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MICROBIAL BIOFILM STUDIES OF THE ENVIRONMENTAL CONTROL AND LIFE SUPPORT SYSTEM WATER RECOVERY TEST FOR SPACE STATION *FREEDOM*

By E.B. Rodgers, D.C. Obenhuber, and T.L. Huff,

Materials and Processes Laboratory
Science and Engineering Directorate

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D.C. Obenhuber,* T.L. Huff,* and E.B. Rodgers

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Marshall Space Flight Center, Alabama 35812

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NASA is developing a water recovery system (WRS) for Space Station *Freedom* to reclaim human waste water for reuse by astronauts as hygiene or potable water. A water recovery test (WRT) currently in progress investigates the performance of a prototype of the WRS. Analysis of biofilm accumulation, the potential for microbially influenced corrosion (MIC) in the WRT, and studies of iodine disinfection of biofilm are reported.

Analysis of WRT components indicated the presence of organic deposits and biofilms in selected tubing. Water samples for the WRT contained acid-producing and sulfate-reducing organisms implicated in corrosion processes. Corrosion of an aluminum alloy was accelerated in the presence of these water samples; however, stainless steel corrosion rates were not accelerated.

Biofilm iodine sensitivity tests using an experimental laboratory-scale recycled water system containing a microbial check valve (MCV) demonstrated that an iodine concentration of 1 to 2 mg/L was ineffective in eliminating microbial biofilm. For complete disinfection an initial concentration of 16 mg/L was required which was gradually reduced by the MCV over 4 to 8 hours to 1 to 2 mg/L. This treatment may be useful in controlling biofilm formation.

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TECHNICAL MEMORANDUM

MICROBIAL BIOFILM STUDIES OF THE ENVIRONMENTAL CONTROL AND LIFE SUPPORT SYSTEM WATER RECOVERY TEST FOR SPACE STATION *FREEDOM*

INTRODUCTION

Microorganisms will, without question, be part of the environment of any space capsule inhabited by humans. NASA's Space Station *Freedom* may be considered as a volume partially closed to materials for a number of years, consisting of living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts (ecosystem). The space station's small volume in zero gravity presents engineers and biologists with unique problems of subsystem design, management, and control that are very different from those encountered in the design for life support on Earth. The assemblage of organisms colonizing the space station, determined predominantly by human activity, is expected to adapt to the environment in ways not entirely predictable from the information derived from human habitations on Earth. Increased surface-to-volume ratio, vastly decreased dilution by air and water, and reduced diversity of living organisms are among the factors that can potentially lead to unique relationships among microorganisms and between microorganisms and the materials and subsystems they colonize.[1]

An important microbiological issue which must be considered in developing a recycling system for potable and hygiene water is the effect of the product water on the recipients. Drinking water for the space station is of particular concern due to the well-established potential for the spread of communicable disease through contaminated drinking water on Earth. Pathogenic microorganisms may enter the water system from environmental contamination or crew contact and may survive in the water for extended periods of time. Contamination of the water system must be considered an unavoidable problem and, therefore, must be dealt with on a continuous basis.

Microbial contamination of water systems is a hazard not only to those who use the water but also to the materials of the systems. Degradation and corrosion can be enhanced by microorganisms attached to surfaces. This form of microbial contamination, termed biofouling or biocorrosion, can lead to premature failure of a system.

NASA's water recovery system (WRS) is part of the larger environmental control and life support system (ECLSS) for Space Station *Freedom* which is designed to integrate critical life support processes such as air and water recovery into a recycling system with minimal size, weight, power, and maintenance requirements. The water recovery test (WRT) reclaims potable water from humidity condensate and hygiene water from a dishwasher, hand wash, whole body shower, clothes washer, and urine collector.[2] Because the accumulation of biofilms and subsequent potential for corrosion of materials within the recycled water system are of concern, microbiological technology is challenged to provide disinfection and monitoring of microbial contamination in the water system. Methods for maintaining acceptable levels of microorganisms in the integrated system are under investigation. Chemical disinfection with iodine is currently the method of choice due to the success in using iodine as a disinfectant in the space shuttle potable water system.[3]

Microbial analyses of bulk water taken from over 35 sampling ports, including hygiene and potable storage tanks, all subsystem sites, and each point of use were performed. Results of completed analyses are reported by Roman et al.[4] Studies discussed here were conducted in the Microbial Ecology Facility at NASA's Marshall Space Flight Center to supplement routine sampling and assist in data verification for the WRT. The types and amounts of microbial waterborne contaminants and biofilms which accumulate in the WRT during the test period were studied, and the use of iodine was investigated as a disinfectant.

BIOFILM CONTAMINATION OF WATER RECOVERY TEST COMPONENTS

Although routine analyses of biofilm formation in the WRT system were not performed, the failure of hardware components or replacement of used components (i.e., filters, valves, tubing, ion exchange cartridges) provided an opportunity to examine these in the laboratory. Microbial accumulation was assessed microscopically using acridine orange direct counts (AODC); cultural methods with R2A agar were used to determine the number of viable microorganisms in the biofilm. Microbial identification was performed on the Biolog Microbial Identification System and the Minitek Identification System. Chemical analysis of organic deposits was conducted by chemical oxygen demand to assess the potential for biofilm formation.[5]

Examination of the organic removal resins of a spent unibed multifiltration (MF) column, used in preliminary WRS tests, led to isolation of a pure culture of over 1.0E+0.5 CFU/g of *Methylobacter* sp. Pure cultures of the same organism were isolated from three additional MF columns at similar locations within the columns. No microorganisms were isolated from either of two of the newest generation MF columns. All columns examined contained iodinated resins designed to disinfect the cartridge at both the input and output ends. Biofilm accumulation was suspected at the site of iodine resistant microbial contamination within the MF cartridges.

Biofilm containing viable microorganisms on components used during the 28-day WRT appeared to be minimal (table 1). Only three samples taken from the WRT contained recoverable microorganisms. Chemical analysis showed significant organic deposition on some components, and although quantification of organic deposit is not a requirement for the WRS and therefore is not evaluated relative to system performance, it may influence bacterial survival in biofilms. Bacteria recovered from the disinfected portions of the water system (reverse osmosis (RO) tubing and RSR01 sample needle) were identified as *Clavibacter michiganense*. These findings suggest that this organism survived disinfection within the organic deposits on the internal surfaces of the components. Though *C. michiganense* is a common water organism and not considered to be pathogenic, the potential for the survival of known pathogens in the presence of iodine as a disinfectant must be further studied.[6]

Table 1 presents data compiled during the ECLSS-WRT for selected components removed from the system during testing. Analysis consisted of microbial counts and chemical oxygen demand (COD) on tubing or inlet sections of each component. Each item was cut to approximately 1 to 2 inches in length, swabbed aseptically for microbial counts on R2A (28 °C, 21 days), and assayed for COD by the closed reflux colorimeter method (5220D).

Table 1. Analysis of ECLSS-WRT biofilm samples.

Description	mg Carbon/cm^2	CFU/cm^2
Washers from PVM41 and 42	0.2237	0
RPSA piping (1.5 in)	>10.00	0
MCV (PVB01)	>10.00	0
Chiller tube	>10.00	1.0E+02
MF unibed (10200)	3.7019	0
Needle valves (KVM09 and 06)	0.7792	0
RO tubing	3.3852	1.0E+06
RO unibed (10200)	>10.00	0
RO unibed (10203)	3.5916	0
Sample needle (HSL09)	>10.00	0
Sample needle (RSR01)	1.0218	1.2E+02
Sample needle (HSL08)	5.9912	0
RO unibed (10210)	4.3470	0
Diaphragm (UP01)	–	0
RO prefilter	–	0
RO unibed (10206)	3.7019	0
RPSA tubing	–	0

A section of a chiller tube did not receive disinfectant and therefore contained a variety of bacterial types. The predominant isolates were identified as *Pseudomonas pickettii* and *Pseudomonas cepacia*. These bacteria are also commonly found in water and could be expected to survive for long periods of time in a water system.

Samples of tubing from waste hygiene water processing systems (waste hygiene RO and urine pretreatment assemblies) were examined. The RO tubing contained bacteria and large segmented fungi (some branching) with little detectable biofilm. The presence of these fungi may play a role in premature system failure by physical blockage and production of corrosive by-products. Microscopic observation indicated that urine pretreatment tubing contained large numbers of bacteria in well-defined biofilms.

When a microbial check valve (MCV) (an iodinated resin cartridge designed to maintain a stable concentration of iodine in the water)[7] from the WRT potable water system was replaced due to excessive back pressure, the MCV was backflushed and the nature of the clogging material was investigated. Metal filings and amorphous translucent material of probable organic composition were found. Epifluorescent staining of presumed organic material demonstrated the presence of numerous microorganisms that could not be cultured using R2A or tryptic soy broth. These preliminary data suggest a relationship between accumulation of organic materials and the formation of biofilm in the WRT.

Two additional MCV's were backflushed with 1 liter sterile water which was collected in sterile bottles containing sodium thiosulfate. Epifluorescent staining of the samples indicated approximately 1.0E+05 cells per 100 mL, the lower detection limit of the procedure. Two types of organisms were isolated on R2A and BHI (10 percent) from one of the samples. *Methylobacter* sp. and an unidentified gram positive coccus were recovered. Debris removed with the backflushings had no observable bacteria associated with it. Biofilm recovered from a backflushed

MCV may or may not have originated in the MCV, though in either case, it demonstrates that even in the presence of 2 to 4 mg/L iodine there is the potential for biofilm accumulation.

Swab samples were taken from the disassembled urine processor, the thermoelectrically integrated membrane evaporation subsystem (TIMES), during water recovery testing. Sites examined included inlet and outlet manifolds of the hollow fiber bundles, hollow fibers, and the surrounding inner tank surface. Two organisms were isolated, one of which, *Klebsiella pneumoniae*, was found at all sites. *Pseudomonas aeruginosa* was isolated from the hollow fibers and inner tank surface. Data from WRT microbial samples also indicated the presence of these species. Neither of these organisms was isolated from the product water during the test period.

BIOCORROSION

Environmental microorganisms have been shown to play a role in the corrosive processes which can occur in water systems. These microorganisms occupy an ecological niche which makes them unlikely candidates as human pathogens, though the result of microbially induced or enhanced corrosion can significantly affect health and safety by reducing the reliability of water systems. The role of opportunistic pathogens in corrosion processes is less well studied despite the ability of many to survive under anaerobic conditions. Although anaerobic corrosion usually implies the removal of hydrogen at cathodic sites (depolarization) by sulfate reducing bacteria such as *Desulfovibrio* sp., there are many other anaerobic reactions that can consume hydrogen. For example, the consumption of hydrogen in the reduction of nitrates to nitrites is a reaction carried out by all members of the *Enterobacteriaceae*. The nontraditional role of these organisms in both the accelerated corrosion of stainless steel and titanium, and human health and safety, requires investigation.

Commercially available media were used to determine the presence of microorganisms in WRT samples potentially capable of influencing corrosion. Positive indicators of microbially influenced corrosion were observed primarily in waste accumulator tank water, RO post-sterilization assembly effluent water, and product water from a urine processor (vapor compression distillation). Media for the detection of sulfate reducing bacteria and acid producing bacteria were positive in waste accumulator tank water. This water increased the corrosion rate in an aluminum alloy (2219-T87) as measured by resistance polarization using an EG&G 350A laboratory apparatus. Scanning electron microscopy verified pitting of the metal adjacent to forming biofilms. Energy dispersive x-ray analysis of the pits revealed high concentrations of sulfur (sulfide formation) which may have depolarized the cathodic reaction, accelerating the corrosion rate. Reflectance Fourier transmission infrared spectra revealed carboxyl groups present on the surface of the coupons, indicating the presence of organic acids, which may also increase corrosion. It is noted that aluminum is not a candidate material for the WRS. Biofilm formation was evident on 316L stainless steel but no corrosive activity was found. Opportunistic enteric bacteria were associated with organisms known to exhibit acid producing and sulfate reducing activity from the source water. These included *Klebsiella* sp., *Citrobacter* sp., and *Enterobacter* sp.

DISINFECTION

Iodine is the disinfectant currently used in the WRT system. In the potable and facility water, iodine is maintained at levels between 0.5 and 4 mg/L by microbial check valves. During the WRT, processor hardware and iodine disinfection using MCV's was generally effective, but occasionally did not maintain the concentration of bacteria to the required level of <1 colony forming unit (CFU) per 100 mL when the residual iodine concentration approached the minimum acceptable level. Mean bacterial densities in limited samples from the hygiene accumulator tank, which holds unprocessed waste water, and the potable tanks were greater than 7.0E+8 CFU/100 mL and fewer than 1 CFU/100 mL, respectively. Thus, the number of bacteria in the WRT appeared to be effectively reduced 8 orders of magnitude from input to product water. Opportunistic pathogens isolated from the waste hygiene tank on chocolate agar include *Klebsiella* sp., *Enterobacter* sp., *Citrobacter freundii, Serratia odorifera,* and *Escherichia coli.* These organisms were absent in the potable tank.

Iodine Sensitivity Studies

Iodine sensitivity studies were conducted on the *Methylobacter* sp. isolated from the unibed columns to determine their level of resistance. The test of iodine sensitivity of *Methylobacter* sp. was conducted in 16- by 150-mm test tubes containing 10 mL of distilled deionized water (18 MΩ). Each tube received approximately 1.0E+06 cells in 0.1 mL 10-percent BHI growth medium and shaken at 28 °C for 4 weeks. Periodically, three replicate tubes were analyzed for iodine sensitivity by aseptically removing the water in the tube and replacing it with sterile distilled water. Saturated iodine solution was added to produce a final concentration which ranged from 0 to 60 mg/L. After a 10-min exposure, the iodine was neutralized with sodium thiosulfate. BHI medium was then added to each tube to a final concentration of 10 percent, and the tubes were incubated at 28 °C for 21 days. Visible growth in the tubes was recorded as positive resistance to iodine disinfection.

The freshly grown bacterial isolate was sensitive to 1- to 2-mg/L iodine with a 1-min exposure. The same isolate maintained for over 4 weeks in glass test tubes at 28 °C showed increased resistance to iodine disinfection by surviving exposure to 10-mg/L iodine after 1 week and 30 mg/L after 4 weeks with a 10-min exposure time (fig. 1). Tests of a 4-week old biofilm produced by this microorganism demonstrated that at least one CFU survives after 1.5-h exposure to 16-mg/L iodine. This increased resistance may be the result of biofilm formation.

Biofilm Testbed

A laboratory scale closed-loop water system referred to as the biofilm testbed (BT) was constructed for controlled studies of biofilm formation in the presence of iodine disinfectant. The BT contained 128 removable coupons to monitor the formation of microbial biofilm. Distilled deionized water (12 L, 18 MΩ) was added to the BT and allowed to circulate untreated for 7 days to establish a biofilm. An MCV was installed on day 7. The water and biofilm coupons were monitored for microorganisms using R2A agar and 100-percent BHI broth. Biofilm was assayed for viable bacteria by removing three coupons, immersing each coupon in a tube of 10-percent BHI, and incubating at 28 °C for 21 days. Observable growth in a coupon tube was

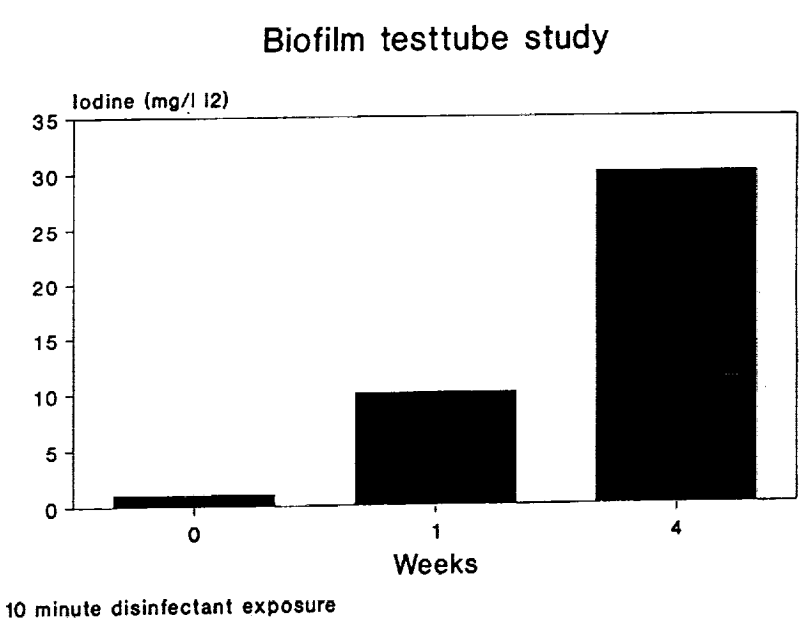

Figure 1. Iodine sensitivity of unibed isolate.

recorded as a positive indication of the resistance of the biofilm microorganisms to the iodine disinfection. Increasing amounts of saturated iodine solution (278 mg/L I_2) were added periodically to the system to temporarily supplement the MCV iodine output. Biofilm sensitivity was assayed immediately before and after each addition by coupon removal. Percent survival represents the percent of all assayed coupons exhibiting growth.

When a microbial check valve removed from the WRT was incorporated into the testbed, stable concentrations of iodine in the range of 1 to 2 mg/L were not effective at eliminating microbial biofilm. When iodine was increased to 16 mg/L, the microbial concentrations in the biofilm of less than 1 CFU were achieved (fig. 2). Throughout the test, microbial counts in the water averaged <1 CFU/100 mL. In a subsequent test, hygiene accumulator waste water was added to the BT in an attempt to recontaminate the system. Detectable levels in the biofilm of greater than 1 CFU were achieved only after the addition of 1 L of waste water.

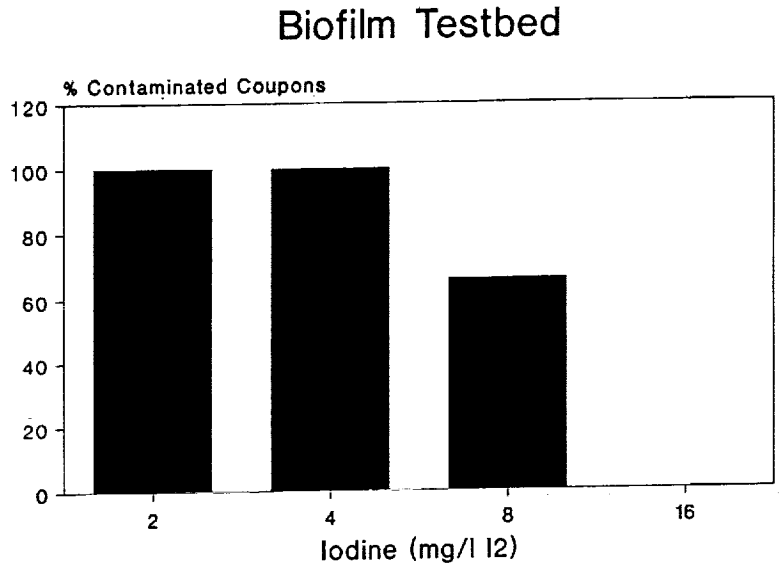

Figure 2. Biofilm testbed study on microbial check valve iodine disinfection.

These preliminary data suggest that iodine disinfection by use of an MVC is not sufficient to eliminate existing biofilm, though the onset of biofilm formation may be delayed by thorough disinfection prior to microbial contamination and the maintenance of an adequate level of disinfectant (1 to 2 mg/L) at all times. Short term high doses of iodine may be useful in controlling biofilm formation in the water system. This treatment may be achievable in the WRT without system modification by using short-term heating of MCV input water to temporarily increase the iodine output and effectively disinfect the system. Continued tests of microbial biofilms will define the minimum duration of iodine exposure at increased concentration for effective microbial elimination.

IMPLICATIONS FOR FUTURE STUDIES

Examination of WRT components, their associated biofilms and microflora, and preliminary laboratory analysis of the efficacy of iodine as a disinfectant, have demonstrated that biofilm contamination was present in the WRT. Though biofilm formation was not extensive in the components analyzed, pathogens were isolated from disinfected subsystems and indicators of MIC were present. Iodine sensitivity studies demonstrated the disinfection resistance of microbial biofilm. This work provided a foundation for the design of further studies to be carried out in the Microbial Ecology Facility. The discovery of biofilms on internal surfaces of material in the WRT system has led to plans for study of the relationship between biofilms, materials, and disinfectants. The development of biofilm will be investigated in relation to two materials (316L stainless steel and titanium) and two disinfection methods (iodination and ozonation) in bench-scale recycling water systems. The optimal conditions for minimizing the rate of biofilm formation will be studied. Attempts are underway to improve the efficiency of sampling biofilm (including anaerobic species) for analysis and testing. Alternative methods are being analyzed as means of determining biofilm accumulation. The technology for rapid monitoring of the accumulation of biofilm is being studied in terms of both biomass and taxonomic identity.

In addition, once biofilms are formed, it is extremely difficult to remove them without changing (often deleteriously) the structure of the substrate material or the chemistry of the overlying water. Routine methods for removing biofilm and cleaning a biologically contaminated water system are being sought. Based on data gathered from the influence of primary system parameters on biofilm formation, a predictive model will be developed which can be used to determine potential sites of pathogen accumulation and enhanced microbially influenced corrosion.

REFERENCES

1. Rodgers, E.B.: "The Ecology of Microorganisms in a Small Closed System: Potential Benefits and Problems for Space Station." NASA Technical Memorandum TM-86563, 1986.

2. Bagdigian, R.M., Traweek, M.S., Griffith, G.K., and Griffin, M.R.: "Phase III Integrated Water Recovery Testing at MSFC: Partially Closed-Hygiene Loop and Open-Potable Loop Results and Lessons Learned." Presented at the 21st Intersociety Conference on Environmental Systems, San Francisco, California 1991.

3. Willis, C.E., and Shultz, J.R.: "Spacecraft Water System Disinfection Technology: Past, Present, and Future Needs." 17th Intersociety Conference on Environmental Systems, Seattle, Washington, July 1987.

4. Roman, M., Wilson, M.E., Jackson, N.E., Gauthier, J.J., Kilgore, B.A., Huff, T.L., Obenhuber, D.C., and Terrell, D.W.: "Microbial Distribution in the ECLSS Water Recovery Test Conducted at MSFC." Presented at the 21st Intersociety Conference on Environmental Systems, San Francisco, California, 1991.

5. Cleseri, L.S., Greenberg, A.E., Trussell, R.R., and Franson, M.H.: "Standard Methods for the Examination of Water and Waste Water." 17th edition, American Public Health Association, Washington, DC, 1989.

6. Craven, D.E., Moody, B., Connolly, M.G., Kollisch, N.R., Stottmeier, K.D., and McCabe, W.R. (eds.): "Pseudobacteremia Caused by Povidone-Iodine Solution Contaminated With *Pseudomonas cepacia*." New England Journal of Medicine, vol. 305, 1981, pp. 621–623.

7. Taylor, S.L., Fina, L.R., and Lambert, J.L.: "New Water Disinfectant: An Insoluable Quarternary Ammonium Resin-Triiodide Combination That Releases Bactericide on Demand." Applied Microbiology, vol. 20 (5), 1970, pp. 720–722.

APPROVAL

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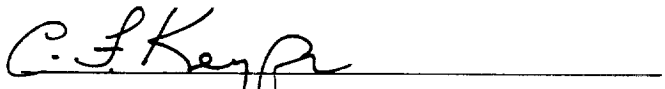

PAUL H. SCHUERER
Director, Materials and Processors Laboratory

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