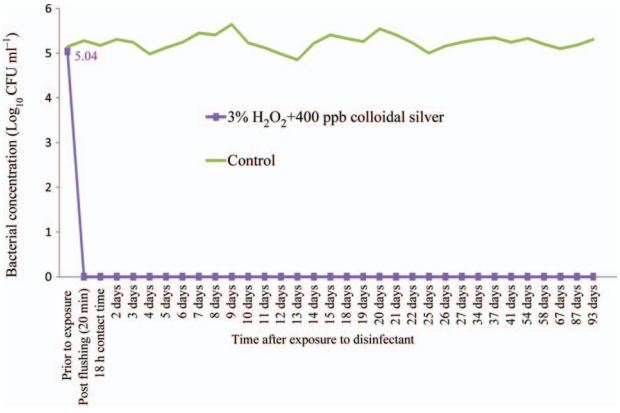

Figure 3. Bacterial concentrations recovered from hose after exposure to a mixture of 3% H_2O_2 and 400 ppb colloidal silver.

Control

The bacterial concentration of the hose that was not exposed to any disinfectants fluctuated around 100,000 CFU ml^{-1} (Log_{10} CFU $ml^{-1} = 5$) throughout the duration of the study (Figures 2–4). At the conclusion of the study, *C. pauculus* and *B. multivorans* were the predominant species remaining in the control hose at an approximate 8:2 ratio. The identifications of the bacteria were confirmed by sequencing.

Discussion

The data demonstrate that a single flush for an extended contact time of 18 h with either 6% H_2O_2

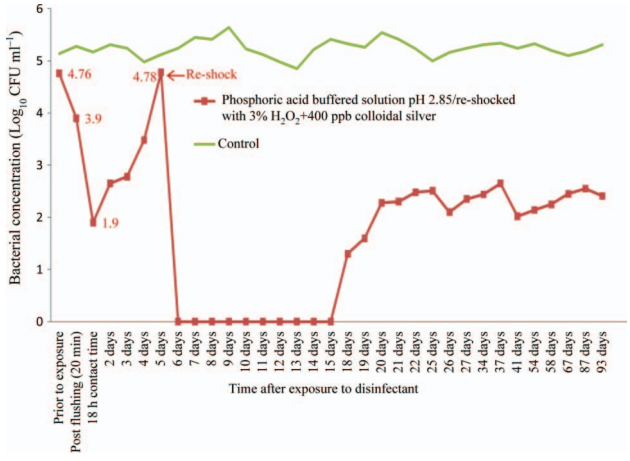

Figure 4. Bacterial concentrations recovered from hose after exposure to phosphoric acid buffered solution and then re-shocked with a 3% H_2O_2 and 400 ppb colloidal silver solution.

solution or a mixture of 3% H_2O_2 and 400 ppb colloidal silver effectively reduced the bacterial concentrations in 5-day-old biofilms formed in a spacecraft portable water system component to <1 CFU ml^{-1} for up to 3 months. The data also show that a phosphoric acid buffered solution with a pH of 2.85 was unsuccessful in reducing the concentration of sessile bacteria, and subsequent exposure of the pH treated hose with a mixture of H_2O_2 and colloidal silver remained ineffective. The ineffectiveness could be due to an inadvertent extended incubation time and/or a selection of entrenched biofilms resulting from the unsuccessful initial exposure of the hose to the phosphoric acid buffered solution.

At the time of the experiment, only four hoses were available. Due to a significant time constraint that was placed in identifying and testing a suitable alternative disinfectant to reduce the bacterial concentration in the ISS potable water system, a repeat of the disinfection experiment was not feasible at the time. For on-going studies to determine the efficacy of different concentrations of H_2O_2 with various flush and contact times on 3 month old biofilms in the corrugated SS flex hoses, an adequate number of hoses are available and inoculated to ensure that the disinfection assays are performed in triplicate.

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