Characterization of Volume F trash from four recent STS missions: microbial occurrence, numbers, and identifications

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The fate of space-generated solid wastes, including trash, for future missions is under consideration by NASA. Several potential treatment options are under active technology development. Potential fates for space-generated solid wastes: Storage without treatment; storage after treatment(s) including volume reduction, water recovery, sterilization, and recovery plus recycling of waste materials. For this study, a microbial characterization was made on trash returned from four recent STS missions. The material analyzed were 'Volume F' trash and other bags of accompanying trash. This is the second of two submitted papers on these wastes. This first one covered trash content, weight and water content. Upon receipt, usually within 2 days of landing, trash contents were catalogued and placed into categories: drink containers, food waste, personal hygiene items, and packaging materials, i.e., plastic film and duct tape. Microbial counts were obtained with cultivatable counts on agar media and direct counts using Acridine Orange fluorescent stain (AODC). Trash bag surfaces, 25 cm^2, were also sampled. Direct counts were ~ 1 x 10^6 microbes cm^{-2} and cultivatable counts ranged from 1 x 10^1 to 1 x 10^4 microbes cm^{-2}. Aerobic microbes, aerobic sporeformers, and yeasts plus molds were common for all four missions. Waste items from each category were placed into sterile ziplock bags and 1.5 L sterile DI water added. These were then dispersed by hand shaking for 2 min. prior to inoculation of count media or determining AODC. In general, cultivatable microbes were found in drinks, food wastes, and personal hygiene items. Direct counts were usually higher than cultivatable counts. Some pathogens were found: *Staphylococcus auerus*, *Escherichia coli* (fecal wastes). Count ranges: drink pouches – AODC 2 x 10^6 to 1 x 10^8 g_{fw}^{-1}; cultivatable counts variable between missions; food wastes: Direct counts were close to aerobic plate counts. Counts ranged from 10^6 to 10^9 per g_{fw}. Identities of isolates from cultivation media were obtained using a Biolog Microbial ID System or microSEQ molecular ID methodology using an ABI3130 gene analyzer.

Nomenclature

APC = Aerobic plate count or the number of bacterial colonies counted on agar plates incubated aerobically times a dilution factor and calculated per unit of sample.
AnPC = Anaerobic plate count or the number of bacterial colonies counted on agar plates incubated anaerobically times a dilution factor and calculated per unit of sample.
CFU = Colony forming units
EPC = Estimated plate count. Based on plates with a "to numerous to count" result.

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HT APC = Heat treated Aerobic plate count or the number of bacterial colonies counted on agar plates incubated aerobically times a dilution factor and calculated per unit of sample from sample diluent that is subjected to heat treatment of 85°C for 15 minutes thereby selecting for heat resistant spore forming bacteria.

HT AnPC = Heat treated Anaerobic plate count or the number of bacterial colonies counted on agar plates incubated anaerobically times a dilution factor and calculated per unit of sample from sample diluent that is subjected to heat treatment of 85°C for 15 minutes thereby selecting for heat resistant spore forming bacteria that grow anaerobically.

Y + M = Yeast and mold colonies counted on inhibitory mold agar.

WMS = Waste Management Systems.

I. Introduction

THE Waste Management Systems (WMS) element of the Life Support and Habitation Systems program is responsible for the development of technologies and approaches to manage the numerous types of solid waste materials generated in future human space flight. Currently, STS and ISS utilize simple waste management methods, where trash is stored, and either burned during Earth reentry (Russian Progress vehicles) or returned to Earth (STS). Future long-duration missions will require more sophisticated methods for in-situ processing, storage and disposal of wastes. The WMS element is therefore engaged in designing, developing and testing technologies that: ensure the protection of the health and well-being of the crew; optimize waste storage volume; minimize crew handling; recover resources; and meet planetary protection guidelines.

WMS has a number of solid waste treatment technologies that are, or have been, under development. The goals of these treatments are to (1) reduce the volume of the waste because storage space is very limited on space vehicles, (2) the remove and recover water because many wastes contain water and easily biodegraded organic compounds from food wastes and crew feces, (3) stabilize and make wastes safe for the crew and harmless to the environment, (4) contain waste to isolate it from the crew and the rest of the world, and dispose of the contained waste, and (5) process the waste for reuse of resources within the stored waste. Because a major reason behind goals (2), (3), and (4) are to eliminate hazards to crew caused by the presence of pathogenic or otherwise deleterious microorganisms in solid wastes, our efforts at KSC have been to provide support to WMS process technologies that have been designed to eliminate microbiological hazards. These technologies have been selected because they either remove and recover water, which microbes need to survive and grow, or they sterilize the solid waste, usually though heat.

The role of the WMS microbiological support projects at KSC have been to characterize the microflora present in space-generated solid wastes such as food wastes, crew fecal wastes, and other wet organic wastes. These wastes typically contain easily biodegraded organic compounds that support microbial growth and proliferation. If solid wastes remain untreated or unprocessed and are then placed into storage, over time the labile organic components in the waste will likely be responsible for both microbial proliferation and microbial byproduct production of noxious odors.

Two studies at KSC in FY07[1] and FY08 (unpublished), respectively, have examined the microbial characterization of food wastes in simulated space mission trash, i.e., for a Lunar Base. In the KSC-WMS FY07 project, food wastes were inoculated with material from volunteer / donor mouth scrubbing. Body wipes, in lieu of a shower, from volunteer donors, disinfectant and wet wipes of facility urinals and commodes at the Space Life Sciences Laboratory (SLSL) at KSC, and dry wipes of SLSL laboratory tabletop surfaces were also added to the simulated waste after placing the wipes into a ziplock bags, which were then sealed. At the time, we felt that these inocula would 'simulate' crew handling of food wastes and exposure of wastes to other space habitats components.. However, the results of the FY07 study[1] indicated that few human pathogens were present in the wastes, thus we wondered if the inocula might not be very representative. During these studies, we had access to the wet waste from the Volume F trash returned on each STS mission, but resources were not available to process these wastes for our microbiological studies. This all changed this past year as both access and resources could be used.

The primary goal of the WMS microbiological support projects at KSC for the results reported in this paper was microbial characterization of the STS Volume F trash. However, we also had the opportunity to characterize, or survey, the contents of the trash in relation to total wet weights, water content, plastic film content, and to photodocument the trash contents. A companion paper reports our findings on this physical characterization of the Volume F, and other, wet trash from four recent shuttle missions (reference not yet determined by AIAA). This present paper reports our results of the microbiological characterization of this same trash from STS 129 – 132 missions.

II. Materials and Methods

A. Approach

Voume F wet trash and other large ziplock plastic bags, which also contained trash items, are generated on each STS mission, whether to the International Space Station (ISS) or not. As noted by Kish, et al.[1], waste storage aboard the orbiter consists of the Volume F compartment for wet treash and includes mealtime wastes such as leftover food and drink and the associated food packaging, personal hygiene articles, toilet wipes (termed "elbow packs" because of their shape), and Maximum Adsorption Garments (MAGs) worn by the crew during launch and extravehicular activities (EVA). The Volume F trash from four recent STS missions were available for this report and mission specifics are shown in Table 1.

Table 1. Mission information for Shuttle Volume F trash characterized in this study. Each mission had 3 EVAs / space walks with 2 crew members per EVA.

Shttle Mission	Crew Size,	Launch Date	Landing Date	Mission duration
STS 129	6	16-Nov-09	27-Nov-09	10 days, 19 hours, 16 minutes, 13 seconds
STS 130	6	08-Feb-10	21-Feb-10	13 days, 18 hours, 6 minutes, 24 seconds
STS 131	7	05-Apr-10	20-Apr-10	15 days, 2 hours, 47 minutes, 10 seconds
STS 132	6	14-May-10	26-May-10	11 days, 18 hours, 29 minutes, 9 seconds

B. Sequence of sampling events for each shuttle landing at KSC

Upon notification by shuttle personnel, the Volume F trash 'package' was picked up from landing support personnel. Total weight of the entire Volume F trash was determined. As the Volume F trash bag was opened, the contents were catalogued and each item was placed into an appropriate category or subcategory. The broad categories were determined during the cataloging of the first Volume F trash sample received. On subsequent missions, the list was modified as needed. Attempts were made to determine the amount/weight of plastic film in the total trash. Wet weights of each trash category were determined, and a subsample was weighed, dried (70 oC until dry) , and weighed again to determine the amount of water in each category. Aseptically obtained a representative subsample of each category of waste likely to contain microorganisms. Microbiological analyses were performed on these aseptically obtained subsamples.

C. Sample preparation.

Waste items that were to be sampled for microbiological analyses were placed into sterile gallon ziplock bags and 1.5 L of sterile deionized water was added. The bag contents and water were mixed / shaken by hand for 2 minutes (10-2 dilution) and a 10-fold dilution series was prepared from the trash-water mixture. These dilutions were then used to obtain: acridine orange direct counts, numbers of cultivatable total aerobic and anaerobic bacteria, cultivatable gram positive spore forming bacteria, selected cultivatable bacteria (*Staphylococcus aureus,Coliforms and Escherichia coli*); and cultivatable fungi (yeast and molds).

D. Microbiological analyses

1. Microbial Load via Acridine Orange Direct Count (AODC).

0.5 ml 0.2µm-filtered 37% formalin was added per 9.5 ml of blended, diluted trash-DI sample to fix cells for the AODC protocol. NOTE: Formalin-fixed samples were stored @ 4°C for up to two weeks in a 4 oC refrigerator until filtered for enumeration. Formalin-fixed samples were sonicated, diluted into 0.2µm-filtered dI-H2O, stained with Acridine Orange, and filtered onto 25-mm (diameter), 0.2µm (pore size) black polycarbonate filters for enumeration. Some samples required serial dilution to 10^{-1} - 10^{-3} for accurate enumeration.

2. Cultivatable total aerobic and anaerobic bacteria.

A dilution series of blended, trash-DI samples was plated onto R2A agar which was incubated aerobically and anaerobically (AnaeroPack System, Mitsubishi Gas Chemical Co. Tokyo, Japan) at 30°C for 48 hours before enumeration.

3. Cultivatable gram positive spore forming bacteria.

Counts of gram positive spore forming bacteria were determined after heat shock treatment (80 °C for 15 minutes) of the blended, trash-DI samples and this treated dilution was plated onto R2A agar (incubated aerobically and anaerobically) at 30°C for 48 hours before enumeration.

4. Selected cultivatable bacteria.

Samples were screened for potential pathogens by plating blended samples on selective media, S. aureus was isolated on Mannitol Salt agar(MSA) (Difco) and Staph Express petri film (3M) and E. coli and coliforms on E. coli/ coliform petrifilm (3M) ..

5. Cultivatable fungi.

Yeast and fungal counts that occurred in the blended trash-DI samples. were obtained using Inhibitory Mold Agar (IMA)(Difco) .

6. Bacterial and Fungal isolate identification

All colonies that grew on any of the media where further isolated and identified by either the Biolog micro ID system by the inoculation of pure cultures into GEN III plates (bacteria identification), or the plates specific for yeast and filamentous fungi per manufacturers instructions. For isolates that were not identified by the Biolog, additional ID tests were run using the MicroSeq® D2 LSU rDNA Fungal Sequencing and the MicroSeq® 500 16s rDNA Bacterial Sequencing identification kits (ABI) following the manufacturer's recommended protocol. For these IDs, DNA was isolated from cultivated microbes using the PrepMan™ Ultra Sample Preparation Reagent (ABI) and diluted 1:100. The PCR Module from the kit used approximately 25 ng of genomic DNA on the BioRad C1000 thermocycler. The PCR thermocycling conditions were: 95°C for 10 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds, and finished with 72°C for 10 minutes. PCR product was run on a SYBR Safe (Invitrogen) 2% agarose gel (Sigma) with the Benchtop pGEM® DNA markers (Promega) and visualized for quality and size. 5 µL of the PCR product was then purified with 2 µL of ExoSAP-IT® (USB) in duplicate. The 7µL of purified sample was then processed through the sequencing module of the kit. The cycle sequencing thermocycling conditions were: 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The excess dye terminators and primers were removed the cycle sequencing reaction with the DyeEx 2.0 Spin Kit (Qiagen). 7.5 µL of Hi-Di™ Formamide (ABI) was loaded with 7.5 µL of the purified product on the ABI 3130 Genetic Analyzer. Results were analyzed with the MicroSeq Analysis Software (ABI).

III. Results and Discussion

A. Surface sampling.

For each STS mission, a variety of surfaces, including the interior and exterior surfaces of the bags containing trash, were sampled using sterile sampling sponges (Biotrace International, www.biotrace.com) wetted with sterile phosphate buffered saline (PBS). 100 cm^2 total surface area was sampled per item by swabbing four separate areas using a sterile 25 cm^2 metal template and the same sampling sponge. Sampling sponges were then placed into sterile sample bags and stored at 4 °C until microbiological analaysis. The day following acquisition of surface samples, 50 mL of sterile PBS was added to each sample bag. Bags were shaken vigorously for two minutes before plating the diluted surface sample on various agar media.

Cultivatable microbes were present in a number of these surface samples (Fig 1). Acridine Orange direct counts (AODC) of the diluted surface samples are also shown on these graphs. The number of surfaces sampled for each mission and the number that were positive for cultivatable microbes were: STS 129, 16 surfaces, 9 positive; STS 130, 14 surfaces, 12 positive; STS 131, 11 surfaces, 9 positive; and STS 132, 14 surfaces with only one positive for growth of cultivatable microbes. The direct count method showed a variety of microbial cellular morphologies were present on each surface. AODC were close to 1 x 10^6 microbes cm^{-2} sampled. The counts of cultivatable microbes ranged between ~1 x 10^1 and ~1 x 10^4 per cm^2. This difference between direct counts and cultivatable counts is a common occurrence in environmental samples. A curious microscopic observation was that small yeast-like cells, ~ 4 µm in diameter, were often observed. This was not backed up by the cultivatable counts of yeast and molds on the IMA medium.

The occurrence of aerobic heterotrophs on plate count agar (APC, on R2A medium) was the most common among cultivatable microbes across different STS mission trash samples. The near absence of cultivatable microbes on the trash bag surfaces of STS 132 is hard to explain, given that the trash from the other three missions had a high number of positives for the surfaces that were sampled. Another interesting finding is the occurrence of a larger variety of cultivatable microbes on STS 131 trash surfaces. Aerobic plate counts (APC), heat-treated aerobic plate counts (HT-APC), which looks for aerobic spore-forming bacteria, and yeast + molds (Y + M) were common for all

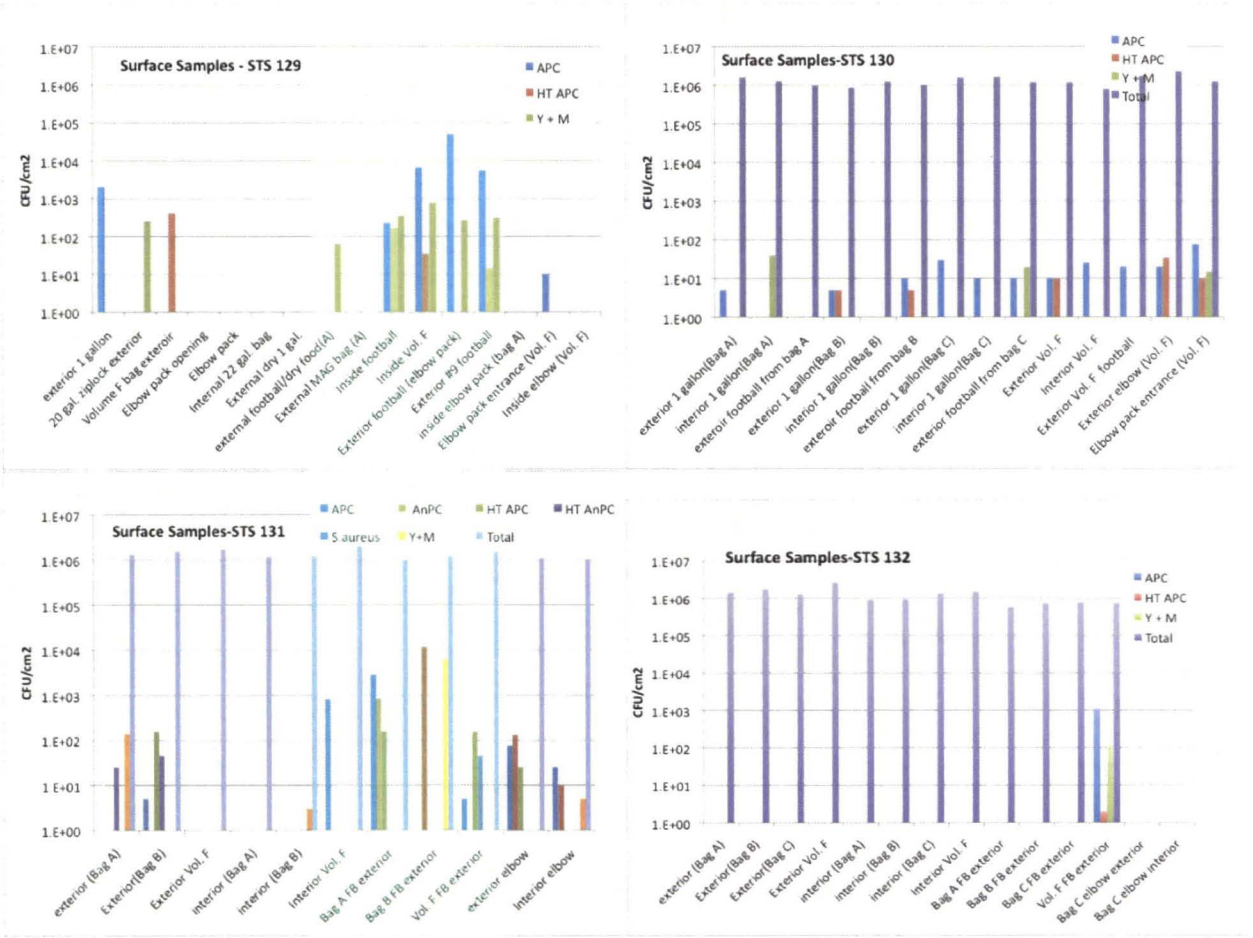

Figure 1. Bar graphs of the number of microorganisms found on the internal and external surfaces of plsstic-film bags in the trash of four STS missions. Shown are direct conts (Acirdine Orange fluorescent stain direct counts) and cultivatable counts on a variety of media.

four missions. However, only STS 132 showed cultivatable counts for anaerobic place counts (AnPC), heat-treated anaerobic plate counts (HT-AnPC), and *Staphylococcus aureus* (on Mannitol Salts Agar).

To our knowledge, this is the only microbiological characterization of exterior and exterior surfaces of space wet trash. Kish, et al.[1] sampled individual trash items in STS 105 Volume F trash including food (mushroom soup and shrimp cocktail), plastic (duct tape and plastic packaging), drink straws, toilet wipes, and the bulk liquid that accumulated in the bottom of the main Volume F bag. For STS 108 only the bulk liquid was sampled for microbial analyses. In a follow up study to this one, Peterson, et al.[2] reported counts for STS 109 and 110 during a storage study, but again they sampled only the bulk liquid. In their defense, the main purpose of this study was to monitor the volatile organic compounds that came off of the trash during a 9 month post-landing storage period

B. Microbial Characterization of Trash Content According to Source: Trash Category over 4 different STS missions.

The trash was divided into different categories to examine the differences in microbial content for different types of trash. The categories of microbiological interest were: drink pouches (i.e., containers) – Fig. 2, food wastes – Fig. 3, and personal hygiene wastes – Fig. 4. Each figure is organized the same, with cultivatable microbes and, where data exist, direct counts for STS 129 (upper left), STS 130 (upper right), STS 131 (lower left) and STS 132 (lower right). Another data division is the trash source bag for each mission: STS 129 – Bag A and Volume F trash; STS 130 – Bags A, B, and C and the Volume F bag; STS 131 – Bags A and B and the Volume F bag; and STS 132 – Bags A, B, and C and the Volume F bag. Also note that if no counts were obtained with a particular medium, then

those results are left off of the figures, even though all we can say is that the numbers were below our detection limit, which usually was 10 per mL of diluted sample.

1. Drink pouches (Fig. 2)

Total direct counts of drink pouches were not done for STS 129 samples but were added for the other three missions. For the drink pouches these counts mostly ranged between 107 and 108 per g(wet weight) for all trash bags except Bag A for STS 130 where it was ~ 2 x 106 g-1. Drink pouches for all four STS missions had cultivatable aerobic plate counts (APC) and counts of yeast plus molds (Y + M). Hetertrophic aerobic bacteria, APC counts, were lowest for STS 130 and highest for STS 132. Yeast and mold counts were mostly lower than APC counts. Drink pouches from STS 131 were the only ones that had cultivatable counts of anaerobic and spore forming bacteria (both aerobic and anaerobic) and of Staphylococcus aureus. The S. aureus counts were obtained on selective media, Mannitol Salts Agar, that was used to detect the presence of this microorganism. Very low counts of aerobic spore formers were also detected in / on STS 132 drink pouches. Many drinks contain sugars, which provide energy and growth sources for many different bacteria, so the detection of viable cells on these containers was expected. The presence of S. aureus may be bothersome, but this bacterium can be found in / on many environments, including human skin.

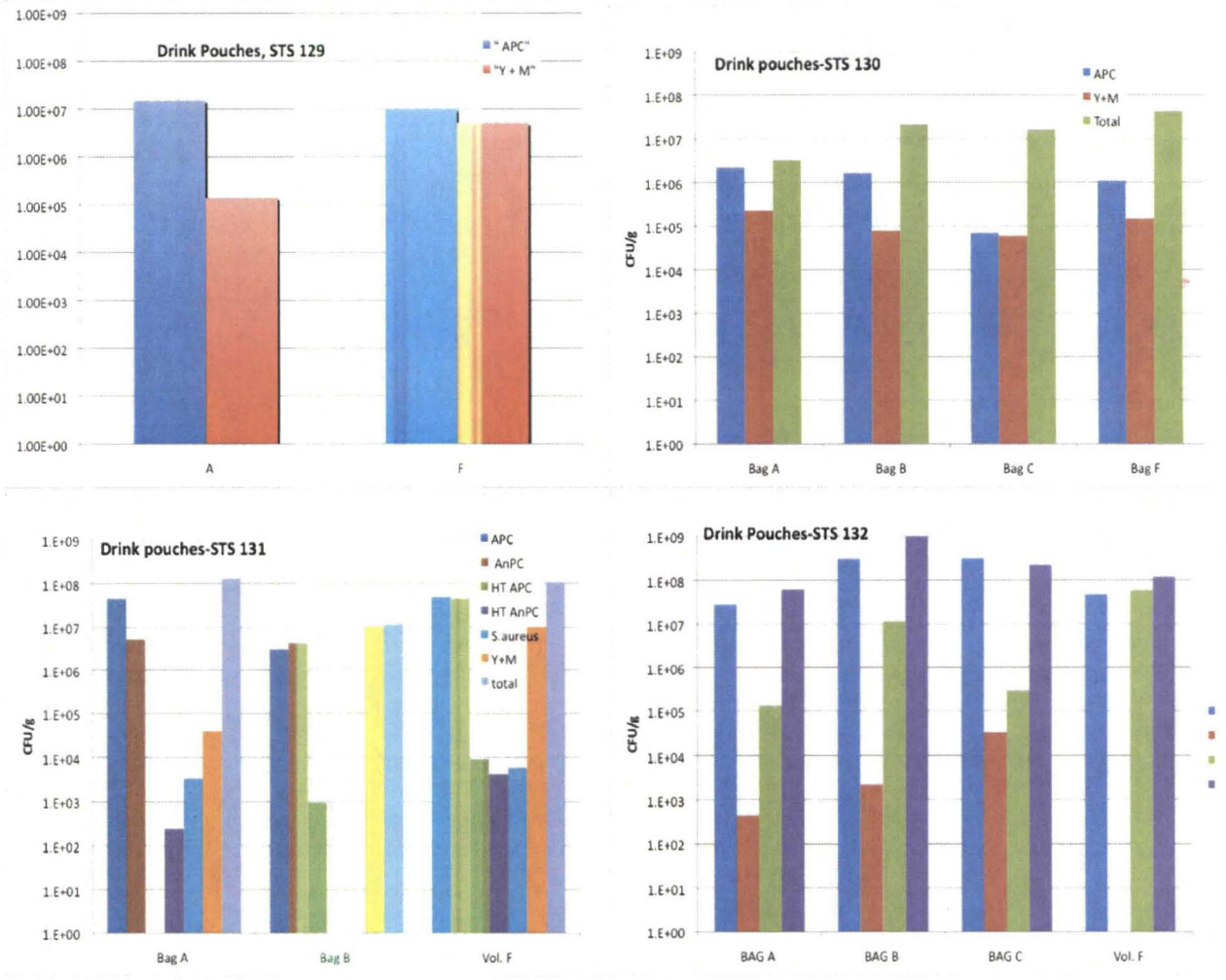

Figure 2. Direct total counts and cultivatable counts of microorganisms in or on drink pouches returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

2. *Food wastes, including food containers (Fig. 3)*

Because FY07 and FY08 projects at KSC examined the fate, including growth and proliferation, of microbes on simulated space mission food wastes, our main interest in characterization of STS trash was the food wastes. Figure 3 shows the numbers of microbes, both cultivatable and direct counts, in the food trash of the four STS missions. In general, the direct counts (not done for STS 129) were closer to aerobic plate counts than for the drink pouches. The reason for this could be the availability and amount of readily biodegradable compounds in the food when compared with the drinks. The range of direct counts were somewhat different for STS 130, between 10^6 and 10^7 per g(wet weight), than for STS 131, between 10^7 and 10^9 per g(wet weight), and STS 132, between 10^6 and 10^9 per g(wet weight).

For the cultivatable plate counts, both APC (hetertrophic aerobic plate counts) and Y + M (yeast plus molds) were present in all missions and all trash bags A – F. Again, in general, heterotroph counts were greater than yeasts and molds. And, once again, the cultivatable counts for STS 131 showed up on more test media than for the other three missions. Although the counts were low, ~ 10^2 and 10^4 , respectively, for Bag A and Volume F, *S. aureus* was detected in STS 131 food wastes. In addition, both aerobic and anaerobic spore-forming bacteria were found in cultivatable counts in Bag A and Volume F trash for STS 131 and aerobic spore-formers in all but Volume F bags from STS 132.

For comparison, two food items were sampled by Kish et al.[1] for STS 105 Volume F trash. The mushroom soup had ~ 5 x 10^8 total counts (AODC), 1 x 10^5 cells g(dry weight)$^{-1}$ aerobic microbes and ~ 5 x 10^5 cells g(dry weight)$^{-1}$ anaerobic cells. The shrimp cocktail from STS 105 had higher numbers at 1 x 10^9 total counts (AODC), 4 x 10^6 cells g(dry weight)$^{-1}$ aerobic microbes and 1 x 10^6 cells g(dry weight)$^{-1}$ anaerobic cells.

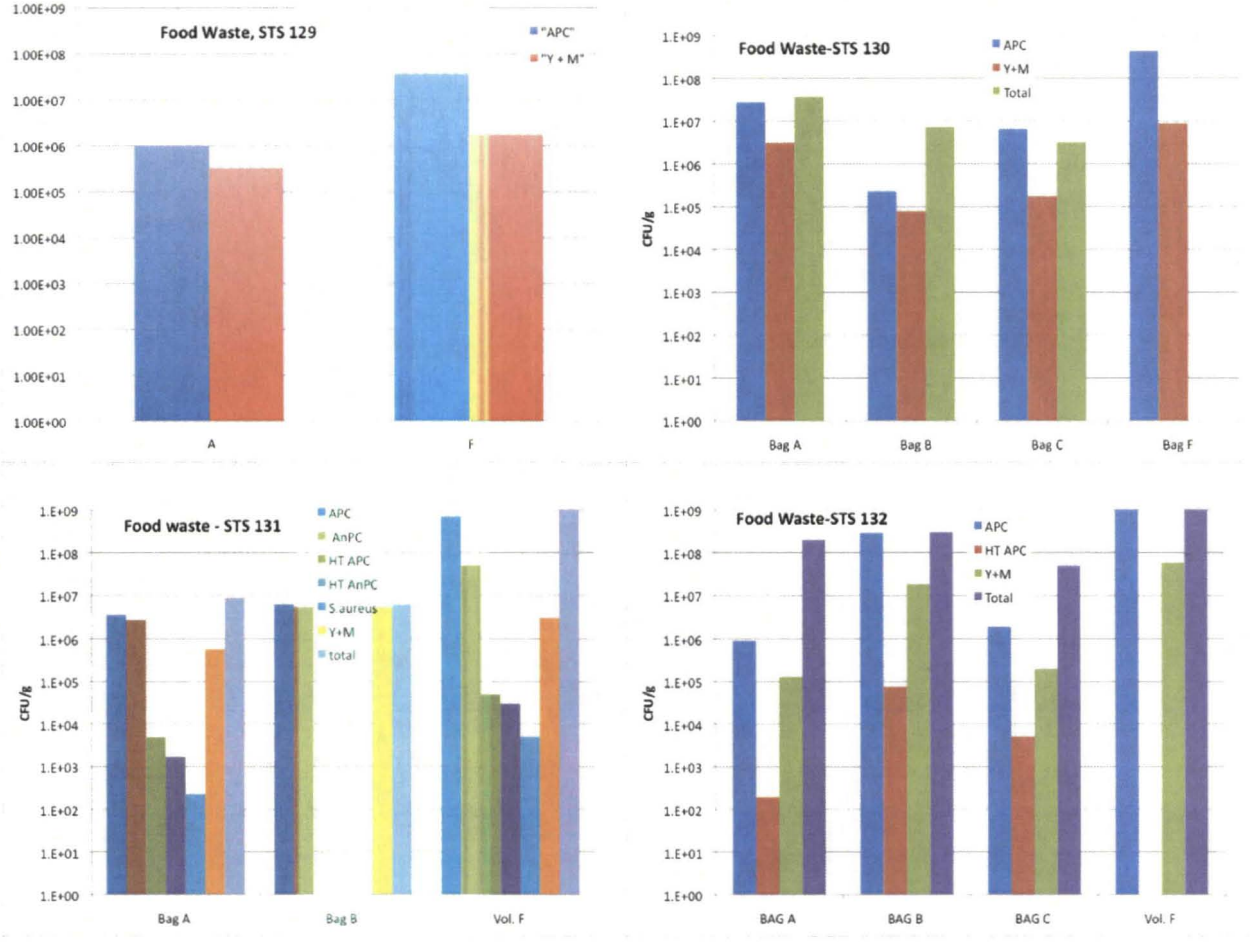

Figure 3. Direct total counts and cultivatable counts of microorganisms in food waste trash or on food containers returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

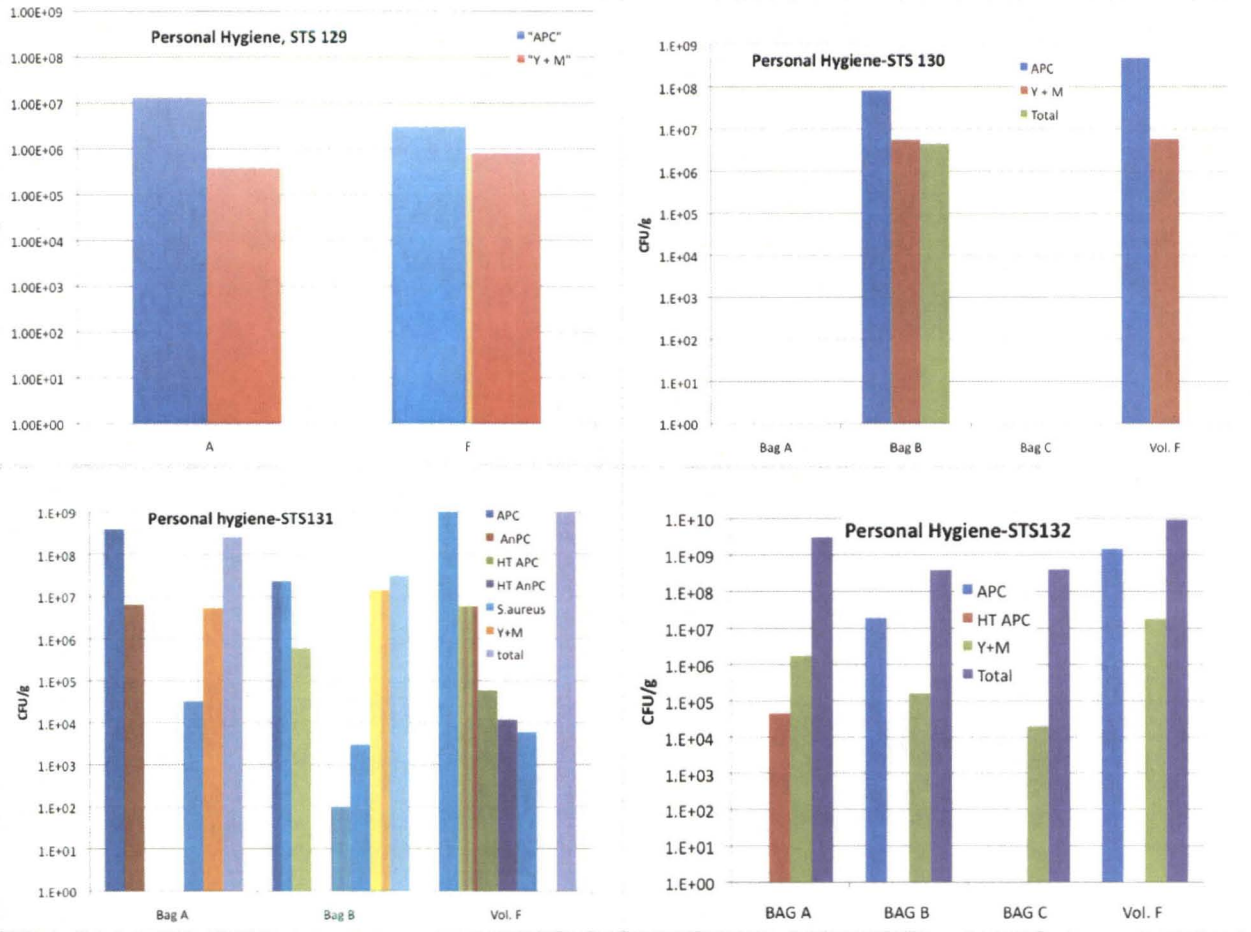

Figure 4. Direct total counts and cultivatable counts of microorganisms in personal hygiene wastes returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

3. Personal hygiene wastes (Fig. 4)

The category of personal hygiene includes wipes, meal napkins, etc. that were found in the waste and that didn't clearly belong to drink pouches or food waste. Although the MAGs and Elbow packs could be included in this category, we were not able to obtain adequate samples from the MAGS because the more diluent we added the more that was absorbed. The Elbow packs were clearly toilet wastes and the several that were sampled early in the study did not yield very high counts. Both MAGs and Elbow packs were contained in separate, duct-tape wrapped football packages to keep the contents well isolated from the other trash. As the likelihood that these footballs would ever be knowingly opened would be low, we felt it was more prudent to focus our attention on the other waste categories.

As with the food waste and drink pouch categories that were sampled for microbial characterization, personal hygiene wastes also contained aerobic heterotrophs (APC) and yeast and molds (Y + M) (Fig. 4). Of note, however, is the absence of any cultivatable microbes in Bags A and C from STS 130. Direct counts, when present, were high, However, direct counts are missing for a number of the samples because fine, small, non-staining particulates were present in high numbers and made direct counting of cells difficult or impossible for these samples. When samples were countable, direct counts ranged from 10^7 to 10^{10} per g. Counts of heterotrophs, APC, ranged between absent to 10^7 to 10^9 per g. The cultivatable counts for STS 131 again showed the presence of *Staph. aureus* and anaerobic spore-formers which were not present in STS 129, 130 or 132. Aerobic spore formers were present in STS 130 and STS 132 personal hygiene wastes.

By comparison, the results of Kish et al. for toilet wipes, presumably from elbow packs, were ~ 6 x 10^8 cells g(dryweight)$^{-1}$ for total counts and 1 x 10^8 cells g(dryweight)$^{-1}$ for both aerobic and anaerobic microbes.

Table 2. Identified bacterial isolates from trash returned on STS 129-132.				
Trash source	STS 129	STS 130	STS 131	STS 132
Personal Hygiene wastes	*Staphylococcus aureus, Bacillus subtilis ss subtilis, Staphylococcus sp Enterobacter aerogenes*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus epidermidis Bacillus subtilis ss subtilis*	*Curtobacterium spp Sphingomonas sanquinis Enterobacter pyrinus*
Food wastes	*Bacillus spp.*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus saprophyticus*	*Bacillus pumilus Sphingomonas sanquinis*
Drink pouches	*Bacillus subtilis ss subtilis*	*Enterococcus pseudoavium Burkholderia cepacia*	*Staphylococcus aureus*	*Enterobacter pyrinus Citrobacter spp Sphingomonas sanquinis Burkholderia multivorans Enterobacter pyrinus*
External trash bag surfaces	*Bacillus amyloliquifaciens Bacillus pumilus*	*Microbacterium marytipicum Bacillus amyloliquifaciens*	*Paenibacillus pabuli*	*Bacillus amyloliquifaciens Burkholderia pyrrocinia*
Internal trash bag surfaces	*Bacillus subtilis ss subtilis*	Isolates were not identified	*Bacillus subtilis ss subtilis,*	Isolates were not identified
MAGS/elbow pack contents	*E. coli, Citrobacter murliniae*	No sample	No sample	*Shigella flexneri*

4. Identification of microorganisms obtained from cultivatable plate count media from STS missions and different waste categories. (Tables 2 and 3)

The bacterial species that were identified by the Biolog ID system or by the MicroSEQ procedure are presented in Table 2. Nearly all of these microbes could be part of a normal human microflora and can be isolated from the environment. Many of the named organisms could be opportunistic pathogens, i.e., a possible pathogen, for immune compromised hosts, such as crew members in a microgravity habitat.

Three known pathogenic bacteria were isolated from STS trash samples. *Staphylococcus aureus* was isolated from personal hygiene waste from STS 129 and STS 131 and from food waste and drink pouches from STS 131. *Escherichia coli* was found in MAG/elbow pack contents from STS 129 and *Shigella flexneri* was identified from isolates obtained from MAG/elbow pack contents of STS 132. None of these could be considered unexpected. S. aureus is a common microbe on human skin and the *E. coli* and *Shigella* sp. are enteric bacteria that can be found in human feces. Of interest amonth the IDs are a number of species of the *Bacillus* genus. These are spore-forming bacteria and most are resistant to desiccation, heat, dehydration, and other stresses that might be used by WMS waste processing systems to limit or eliminate bacteria. We would recommend that an assessment of the survival of *Bacillus* be one of the criteria used during testing of the effectiveness of candidate solid waste treatment technologies.

Regarding the fungi that were identified from various trash samples (Table 3), nearly all of the identified genera are common environmental inhabitants. These would include *Rhodotorula* (soil, water, air), *Fusarium*[5] (soil), *Cladosporium* (indoor-outdoor mold and on plants), *Aspergillus* (common contaminants of starchy foods), *Cryptococcus* (majority of species live in the soil and are not harmful to humans), and *Penicillium* (ubiquitous soil fungus). *Cryptococcus laurentii* and *Cryptococcus albidus* have been known to occasionally cause moderate-to-severe disease in human patients with compromised immunity[6]. *Candida* are almost universal on normal adult skin and *albicans* is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal, and female genital tracts which cause no disease.

If storage of space-generated trash, such as the Volume F wet trash, is the only 'treatment' option under consideration for future space missions, then we recommend that food wastes be placed immediately into storage and the containers immediately sealed to prevent crew exposure to dangerous levels of cross-contaminating pathogens,. We believe that a better treatment option would be to limit microbial growth through immediate dehydration of food, or other, wastes or immediate sterilization of these wastes. The results reported here can be used to determine requirements and criteria for NASA Waste Management Systems. These methods and resulting data will provide a basis for testing technologies for the ability to limit contaminant survival, growth and proliferation.

IV. Conclusion

The microflora of the Volume F wet trash returned on four recent US Space Shuttle missions have been characterized. STS trash wastes have an abundance of easily biodegraded compounds that can support the growth of microorganisms and the research presented here shows that large numbers of bacteria and fungi have taken advantage of this readily available nutrient source to proliferate.

If storage of these wastes is the only 'treatment' option, then, to prevent crew exposure to dangerous levels of cross-contaminating pathogens, we recommend that food wastes be placed immediately into storage and the containers immediately sealed. We believe that a better treatment option would be to limit microbial growth through immediate dehydration of food, or other, wastes and/or immediate sterilization of these wastes. The results reported here can be used to determine requirements and criteria for NASA Waste Management Systems. These methods and resulting data will provide a basis for testing technologies for the ability to limit contaminant survival, growth and proliferation.

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Title

Characterization of Volume F trash from four recent STS missions: weights, categorization, water content

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Characterization of Volume F trash from four recent STS missions: microbial occurrence, numbers, and identifications

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Introduction

- KSC project – Microbial Characterization of Solid Wastes
- Provide microbiological support of
  - Waste Management Systems element at ARC
  - NASA's Life Support and Habitation Systems program at JSC
- WMS role
  - Develop technologies and approaches to manage numerous types of solid wastes materials generated in future, long duration human space missions
  - Protect crew health & well being, optimize waste storage volume, minimize crew handling, recover resources, meet planetary protection guidelines

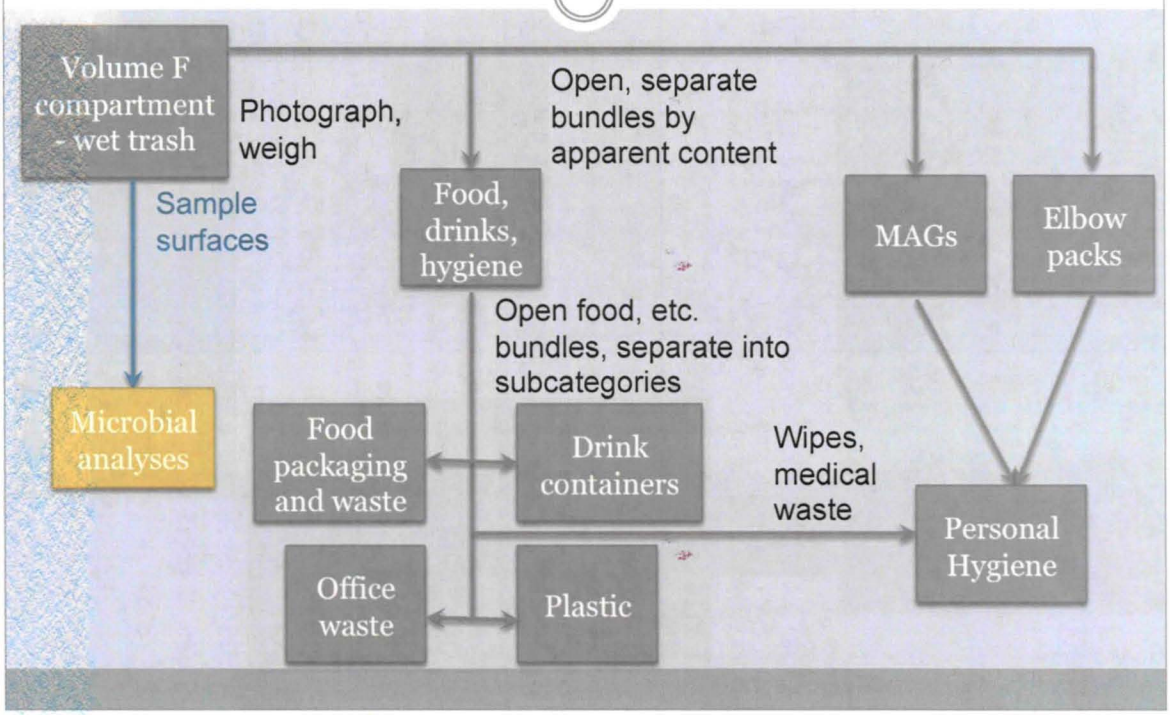
Mission information for Shuttle Volume F trash characterized in this study

Shuttle Mission	Crew Size	Launch Date	Landing Date	Mission duration
STS 129	6	16-Nov-09	27-Nov-09	10 d, 19 h
STS 130	6	08-Feb-10	21-Feb-10	13 d, 18 h
STS 131	7	05-Apr-10	20-Apr-10	15 d, 2 h
STS 132	6	14-May-10	26-May-10	11 d, 18 h

Sequence of events after each landing

- Pick up Volume F compartment wet trash from landing support personnel, usually within 48 hrs of landing
- Store trash at room temperature
  - 1 – 3 days until processed and characterized.
- Process and characterize trash
  - Determine wet weights
  - Open exterior bags and catalog contents. Photograph.
  - Trash bundles ('footballs') aseptically opened and contents sorted, cataloged, and weighed, by category
  - Subsamples from each category were taken for dry weight determination and microbiological analyses.

Trash categorization flow chart

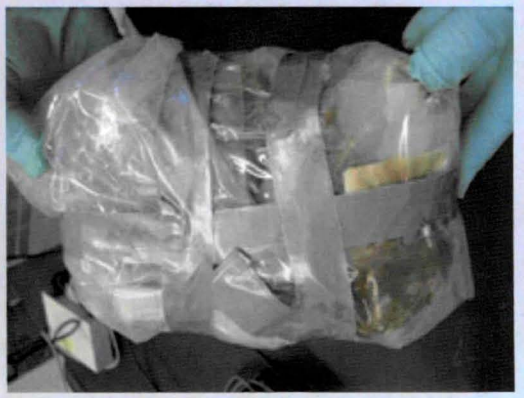


Photographs: main Volume F bag and other trash bag

Photographs: Characterizing and cataloging trash bundles

Mixture of different types

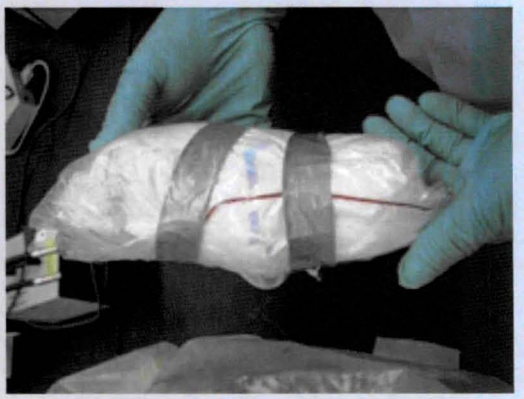
Food, drinks, wipes, etc.



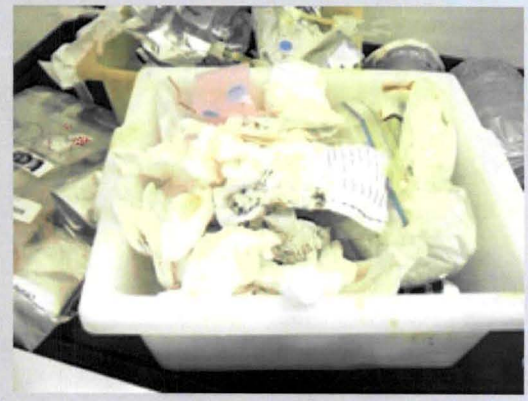
Photographs: Characterizing and cataloging trash bundles

Maximum Absorbancy Garment (MAG)

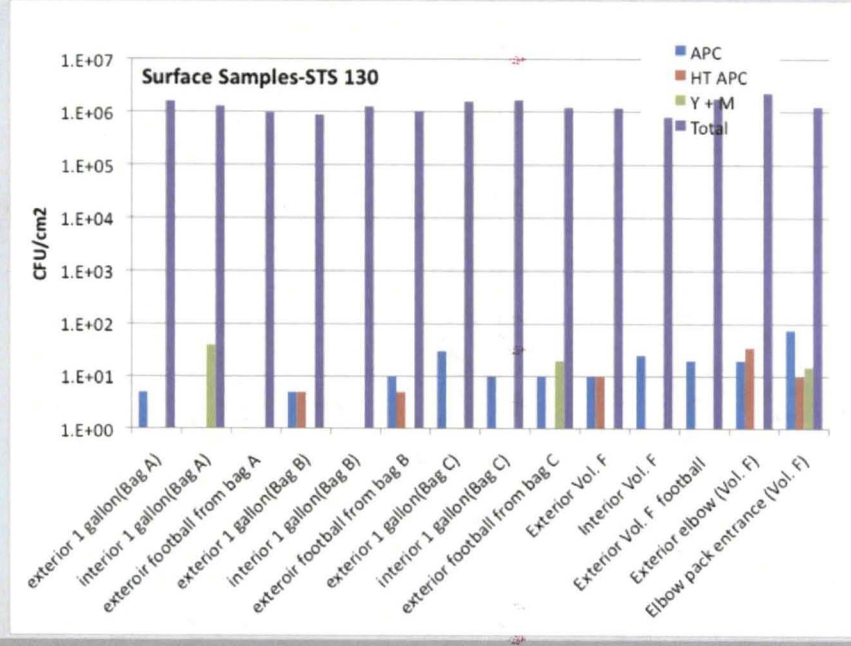
'Elbow' pack - toilet wipes, etc.



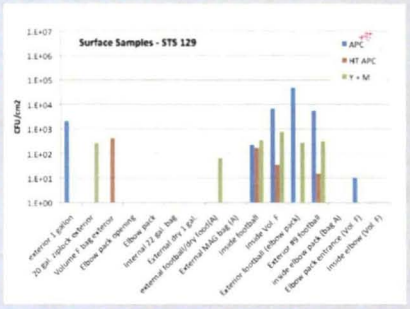
Photographs: Separation of bundle contents into categories

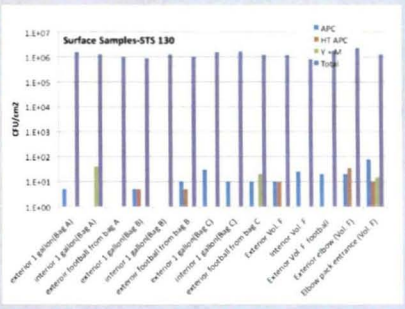


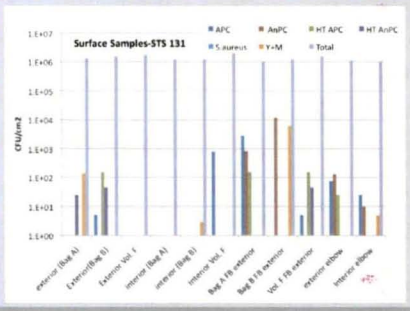
Surface Samples, STS 130

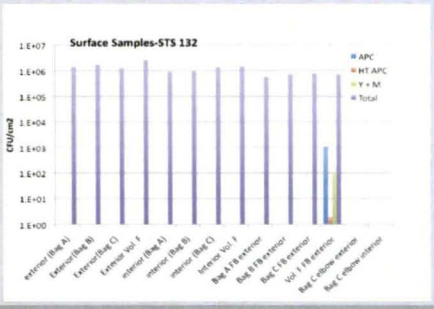


Surface samples

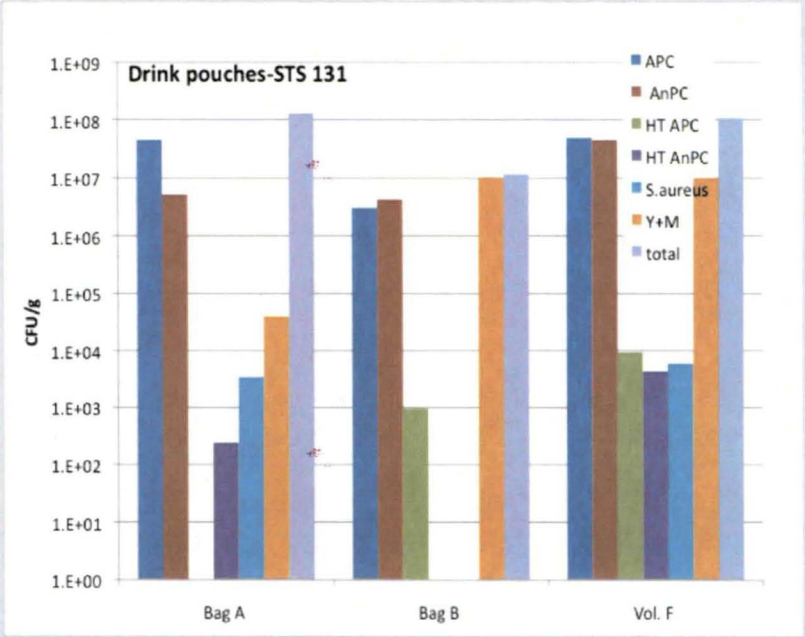




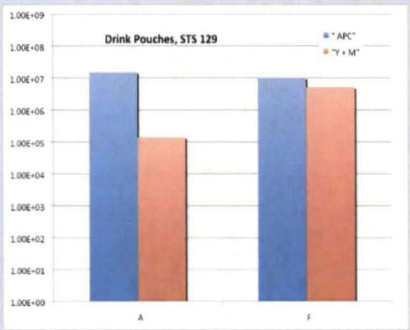


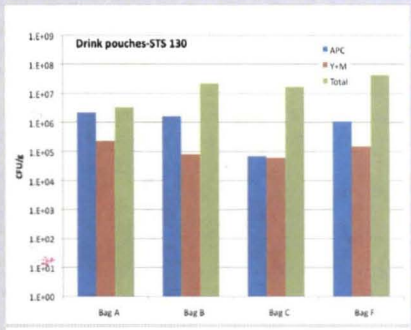


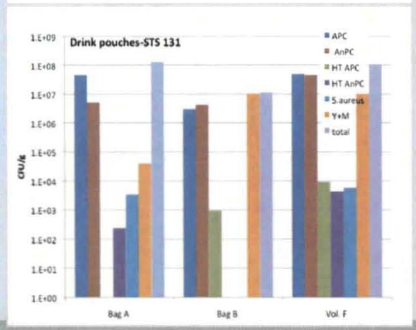
Drink pouches, STS 131

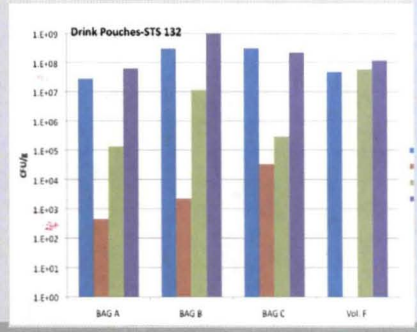


Drink containers

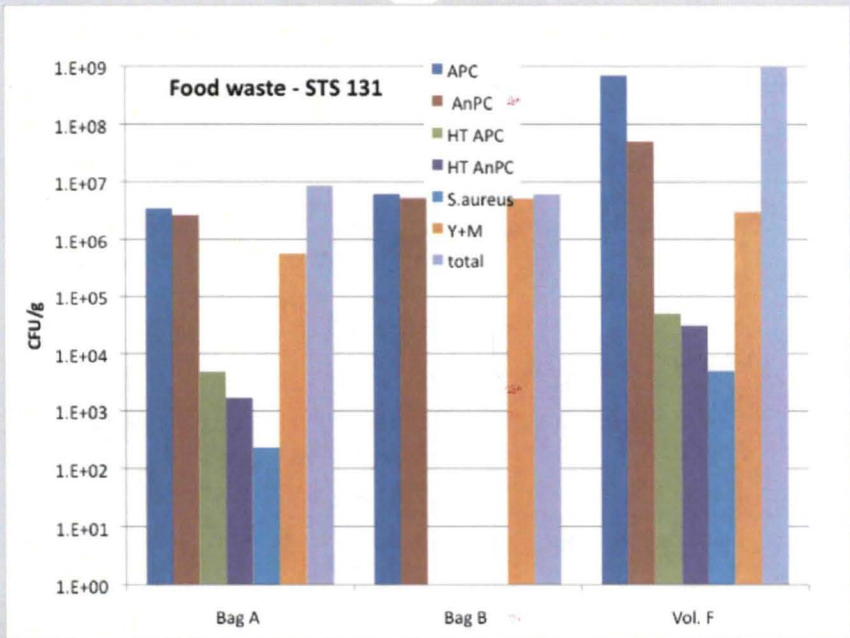




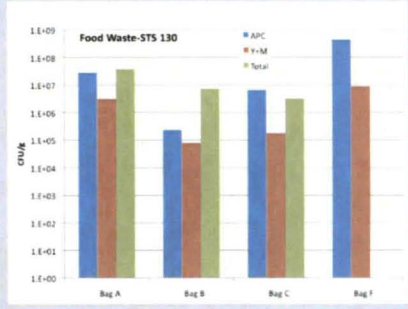


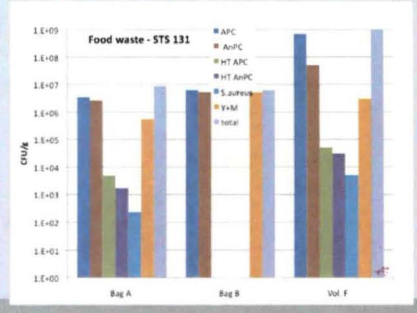


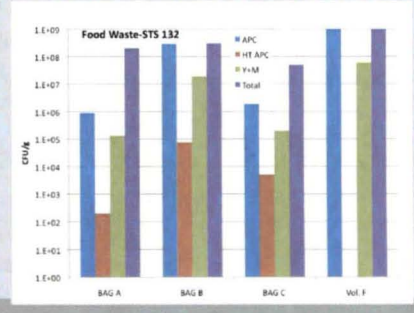
Food waste, STS 131



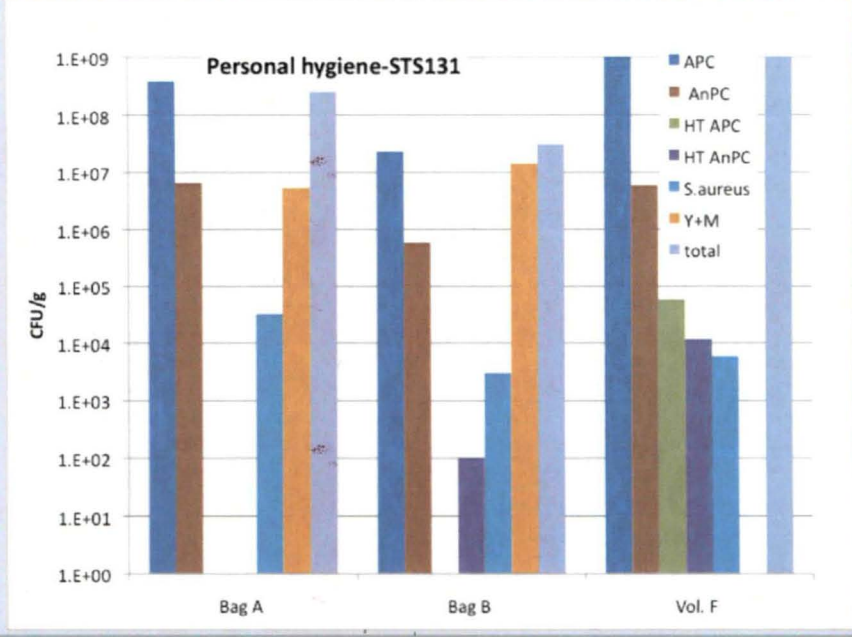
Food packaging and waste

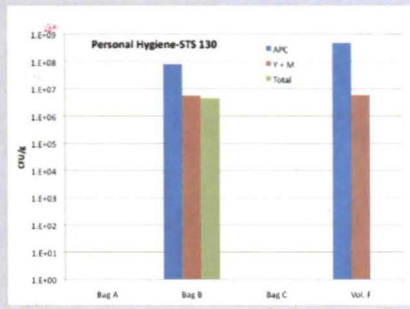


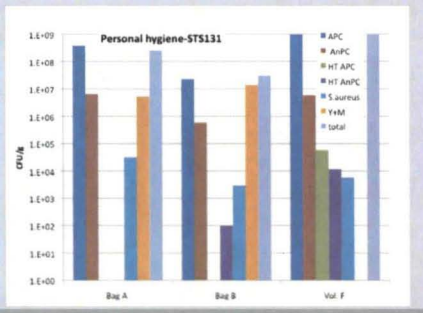


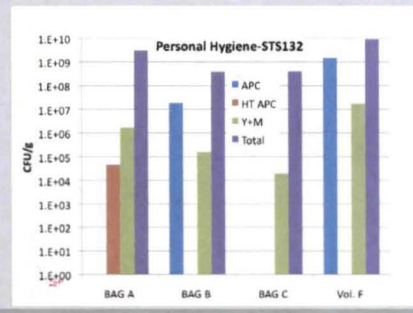


Personal hygiene waste, STS 131









Identified bacteria isolated from STS trash

Table 2. Identified bacterial isolates from trash returned on STS 129-132.

Trash source	STS 129	STS 130	STS 131	STS 132
Personal Hygiene wastes	*Staphylococcus aureus, Bacillus subtilis ss subtilis, Staphylococcus sp Enterobacter aerogenes*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus epidermidis Bacillus subtilis ss subtilis*	*Curtobacterium spp Sphingomonas sanquinis Enterobacter pyrinus*
Food wastes	*Bacillus spp.*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus saprophyticus*	*Bacillus pumilus Sphingomonas sanquinis*
Drink pouches	*Bacillus subtilis ss subtilis*	*Enterococcus pseudoavium Burkholderia cepacia*	*Staphylococcus aureus*	*Enterobacter pyrinus Citrobacter spp Sphingomonas sanquinis Burkholderia multivorans Enterobacter pyrinus*
External trash bag surfaces	*Bacillus amyloliquifaciens Bacillus pumilus*	*Microbacterium marytipicum Bacillus amyloliquifaciens*	*Paenibacillus pabuli*	*Bacillus amyloliquifaciens Burkholderia pyrrocinia*
Internal trash bag surfaces	*Bacillus subtilis ss subtilis*	Isolates were not identified	*Bacillus subtilis ss subtilis,*	Isolates were not identified
MAGS/elbow pack contents	*E. coli, Citrobacter murliniae*	No sample	No sample	*Shigella flexneri*

Fungal ID's, various trash samples

Fungal genera Ided	Common inhabitant of:
• *Rhodotorula*	Soil, water, air
• *Fusarium*	Soil
• *Cladosporium*	Indoor/outdoor mold, plants
• *Cryptococcus*	Soil, majority not harmful to humans
• *Aspergillus*	Common, starchy foods
• *Penicillium*	Ubiquitous soil fungus
• *Candida*	

Conclusions

- The microflora of the Volume F wet trash returned on four recent US Space Shuttle missions were characterized.
- STS trash wastes have an abundance of easily biodegraded compounds that can support the growth of microorganisms and the research presented here shows that large numbers of bacteria and fungi have taken advantage of this readily available nutrient source to proliferate.

Recommendations

- If trash storage is the only 'treatment' option
  - Food wastes be placed immediately into storage and seal containers to prevent crew exposure to pathogens.
- A better treatment option -- limit microbial growth
  - Immediate dehydration of food, or other, wastes
  - Immediate sterilization of these wastes.
- Use these results to determine requirements and criteria for NASA Waste Management Systems.
- Use methods and data to provide a basis for testing waste treatment technologies for the ability to limit contaminant survival, growth and proliferation.

Acknowledgments

- This research was supported by NASA Life Support and Habitation Systems (ne: Exploration Life Suport) project (JSC) through the Waste Management Systems element (ARC)

Characterization of Volume F trash from four recent STS missions: microbial occurrence, numbers, and identifications

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The fate of space-generated solid wastes, including trash, for future missions is under consideration by NASA. Several potential treatment options are under active technology development. Potential fates for space-generated solid wastes: Storage without treatment; storage after treatment(s) including volume reduction, water recovery, sterilization, and recovery plus recycling of waste materials. For this study, a microbial characterization was made on trash returned from four recent STS missions. The material analyzed were 'Volume F' trash and other bags of accompanying trash. This is the second of two submitted papers on these wastes. This first one covered trash content, weight and water content. Upon receipt, usually within 2 days of landing, trash contents were catalogued and placed into categories: drink containers, food waste, personal hygiene items, and packaging materials, i.e., plastic film and duct tape. Microbial counts were obtained with cultivatable counts on agar media and Acridine Orange direct counts (AODC). Trash bag surfaces, 25 cm^2, were also sampled. Direct counts were ~ 1 x 10^6 microbes cm^{-2} and cultivatable counts ranged from 1 x 10^1 to 1 x 10^4 microbes cm^{-2}. Aerobic microbes, aerobic sporeformers, and yeasts plus molds were common for all four missions. Waste items from each category were placed into sterile ziplock bags and 1.5 L sterile de-ionized water added. These were then dispersed by hand shaking for 2 min. prior to inoculation of count media or determining AODC. In general, cultivatable microbes were found in drinks, food wastes, and personal hygiene items. Direct counts were usually higher than cultivatable counts. Some pathogens were found: *Staphylococcus auerus* and *Escherichia coli* (fecal wastes). Count ranges: drink pouches – AODC 2 x 10^6 to 1 x 10^8 g_{fw}^{-1}; cultivatable counts variable between missions; food wastes: Direct counts were close to aerobic plate counts. Counts ranged from 10^6 to 10^9 per g_{fw}. Identities of isolates from cultivation media were obtained using a Biolog Microbial ID System or microSEQ molecular ID methodology using an ABI3130 gene analyzer.

Nomenclature

APC = Aerobic plate count or the number of bacterial colonies counted on agar plates incubated aerobically times a dilution factor and calculated per unit of sample.
AnPC = Anaerobic plate count or the number of bacterial colonies counted on agar plates incubated anaerobically times a dilution factor and calculated per unit of sample.
CFU = Colony forming units
EPC = Estimated plate count. Based on plates with a "to numerous to count" result.
HT APC = Heat treated Aerobic plate count or the number of bacterial colonies counted on agar plates incubated

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aerobically times a dilution factor and calculated per unit of sample from sample diluent that is subjected to heat treatment of 85°C for 15 minutes thereby selecting for heat resistant spore forming bacteria.

HT AnPC = Heat treated Anaerobic plate count or the number of bacterial colonies counted on agar plates incubated anaerobically times a dilution factor and calculated per unit of sample from sample diluent that is subjected to heat treatment of 85°C for 15 minutes thereby selecting for heat resistant spore forming bacteria that grow anaerobically.

Y + M = Yeast and mold colonies counted on inhibitory mold agar.

WMS = Waste Management Systems.

I. Introduction

THE Waste Management Systems (WMS) element of the Life Support and Habitation Systems program is responsible for the development of technologies and approaches to manage the numerous types of solid waste materials generated in future human space flight. Currently, STS and ISS utilize simple waste management methods, where trash is stored, and either burned during Earth reentry (Russian Progress vehicles) or returned to Earth (STS). Future long-duration missions will require more sophisticated methods for in-situ processing, storage and disposal of wastes. The WMS element is therefore engaged in designing, developing and testing technologies that: ensure the protection of the health and well-being of the crew; optimize waste storage volume; minimize crew handling; recover resources; and meet planetary protection guidelines.

WMS has a number of solid waste treatment technologies that are, or have been, under development. The goals of these treatments are to (1) reduce the volume of the waste because storage space is very limited on space vehicles, (2) the remove and recover water because many wastes contain water and easily biodegraded organic compounds from food wastes and crew feces, (3) stabilize and make wastes safe for the crew and harmless to the environment, (4) contain waste to isolate it from the crew and the rest of the world, and dispose of the contained waste, and (5) process the waste for reuse of resources within the stored waste. Because a major reason behind goals (2), (3), and (4) are to eliminate hazards to crew caused by the presence of pathogenic or otherwise deleterious microorganisms in solid wastes, our efforts at KSC have been to provide support to WMS process technologies that have been designed to eliminate microbiological hazards. These technologies have been selected because they either remove and recover water, which microbes need to survive and grow, or they sterilize the solid waste, usually though heat.

The role of the WMS microbiological support projects at KSC have been to characterize the microflora present in space-generated solid wastes such as food wastes, crew fecal wastes, and other wet organic wastes. These wastes typically contain easily biodegraded organic compounds that support microbial growth and proliferation. If solid wastes remain untreated or unprocessed and are then placed into storage, over time the labile organic components in the waste will likely be responsible for both microbial proliferation and microbial byproduct production of noxious odors.

Two studies at KSC in FY07[1] and FY08 (unpublished), respectively, have examined the microbial characterization of food wastes in simulated space mission trash, i.e., for a Lunar Base. In the KSC-WMS FY07 project, food wastes were inoculated with material from volunteer / donor mouth scrubbing. Body wipes, in lieu of a shower, from volunteer donors, disinfectant and wet wipes of facility urinals and commodes at the Space Life Sciences Laboratory (SLSL) at KSC, and dry wipes of SLSL laboratory tabletop surfaces were also added to the simulated waste after placing the wipes into a ziplock bags, which were then sealed. At the time, it was felt that these inocula would 'simulate' crew handling of food wastes and exposure of wastes to other space habitats components.. However, the results of the FY07 study[1] indicated that few human pathogens were present in the wastes, thus, the inocula might not be very representative. During these studies, access to the wet waste from the Volume F trash returned on each STS mission was available, but resources were to process these wastes for microbiological studies were not. This all changed this past year as both access and resources could be used.

The primary goal of the WMS microbiological support projects at KSC for the results reported in this paper was microbial characterization of the STS Volume F trash. However, the opportunity to characterize, or survey, the contents of the trash was also carried out in relation to total wet weights, water content, plastic film content, and to photodocument the trash contents. A companion paper reports the findings on this physical characterization of the Volume F, and other, wet trash from four recent shuttle missions (reference not yet determined by AIAA). This present paper reports our results of the microbiological characterization of this same trash from STS 129 – 132 missions.

II. Materials and Methods

A. Approach

Voume F wet trash and other large ziplock plastic bags, which also contained trash items, are generated on each STS mission, whether to the International Space Station (ISS) or not. As noted by Kish, et al.[1], waste storage aboard the orbiter consists of the Volume F compartment for wet treash and includes mealtime wastes such as leftover food and drink and the associated food packaging, personal hygiene articles, toilet wipes (termed "elbow packs" because of their shape), and Maximum Adsorption Garments (MAGs) worn by the crew during launch and extravehicular activities (EVA). The Volume F trash from four recent STS missions were available for this report and mission specifics are shown in Table 1.

Table 1. Mission information for Shuttle Volume F trash characterized in this study. Each mission had 3 EVAs / space walks with 2 crew members per EVA.

Shttle Mission	Crew Size,	Launch Date	Landing Date	Mission duration
STS 129	6	16-Nov-09	27-Nov-09	10 days, 19 hours, 16 minutes, 13 seconds
STS 130	6	08-Feb-10	21-Feb-10	13 days, 18 hours, 6 minutes, 24 seconds
STS 131	7	05-Apr-10	20-Apr-10	15 days, 2 hours, 47 minutes, 10 seconds
STS 132	6	14-May-10	26-May-10	11 days, 18 hours, 29 minutes, 9 seconds

B. Sequence of sampling events for each shuttle landing at KSC

Upon notification by shuttle personnel, the Volume F trash 'package' was picked up from landing support personnel. Total weight of the entire Volume F trash was determined. As the Volume F trash bag was opened, the contents were catalogued and each item was placed into an appropriate category or subcategory. The broad categories were determined during the cataloging of the first Volume F trash sample received. On subsequent missions, the list was modified as needed. Attempts were made to determine the amount/weight of plastic film in the total trash. Wet weights of each trash category were determined, and a subsample was weighed, dried (70°C until dry) , and weighed again to determine the amount of water in each category. Aseptically obtained a representative subsample of each category of waste likely to contain microorganisms. Microbiological analyses were performed on these aseptically obtained subsamples.

C. Sample preparation.

Waste items that were to be sampled for microbiological analyses were placed into sterile gallon ziplock bags and 1.5 L of sterile deionized water was added. The bag contents and water were mixed / shaken by hand for 2 minutes (10^{-2} dilution) and a 10-fold dilution series was prepared from the trash-water mixture. These dilutions were then used to obtain: acridine orange direct counts, numbers of cultivatable total aerobic and anaerobic bacteria, cultivatable gram positive spore forming bacteria, selected cultivatable bacteria (*Staphylococcus aureus,Coliforms and Escherichia coli*); and cultivatable fungi (yeast and molds).

D. Microbiological analyses

1. Microbial Load via Acridine Orange Direct Count (AODC).

0.5 ml 0.2µm-filtered 37% formalin was added per 9.5 ml of blended, diluted trash-DI sample to fix cells for the AODC protocol. NOTE: Formalin-fixed samples were stored @ 4°C for up to two weeks in a 4°C refrigerator until filtered for enumeration. Formalin-fixed samples were sonicated, diluted into 0.2µm-filtered dI-H2O, stained with Acridine Orange, and filtered onto 25-mm (diameter), 0.2µm (pore size) black polycarbonate filters for enumeration. Some samples required serial dilution to 10^{-1} - 10^{-3} for accurate enumeration.

2. Cultivatable total aerobic and anaerobic bacteria.

A dilution series of blended, trash-DI samples was plated onto R2A agar which was incubated aerobically and anaerobically (AnaeroPack System, Mitsubishi Gas Chemical Co. Tokyo, Japan) at 30°C for 48 hours before enumeration.

3. Cultivatable gram positive spore forming bacteria.

Counts of gram positive spore forming bacteria were determined after heat shock treatment (80 °C for 15 minutes) of the blended, trash-DI samples and this treated dilution was plated onto R2A agar (incubated aerobically and anaerobically) at 30°C for 48 hours before enumeration.

4. Selected cultivatable bacteria.

Samples were screened for potential pathogens by plating blended samples on selective media, S. aureus was isolated on Mannitol Salt agar(MSA) (Difco) and Staph Express petri film (3M) and E. coli and coliforms on E. coli/ coliform petrifilm (3M) ..

5. Cultivatable fungi.

Yeast and fungal counts that occurred in the blended trash-DI samples were obtained using Inhibitory Mold Agar (IMA)(Difco) .

6. Bacterial and Fungal isolate identification

All colonies that grew on any of the media where further isolated and identified by either the Biolog micro ID system by the inoculation of pure cultures into GEN III plates (bacteria identification), or the plates specific for yeast and filamentous fungi per manufacturers instructions. For isolates that were not identified by the Biolog, additional ID tests were run using the MicroSeq® D2 LSU rDNA Fungal Sequencing and the MicroSeq® 500 16s rDNA Bacterial Sequencing identification kits (ABI) following the manufacturer's recommended protocol. For these IDs, DNA was isolated from cultivated microbes using the PrepMan™ Ultra Sample Preparation Reagent (ABI) and diluted 1:100. The PCR Module from the kit used approximately 25 ng of genomic DNA on the BioRad C1000 thermocycler. The PCR thermocycling conditions were: 95°C for 10 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds, and finished with 72°C for 10 minutes. PCR product was run on a SYBR Safe (Invitrogen) 2% agarose gel (Sigma) with the Benchtop pGEM® DNA markers (Promega) and visualized for quality and size. 5 µL of the PCR product was then purified with 2 µL of ExoSAP-IT® (USB) in duplicate. The 7µL of purified sample was then processed through the sequencing module of the kit. The cycle sequencing thermocycling conditions were: 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The excess dye terminators and primers were removed the cycle sequencing reaction with the DyeEx 2.0 Spin Kit (Qiagen). 7.5 µL of Hi-Di™ Formamide (ABI) was loaded with 7.5 µL of the purified product on the ABI 3130 Genetic Analyzer. Results were analyzed with the MicroSeq Analysis Software (ABI).

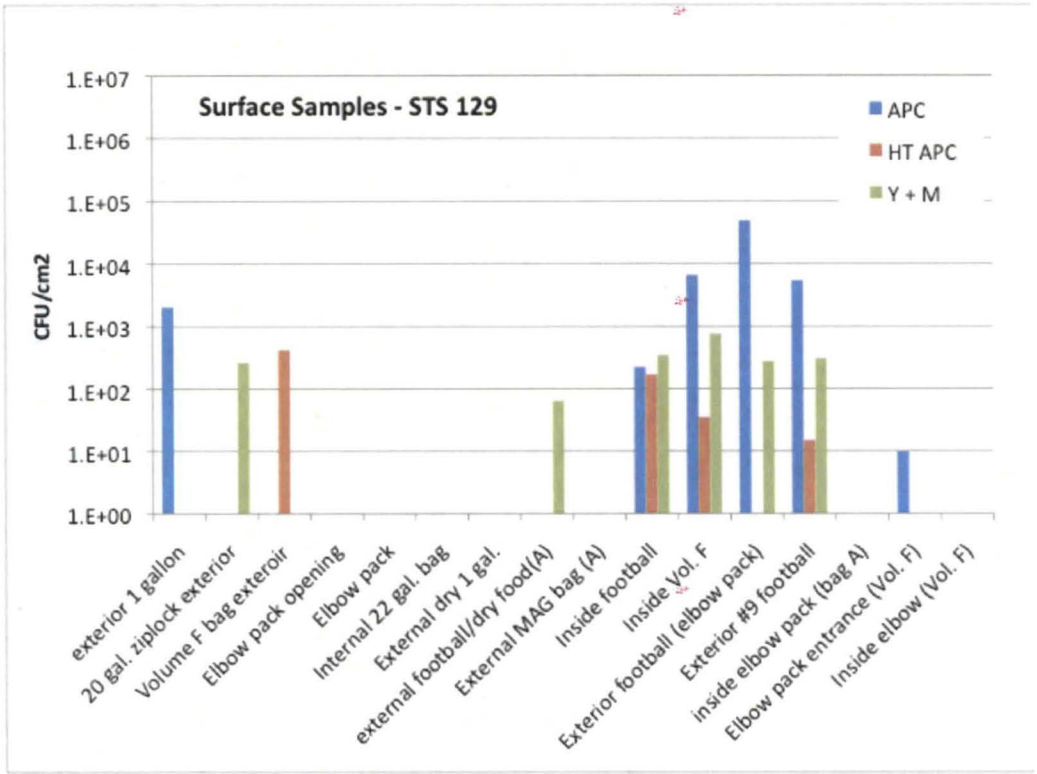
III. Results and Discussion

A. Surface sampling.

For each STS mission, a variety of surfaces, including the interior and exterior surfaces of the bags containing trash, were sampled using sterile sampling sponges (Biotrace International, www.biotrace.com) wetted with sterile phosphate buffered saline (PBS). 100 cm^2 total surface area was sampled per item by swabbing four separate areas using a sterile 25 cm^2 metal template and the same sampling sponge. Sampling sponges were then placed into sterile sample bags and stored at 4 °C until microbiological analysis. The day following acquisition of surface samples, 50 mL of sterile PBS was added to each sample bag. Bags were shaken vigorously for two minutes before plating the diluted surface sample on various agar media.

Cultivatable microbes were present in a number of these surface samples (Figs 1a and 1b). Acridine Orange direct counts (AODC) of the diluted surface samples are also shown on these graphs. The number of surfaces sampled for each mission and the number that were positive for cultivatable microbes were: STS 129, 16 surfaces, 9 positive; STS 130, 14 surfaces, 12 positive; STS 131, 11 surfaces, 9 positive; and STS 132, 14 surfaces with only one positive for growth of cultivatable microbes. The direct count method showed a variety of microbial cellular morphologies were present on each surface. AODC were close to 1 x 10^6 microbes cm^{-2} sampled. The counts of cultivatable microbes ranged between ~1 x 10^1 and ~1 x 10^4 per cm^2. This difference between direct counts and cultivatable counts is a common occurrence in environmental samples. A curious microscopic observation was that small yeast-like cells, ~ 4 µm in diameter, were often observed. This was not backed up by the cultivatable counts of yeast and molds on the IMA medium.

The occurrence of aerobic heterotrophs on plate count agar (APC, on R2A medium) was the most common among cultivatable microbes across different STS mission trash samples. The near absence of cultivatable microbes on the trash bag surfaces of STS 132 is hard to explain, given that the trash from the other three missions had a high number of positives for the surfaces that were sampled. Another interesting finding is the occurrence of a larger variety of cultivatable microbes on STS 131 trash surfaces. Aerobic plate counts (APC), heat-treated aerobic plate counts (HT-APC), which looks for aerobic spore-forming bacteria, and yeast + molds (Y + M) were common for all



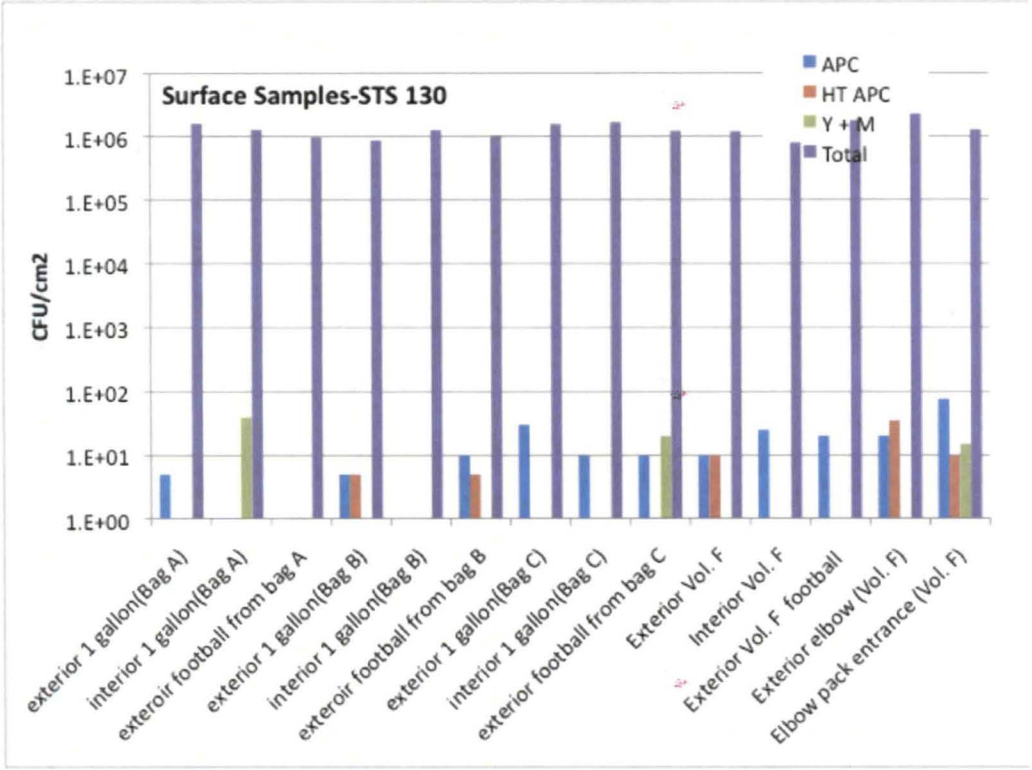


Figure 1a. Bar graphs of the number of microorganisms found on the internal and external surfaces of plastic-film bags in the trash of two Shuttle missions, STS 129 and STS 130. Shown are direct counts (Acridine Orange fluorescent stain direct counts) and cultivatable counts on a variety of media.

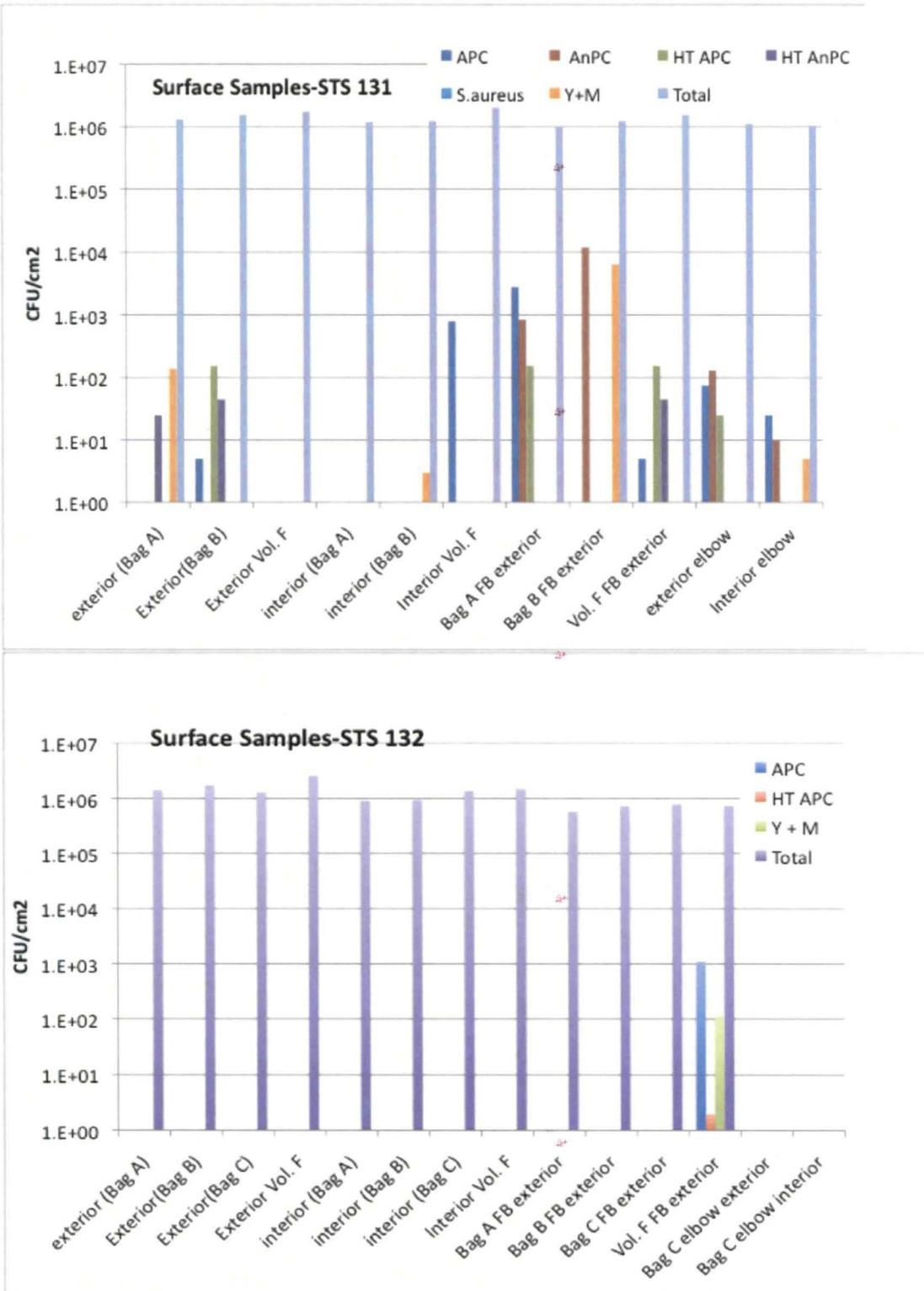


Figure 1b. Bar graphs of the number of microorganisms found on the internal and external surfaces of plastic-film bags in the trash of two Shuttle missions, STS 131 and STS 132. Shown are direct counts (Acridine Orange fluorescent stain direct counts) and cultivatable counts on a variety of media.

four missions. However, only STS 132 showed cultivatable counts for anaerobic place counts (AnPC), heat-treated anaerobic plate counts (HT-AnPC), and *Staphylococcus aureus* (on Mannitol Salts Agar).

To our knowledge, this is the only microbiological characterization of exterior and exterior surfaces of space wet trash. Kish, et al.[1] sampled individual trash items in STS 105 Volume F trash including food (mushroom soup and shrimp cocktail), plastic (duct tape and plastic packaging), drink straws, toilet wipes, and the bulk liquid that accumulated in the bottom of the main Volume F bag. For STS 108 only the bulk liquid was sampled for microbial analyses. In a follow up study to this one, Peterson, et al.[2] reported counts for STS 109 and 110 during a storage study, but again they sampled only the bulk liquid. In their defense, the main purpose of this study was to monitor the volatile organic compounds that came off of the trash during a 9 month post-landing storage period

B. Microbial Characterization of Trash Content According to Source: Trash Category over 4 different STS missions.

The trash was divided into different categories to examine the differences in microbial content for different types of trash. The categories of microbiological interest were: drink pouches (i.e., containers) – Fig. 2, food wastes – Fig. 3, and personal hygiene wastes – Fig. 4. Each figure is organized the same, with cultivatable microbes and, where data exist, direct counts for STS 129 (upper left), STS 130 (upper right), STS 131 (lower left) and STS 132 (lower right). Another data division is the trash source bag for each mission: STS 129 – Bag A and Volume F trash; STS 130 – Bags A, B, and C and the Volume F bag; STS 131 – Bags A and B and the Volume F bag; and STS 132 – Bags A, B, and C and the Volume F bag. Also note that if no counts were obtained with a particular medium, then those results are left off of the figures, even though all that can be said is that the numbers were below the detection limit, which usually was 10 per mL of diluted sample.

1. Drink pouches (Fig. 2)

Total direct counts of drink pouches were not done for STS 129 samples but were added for the other three missions. For the drink pouches these counts mostly ranged between 10^7 and 10^8 per g(wet weight) for all trash bags except Bag A for STS 130 where it was ~ 2 x 10^6 g-1. Drink pouches for all four STS missions had cultivatable aerobic plate counts (APC) and counts of yeast plus molds (Y + M). Hetertrophic aerobic bacteria, APC counts, were lowest for STS 130 and highest for STS 132. Yeast and mold counts were mostly lower than APC counts. Drink pouches from STS 131 were the only ones that had cultivatable counts of anaerobic and spore forming bacteria (both aerobic and anaerobic) and of Staphylococcus aureus. The S. aureus counts were obtained on selective media, Mannitol Salts Agar, that was used to detect the presence of this microorganism. Very low counts of aerobic spore formers were also detected in / on STS 132 drink pouches. Many drinks contain sugars, which provide energy and growth sources for many different bacteria, so the detection of viable cells on these containers was expected. The presence of *S. aureus* may be bothersome, but this bacterium can be found in / on many environments, including human skin.

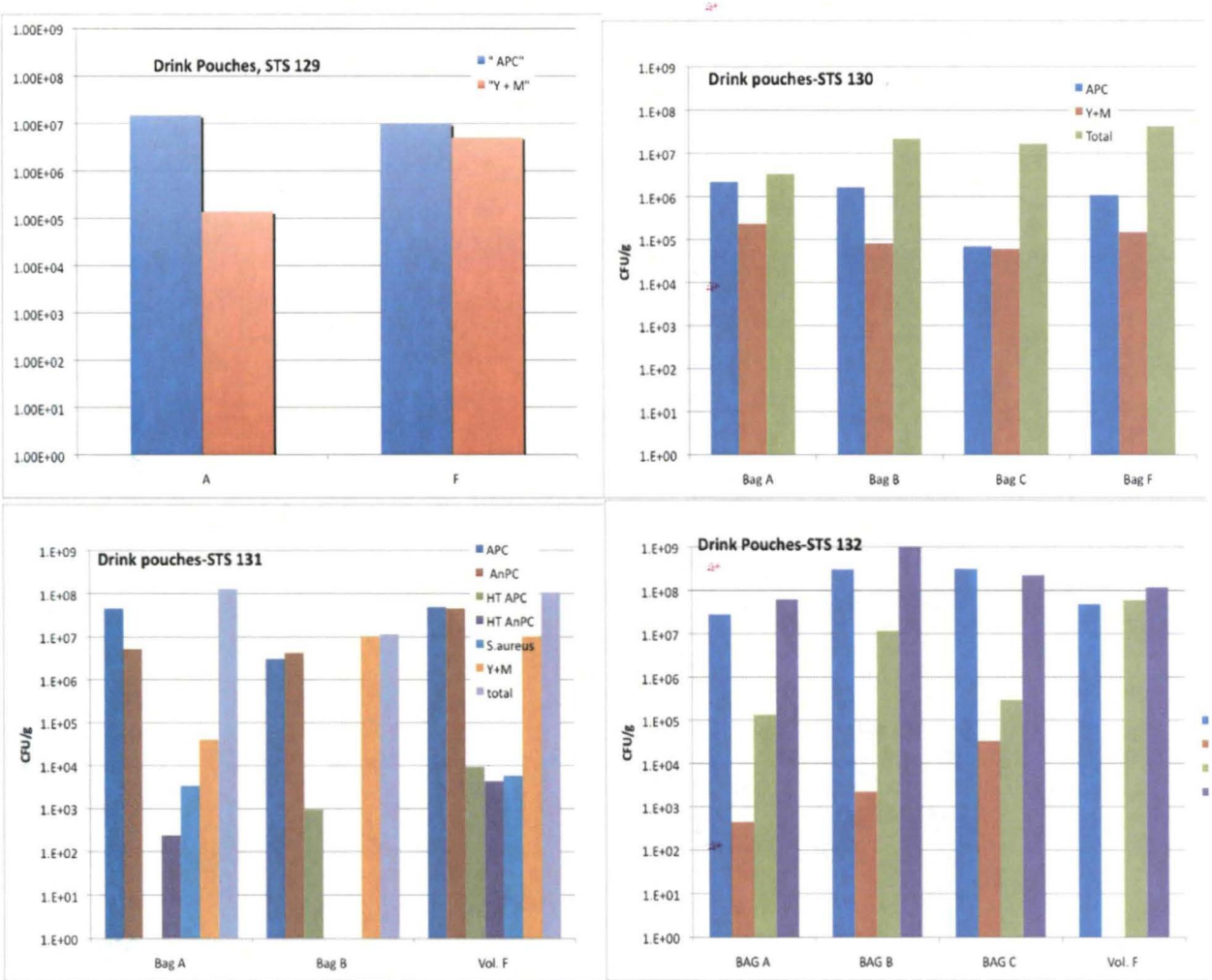


Figure 2. Direct total counts and cultivatable counts of microorganisms in or on drink pouches returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

2. Food wastes, including food containers (Fig. 3)

Because FY07 and FY08 projects at KSC examined the fate, including growth and proliferation, of microbes on simulated space mission food wastes, our main interest in characterization of STS trash was the food wastes. Figure 3 shows the numbers of microbes, both cultivatable and direct counts, in the food trash of the four STS missions. In general, the direct counts (not done for STS 129) were closer to aerobic plate counts than for the drink pouches. The reason for this could be the availability and amount of readily biodegradable compounds in the food when compared with the drinks. The range of direct counts were somewhat different for STS 130, between 10^6 and 10^7 per g(wet weight), than for STS 131, between 10^7 and 10^9 per g(wet weight), and STS 132, between 10^6 and 10^9 per g(wet weight).

For the cultivatable plate counts, both APC (hetertrophic aerobic plate counts) and Y + M (yeast plus molds) were present in all missions and all trash bags A – F. Again, in general, heterotroph counts were greater than yeasts and molds. And, once again, the cultivatable counts for STS 131 showed up on more test media than for the other three missions. Although the counts were low, ~ 10^2 and 10^4 , respectively, for Bag A and Volume F, *S. aureus* was detected in STS 131 food wastes. In addition, both aerobic and anaerobic spore-forming bacteria were found in cultivatable counts in Bag A and Volume F trash for STS 131 and aerobic spore-formers in all but Volume F bags from STS 132.

For comparison, two food items were sampled by Kish et al.[1] for STS 105 Volume F trash. The mushroom soup had ~ 5 x 10^8 total counts (AODC), 1 x 10^5 cells g(dry weight)$^{-1}$ aerobic microbes and ~ 5 x 10^5 cells g(dry weight)$^{-1}$ anaerobic cells. The shrimp cocktail from STS 105 had higher numbers at 1 x 10^9 total counts (AODC), 4 x 10^6 cells g(dry weight)$^{-1}$ aerobic microbes and 1 x 10^6 cells g(dry weight)$^{-1}$ anaerobic cells.

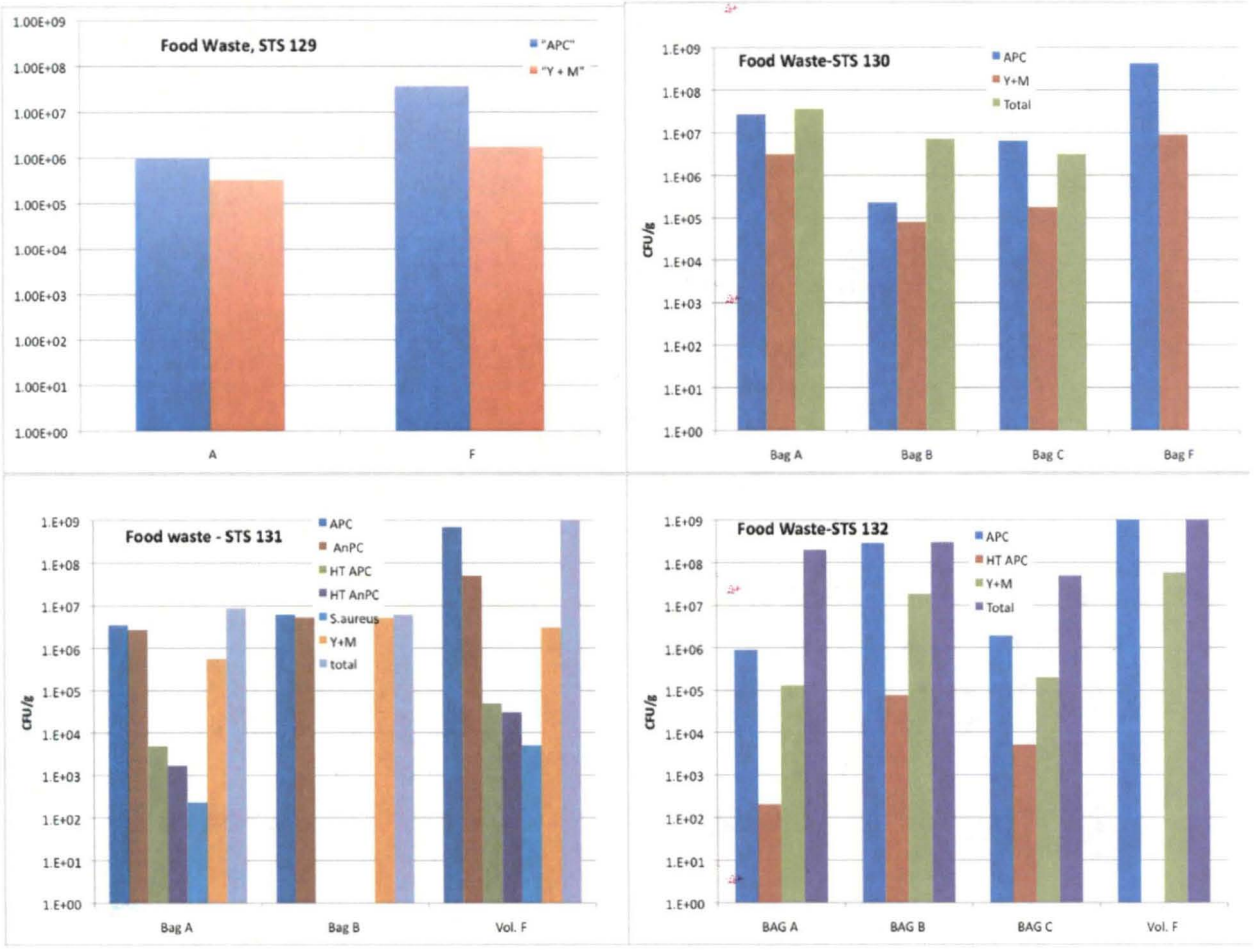


Figure 3. Direct total counts and cultivatable counts of microorganisms in food waste trash or on food containers returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

3. Personal hygiene wastes (Fig. 4)

The category of personal hygiene includes wipes, meal napkins, etc. that were found in the waste and that didn't clearly belong to drink pouches or food waste. Although the MAGs and Elbow packs could be included in this category, adequate samples from the MAGS were difficult to obtain because the more diluent that was added, the more that was absorbed. The Elbow packs were clearly toilet wastes and the several that were sampled early in the study did not yield very high counts. Both MAGs and Elbow packs were contained in separate, duct-tape wrapped football packages to keep the contents well isolated from the other trash. As the likelihood that these footballs would ever be knowingly opened would be low, it was felt a more prudent approach would be to focus attention on the other waste categories.

As with the food waste and drink pouch categories that were sampled for microbial characterization, personal hygiene wastes also contained aerobic heterotrophs (APC) and yeast and molds (Y + M) (Fig. 4). Of note, however, is the absence of any cultivatable microbes in Bags A and C from STS 130. Direct counts, when present, were high, However, direct counts are missing for a number of the samples because fine, small, non-staining particulates were present in high numbers and made direct counting of cells difficult or impossible for these samples. When samples were countable, direct counts ranged from 10^7 to 10^{10} per g. Counts of heterotrophs, APC, ranged between absent to

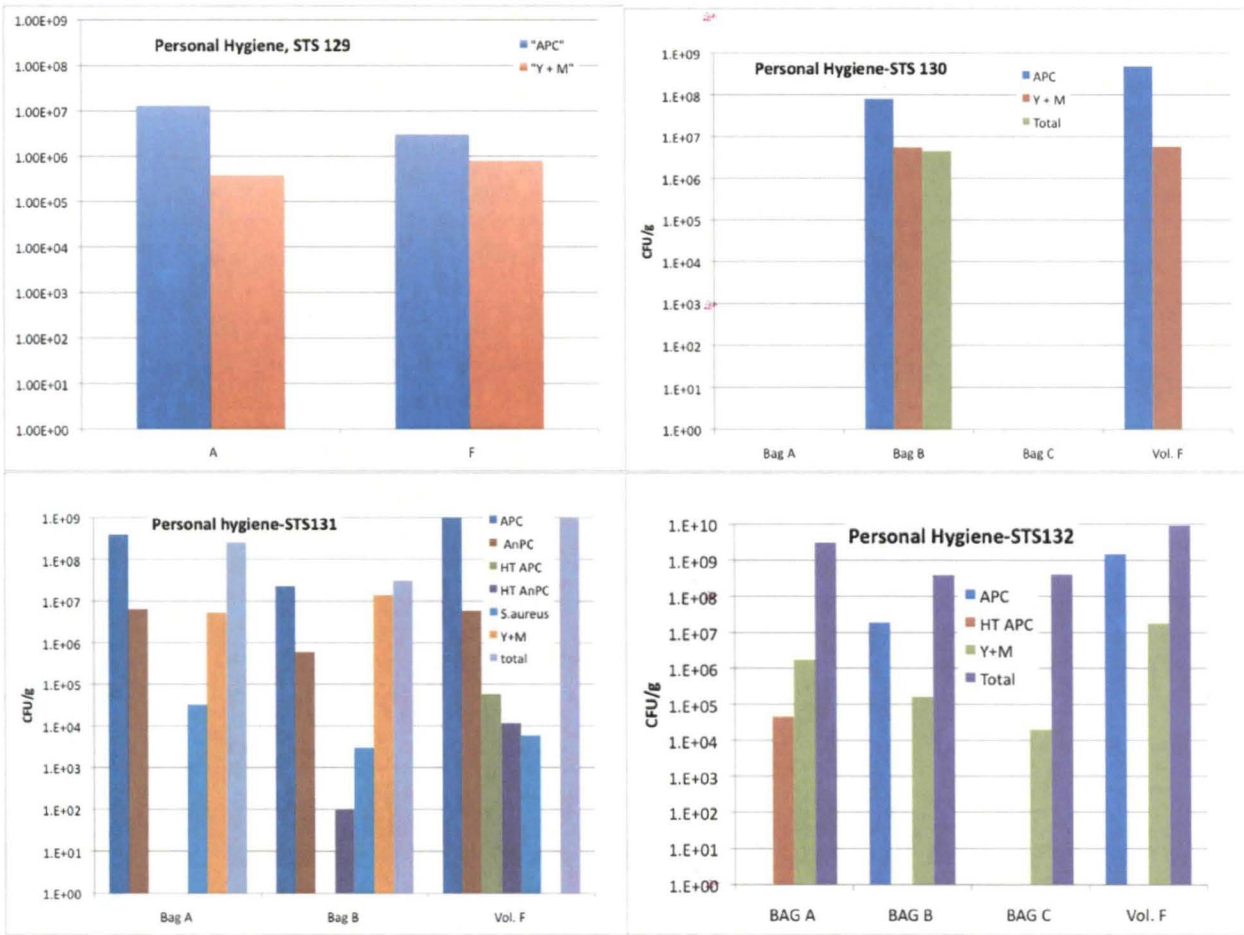


Figure 4. Direct total counts and cultivatable counts of microorganisms in personal hygiene wastes returned on STS flights 129, 130, 131, and 132. Besides the main trash source, Volume F trash, additional plastic bags of trash were also sampled (labeled A, B, or C).

10^7 to 10^9 per g. The cultivatable counts for STS 131 again showed the presence of *S. aureus* and anaerobic spore-formers which were not present in STS 129, 130 or 132. Aerobic spore formers were present in STS 130 and STS 132 personal hygiene wastes.

By comparison, the results of Kish et al. for toilet wipes, presumably from elbow packs, were ~ 6 x 10^8 cells g(dry weight)$^{-1}$ for total counts and 1 x 10^8 cells g(dry weight)$^{-1}$ for both aerobic and anaerobic microbes.

4. *Identification of microorganisms obtained from cultivatable plate count media from STS missions and different waste categories. (Tables 2 and 3)*

The bacterial species that were identified by the Biolog ID system or by the MicroSEQ procedure are presented in Table 2. Nearly all of these microbes could be part of a normal human microflora and can be isolated from the environment. Many of the named organisms could be opportunistic pathogens, i.e., a possible pathogen, for immune compromised hosts, such as crew members in a microgravity habitat.

Three known pathogenic bacteria were isolated from STS trash samples. *Staphylococcus aureus* was isolated from personal hygiene waste from STS 129 and STS 131 and from food waste and drink pouches from STS 131. *Escherichia coli* was found in MAG/elbow pack contents from STS 129 and *Shigella flexneri* was identified from isolates obtained from MAG/elbow pack contents of STS 132. None of these could be considered unexpected. *S. aureus* is a common microbe on human skin and the *E. coli* and *Shigella* sp. are enteric bacteria that can be found in human feces. Of interest among the IDs are a number of species of the *Bacillus* genus. These are spore-forming bacteria and most are resistant to desiccation, heat, dehydration, and other stresses that might be used by WMS waste processing systems to limit or eliminate bacteria. An assessment of the survival of *Bacillus* is recommended

Table 2. Identified bacterial isolates from trash returned on STS 129-132.				
Trash source	STS 129	STS 130	STS 131	STS 132
Personal Hygiene wastes	*Staphylococcus aureus, Bacillus subtilis ss subtilis, Staphylococcus sp Enterobacter aerogenes*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus epidermidis Bacillus subtilis ss subtilis*	*Curtobacterium spp Sphingomonas sanquinis Enterobacter pyrinus*
Food wastes	*Bacillus spp.*	*Enterococcus pseudoavium*	*Staphylococcus aureus Staphylococcus saprophyticus*	*Bacillus pumilus Sphingomonas sanquinis*
Drink pouches	*Bacillus subtilis ss subtilis*	*Enterococcus pseudoavium Burkholderia cepacia*	*Staphylococcus aureus*	*Enterobacter pyrinus Citrobacter spp Sphingomonas sanquinis Burkholderia multivorans Enterobacter pyrinus*
External trash bag surfaces	*Bacillus amyloliquifaciens Bacillus pumilus*	*Microbacterium marytipicum Bacillus amyloliquifaciens*	*Paenibacillus pabuli*	*Bacillus amyloliquifaciens Burkholderia pyrrocinia*
Internal trash bag surfaces	*Bacillus subtilis ss subtilis*	Isolates were not identified	*Bacillus subtilis ss subtilis,*	Isolates were not identified
MAGS/elbow pack contents	*E. coli, Citrobacter murliniae*	No sample	No sample	*Shigella flexneri*

by this paper's authors to be one of the criteria used during testing of the effectiveness of candidate solid waste treatment technologies.

Regarding the fungi that were identified from various trash samples (Table 3), nearly all of the identified genera are common environmental inhabitants. These would include *Rhodotorula* (soil, water, air), *Fusarium*[5] (soil), *Cladosporium* (indoor-outdoor mold and on plants), *Aspergillus* (common contaminants of starchy foods), *Cryptococcus* (majority of species live in the soil and are not harmful to humans), and *Penicillium* (ubiquitous soil fungus). *Cryptococcus laurentii* and *Cryptococcus albidus* have been known to occasionally cause moderate-to-severe disease in human patients with compromised immunity[6]. *Candida* are almost universal on normal adult skin and *Candida albicans* is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal, and female genital tracts which cause no disease.

If storage of space-generated trash, such as the Volume F wet trash, is the only 'treatment' option under consideration for future space missions, then the authors recommend that food wastes be placed immediately into storage and the containers immediately sealed to prevent crew exposure to dangerous levels of cross-contaminating pathogens. A better treatment option would be to limit microbial growth through immediate dehydration of food, or other, wastes or immediate sterilization of these wastes. The results reported here can be used to determine requirements and criteria for NASA Waste Management Systems. These methods and resulting data will provide a basis for testing technologies for the ability to limit contaminant survival, growth and proliferation.

IV. Conclusion

The microflora of the Volume F wet trash returned on four recent US Space Shuttle missions have been characterized. STS trash wastes have an abundance of easily biodegraded compounds that can support the growth of microorganisms and the research presented here shows that large numbers of bacteria and fungi have taken advantage of this readily available nutrient source to proliferate.

If storage of these wastes is the only 'treatment' option, then, to prevent crew exposure to dangerous levels of cross-contaminating pathogens, the authors recommend that food wastes be placed immediately into storage and the containers immediately sealed. A better treatment option would be to limit microbial growth through immediate dehydration of food, or other, wastes and/or immediate sterilization of these wastes. The results reported here can be used to determine requirements and criteria for NASA Waste Management Systems. These methods and resulting data will provide a basis for testing technologies for the ability to limit contaminant survival, growth and proliferation.

Acknowledgments

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