4.3

Microbiology

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Introduction

As NASA prepares for long-term missions aboard the International Space Station (ISS) and spacecraft destined for Mars, self-contained/closed chambers on Earth have become important test beds for microbiological evaluations. The insight gained from these studies directly benefits NASA as this knowledge is incorporated into the development of monitoring systems and countermeasures against microbiological contamination problems unique to long-duration space missions.

The microbiological study of these chambers and their crew also addresses many Earth-based concerns. Many office buildings are semiclosed systems, which can develop air and surface contamination (3, 5). Commonly referred to as "sick building syndrome," contamination in these facilities may affect up to 30% of new and remodeled buildings worldwide (6). The United States Navy has also investigated the problems associated with microbial growth in semiclosed systems to determine the potential health risks during long-term submarine missions (2, 4). While studies of office buildings and submarines give some insight into changes in the microbial levels and diversity created by an artificial ecosystem, all terrestrial models eventually have access to fresh air and water and can be thoroughly disinfected. The NASA closed-chamber studies provided the unique opportunity to evaluate undisturbed changes in microbial ecology and its relationship to the crew.

During the chamber studies, the primary objective of microbiological evaluations was to ensure crew health by monitoring microbial levels and changes in microbial ecology. In every phase of these studies, the scope of the analyses included the air, potable water, and surfaces that the crew directly contacted. The surfaces that were sampled included not only smooth surfaces, but also carpeting which builds high microbial levels and is difficult to disinfect. In addition, the ability of viral contaminants to survive the water treatment system was evaluated prior to the Phase II test using bacteriophages MS-2 and PDR-1 (1). Monitoring microbial concentrations and diversity provided a way to assess other problems that could affect life support and other systems, such as microbial degradation of materials and the potential fouling of process lines. The use of in-line coupons

provided a way to determine both the type of microorganism in the flow systems and the effect of these organisms on in-line materials.

Evaluations of the air, potable water, and surfaces were performed using standard culturing methodologies and biochemical identification. Physical techniques were applied to retrieve microbial samples from carpets, rugs, and biofilm evaluations of water lines from metal coupons that were then assayed for microbial concentration and identity. The viral challenge of the water reclamation system used standard culturing methodologies combined with plaque enumeration to determine phage survival.

The microbial concentration and diversification in the air and surface samples varied dramatically over time at any given sampling site. Evidence suggested that these changes were directly related to crew activities. These activities range from obvious activities such as cleaning to innocuous activities such as walking through the chamber. Microbial contamination of the air, especially fungal contamination, was maintained at minimal levels, possibly resulting from the complex air revitalization system. In general, the potable water system was successfully disinfected with iodine. The average bacterial concentration was generally kept below the NASA specification throughout the studies. After 60 days during Phase III, the unexpected emergence of various gram-positive *Bacillus* species as the dominant flora suggested potential long-term contamination problems.

Samples extracted from carpets and rugs indicated high levels of microorganisms, although the concentration would on occasion drop dramatically, possibly due to housekeeping patterns. The evaluation of metal coupons in a heat exchanger line indicated bacterial biofilm formation. The coupons were coated with a biocide that limited biofilm buildup initially, but inevitably did not prevent bacterial colonization. Lipid analysis of the coupons suggested viable bacteria were not detectable, but did indicate a bacterial presence.

In order to understand the risk of infectious disease among crewmembers during the chamber studies, microbial samples were collected from the throat, nose, urine, and feces of each crewmember upon entering and exiting. A second throat swab was collected from each crewmember for a viral culture. This data also allowed the identification of microorganisms that may not be considered normal human flora.

The information provided by these studies suggests that long-term space flight can be accomplished and that a unique environmental equilibrium between humans and microbial flora can be maintained. The living environment is in many ways healthier than the conditions found on Earth. However, the potential for long-term microbial contamination is also suggested by this data. The possibility of long-term contamination requires further study to ensure the health of the crew and the operational function of life support systems.

Methods

Microbial Control During Operations

During all phases of the chamber studies, potable water was disinfected with iodine targeted at 2 to 4 ppm. During scheduled and contingent housekeeping, surfaces were cleaned with benzalkonium chloride antiseptic towelettes (PDI, Orangeburg, NY). No HEPA (high-efficiency particulate arresting) or equivalent filter was attached to the air systems.

Sites for Water, Air, and Surface Samples

During Phase I, potable water was sampled directly from fully filled storage tanks. A single air sample site monitored air quality. Surface sample sites included the air return vent, air intake vent, bed rail, desk surface, urinal, cabinet door, sink edges, air conditioner, microwave, and rug. During Phase I, microbial analyses were performed on several sites in the Variable Pressure Growth Chamber (VPGC). Heterotrophic plate counts (HPC) were assessed from the distilled water source, nutrient sources (A and B), the water supply tanks (A and B), and two condensate sources. Two in-line coupons from the water system were analyzed for bacterial and fungal accumulation. Plant samples from the VPGC were evaluated for microbial concentration and identity. Surface samples at four separate air-return sites were evaluated. A single air sample source in the VPGC also was evaluated during the course of this study.

During Phases II, IIa, and III, potable water was supplied by separate water tanks (A, B, C, and D) which were periodically replenished and disinfected with iodine. HPC of potable water samples were taken initially and when the tanks were replenished. Air samples during these three phases were collected from the same locations on every floor of the Life Support Systems Integration Facility (LSSIF) (Figure 4.3-1). Surface samples were collected at similar locations during the final three phases. Minor adjustments were made depending on physical changes within the LSSIF.

Air Sample Preparation and Analysis

Air samples were collected inside the chambers with a Burkard air sampler (Figure 4.3-2), which impacted 84.9 liters of air onto either trypticase soy agar (Remel, Lenexa, KS) for bacteria or rose bengal agar (Difco, Ann Arbor, MI) for fungi. Trypticase soy agar plates were incubated at 35°C for 48 hours; rose bengal agar plates were incubated for five days at 30°C. Bacterial and fungal colonies were counted. Morphologically different bacterial colony types were streaked on blood agar plates for isolation and identification. Morphologically different fungal colony types were streaked on Sabouraud-dextrose agar (Difco, Ann Arbor, MI) for isolation and identification.

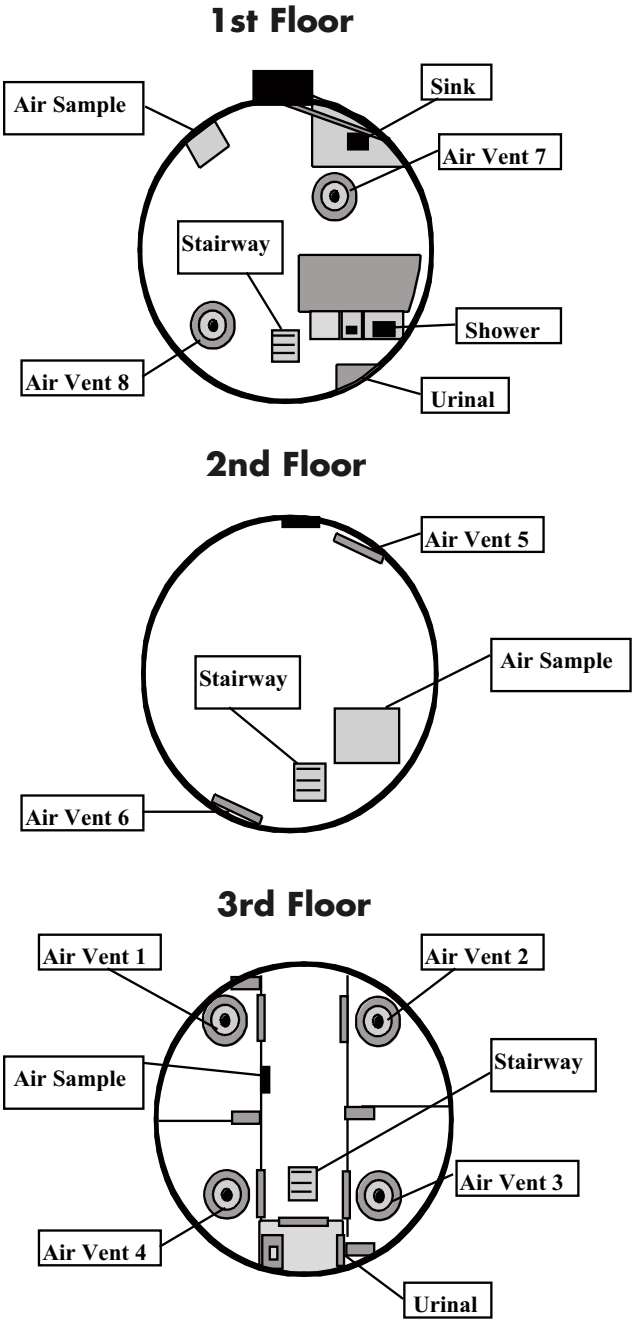

Figure 4.3-1 *Microbiology sampling sites in the Life Support Systems Integration Facility*

Figure 4.3-2 *Burkard air sampler being prepared for sample collection*

Surface Sample Preparation and Analysis

Surface samples were collected by swabbing a 25 cm^2 area with a moistened calcium alginate swab (Figure 4.3-3), which then was placed in 3 ml of trypticase soy broth (Remel, Lenexa, KS). The swabs in broth were vortexed, and the suspension was plated on trypticase soy agar for bacteria and rose bengal agar for fungi. Plates were incubated for enumeration and identification as described for air sampling.

Figure 4.3-3 *Collection of a surface sample from an air vent*

Potable Water Preparation and Analysis

Aliquots of 100 ml were passed through 0.22 µm membrane filters (Millipore, Bedford, MA). For HPC, the filter was placed on a pad saturated with TGE (trypticase/glucose/yeast extract, Difco, Ann Arbor, MI) or R2A broth (Remel, Lenexa, KS), and incubated at 35°C for 48 hours. Potable water specifications were based upon the current NASA specification at the time of the respective study. During Phases I and II, the specification was one colony forming unit (CFU) per 100 ml. During Phases IIa and III, the specification had been increased to 100 CFU per 100 ml.

Microbial Identification

All bacterial isolates were identified with either a Biolog Automated Identification System (Biolog, Hayward, CA) or a VITEK Identification System (bioMérieux, Hazelwood, MO). Fungi were identified microscopically by their morphological characteristics.

Coupon Analysis in the VPGC During Phase I

A manifold holding 30 stainless steel coupons was positioned in the flow stream to allow a representative flow of fluid across the coupon surface. The coupons were removed from the manifold and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria.

Bacterial concentrations of this buffer were determined by HPC using serial dilutions.

Coupon Analysis for Phospholipid Fatty Acid During Phase II

Stainless steel coupons in the manifold were removed and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria. Samples were shipped to Microbial Insights (Rockford, TN) for phospholipid fatty acid (PLFA) determination.

Coupon Analysis of the Condensate Heat Exchanger (CHX) During Phase IIa

Stainless steel coupons were place in the CHX system before the start of Phase IIa. Two coupons were removed for microbiological analysis at the start of the test (day 0), day 2, day 30, and day 60. The coupons were removed from the manifold and gently rinsed with deionized water before sonication in 90 ml of phosphate buffer (pH 7.0) to remove sessile bacteria. Bacterial concentrations of this buffer were determined by HPC analysis following serial dilutions.

Assays for Viral Challenge

Bacteriophage concentrates containing 1×10^9 plaque forming units (PFU) per 100 ml were used to inoculate the water recovery system (WRS) at the urine and wastewater collection tanks prior to the Phase II test (1). One-liter samples were collected at a variety of sources including the urine and wastewater collection tanks after disinfection with Oxone™ and sulfuric acid and downstream after vapor-compression distillation (VCD), after ultrafiltration/reverse osmosis (UF/RO), and after the aqueous phase catalytic oxidation system (APCOS). Samples were split to determine HPC and PFU. PFU were determined by first filtering 500 ml through a 0.45 μm cellulose acetate filter. The filtrate was plated in serial dilutions with phosphate-buffered saline. Host cells, *Escherichia coli* (MS-2) and *Salmonella typhimurium* (PRD-1), were grown in trypticase soy broth for 3 to 5 hours at 37°C, added to 1.5% agar, then inoculated with the viral samples. PFU were counted after 24 hours.

Assays for Crew Microbiology

Crew samples were obtained before and after the tests. These consisted of throat, nose, urine, and fecal samples. Throat samples were collected by swabbing the posterior pharyngeal vault of the crewmembers with the swab from the Culturette device (Becton-Dickinson). Nasal samples were collected by using the swab from the Culturette device moistened with phosphate buffer to swab both nares. Clean-catch midstream urine samples were collected in 4 oz. sterile specimen containers. Fecal specimens were collected in commode containers and transferred to bacterial transport medium for culture and to sodium acetate-acetic acid formalin fixative for ova and parasite examination. Quantification and isolation of organisms were performed by plating each sample onto selected media.

Bacterial cultures were incubated at 35°C and examined after 48 hours, and organisms were identified using the VITEK Identification System. Fungal cultures were incubated at 25°C and examined after five days, and organisms were identified by microscopic examination. Each culture was examined for the presence of medically significant organisms, and antibiotic susceptibilities were performed on the isolates. During Phase II, throat and nasal samples were collected by the crewmembers on day 7 and day 22. Samples were examined for bacteria and fungi, and the fecal samples were examined for ova and parasites.

Findings

Phase I

While the duration of Phase I was only 15 days, increases in surface colonization were apparent as nine of the 10 surface sites displayed over a four-log increase in bacterial concentration during the final week (Figure 4.3-4). Five of the 10 surface sites displayed a six-log increase. Of the bacteria identified, *Clavibacter* and *Curtobacterium* were the predominant genera. Fungal concentrations did not reflect the sharp increase seen with bacteria. A wide variety of fungal genera were identified including *Aspergillus* species, *Penicillium* species, *Acinetobacter* species, *Acremonium* species, *Microsporium* species, and Hyphomycetes.

No culturable bacteria were isolated from the initial samples from the Phase I potable water supply. However, on day 7, bacterial concentration increased to 230 CFU/ml. After 15 days, bacterial concentration decreased to nondetectable levels. Coliform bacteria were never detected during the study. *Burkholderia pickettii, Clavibacter michiganense,* and an unidentified *Clavibacter* species were the only bacteria isolated from potable water samples.

Measurement of biofilm formation on in-line coupons in the VPGC indicated both bacterial and fungal adhesion, although no pattern of progressive contamination was discernable (Table 4.3-1). This steady concentration of both bacterial and fungal levels was reflected in various liquid samples analyzed from the VPGC (Table 4.3-2) (Table 4.3-3). Microbial speciation of liquid samples indicated a wide variety of organisms, although none were medically significant (Table 4.3-4). Fungal levels from VPGC surface samples were relatively stable; however, bacterial concentrations increased dramatically, exceeding 10^8 CFU/cm^2 on certain air vents during crew egress. Identification of VPGC surface microorganisms indicated only common flora of no specific medical importance.

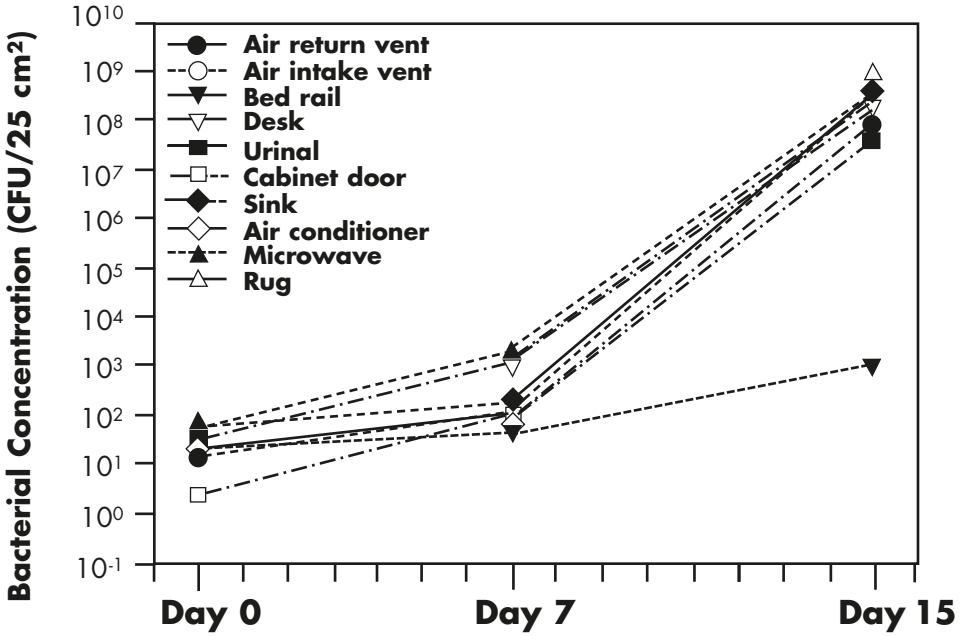

Figure 4.3-4 *Bacterial concentration (CFU/cm²) from Phase I surface samples*

Table 4.3-1 *Microbial adhesion (CFU/coupon) from in-line coupons from the VPGC before and after Phase I*

Date	Bacteria		Fungi	
	Coupon A	Coupon B	Coupon A	Coupon B
6/29/1995	1.0×10^7	1.1×10^8	3.4×10^2	2.0×10^2
6/29/1995	0	0	2.3×10^1	2.0×10^1
7/7/1995	7.7×10^6	1.0×10^6	1.1×10^2	6.0×10^1
7/10/1995	2.7×10^6	4.51×10^3	1.1×10^2	6.1×10^1
8/8/1995 (Phase I egress)	2.0×10^4	2.5×10^4	1.0×10^2	3.0×10^2
9/13/1995	2.9×10^5	1.5×10^6	3.6×10^2	3.6×10^2

Table 4.3-2 *Bacterial concentration (CFU/ml) of liquid samples from the VPGC before, during, and after Phase I*

Date	Deionized Water	Nutrient Source A	Nutrient Source B	Water Supply A	Water Supply B	Condensate Tank A	Condensate Tank B
6/29/95	1.1×10^2	5.6×10^5	1.3×10^6	NC	NC	NC	NC
6/29/95	1.4×10^2	1.0×10^5	9.7×10^2	NC	NC	NC	NC
7/7/95	2.4×10^2	9.5×10^4	6.0×10^5	NC	NC	NC	NC
7/10/95	1.4×10^2	3.3×10^5	5.5×10^5	5.0×10^2	5.6×10^2	2.8×10^6	4.0×10^3
7/18/95	NC	NC	NC	NC	NC	7.0×10^2	1.4×10^3
7/21/95	NC	NC	NC	NC	NC	4.7×10^2	4.5×10^2
7/24/95	1.6×10^2	2.8×10^4	3.0×10^4	3.4×10^4	6.0×10^4	3.1×10^4	2.0×10^5
7/31/95	1.1×10^2	1.7×10^4	5.3×10^4	7.2×10^4	4.0×10^4	1.1×10^5	1.0×10^5
8/8/95 (Phase I egress)	7.8×10^2	3.5×10^4	6.3×10^4	1.2×10^6	1.4×10^5	1.3×10^4	4.2×10^5
9/13/95	5.8×10^2	1.0×10^5	1.0×10^5	3.7×10^5	1.2×10^5	2.9×10^5	5.9×10^4

NC = Not collected

Table 4.3-3 *Fungal concentration (CFU/ml) of liquid samples from the VPGC before, during, and after Phase I*

Date	Deionized Water	Nutrient Source A	Nutrient Source B	Water SupplyA	Water Supply B	Condensate Tank A	Condensate Tank B
6/29/95	0	1.4×10^2	1.1×10^2	NC	NC	NC	NC
6/29/95	7.5×10^0	7.5×10^0	7.5×10^0	NC	NC	NC	NC
7/7/95	3.8×10^1	2.0×10^0	2.3×10^1	NC	NC	NC	NC
7/10/95	7.5×10^0	2.3×10^1	3.0×10^1	5.0×10^1	5.0×10^1	2.0×10^1	4.5×10^1
7/18/95	NC	NC	NC	NC	NC	0	0
7/21/95	NC	NC	NC	NC	NC	3.8×10^1	2.3×10^1
7/24/95	7.5×10^0	4.5×10^1	2.3×10^1	4.0×10^1	4.0×10^1	1.5×10^1	7.5×10^0
7/31/95	7.5×10^0	2.3×10^1	1.5×10^1	1.5×10^1	4.0×10^1	7.5×10^1	2.3×10^1
8/8/95	0	3.0×10^1	4.0×10^1	0	7.5×10^0	1.5×10^1	6.0×10^1
9/13/95	0	7.5×10^0	3.6×10^2	0	3.0×10^1	4.5×10^1	2.3×10^2

NC = Not collected

Table 4.3-4 *Microbial colonies isolated from the VPGC liquid samples during Phase I*

	Nutrient A	Nutrient B	Air Sample	Supply Tank A	Supply Tank B	Condensate A	Condensate B
Bacteria	*Acidovorax delafieldie, Bacillus* sp., *Clavibacter michiganense, Comomonas terrigena, Rhizobium loti, Rhizobium meliloti, Vibrio cyclosites*	*Acidovorax facilis, Curtobacterium flaccumfaciens, Variovorax paradoxus, Vibrio cyclosites*	*Bacillus* sp., *Cladosporium* sp., *Clavibacter michiganense*	*Burkholderia picketti, Comomonas acidovorans, Variovorax paradoxus*	*Cladosporium* sp., *Rhizobium loti, Salmonella* sp., *Variovorax paradoxus*	*Cladosporium* sp., CDC grp IVC-2, *Salmonella* sp.	*Burkholderia cepacia, Comomonas acidovorans, Rhizobium leguminosarum*
Fungi	*Acremonium* sp., *Fusarium* sp.	*Acremonium* sp., *Epicoccum* sp.	*Acremonium* sp., Hyphomycete, *Penicillum* sp.	*Acremonium* sp., Hyphomycete, *Fusarium* sp.	Hyphomycete, *Fusarium* sp.	*Acremonium* sp., Hyphomycete, *Fusarium* sp.	*Fusarium* sp., *Monilia* sp., *Cladosporium* sp.
	Distilled Water	**Air Intake A**	**Air Intake B**	**Coupon A**	**Coupon B**	**Rhizoplane A**	**Rhizoplane B**
Bacteria	*Burkholderia picketti, Rhizobium meliloti*			*Curtobacterium flaccumfacien, Clavibacter michiganense, Alcaligenes* sp.	*Rhizobium meliloti, Rhizobium loti, Aureobacterium saperdae, Rhodococcus aichiensis, Alcaligenes xylosoxydans*	*Pseudomonas putida, Pseudomonas* sp.	*Alcaligenes xylosoxydans, Xanthomonas maltophilia, Pseudomonas* sp., *Alcaligenes* sp.
Fungi	*Fusarium* sp.	*Fusarium* sp., *Penicillum* sp., *Aspergillus* sp.,	*Fusarium* sp., *Penicillum* sp., *Aspergillus* sp.,	*Acremonium* sp., *Fusarium* sp., *Rhodotorula glutinis, Trichoderma* sp.	*Fusarium* sp., *Acremonium* sp., *Trichoderma* sp.	*Fusarium* sp., *Trichoderma* sp., Hyphomycete, *Acremonium* sp.	*Acremonium* sp., Hyphomycete, *Penicillum* sp., *Trichoderma* sp., *Paecilomyces* sp.

Phase II

Patterns of bacterial and fungal colonization on surface samples during Phase II varied depending on the sampling site. The greatest numbers of bacteria were found at sample sites near wet areas such as the sink, urinal, and shower. These levels fluctuated more than two-log fold with no apparent pattern (Figure 4.3-5). The greatest numbers of fungi were found on the air vents and carpet samples. A subtle increasing trend in microbial concentration was observed during Phase II.

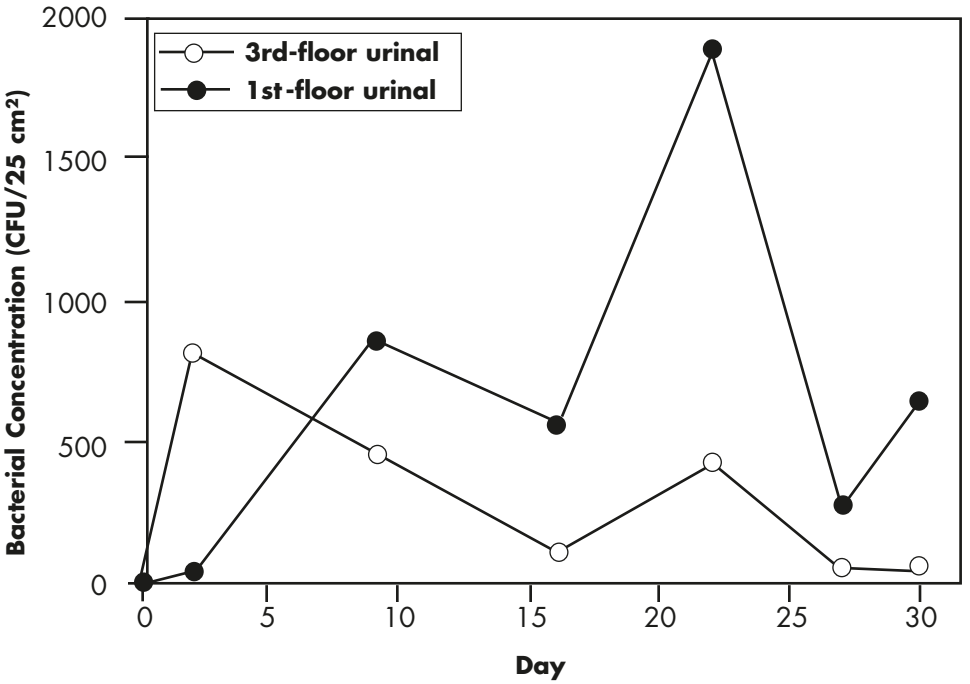

Figure 4.3-5 *First- and third-floor bacterial contamination (CFU/cm²) at urinals during Phase II*

Bacterial and fungal concentrations in air samples were consistently low, never exceeding 332 CFU/m³ for total bacteria and 339 CFU/m³ for total fungi. No trends were apparent based upon the day collected or location of the air sample.

During Phase II, no coliforms or anaerobic bacteria were isolated in the potable water system. Bacterial concentration generally remained at or below NASA specifications (Figure 4.3-6). Several bacterial species were isolated from the potable water tanks including *Sphingomonas paucimobilis, Burkholderia picketti, Stentrophomonas maltophilia,* and *Pseudomonas vesicularis*.

Bacterial viruses MS-2 and PRD-1 were injected into the WRS at the urine and wastewater collection tanks prior to Phase II to evaluate the ability of the WRS to remove or inactivate viral particles and prevent transmission to recovered potable water. Oxone/sulfuric acid and vapor compression distillation dramatically decreased the viral titer from 5.5×10^9 PFU/100 ml (MS-1) and 3.7×10^9 PFU/100 ml (PRD-1) to less than 1 PFU/100 ml. In comparison, HPC for these samples did not significantly decrease, remaining between 100 to 500 CFU/100 ml. The reverse osmosis unit also decreased viral density from 9.3×10^8 PFU/100 ml (MS-1) and 4.3×10^8 PFU/100 ml (PRD-1) to less than 1 PFU/100 ml. Bacterial concentration

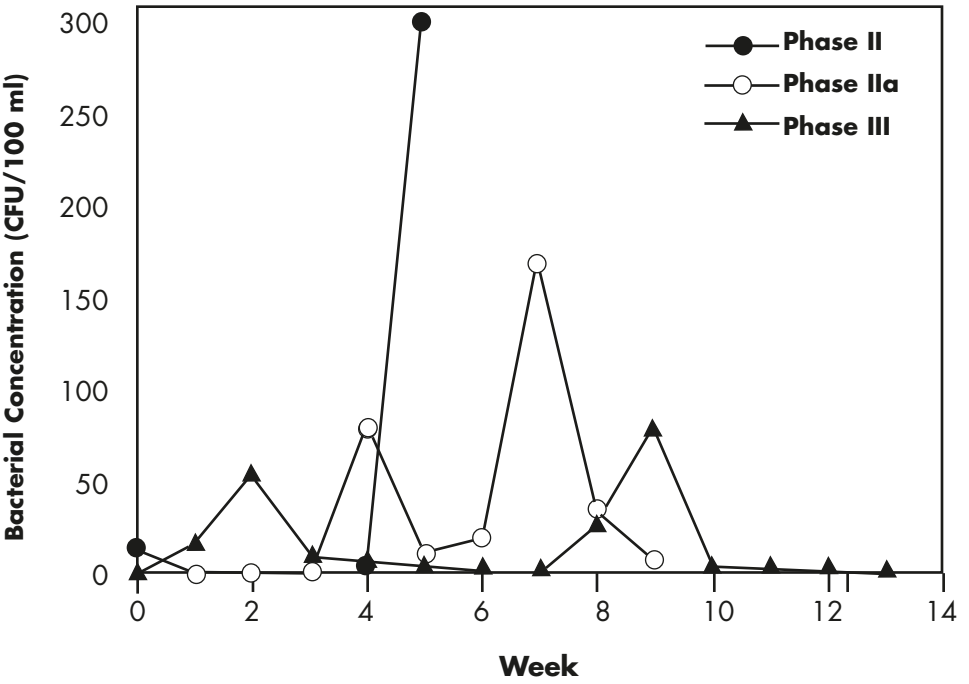

Figure 4.3-6 *Bacterial concentration (CFU/100 ml) in potable water during Phases II, IIa, and III*

decreased approximately one log, including a complete removal of all coliform bacteria. The retention of bacteria downstream of the reverse osmosis unit is possibly the result of contamination of the unit prior to installation. In combination, the units of the WRS removed all detectable MS-1 and PRD-1 viral particles.

Stainless steel coupons from Phase II were analyzed for PLFA content. The coupon that was in the final potable water system water line indicated viable bacteria, although it did have residue lipids that are indicative of gram-negative bacteria, gram-positive bacteria, and eukaryotes. Biomass, as measured by PLFA, was relatively low in the potable water line at 46 picomoles PLFA/coupon. Processing lines, such as the water from the UF/RO unit, contained diverse microbial communities with PLFA primarily from gram-negative bacteria. The total biomass per coupon from the UF/RO was 359 picomoles PLFA/coupon. Biomarkers indicative of gram-positive and sulfate-reducing bacteria were detected in the process line leading to the UF/RO unit that also contained very high levels of biomass at 3322 picomoles PLFA/coupon.

The crew microbiology results from Phase II were collected from eight subjects, and numbers 1, 2, 3, and 4 were chosen as crewmembers (Table 4.3-5). *Staphylococcus aureus* was recovered from the nose of crewmember 1 at entry, day 7, and day 22. *Klebsiella pneumoniae* was isolated from the nasal swab of crewmember 1 on day 30 (exit) and also from crewmember 2 on day 7 and day 30. *Streptococcus agalactiae* was isolated in low numbers from one urine sample on day 30. *Pseudomonas aeruginosa* was isolated from the throat swab of crewmember 4 on day 30. *Candida albicans* was isolated from crewmember 2's fecal sample at pre-entry and from the urine and fecal sample on day 30. It was also isolated from the throat and feces of crewmember 3 at pre-entry and from the feces on day 30. *Candida albicans* was isolated from the throat and urine of crewmember 4 at pre-entry and from the urine and feces on day 30.

Table 4.3-5 *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
1	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, non-hemolytic No fungi isolated	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus* species, not *aureus* *Corynebacterium* species	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus aureus* *Staphylococcus* species, not *aureus* *Cladosporium* species	*Staphylococcus aureus*	*Staphylococcus aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Klebsiella pneumoniae* No fungi isolated
	Urine	No bacteria isolated No fungi isolated	No sample collected	No sample collected	*Streptococcus agalactiae* No fungi isolated
	Feces	Normal enteric flora *Aspergillus* species	No sample collected	No sample collected	Normal enteric flora *Trichosporon* species
2	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Staphylococcus* species, not *aureus* *Neisseria* species *Cladosporium* species	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species No fungi isolated

Table 4.3-5 continued *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
2	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Klebsiella pneumoniae*	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Klebsiella pneumoniae*	*Staphylococcus aureus* *Corynebacterium* species No fungi isolated
	Urine	*Enterococcus faecalis* *Staphylococcus* species, not *aureus* No fungi isolated	No sample collected	No sample collected	*Staphylococcus* species, not *aureus* *Candida albicans*
	Feces	Normal enteric flora *Candida albicans* *Rhodotorula rubra* *Penicillium* species	No sample collected	No sample collected	Normal enteric flora *Candida albicans*
3	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus aureus* *Corynebacteria* species *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Staphylococcus* species, not *aureus* *Neisseria* species	*Streptococcus* species, alpha-hemolytic *Micrococcus* species *Staphylococcus aureus*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, non-hemolytic *Neisseria* species *Haemophilus parainfluenzae* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* *Lactobacillus* species No fungi isolated	No sample collected	No sample collected	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans* *Rhodotorula rubra* *Penicillium* species	No sample collected	No sample collected	Normal enteric flora *Candida albicans*
4	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Neisseria* species, *Streptococcus* species, nonhemolytic	*Streptococcus* species, alpha-hemolytic *Pseudomonas aeruginosa*	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Bacillus* species No fungi isolated

Table 4.3-5 continued *Microorganisms isolated from Phase II crewmembers*

		SAMPLE PERIOD			
Crewmember	Sample	Pre-entry	Day 7/8	Day 22	Exit – Day 30
4	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species	*Corynebacterium* species *Staphylococcus* species, not *aureus*	*Micrococcus* species *Corynebacterium* species *Staphylococcus* species, not *aureus* *Aspergillus* species
	Urine	*Staphylococcus* species, not *aureus* *Enterococcus faecalis* *Candida albicans*	No sample collected	No sample collected	*Streptococcus* species, non-hemolytic *Staphylococcus* species *Lactobacillus* species *Candida albicans*
	Feces	Normal enteric flora No fungi isolated	No sample collected	No sample collected	Normal enteric flora *Candida albicans*

Crewmember (Backup)	Sample	Pre-entry
5	Throat	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species *Haemophilus parainfluenzae* *Neisseria* species *Bacillus* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Streptococcus* species, alpha-hemolytic *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans* No ova or parasites observed
6	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Micrococcus* species *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Epicoccum nigrum* *Acremonium* species
	Urine	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora No fungi isolated

Crewmember (Backup)	Sample	Pre-entry
7	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*
	Nasal	No bacteria isolated No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*
8	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus* species, not *aureus* *Haemophilus* species, not *influenzae* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Bacillus* species *Staphylococcus aureus* *Corynebacterium* species *Cladosporium* species
	Urine	*Enterococcus faecalis* *Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora No fungi isolated

Phase IIa

The greatest numbers of bacteria during Phase IIa were found at sample sites near wet areas such as the sink, urinal, and shower, and the greatest numbers of fungi were found on the air vents and carpet samples. The numbers of microorganisms detected in surface and air samples remained relatively constant for Phase IIa. The second floor, which contained only air revitalization equipment, had the fewest bacteria and fungi during the Phase IIa study. The highest numbers of fungal species were found before and after crew entry and exit. The number of fungal genera isolated from all samples ranged from zero to five during habitation, with the most common genera being *Penicillium, Cladosporium,* and *Aspergillus.* The bacterial levels from carpet decreased during the Phase IIa study, while the fungal levels remained consistent.

While air samples collected on the first and third floors had similar bacterial concentrations, samples from the second floor had fewer bacteria. Levels of airborne fungi were greatest on the third floor. The genera *Penicillium, Cladosporium,* and *Aspergillis* were commonly collected.

During Phase IIa, no coliforms or anaerobic bacteria were isolated in the potable water system. Bacterial concentration generally remained below 100 CFU/100 ml (Figure 4.3-6). The primary isolate during Phase IIa was *Burkholderia cepacia*. Other species included *Burkholderia picketti, Acenitobacter calcaoceticus, Burkholderia pseudomallei,* and *Staphylococcus saprophyticus*.

During Phase IIa, an evaluation was performed of biofilm accumulation on metal coupons exposed to condensate in the air handling system. Coupons were either uncoated or coated with a biocidal coating. The coated coupons had less bacterial growth than uncoated coupons (Table 4.3-6). The numbers of attached bacteria increased with time on both types of coupons. The types of bacteria found on both coupon types were common water-associated species including *Bacillus brevis, Burkholderia picketti, Methylobacterium rhodinum,* and *Sphingomonas paucimobilis.* The diversity of bacterial flora on the coated coupons was less compared to the uncoated coupon. The biocide coating also reduced the loading on coupons by at least two-log fold after 60 days of exposure to the Phase IIa condensate, although the biocide coating did not eliminate biofilm formation. The bacterial numbers for the day 30 coupons had a predominance of *Bacillus* on both coupon types, which may result from a resistance of spores to the biocide. Fungal loading seemed to be less affected by the coupon coating than the bacteria, which like bacterial spores may be a result of reduced sensitivity of fungal spores to biocide action. The most predominant genera included *Aspergillis, Penicillium,* and *Trichosporon*. The initial increase in fungal concentration from day 2 to day 30 was followed by a subsequent decrease at day 60.

Table 4.3-6 *Microbial concentration on stainless steel coupons in the CHX condensate biofilms during Phase IIa*

	Bacterial Count (CFU/coupon)		**Fungal Count (CFU/coupon)**	
Day	Coated	Uncoated	Coated	Uncoated
0	NG	NG	NG	NG
2	NG	6.3×10^4	NG	NG
30	2.0×10^3	3.0×10^3	3.8×10^3	1.2×10^4
60	3.6×10^4	2.4×10^6	6.0×10^1	5.3×10^3

NG = No growth

Crew microbiology results from Phase IIa were collected from eight subjects, and numbers 1, 2, 3, and 4 were chosen as crewmembers (Table 4.3-7). *Staphylococcus aureus* was recovered from the throat swab of crewmembers 3 and 4 at pre-entry. It was also recovered from the nasal swab of crewmember 1 and from the throat swabs of crewmembers 2 and 3 on day 60 (exit). *Candida albicans* was recovered at pre-entry from the feces of crewmember 2 and from the throat swab and feces of crewmembers 3 and 4. It was recovered on day 60 from the throat swabs of crewmembers 1, 2, and 3 and from the nasal swab of crewmember 4.

Table 4.3-7 *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
1	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus* species, not *aureus* No fungi isolated	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Candida albicans*
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Staphylococcus aureus* No fungi isolated
	Urine	*Corynebacterium* species No fungi isolated	No bacteria isolated No fungi isolated
	Feces	Normal enteric flora *Rhodotorula* species *Trichosporon* species	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
2	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic No fungi isolated	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Corynebacterium* species *Staphylococcus aureus* *Candida albicans*
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Micrococcus* species No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated
	Urine	*Lactobacillus* species *Corynebacterium* species *Staphylococcus* species No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected
3	Throat	*Corynebacterium* species *Staphylococcus aureus* *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Staphylococcus aureus* *Candida albicans*
	Nasal	*Staphylococcus* species, not *aureus* No fungi isolated	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Staphylococcus* species, not *aureus* No fungi isolated	*Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit – Day 60
4	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Staphylococcus aureus* *Candida albicans*	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated	No bacteria isolated *Candida albicans*
	Urine	No bacteria isolated No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Crewmember (Backup)	Sample	Pre-entry	Crewmember (Backup)	Sample	Pre-entry
5	Throat	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Enterobacter gergoviae* No fungi isolated	6	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Corynebacterium* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Micrococcus* species *Klebsiella pneumoniae* No fungi isolated		Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Staphylococcus aureus* No fungi isolated
	Urine	No bacteria isolated No fungi isolated		Urine	No sample collected
	Feces	Normal enteric flora No fungi isolated No ova or parasites seen		Feces	No sample collected

Table 4.3-7 continued *Microorganisms isolated from Phase IIa crewmembers*

Crewmember (Backup)	Sample	Pre-entry	Crewmember (Backup)	Sample	Pre-entry
7	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Staphylococcus* species, not *aureus* No fungi isolated	8	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species *Staphylococcus* species, not *aureus* No fungi isolated
	Nasal	*Staphylococcus aureus* *Staphylococcus* species, not *aureus*		Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species
	Urine	No bacteria isolated No fungi isolated			*Streptococcus* species, alpha-hemolytic No fungi isolated
	Feces	Normal enteric flora *Candida albicans*		Urine	*Lactobacillus* species *Staphylococcus* species No fungi isolated
				Feces	Normal enteric flora No fungi isolated

Phase III

Air, surfaces, and carpet were sampled immediately before entry (day 0), after 2, 25, 45, 65 days, and upon egress (day 91). A large number of bacterial species were detected on day 0 before closing the chamber but not isolated again throughout the study. These included several bacteria of medical importance, such as *Klebsiella* species*, Serratia marcescens,* and *Enterobacter gergoviae*.

In general, microbial levels from most of the surfaces were low. Dramatic changes in bacterial levels were exemplified by the third-floor air vent, third-floor sink, and first-floor urinal (Table 4.3-8). Fungal levels on tested surfaces fluctuated, although they remained low throughout the test. The only exception was a slight increase on day 65 at the third-floor sink. Carpet samples displayed relatively low bacterial counts on day 0, then rose rapidly at all sites, with counts remaining over 10^7 CFU/m^2 throughout the majority of the test. Fungal levels in carpet samples were initially high but decreased below 10^2 CFU/m^2 after day 45 and did not increase even upon egress (day 91). The bacteria identified from surface samples included a wide diversity of genera including *Bacillus, Corynebacterium, Staphylococcus,* and *Micrococcus*. No fungal genera appeared to dominate the surface flora. The wide variety of microbial flora collected was illustrated by analysis of the first-floor carpet (Table 4.3-9).

Table 4.3-8 *Microbial contamination (CFU/cm²) at selected surface sites during Phase III*

		Day					
Sample site		**0**	**2**	**25**	**45**	**60**	**91**
Third-floor air vent	Bacteria	15	30	0	0	8	1,500
	Fungi	0	0	0	0	0	15
Third-floor sink	Bacteria	30	1,600	170	680	1,700	210
	Fungi	0	0	75	NC	315	30
First-floor urinal	Bacteria	7	8	8	8	40	900
	Fungi	0	0	0	0	0	30

NC = not collected

Table 4.3-9 *Microbial diversity and occurrence identified from the first-floor carpet during Phase III*

	Day					
Bacteria	**0**	**2**	**25**	**45**	**65**	**91**
Bacillus sp.	X	X		X		X
Bacillus azotoformans					X	
Bacillus brevis	X	X	X			X
Bacillus coagulans				X		
Bacillus licheniformis	X	X		X	X	X
Bacillus megaterium	X	X				
Bacillus mycoides	X					
Bacillus pasturii	X					
Bacillus pumilus	X	X		X		
Bacillus sphaericus		X				
Bacillus subtilis varglobigii	X					
Chryseomonas luteola	X					
Corynebacterium sp.		X	X	X		X
Corynebacterium afermentanis				X	X	
Corynebacterium aquaticum A	X					
Corynebacterium pseudodiptherticium			X			
Enterobacter agglomerius grp 3B		X				
Enterobacter gergoviae	X	X				
Erysipelothrix rhasiophthiae/tonsialum						X
Kingell kingae		X				
Rothia dentrocarios		X				

Table 4.3-9 continued *Microbial diversity and occurrence identified from the first-floor carpet during Phase III*

	Day					
Bacteria	**0**	**2**	**25**	**45**	**65**	**91**
Serratia marcesans	X					
Staphylococcus capitis					X	
Staphylococcus capitis ss *ureolyticus*		X	X			
Staphylococcus caprae					X	
Staphylococcus epidermidis A			X	X		
Staphylococcus haemolyticas				X		
Staphylococcus simulans						X
Fungi						
Acremonium sp.		X		X		
Alternaria sp.	X					
Aspergillus sp.			X		X	
Aspergillus flaus					X	
Candida parapulois				X		
Cladosporium sp.	X	X			X	
Cryptococcus laurentii						X
Curvularia sp.			*X*			
Fusarium sp.			X			
Hyphomycete	X			X		
Penicillium sp.		X				
Rhodotorula sp.	X	X				
Unidentified yeast	X	X				

Throughout the testing period, air sampling indicated low bacterial counts, with the exception of a sharp increase at all sites on day 25. The only bacterial species common to all air samples on day 25 was *Staphylococcus hominis*, a common skin flora. Fungal counts in air samples displayed low levels except for the pre-entry samples on day 0. The identification of bacterial isolates indicated that most bacteria found in the air, surface, and carpet were from the genera *Bacillus, Corynebacterium,* and *Staphylococcus*. The total number of bacterial species in the chamber, as a whole, decreased rapidly after two days but leveled off after day 25. Individual bacterial species fluctuated. The fungal genera *Penicillium, Cladosporium,* and *Aspergillis* were predominant.

Burkholderia cepacia, the most common isolate from Phases I and IIa studies, was again detected in the potable water tank samples, but it was not the most common isolate. In Phase III, *Flavobacterium meningosepticum,* a bacterium that is nonpathogenic in adults, was detected most often. This bacterium was not isolated in other studies. Total heterotrophic counts were within specification during Phase III testing, with the exception of one slight overage of 108 CFU/100 ml (Figure 4.3-6). No coliforms or anaerobes were detected in any of the water samples analyzed during the testing period. On and after day 61, various *Bacillus* species were detected in the water system.

In crew microbiology results from Phase III, *Candida albicans* was recovered pre-entry from the throat swabs and feces of crewmembers 3 and 4 and from the feces of crewmember 8 (Table 4.3-10). On day 91 (exit), *Escherichia coli* and *Enterococcus faecalis* were recovered from the crewmember 2 urine sample in low numbers.

Table 4.3-10 *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
1	Throat	*Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Neisseria* species No fungi isolated	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Corynebacterium* species No fungi isolated
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Cladosporium* species *Penicillium* species Hyphomycete	*Staphylococcus* species, not *aureus* *Corynebacterium* species No fungi isolated
	Urine	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Lactobacillus* species No fungi isolated	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans* *Trichosporon* species	No sample collected

Table 4.3-10 continued *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
2	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species No fungi isolated	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Corynebacterium* species No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species *Streptomyces* species	*Neisseria* species *Streptococcus* species, alpha-hemolytic No fungi isolated
	Urine	*Lactobacillus* species *Staphylococcus* species No fungi isolated	*Escherichia coli* *Enterococcus faecalis* *Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora	Normal enteric flora with few *Staphylococcus aureus* No fungi isolated

Table 4.3-10 continued *Microorganisms isolated from Phase III crewmembers*

		SAMPLE PERIOD	
Crewmember	Sample	Pre-entry	Exit - Day 90
3	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Candida albicans*	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Staphylococcus aureus* No fungi isolated
	Nasal	*Staphylococcus* species, not *aureus* *Corynebacterium* species *Cladosporium* species	*Corynebacterium* species *Staphylococcus* species, not *aureus* *Aspergillus* species
	Urine	No sample collected	*Corynebacterium* species No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected
4	Throat	*Streptococcus* species, alpha-hemolytic *Neisseria* species *Streptococcus* species, nonhemolytic *Candida albicans*	*Neisseria* species *Streptococcus* species, alpha-hemolytic *Streptococcus* species, nonhemolytic *Corynebacterium* species No fungi isolated
	Nasal	*Corynebacterium* species *Staphylococcus* species, not *aureus* No fungi isolated	*Staphylococcus aureus* *Corynebacterium* species No fungi isolated
	Urine	*Corynebacterium* species *Staphylococcus* species *Lactobacillus* species No fungi isolated	*Staphylococcus* species, not *aureus* No fungi isolated
	Feces	Normal enteric flora *Candida albicans*	No sample collected

Discussion

The information collected during the closed-chamber studies strongly indicates a connection between the concentration and diversity of the microbial flora and the presence of the crew and plant life. The presence and activity of the support personnel before or after each study caused microbial levels to fluctuate and often increase. In addition, the presence of medically significant bacteria before Phase III, but not during the study, suggests the contribution of microorganisms by human interaction before the study. Sharp fluctuations in microbial concentrations, such as the day 25 increase during Phase III, was quite possibly due to human activity, although an exact cause has not been established. The microbial flora during the Phase I study dramatically shows the influence of plants in an isolated environment, as the predominant bacterial species were mostly plant-associated bacterial genera such as *Clavibacter* and *Curtobacterium*. The connection between microorganisms and other life does not imply as great of an influence. While bacterial loads in both the habitation and plant growth chambers during Phase I increased with length of human presence, the fungal loads decreased during the same period. In addition, microbial loads in Phases II, IIa, and III followed unique patterns of changes in concentration over time. The trends in microbial concentration and diversity confirmed the need for standards to insure microbial control, but also suggested a futility in attempts to "sterilize" the environment prior to occupancy.

Surface samples taken from areas with direct contact with water, such as sinks, were compared to samples from air vents which remained relatively dry. While, intuitively, the wet areas should have higher microbial populations than the drier areas, neither of these sets of surfaces consistently maintained higher microbial counts based upon a comparison of Phase IIa and Phase III data (Table 4.3-11). Compared to the overall surface sample averages, certain wet sites did display high bacterial levels during both studies, such as samples from the sink which averaged 20.87 ± 9.26 CFU/cm^2 during Phase IIa and 41.50 ± 14.77 CFU/cm^2 during Phase III. Other wet sites displayed high levels in one study, but not both. For example, samples from the first-floor urinal displayed high levels during Phase IIa, averaging 34.10 ± 12.12 CFU/cm^2, but only averaging 0.63 ± 0.33 CFU/cm^2 during Phase III. The inconsistency of the comparison of wet and dry areas was probably the result of housekeeping patterns among the crews. Surface samples taken from carpet displayed high microbial levels, reinforcing the need for judicious use of carpeting in self-contained systems.

Table 4.3-11 *Average microbial concentration and diversity from surface samples during Phases IIa and III*

	Phase IIa		Phase III	
Site	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**
Air Vents				
Average Level (CFU/cm^2)	11.5 ± 3.0	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
Average Species or Genera (CFU/cm^2)	10.9 ± 1.6	3.1 ± 0.4	3.5 ± 1.1	1.9 ± 0.6
Wet Areas				
Average Level (CFU/cm^2)	16.7 ± 4.2	0.03 ± 0.02	10.1 ± 4.6	1.1 ± 0.7
Average Species or Genera (CFU/cm^2)	10.2 ± 0.9	0.6 ± 0.4	8.6 ± 2.4	2.0 ± 0.6

In both studies, certain bacterial species dominated both air and surface samples. Of the 42 species isolated in air and surface samples throughout all of Phase IIa, 15 were isolated at more than one site and 10 were isolated at more than two. Seven of these species were isolated on every floor of the chamber. Of the 31 species isolated in air and surface samples throughout all of Phase III, 12 were isolated at more than one site and six were isolated at more than two. Only four of these species were isolated on every floor of the chamber. Certain bacterial species, such as *Bacillus brevis* during Phase IIa, were isolated from surface samples on every floor, but never from air samples. Other bacteria, such as *Staphylococcus capitis* during Phase III, were found in air samples but not isolated from every floor. These findings indicate that bacteria are transient in nature and do not necessarily maintain their presence indefinitely.

The pattern of bacterial appearance during the studies varied. Of the common isolates, most could be detected in at least one site during each sampling period. However, certain bacteria were detected during only one sampling period, such as the detection of *Bacillus azotoformans* on all three floors during day 65 of Phase III. No particular microbial species were associated with either the dryer air vents or the wet areas. The pattern of bacterial dominance and unexpected appearance were likely the result of cleaning patterns and human traffic.

The identification of fungal isolates indicated a large variety of contaminants with no single genera dominating the environment throughout the test. However, the genera *Penicillium, Cladosporium,* and *Aspergillis* were common during all studies. The total number of genera decreased over time, although no particular

fungi appeared to endure better than the others. As with the pattern displayed by the bacteria, the presence of fungi over time appeared to be affected by cleaning patterns and human traffic. The efficiency of the air system to remove fungi from the air, and subsequently surface samples, may have also contributed to the decreased fungal levels.

The lack of proliferation of bacterial species and numbers may also be a function of their interaction within the community. A nonlinear relationship between bacterial concentration and diversity on surface samples during Phase IIa and Phase III suggests a repression of the proliferation of new species after bacterial concentration reaches a certain limit (Figure 4.3-7). For these studies, the concentration where repression began was 5 to 10 CFU/cm^2.

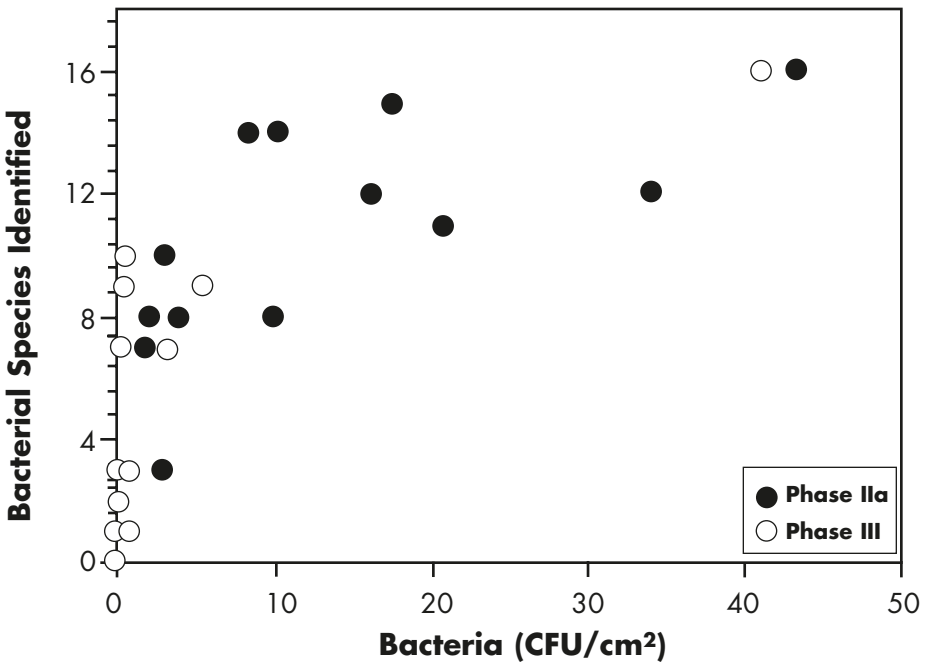

Figure 4.3-7 *Bacterial concentration (CFU/cm^2) versus diversity at surface sampling sites during Phases IIa and III*

Potable water concentrations were affected by varying disinfectant levels, and thus microbial trend analysis was difficult. Bacterial diversity in the potable water from the Phase III study was greater than other studies. For example, during Phase IIa *Burkholderia cepacia,* isolated in 39.4% of the samples, was the predominant bacterial contamination during the Phase IIa study. Only five other bacterial species were detected and appeared in 13.5% of the samples. During Phase III, *Flavobacterium meningosepticum* (found in 33.3% of the samples), *Stentrophomonas*

maltophilia (11.8%), *Burkholderia cepacia* (11.8%), and several species of *Bacillus* (11.8%) were most commonly detected. The identified *Bacillus* species, *subtilis*, *licheniformis*, and *pumilus*, were detected only after day 60. Since no gram-positive rods were detected during the 60-day Phase IIa water study, their presence in Phase III may be the result of either changes in the water systems, levels of disinfection, or the additional 31-day duration of the Phase III study.

Overall the microbiota isolated from the crew was characteristic of healthy individuals. Organisms such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are opportunistic pathogens that may cause disease in immunocompromised hosts but not healthy individuals. *Staphylococcus aureus* is frequently a resident of the nasal passages, and *Candida albicans* is a ubiquitous yeast that is found as normal flora of the alimentary tract and mucocutaneous membranes. No clinical symptoms were experienced by the crewmembers as a result of the presence of any of these organisms. No ova or parasites were seen in any of the crewmembers, and all viral cultures were negative. No significant changes were found in the body flora during Phases II, IIa, or III.

Several studies focused on potential biodegradation and methods of prevention. Coating the stainless steel coupons from Phase I with biocides temporarily controlled microbial biofilm formation. However, the coating did not eliminate biofilm formation. The major organism in all the samples taken during this study was *Methylobacterium*, a common water bacterium. During Phase II, biofilm-forming bacteria were identified using PLFA. These bacteria were controlled with iodine disinfection, although the iodine did not remove the biological materials from the coupon surface as measured with the lipid analysis.

In addition, the ability of viral contaminants to survive the water treatment system was evaluated using bacteriophages MS-2 and PDR-1. These viruses were chosen because of their similarity to the human enterovirus (MS-2) and rotavirus (PRD-1). Both of these viruses have been used in other municipal studies as they absorb poorly to flocculated material and thus must be removed by the filtration mechanisms within the water purification system. The advanced water purification techniques completely removed the viral particles from the recycled potable water to below the detection limit of this assay.

SIGNIFICANCE

These studies confirm a generally accepted relationship between microorganisms and other living organisms in a closed ecosystem. Disinfection and cleaning patterns changed this equilibrium as displayed by shifts in both microbial concentration and identity. In a small ecosystem, such as the chambers, the relationship is more dramatically affected by the actions of participants and thus more difficult to control. This understanding applies not only to NASA spacecraft, but also other small confined areas such as office buildings. Further complicating the ecosystem is the microbial interrelationship. On surfaces, these studies suggest a competitive

inhibition that limits bacterial diversity. This finding could be of great importance in decontamination, as a sterile surface may be a more fertile breeding ground than expected for different, possibly pathogenic microorganisms. The number of potential microorganisms at any given site is large, and thus continued sampling and analysis must continue to gain a better understanding of the diversity of microbial flora, their interrelationship, and the effect of human activities on the consortium.

These studies showed the ability of advanced water recovery systems to microbially purify water to potable quality, including the removal of viruses. However, the purification process appears to be susceptible to bacterial biofilm formation and potential corrosion problems. The appearance of gram-positive bacteria after 60 days during Phase III suggests a major flora change. Future work should focus on the effect of recycling water on microbial flora after extended periods of time.

Perhaps the most intriguing need for future research may be the changes that occur in the microorganism in a confined environment. Both bacteria and fungi adapt to changes in their environment, and genotypic and phenotypic alterations should be expected. Environmental stresses such as the addition of disinfectants or possibly the proximity of several microbial populations to each other could cause eventual changes in phenotypic characteristics including antimicrobial resistance and virulence. As questions of this nature are answered in closed chambers, the safety and health of the astronauts will be ensured for future long-term missions.

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