

Biostability and Microbiological Analysis of Shuttle Crew Refuse

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ABSTRACT

Microbiological sampling and analysis was performed on the wet waste returned from the STS-105 and STS-108 shuttle missions servicing the International Space Station (ISS). Samples were collected from a variety of materials including plate waste and associated food packaging (which composed the majority of the collected waste), sanitary waste, and loose liquid inside the waste container. Analyses of the microbial loads cultured on both selective and non-selective media and through total bacterial counts by acridine orange direct count (AODC) methods showed high microbial densities in the waste container liquid. Isolates identified included *Klebsiella pneumoniae*, *Serratia marcescens*, *Bacillus* spp., *Salmonella* spp., and *Escherichia coli* (*E.coli*). Dry and ash weights were collected for each sample to determine water and organic content of the materials. Wastes from shuttle flights will continue to be monitored for biostability to establish a baseline measure of waste content, labile organics, and microbial load. The objective is to define the waste stream content and possible stabilization and recovery technologies that may be adapted for long duration missions.

INTRODUCTION

Shuttle trash evaluations have been conducted in the past at Johnson Space Center (JSC) for STS-29, STS-30 and STS-35, and at Kennedy Space Center (KSC) for STS-99 and STS-101. These analyses focused on defining the physical condition of the various waste stream products returned aboard the shuttle orbiter with emphasis on developing waste processing options for future longer-duration spaceflights. Solid wastes were categorized as trash, solid food system waste, human solid waste, and experimental waste [1].

Waste analyses carried out for STS-29, STS-30, STS-35, STS-99, and STS-101 revealed that water accounted for roughly 30% (by mass) of the average 9.89 kg per crew day of waste produced on orbit [2], while packaging material accounted for the majority (~80%) of the trash volume [1].

Waste storage aboard the orbiter consists of the volume F compartment for wet trash and the volume B dry trash compartment. Dry trash includes such materials as discarded office products, plastic packaging, and experimental wastes. Wet trash stored in the volume F includes mealtime wastes such as leftover food and drink (plate waste) and the associated food packaging, personal hygiene articles, and toilet wipes. The toilet wipes are disposed of beside the Waste Collection System (WCS) (toilet) in a plastic disposal bag referred to as an "elbow pack" due to its angular shape. The food and personal hygiene articles are stored within individual trash liners located in areas of trash generation within the crew compartment such as the galley. These liners are collected when full and placed in the appropriate storage compartment. Because trash overflow is stowed in the airlock, storage is adequate for STS missions with duration less than 30 days.

The International Space Station (ISS) is a continually operating on-orbit research platform, and therefore has different waste disposal needs than are experienced on STS missions. Solid waste is generally stored aboard the ISS until it can be returned to Earth. The mass of waste that can be removed from the station in this manner depends on the method of return. The bulk of the station waste (1600 kg [2]) is removed via unmanned Russian

Progress modules, which are sent to the station roughly once a month. The space shuttle can also remove quantities of waste (up to 9000 kg [2]) with when it undocks from the station on ISS servicing missions including an Italian-built Multi-Purpose Logistics Module (MPLM) payload for ferrying equipment and experiments to the ISS. STS missions to the ISS, however, are not evenly spaced with Progress launches. Thus, wastes are stored for varying periods of time aboard the space station with implications for levels of bacterial growth and accompanying decomposition of waste material. Another consideration for ISS waste is the lack of venting ability such as is found aboard the shuttle to dissipate any odors or large volumes of outgassing products generated by the microbial decomposition of stored waste. These factors will affect the waste storage design for future missions of increasing duration.

Microbial analysis of the wet trash was added to existing trash sampling procedures for STS-105 and STS-108 in order to quantify the biological presence in on-orbit waste storage systems.

STS-105 was a mission to the ISS flown aboard the space shuttle Discovery. The crew consisted of the 4 shuttle astronauts, 3 ISS Expedition Two crewmembers being replaced and the 3 ISS Expedition Three crewmembers that replaced them. Discovery was in space for 10 days, roughly 8 days of which they spent docked to the station. During this time, the shuttle and ISS operated as a single entity with the crews moving freely between both spacecraft and utilizing their respective resources, including waste management facilities.

The same was true during the 10 days Endeavor spent docked to the ISS during the first ISS Utilization Flight (UF-1), STS-108. Through the course of this mission, the 4 members of the shuttle crew observed the ISS crew swap bringing the Expedition Four crew to the orbiting laboratory. The shuttle returned to KSC after a nearly 12-day mission with the Expedition 3 crew after 129 days in orbit.

Both STS-105 and STS-108 carried a MPLM, bringing ISS wastes back as well as the shuttle wet wastes stored aboard the volume F compartment of the orbiter middeck. Due to the communal nature of spacecraft use during orbit, the volume F included wet wastes from not only the orbiter crew, but also some from the station crewmembers.

The objective of the STS-105 and STS-108 wet trash investigations was to survey the refuse to establish baseline information regarding the density of the microbial community present in on-orbit waste and the physical condition of the wet waste that contained the community. Once the biological and physical composition of the waste is defined, research and technology development to define strategies for waste stabilization and recovery may commence.

METHODS

APPROACH

The wet trash sampling procedures for STS-105 and STS-108 differed considerably based on our experiences with the first sampling. In both cases, however, the volume F bag was unloaded from the orbiter's middeck and transported to the lab within 5-7 hours after the shuttle landing at KSC's Shuttle Landing Facility. The bag was then weighed and stored overnight for analysis the following day. No ISS wastes were removed from the MPLM for analysis.

The wet trash was inventoried itemizing the number and contents of the individual trash liners held within the larger volume F bag were created for both shuttle waste samplings, and photographic documentation was created to record the overall appearance. The waste was sorted by type of material and the relative proportions of the basic elements of those materials (i.e.: water content, dry weight, ash-free dry weight) were determined.

The microbial community was characterized by density from a number of source materials from inside the volume F, in addition to isolates obtained by enrichment and analyses to yield presumptive identifications.

STS-105

After the initial inventory, we selected a number of samples to represent the major trash components for analysis. Samples of food (mushroom soup and shrimp cocktail), plastic (duct tape and plastic packaging), used plastic straws, toilet wipes, and bulk liquid that had accumulated in the bottom of the volume F bag were taken and placed into labeled, sterile containers (50-mL Falcon tubes for liquid, plastic sample bags for 2 g of solid sample material). The volume of the seepage liquid collected was recorded.

Wet, Dry, and Ash Weights

Wet weights for duplicate samples of the six sample types (straws, duct tape and plastic packaging, mushroom soup, shrimp cocktail, toilet wipes, and accumulated loose liquid) were recorded and later averaged, as were the dry weights of each. Sample materials were dried for 2 days at 70°C. Ash weights were obtained using a Type 30400 Thermolyne Furnace (Barnstead/Thermolyne Corp., Dubuque, Iowa) at 550 °C for 2 days, and the results averaged.

Microbial Sampling and Analyses

Sample Preparation For Microbial Sampling

The nature of the microbial analyses performed required that samples of a non-liquid constitution were either rendered in a liquid form or methods employed to extract the microbial content in the case of samples that could not be liquefied. The duct tape and plastic packaging, as well as the plastic straws, were manually shaken for 3 minutes in 30 mL sterile DI water with 5 mL of 3 mm sterile glass beads (Fisher Scientific Co.). Samples of shrimp cocktail and toilet wipes were added to volumes of sterile DI to yield 1:100 dilutions (weight:volume) and separately placed in a blender on high power for 30 seconds. No further processing was required for the samples of mushroom soup and accumulated loose liquid. The resulting liquids were then used in the microbial analyses described below.

Enumeration of Microbes Originating In Trash Samples

1. Microscopic Methods - Total bacterial cell counts were performed using acridine orange (AO) (Sigma, St. Louis, Mo.). Duplicate 5-mL samples of each material type were first fixed with 1 mL of a 2% 0.22- μ m filtered formalin solution prior to filtration for storage at 4°C. A 1-mL aliquot of an appropriate dilution of each sample was later stained with a 0.1% solution of AO and filtered onto Isopore 0.2- μ m pore diameter black polycarbonate membrane filters (Millipore, Bedford, MA). The filters were air-dried and mounted onto glass microscope slides with low-viscosity microscope immersion oil (Stephens Scientific, Riverdale, NJ). The slides were viewed under epifluorescent illumination using a Zeiss Axioskop 2 plus fluorescence microscope (Carl Zeiss Inc., Thornwood, NY) with a 100 W mercury lamp and Omega filter set XR22 (Omega Optical, Inc., Brattleboro, VT). Ten fields were enumerated and the average obtained for use in conjunction with dry weight data to determine the total number of cells per gram dry weight of sample.

2. Plating Methods - A dilution series was made of each liquefied sample using sterile DI water, and these diluted samples were streaked onto two sets of duplicate Difco R2A medium (Becton Dickinson, Sparks, MD) plates. One set of plates was incubated at 37°C in aerobic conditions, while the other set was incubated under anaerobic conditions in an AnaeroPack™ System 7.0-L anaerobe chamber (Mitsubishi Gas Chemical Co., Inc., Japan). Enumeration of duplicate plates of an appropriate dilution was conducted 48 hours later, and the results averaged. Following examination and enumeration of the colony forming units per gram dry weight (cfu gdw⁻¹) grown on the R2A plates, further analyses were conducted to identify the distinct colony morphologies found.

Representative cfu's from the aerobic plates containing liquefied samples of straws, shrimp cocktail, loose liquid, and toilet wipes were streaked on MacConkey Agar (Becton Dickinson, Sparks, MD) to select for gram negatives and differentiate for lactose fermentation, Columbia CNA medium (Becton Dickinson, Sparks, MD) for selection of gram positives, and 5% Sheep's Blood Agar (SBA) (Becton Dickinson, Sparks, MD) for microbial isolation. Gram stains were then performed on the various colony morphologies to confirm the presumptive classification.

Gram positives were presumptively identified by combining the information gathered by their colony morphology, gram stain results, and catalase analysis followed by subculturing on SBA (in the case of gram-positive cocci).

Gram negatives (both lactose fermenters and non-lactose fermenters) were subjected to a gram stain and oxidase test prior to subculturing onto SBA plates to obtain a pure culture of each colony type for identification in the Vitek Senior (System # 2805 B) system of analysis in conjunction with the Vitek Gram Negative Identification+ (GNI+) Card (both bioMérieux Vitek, Inc., Hazelwood, MI) according to the manufacturer's specifications. Positive identifications using the GNI+ card were reported if there was greater than 75% confidence.

Tests for fecal and total coliforms were performed using the Quanti-Tray/2000™ in conjunction with the Colilert 18® Test Kit reagent products (both by INDEXX Laboratories, Inc., Westbrook, MA) according to the manufacturer's specifications. Results were reported in Most Probable Number (MPN) per gram dry weight.

STS-108

While the collection, weighing, and inventory procedures for STS-108 were identical to those conducted for STS-105, some sampling procedures were varied in light of the observations and data accumulated from the earlier flight. The individual trash liners were separated during the process of inventory based on type of waste: food (including packaging and other mealtime debris), elbow packs, dry plastic, and debris found loose in the volume F bag. It was observed during the analysis of the wet trash from STS-105 that the highest concentration of microbial growth occurred in the loose liquid accumulated in the bottom of the large volume F bag. Thus, the microbial sampling for STS-108 focused solely on the loose liquid.

Wet, Dry, and Ash Weights

Each trash liner contained within the volume F bag was weighed individually to yield data on the percentages of each type of waste by weight and by size (number of trash liners containing this trash type). In addition, wet, dry, and ash weights were obtained for the total contents of five selected representative trash liners containing mostly mealtime debris. Dry weights were obtained after the waste had been placed at 70°C for 10 days. Ash weights were obtained by the same method as was employed for STS-105.

Microbial Sampling

Enumeration of Microbes Originating In Trash Samples

1. Microscopic Methods - A sample of the loose liquid was preserved and enumerated according to the AODC procedure described for the waste sampling of STS-105. Due to the small amount of loose liquid found in Endeavour's volume F bag, only a single sample of the loose liquid diluted in sterile phosphate buffered saline (PBS) was used.

2. Plating Methods - Dilution series were made using sterile 0.2-µm filtered PBS. Spread plates for total bacterial counts were made using R2A media in the same manner as was done for STS-105.

Standard Methods membrane filtration techniques [3] were employed in combination with selective and non-selective media in order to selectively enrich for specific groups of microbes present in the loose-liquid sample. Two types of membrane filters were used depending on the type of microbe to be isolated on the selective media: Pall/Gelman Laboratory Supor® 200 S-Packs (47mm, 0.2 µm) (Pall Corp., Ann Arbor, MI) and EZ-Pak™ Membrane Filter (47mm, 0.45 µm) (Millipore, Bedford, MA) (Table 1). Ten-milliliter aliquots of each sample dilution were filtered under partial vacuum through an appropriate sterile 47-mm gridded membrane filter and subsequently rinsed with portions of sterile DI water. The membranes were then removed from the filtering apparatus and applied to either an agar medium or a sterile support pad saturated with selective media broth in sterilized 50-mm petri dishes. The agar plates were inverted and all the selective media plates were incubated at 37°C, with the exception of plates containing M-FC broth. M-FC plates were sealed in a plastic bag and placed in a 45°C water bath for 24h prior to enumeration according to the manufacturer's guidelines. A duplicate set of Agar F, Agar P, and R2A dilution series plates were also incubated under anaerobic conditions and subsequently enumerated along with those grown under aerobic conditions. Colonies were identified according to the manufacturer's specifications.

Bacterial Type	Prepared Media Name	Filter Type
Total Bacteria	Difco R2A agar (Becton Dickinson, Sparks, MD)	I
Enteric Pathogens	Difco XLD agar (Becton Dickinson, Sparks, MD)	II
<i>Salmonella</i> spp.	BBL Brilliant Green Agar (Becton Dickinson, Cockeysville, MD)	II
<i>Staphylococcus aureus</i>	Mannitol Salt Agar (Remel, Lenexas, KS)	II
<i>Enterococcus</i> spp.	BBL Enterococcosel Agar (Becton Dickinson, Cockeysville, MD)	II
Total Coliform	BBL mEndo Broth (Becton Dickinson, Sparks, MD)	II
Fecal Coliforms, <i>E. coli</i>	mFC Broth Base (Becton Dickinson, Sparks, MD)	II
<i>Pseudomonas aeruginosa</i>	Difco Agar F and Agar P (Becton Dickinson, Sparks, MD)	I
I - Pall/Gelman Laboratory Supor® 200 S-Packs (47mm, 0.2 µm) (Pall Corp., Ann Arbor, MI) II - EZ-Pak™ Membrane Filter (47mm, 0.45 µm) (Millipore, Bedford, MA)		

Table 1. Membrane Filtration Protocols for the Isolation of Microbes Found In The Accumulated Loose Liquid of the Volume F Bag Aboard STS-108.

RESULTS

GENERAL OBSERVATIONS AND DATA

The volume F bags retrieved from the orbiter middeck contained between 25 kg (STS-105) and 40 kg (STS-108) of wastes distributed among individual trash liners (Figure 1). Seventy-two percent of the liners contained food material along with the plastic packaging and straws associated with it. Also included with the mealtime debris



Figure 1. Volume F Bag Access Opening and Trash Liner Filled With Plastic and Plate Wastes

were personal hygiene articles such as toothbrushes, alcohol swabs, and duct tape. The presence of Russian food packages, which were mostly metal containers, in the orbiter waste suggests a potential difference in the content of the microbial community present in the ISS wet trash as compared to that identified in this study for the STS if there is no common procedure for the sterilization and/or preparation of material used in food packaging between participating space agencies.

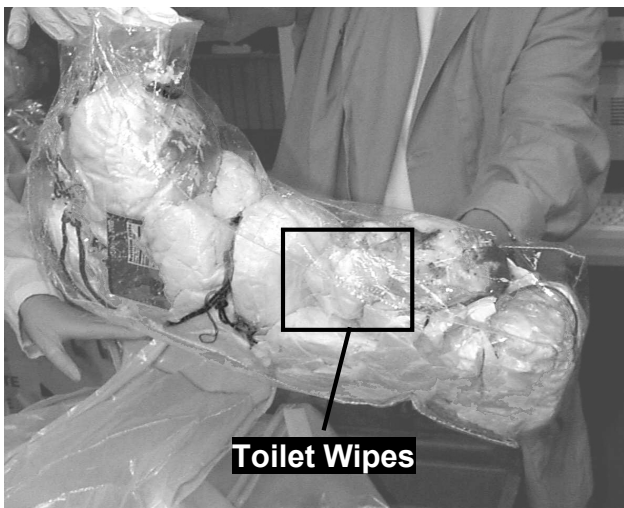


Figure 2. Elbow Pack Containing Toilet Wipes

There was very little residual food material left in the meal pouches, although this varied with the type of food based and the personal preferences of the crewmembers. Shrimp cocktail is one of the more popular dishes on orbit, but there is often cocktail sauce and a few shrimp left unconsumed. There were also a small number of potable water pouches and food packages, some reconstituted and some left in their original form, which were discarded unconsumed in the trash containers. Of particular note was one plastic package of mushroom soup (liquid form) that had developed a large volume of gas.

The other major component of the garbage collected was toilet wastes, which constituted roughly 18% of the trash liners collected. Toilet wastes included the aforementioned “elbow packs” containing wipes (Figure 2). Also included were diapers, or Maximum Absorbency Garments (MAGs), utilized by the shuttle crew during launch and landing, as well any MAGs deposited aboard the orbiter that were worn by shuttle crew members during the two extra-vehicular activities (EVAs) that occurred during STS-105 and single EVA from STS-108.

The types of refuse, whether they were toilet wastes, food wastes, plastic straws, or other plastic material, for the most part were kept in separate bags. We collected loose liquid from the bottom of the volume F bag resulting from seepage of fluid from individual trash liners. Approximately 15 mL of liquid was recovered from the wet waste collection bag following STS-105. In contrast, there was very little (0.5 mL) of loose liquid found accumulated in the bottom of the volume F bag on STS-108. This was most likely due to the presence of an uncontained MAG in the bottom of the bag, which absorbed most of the loose liquid.

WASTE SAMPLE COMPONENTS (WET, DRY, AND ASH WEIGHTS)

Figure 3 shows the relative amounts of the components of various wet waste samples selected for analysis. The food packages were the only trash type to undergo dry weight and ash weight determination from STS-108 since it was observed that food packaging comprised the majority of the trash on both missions. The average

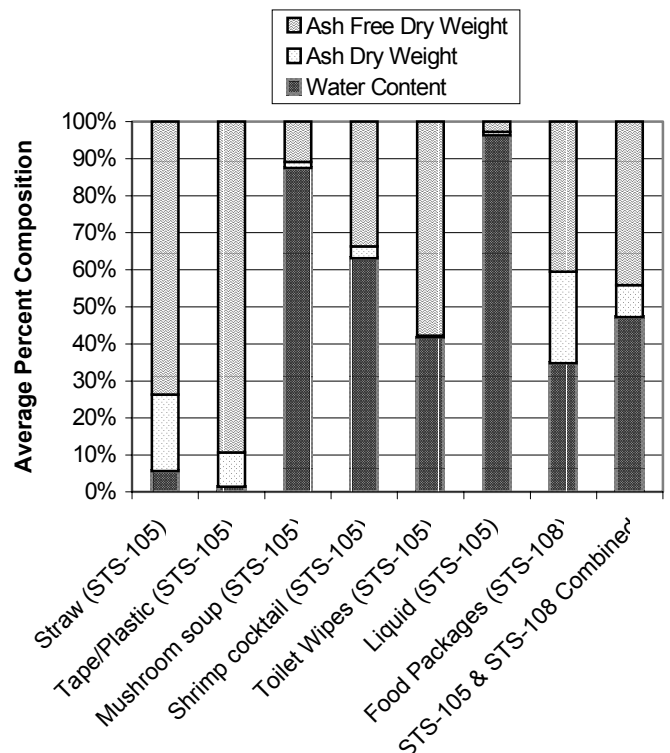


Figure 3. STS-105 and STS-108 Wet Trash Sample Material Composition

percentage of water from STS-105 and STS-108 (42%) was slightly higher than that found on earlier missions studied, which had water content of roughly 30% [2]. This was mostly likely due to the fact that only wet wastes were sampled in this study, whereas past investigations examined the total volume of trash-produced on-orbit. Water used is replenished aboard the shuttle through the use of electricity-generating fuel cells, which produce roughly 3 kg/h of water as a by-product, thereby reducing concerns over water use on shuttle flights. On longer duration flights, however, some water recovery from food trash may be necessary due to mass or energy requirements. The value of the ash free portion in conjunction with the average water content values may represent possible points for resource recovery of organics and/or water on future long-duration missions.

possible contact with the liquid when adding trash to the volume F.

Mission	Cells mL ⁻¹	Cfu mL ⁻¹	Microbial Groups Isolated
STS-105	3.35E+09	>1.00E+08	<i>Serratia marcescens</i> , <i>Klebsiella pneumoniae</i>
STS-108	1.54E+09	2.71E+06	Coliforms, Fecal coliforms, <i>Enterococcus</i> spp., <i>Salmonella</i> spp., <i>E.coli</i>

Table 2. STS-108 Accumulated Liquid Microbiological Content

MICROBIAL DATA

The largest numbers of bacteria on STS-105 were found in the loose liquid accumulated within the volume F bag (Figure 4). It was for this reason that the microbiological

The large number of anaerobes present raises the question of identity and quantity of gaseous products released by these organisms. Accumulation of noxious or toxic gasses (e.g.: mushroom soup package) could be a serious problem for habitats such as the ISS that again lack venting. Also of concern is the consumption of oxygen, which is of critical importance in manned spacecraft, by aerobic bacteria as a part of their metabolism. These questions will need to be examined as part of microbiological studies of the wet trash of future manned spaceflight missions.

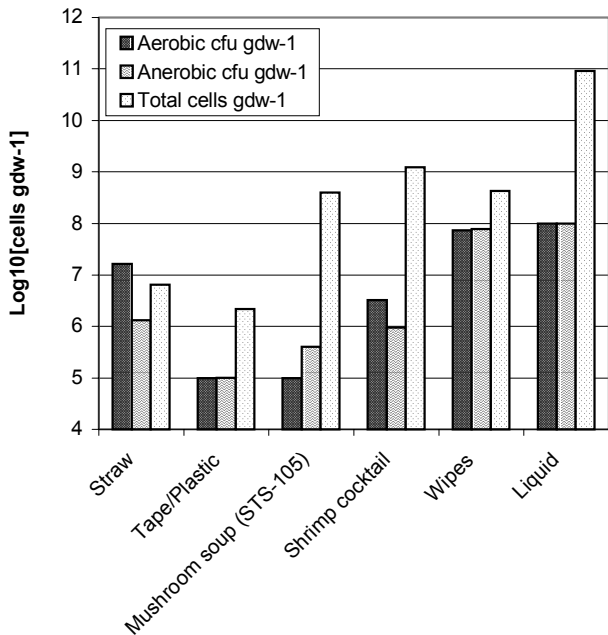


Figure 4. Microbial Density of Wet Trash From STS-105 According To Material Type analyses for STS-108

focused on the liquid, yielding slightly lower concentrations of microbes (Table 2) that may have been a result of the low volume of liquid recovered. This represents a possible source of contamination for the spacecraft and/or crew if containment of the wet wastes is compromised, especially aboard spacecraft that unlike the shuttle have no venting capabilities for stored wastes, such as found in the ISS. The shuttle's wet trash venting capability enables the creation of a partial vacuum in the volume F, which would aid in drawing the seeped liquid away from the opening of the bag, thereby reducing

Microbial isolates selected and identified from the waste of STS-105 included yeasts, *Bacillus* spp., and gram variable rods. Vitek analysis of gram negative bacteria found in the loose liquid accumulated in the volume F bag identified *Serratia marcescens* and *Klebsiella pneumoniae* present in the samples (Table 2). These are both potential human pathogens that can be found in the intestines and respiratory tract, respectively, but are more commonly found in water samples [4]. Extremely high levels of total coliforms were found in samples of shrimp cocktail (>1.31E+06 MPN gdw⁻¹), which could be the result of inoculation from human sources during the process of food consumption. Total and fecal coliforms (both 1.00+05 MPN gdw⁻¹) were also isolated from the wipes contained in one of the elbow pack from STS-105 as could be expected given the nature of the material.

Selective and non-selective media analysis of the loose liquid collected from STS-108 revealed the presence of aerobic and anaerobic organisms that were further classified as coliforms, enteric pathogens, *Enterococcus* spp., *Salmonella* spp. and *E.coli* (Figure 5), which are all human-associated microbes with some species having potential for pathogenesis [4]. Tests for fecal coliforms and *Staphylococcus aureus* (an opportunistic pathogen) in the loose liquid both yielded negative results. The lack of fecal coliforms indicates that the elbow packs maintained containment from the loose liquid accumulated in the STS-108 volume F bag. The identities and densities of the microbes inhabiting on-

orbit wet wastes is important in planning for long-duration spaceflights to identify situations which are potentially hazardous to the crew and or spacecraft, especially if wastes are to be treated (i.e.: resources recovered) rather than stored. Microbial densities also become important in the case of a manned mission to Mars when planetary protection issues must be considered in the design of waste disposal systems in order to establish the levels of containment necessary to prevent biological contamination of a foreign planetary body.

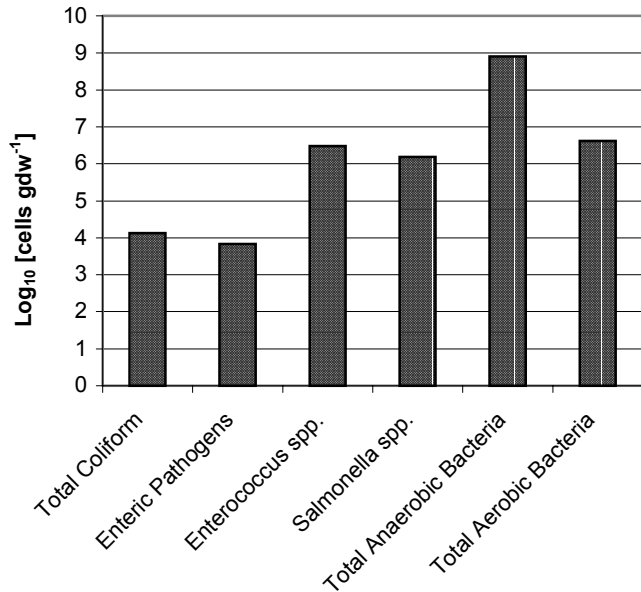


Figure 5. Microbial Density of Various Groups Identified in STS-108 Volume F Accumulated Liquid

CONCLUSION

Wet trash management for long duration spaceflight presents unique challenges in system design. Examinations of wet trash returned from the current short-term (STS) and longer-term (ISS) on-orbit spacecraft enable us to better understand some aspects of stored waste disposal systems. Microbes are present as part of any system that includes biological components such as humans. This initial survey of the shuttle-wet trash from ISS-servicing missions gives some indication of the microbial presence in the waste. This lends a definition of risks associated with resource recovery (mainly of water) from wet trash on longer duration spaceflights.

The next step is to determine what sort of gases are consumed and produced during microbial metabolism and the rates of these processes. This will enable impact evaluations on aspects of waste system design to account for microbial outgassing and use of regulated consumables such as oxygen. The answers to these questions will become increasingly important as we increase the interval of human presence in space.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

STS: Space Transportation System

ISS: International Space Station

AODC: Acridine orange (AO) direct count

JSC: Johnson Space Center

KSC: Kennedy Space Center

WCS: Waste Collection System

MPLM: Multi-Purpose Logistics Module

UF-1: Utilization Flight 1

CFU: Colony Forming Unit

SBA: Sheep's Blood Agar

GNI+: Gram Negative Identification +

PBS: Phosphate Buffered Saline

MPN: Most Probable Number

MAG: Maximum Absorbency Garment

EVA: Extra-Vehicular Activity

