

Microbial Characterization Space Solid Wastes Treated with a Heat Melt Compactor

Richard F. Strayer¹, Mary E. Hummerick², Jeffrey T. Richards³, LaShelle E. McCoy⁴, and Michael S. Roberts⁵.
Engineering Services Contract (ESC, Team Qinetiq-NA, Kennedy Space Center, Florida, 32899

and

Raymond M. Wheeler⁶
Surface Systems Research, NE-S, Kennedy Space Center, FL 32899

The on going purpose of the project efforts was to characterize and determine the fate of microorganisms in space-generated solid wastes before and after processing by candidate solid waste processing. For FY11, the candidate technology that was assessed was the Heat Melt Compactor (HMC). The scope included five HMC product disks produced at ARC from either simulated space-generated trash or from actual space trash, Volume F compartment wet waste, returned on STS 130. This project used conventional microbiological methods to detect and enumerate microorganisms in heat melt compaction (HMC) product disks as well as surface swab samples of the HMC hardware before and after operation. In addition, biological indicators were added to the STS trash prior to compaction in order to determine if these spore-forming bacteria could survive the HMC processing conditions, i.e., high temperature (160 C) over a long duration (3 hrs). To ensure that surface dwelling microbes did not contaminate HMC product disk interiors, the disk surfaces were sanitized with 70% alcohol. Microbiological assays were run before and after sanitization and found that sanitization greatly reduced the number of identified isolates but did not totally eliminate them. To characterize the interior of the disks, ten 1.25 cm diameter core samples were aseptically obtained for each disk. These were run through the microbial characterization analyses. Low counts of bacteria, on the order of 5 to 50 per core, were found, indicating that the HMC operating conditions might not be sufficient for waste sterilization. However, the direct counts were 6 to 8 orders of magnitude greater, indicating that the vast majority of microbes present in the wastes were dead or non-cultivable. An additional indication that the HMC was sterilizing the wastes was the results from the added commercial spore test strips to the wastes prior to HMC operation. Nearly all could be recovered from the HMC disks post-operation and all were showed negative growth when run through the manufacturer's protocol, meaning that the 106 or so spores impregnated into the strips were dead. Control test strips, i.e., not exposed to the HMC conditions were all strongly positive. One area of concern is that the identities of isolates from the cultivable counts included several human pathogens, namely *Staphylococcus aureus*. The project reported here provides microbial characterization support to the Waste Management Systems element of the Life Support and Habitation Systems program.

¹ Research Scientist, Sustainable Systems Research, Mail Code ESC-24, Kennedy Space Center, FL 32899.

² Research Scientist, Sustainable Systems Research, Mail Code ESC-24, Kennedy Space Center, FL 32899.

³ Research Scientist, Sustainable Systems Research, Mail Code ESC-24, Kennedy Space Center, FL 32899.

⁴ Research Scientist, Sustainable Systems Research, Mail Code ESC-24, Kennedy Space Center, FL 32899.

⁵ Research Scientist, Sustainable Systems Research, Mail Code ESC-24, Kennedy Space Center, FL 32899.

⁶ Senior Scientist, Surface Systems Division, Mail Code NE-S, Kennedy Space Center, FL 32899.

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 "too numerous to count" result.
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 NASA Waste Management research and development group at ARC is responsible for the development of technologies and approaches to manage the numerous types of waste materials generated in future human space flight. In past years, this group has been an integral element of the Life Support and Habitation Systems Program. Simple waste management methods have been utilized on STS and ISS, 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 Waste Management Systems group has therefore been 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.

A number of solid waste treatment technologies are under development by the Waste Management element. 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.

Untreated or unprocessed solid wastes / trash components contain organic compounds that are easily biodegraded. The action of microbes on these labile solid waste components is largely responsible for both microbial proliferation, including any food-borne pathogens that may be present, and byproduct odor production. In support of the WMS element, KSC is tasked to determine the effects of solid waste treatment technologies on microbial loads in the wastes before and after treatment. This KSC supporting role is related to item (3) in the preceding paragraph: stabilize and make wastes safe for the crew.

The Heat Melt Compactor (HMC) has been a major, recent focus at ARC and, in both FY10 and FY11, was the main solid waste treatment technology that had products available for microbiological characterization. Human space mission wastes typically contain large percentages of plastic contaminated with wet waste. The HMC, or a similar treatment technology called the Plastic Melt Waste Compactor (PMWC) was developed to be a multi-function means of water recovery and volume reduction of plastic rich trash with the potential for waste stabilization and/or sterilization. To date, the key performance parameters (KPPs) for full success for water recovery and volume reduction have been successfully met. The minimum threshold for success in water recovery was 50% and full success was considered 95%. The minimum threshold for success in volume reduction was 50% with full success defined as 90%.

The HMC project is aimed at development of a volume reduction system that has a lower Equivalent System Mass (a sum of weight, power, volume and crew time) than storage systems that store waste in a hand compacted state. The current focus is on compactors that can have application to both short and long duration missions.

Project Objective: Characterize and determine the fate of microorganisms in space-generated solid wastes before, during (if possible), and after treatment a Heat Melt Compactor (HMC).

II. Materials and Methods

Waste Handling and Storage. Due to the ephemeral nature of microbial communities in some types of environmental samples, waste samples were processed as soon after receipt as possible. Upon receipt, the samples were placed into cold storage at 4 °C. As soon as possible, these samples were removed from cold storage, equilibrated to room temperature for 2 hrs, then processed for microbiological and physical/chemical characterization.

A. Microbial Characterization Methods

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

A portion of all samples for microbiological analyses, i.e., HMC surface samples, HMC product disk surface samples, and the initial dilution of HMC core samples were fixed with 0.2µm-filtered 37% formalin to a final formalin concentration of 2%. These formalin-fixed samples were usually processed within two hours after fixation. However, occasionally they had to be stored at 4°C for up to two weeks until filtered for enumeration. Formalin-fixed samples were sonicated, diluted into 0.2µm-filtered de-ionized water, stained with Acridine Orange, and filtered onto 25-mm (diameter), 0.2µm (pore size) black polycarbonate filters for enumeration. Direct counts of filtered samples were determined using a Zeiss Epi-Fluorescent Axioskop microscope at 1000 x magnification. Microscope software used for counting and observing cells was DP Manager, DP Controller, and Image-Pro Express 6.3.

2. *Cultivation-based total aerobic and anaerobic bacteria.*

A dilution series of samples for microbiological analyses were plated onto R2A agar that were incubated either aerobically and/or anaerobically.

3. *Cultivation-based gram positive spore forming bacteria.*

Counts of gram positive spore forming bacteria were determined after heat treatment (80 °C for 10 minutes) of the blended brine samples and plating of this treated dilution onto R2A agar, then incubated either aerobically and anaerobically.

4. *Cultivation-based selected bacteria.*

The Standard Methods membrane filtration technique was used for enumeration of: *Staphylococcus aureus* (Mannitol Salt Agar and *S. aureus* petri film).

5. *Cultivation-based fungi.*

Fungal and yeast counts that occur in the diluted samples were obtained using Inhibitory Mold Agar (IMA) for fungi in general.

6. *Identification of colony isolates found growing on any agar medium.*

Colonies found growing on any media under cultivation based methods were transferred via streak plating onto to fresh media for isolation, allowed to grow, then this pure clone was used to inoculate Biolog plates for bacterial identification.

III. Results

A. FY10 KSC sampling of HMC product disks

In FY10, the KSC microbial characterization support effort was mostly concerned with determining the best method(s) for aseptic sampling of the interior of HMC product disks. Three heat melt compaction (HMC) disks, prepared (in 2008) from simulated trash composites, were shipped to KSC for microbial sample analyses. In addition, one disk composed of actual space shuttle trash was prepared and shipped late in FY10 along with swab surface samples of the HMC hardware and liquid extracted from the shuttle trash during the compaction process.

Using a hand press, core samples of the HMC disk were successfully obtained (Fig. 5) using a pre-sterilized 1.25 diameter metal punch that had been machined in-house to bevel the cutting edge. The bevel machining was needed to cut a clean core out of the ~2 cm thick HMC product disk. In addition, the external surfaces of the HMC disk were sanitized with 70% ethyl alcohol and sampled with sterile swabs, which were then run through the normal battery of microbiological analyses. Surface sanitization, and sampling to show it had worked, were needed to ensure the interior cores were not contaminated by microbes from the exterior surfaces, which had been subject to handling.

Cores and surface samples were processed to obtain: Microbial load via acridine orange direct count (AODC), cultivatable total aerobic and anaerobic bacteria, cultivatable gram positive spore forming bacteria, selected cultivatable gram negative bacteria, cultivatable fungi.

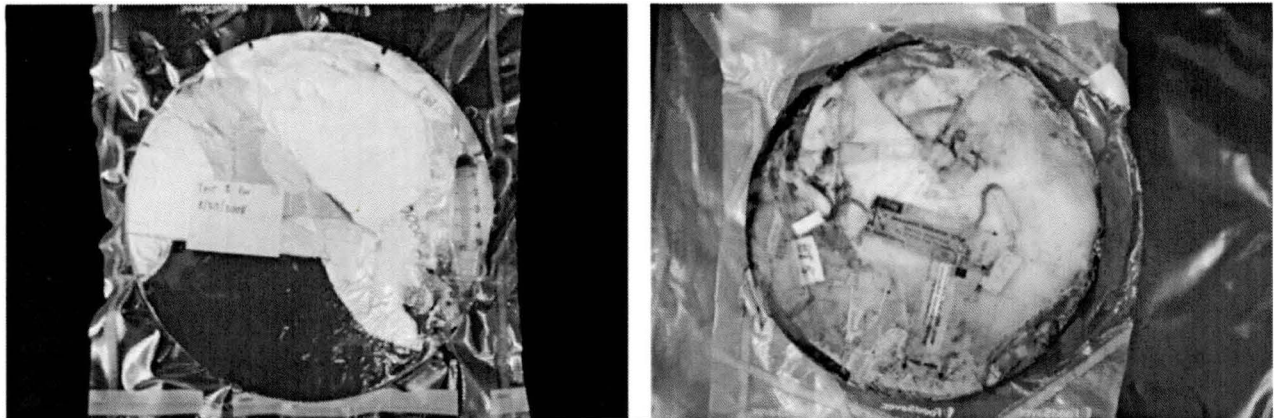


Figure 1. Heat Melt Compaction (HMC) product disks that were part of this FY11 microbial characterization study. Simulated space mission trash was used as the HMC feed material for the top two disks and STS Volume F compartment trash returned on shuttle missions over the past two years was used as the HMC feed material for the bottom three disks.

Densities of cultivable bacteria in the core samples ranged from 100 to 25000 colony-forming units (CFU) per core, while the direct counts indicated much higher numbers, 9.6×10^6 to 3.2×10^8 with a mean of 7.5×10^7 . Only a very small percent of the microbes on the disks were grown and isolated with our culture methods which is a common finding in environmental samples. For those microbes that were cultivatable, identifications were obtained using the Microlog system (Biolog, Hayworth, CA).

Two species of bacteria were isolated from the simulated waste test disks, specifically *Campylobacter curvis/gracialis* and *Bacillus weihenstephanensis*. Five of the core samples taken from the disk composed of actual shuttle trash yielded cultivable bacteria and yeast. *Staphylococcus haemolyticus* is part of normal human skin flora and is considered an opportunistic pathogen i.e. a possible pathogen for immune compromised hosts. The streptococcus isolated is an important commensal organism of the human oral cavity, usually harmless, it can act as an opportunistic pathogen.

ARC scientists and engineers were asked to obtain swab surface samples of the HMC hardware before and after operation using the shuttle trash. The swab samples were returned via overnight express to KSC for microbial analysis. The number of cultivable bacteria and yeast on the HMC surfaces before compaction of the shuttle trash were low, from below the detection limit to 1.2×10^4 cfu cm⁻². Both yeast and bacteria were isolated and identified, all of which are part of normal human microflora or the environment. These same isolates were not found in the shuttle trash disk indicating they were not carried through the compaction process to the final product. Among the IDs were *Bacillus* species, which are spore formers and are more resistant to heat making them more likely to survive the temperatures encountered during the compaction process.

B. HMC product disks included in the FY11 project

For FY11, five HMC product disks were processed for microbial characterization studies. These disks were sent to KSC from scientists and engineers who operate the HMC and are part of the WMS element at ARC. The HMC feed material for two of the disks, labeled Disk 1 and Disk 2 in this paper, was simulated space trash (Hogan, et al.). A photograph of one of these disks is shown on the left in Fig. 1. The feed material for the other three disks, labeled Disk 3, Disk 4, and Disk 4 in this report, was actual space mission solid waste from the STS 130 Volume F compartment trash. This waste had been sent to ARC in FY10 and was characterized with regard to content and microbial analyses. A photograph of one of these disks (Disk 3) is shown on the right in Fig. 1.

1. Surface sampling of HMC product disks to determine if surface sanitization was adequate prior to obtaining internal core samples

Recovery of microorganisms from the interior of any sample is complicated by possible contamination of the interior by exterior dwelling microbes during sample acquisition. In FY10 the problem was addressed by sanitizing the surface of the HMC product disk with 70% ethanol prior to obtaining the sample. After sanitizing the surface, a sterile metal punch was used to cut out core samples from the HMC product disk using a hand press (Fig. 2).

A standard procedure for obtaining microbial surface counts. The surface of the HMC disk was sampled with Sanicult swabs before and after sanitizing and the swab samples were processed for microbial characterization. These results would show the efficacy of the sanitization procedure and indicate if microbes from the exterior

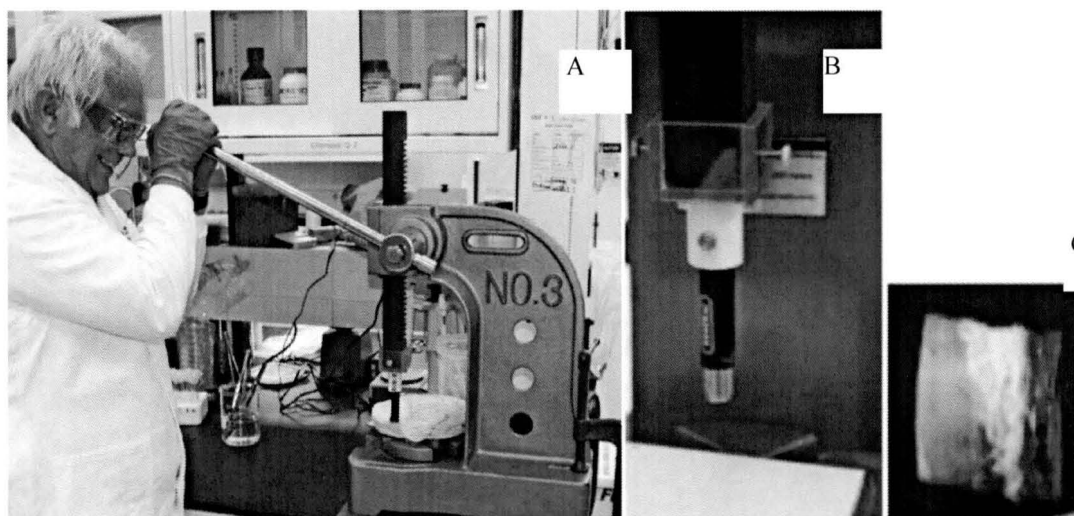


Figure 2. Picture A shows sample procedure using hand press with 1/2 inch hole punch (B) resulting in a core sample (C).

surface might still be present when the HMC core samples were taken. An example of sampling the surface of an HMC disk is shown in the photograph in Figure 3.

Microbial numbers were obtained for the Sanicult swab samples, but they are not useful for the HMC disks made from STS trash. The surface immediately above the spore strips was not sanitized because the effects of 70% ethanol on the spore survival in the test strips was not known. For the standard surface sampling procedure, surfaces are usually normalized to the area sampled, but this area was unknown because as the areas above the spore strips

Table 1. Identities of microbial isolates from HMC disk surface samples

	Bacteria	Fungi
Disk 1*	<i>Micrococcus luteus</i> ATCC=4698, <i>Bacillus niacin</i>	
Before	<i>Staphylococcus hominis</i> ss <i>hominis</i> , <i>Staphylococcus epidermidis</i>	None
Sanitizing		
After	<i>Paenibacillus macerans</i>	<i>Coniothyrium fuckelii</i>
Sanitizing		
Disk 2	<i>Paenibacillus agarexedens</i> , <i>Bacillus simplex</i> , <i>Rummeliibacillus pycnus</i> , <i>Brevibacillus choshinensis</i> , <i>Paenibacillus chitinolyticus</i> , <i>Bacillus alkalitelluris</i> , <i>Paenibacillus chinjuensis</i> , <i>Bacillus circulans</i>	None
Before		
Sanitizing		
After	<i>Bacillus</i> sp	None
Sanitizing		
Disk 3		
Before	<i>Bacillus</i> sp., <i>Staphylococcus epidermidis</i> , <i>Bacillus subtilis</i> ss <i>subtilis</i>	None
Sanitizing		
After	<i>Streptococcus mitis</i> , <i>Streptococcus sanguis</i>	None
Sanitizing		
Disk 4		
Before	<i>Bacillus</i> sp	None
Sanitizing		
After	No Growth	None
Sanitizing		
Disk 5		
Before	No Growth	None
Sanitizing		
After	<i>Bacillus subtilis</i>	None
Sanitizing		

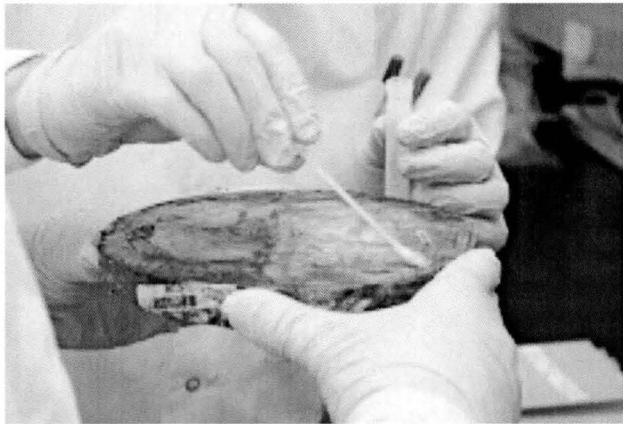


Figure 3. Picture showing the sampling of the surface of an HMC product disk to test for efficacy of surface sanitization with 70% alcohol solution. Surface samples were taken before and after the sanitization procedure.

was not known. Usually the area inside a standard 25 cm² template is swabbed when obtaining surface samples. Counts were obtained for inoculated cultivation media and isolates were obtained and identified (Microlog method, Biolog, Hayward, CA). Results for these IDs for each disk both before and after sanitizing the surface are shown in Table 1.

HMC product disks made from simulated space rash waste, Disk 1 and Disk 2 were made in late FY08 and stored at room temperature until sent to KSC in early FY11. Several additional disks were sent but only these two were sampled because once the disks made fresh from actual space trash were sent, they became the main focus. Prior to surface sanitization, these two disks had a significant number of different bacterial species present on the surfaces. Four different microbial IDs were found in Disk 1 samples and eight different ones on the surface of Disk 2. Sanitization of the surface with 70% ethyl alcohol greatly reduced the number of

identified bacterial isolates to one for each disk. The goal of the sanitization was, of course, zero microbes present after the procedure, but the decrease was significant. The question that remains is whether the post-sanitization surface-dwelling bacteria were also detected in any of the core samples obtained from these disks (see Section 4.4, Table 3).

Also shown in Table 1 are the results of isolate IDs from surface samples from HMC product disks made from actual space solid waste, Volume F compartment trash from STS 131 (Disks 3, 4, and 5 in Table 1). Results of the study show considerably less isolates per disk surface before sanitization than for the older (less freshly made) HMC disks made from simulated solid wastes. Three named bacteria were found on the surfaces of Disk 3, one on Disk 4, and none on Disk 5. Sanitization of the disk surfaces was less than adequate for Disk 3 – two bacteria were still found, good for Disk 4 with no bacteria found, and confusing for Disk 5 where one bacterium was identified after surface sanitization but none were found before. As for Disk 1 and 2 mentioned above, the question that remains is whether the post-sanitization surface-dwelling bacteria were also detected in any of the core samples obtained from these disks (see Table 3).

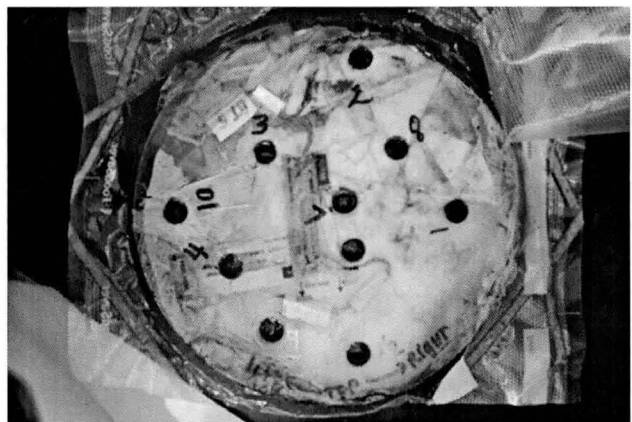
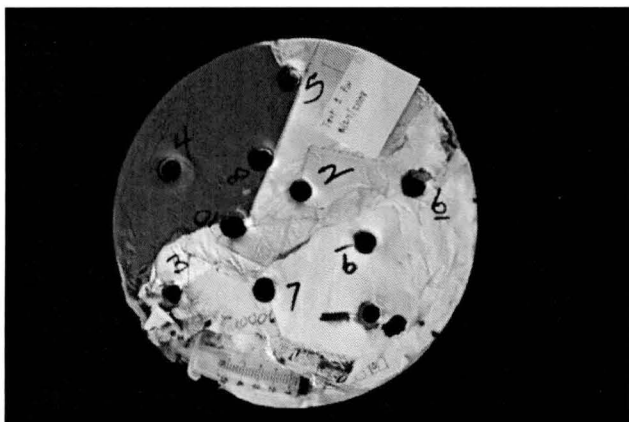


Figure 4. Heat Melt Compaction (HMC) product disks after aseptically sampling 10 cores (from each) using a hand press and sterile 1.25 cm diameter punches. Cores from these disks are shown in Figs. 7 and 8. Microbial content of the cores were determined to assess the parameters for operation of the HMC procedure with regard to sterilizing the wastes.

Surprisingly, for yeasts and molds on the surfaces of any of the five disks, only one was found either before or after surface sanitization.

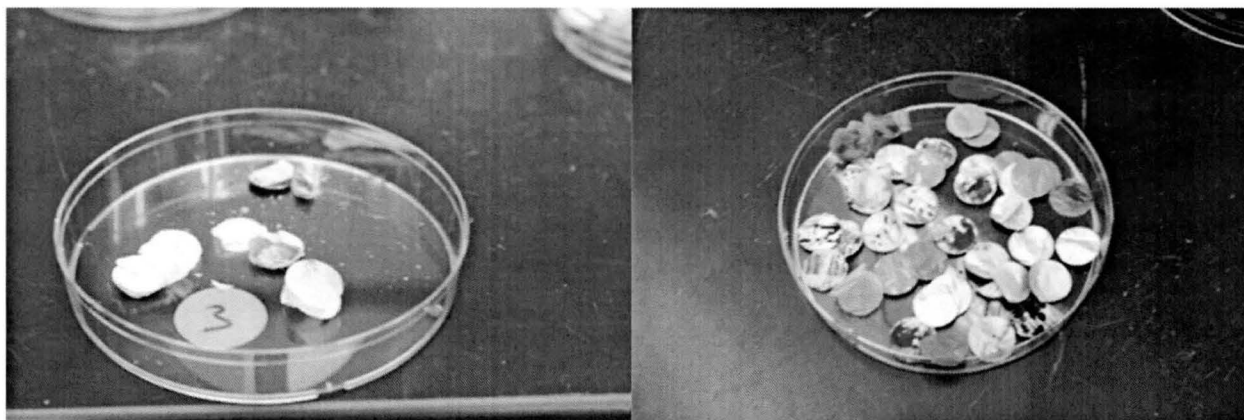


Figure 5. Example separated layers from 1.25 cm diameter cores cut from Heat Melt Compaction (HMC) product disks. These core samples show that the trash had not been totally fused together by the HMC melting the plastic film in the feed material. The core material on the left is from Disk 1, which was made from simulated space solid wastes. The core material on the right is from Disk 3, which was made from actual space solid waste from the Volume F compartment trash returned on STS 130.

2. Core samples from HMC product disks

Ten core samples were cut out of each HMC disk (Fig. 1) using a pre-sterilized 1.25 diameter metal punch that had been machined in-house to bevel the cutting edge. The bevel machining was needed to cut a clean core out of the ~2 cm thick HMC product disk. Fig. 4 shows two HMC disks after cutting out the 10 cores. These are the same disks as shown in Fig. 1 prior to obtaining the cores. The disk on the left, Disk 1, was made from simulated space solid waste / trash and the disk on the right, Disk 3, was made from STS 130 trash.

Close up photographs of example cores from HMC disks are shown in Figs. 5 and 6. Fig. 5 shows cores that had multiple layers of trash that were not fused together by the melting of plastic film by the HMC. Most of the core samples looked like these. The microbial characterization of the cores that followed coring was affected because the cores easily came apart and, thus, more of the interior of the core was exposed than for the fused cores. The core material on the right had separated into layers but it also has been teased apart with sterile tweezers in an attempt to find if the core had cut through any of the spore test strips that were added to the HMC feed material as it was filled.

Fig. 6 shows close-up photographs of examples of cores where the melted plastic had fused. The core on the left, from Disk 1 made from simulated space solid waste, was not completely fused as individual layers and some

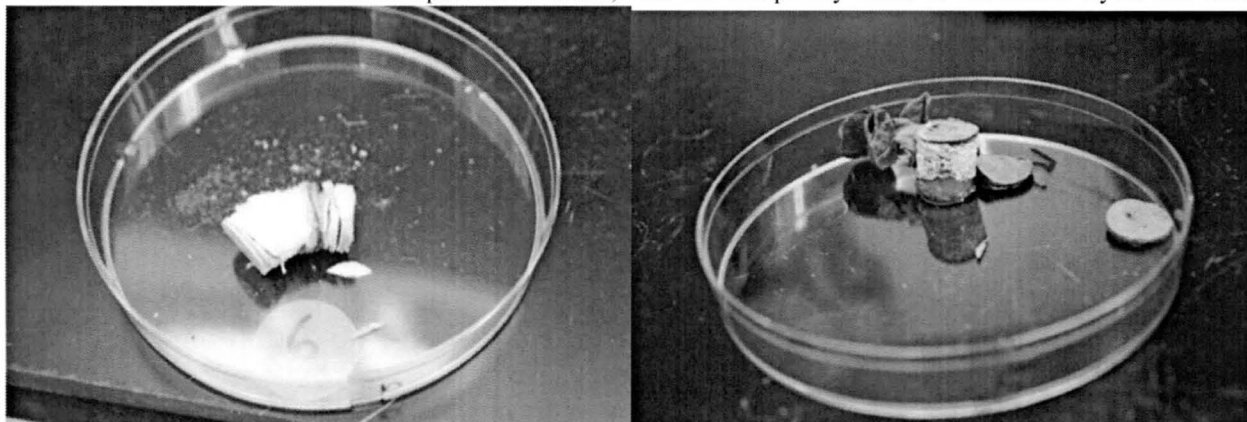


Figure 6. Examples of fused plastic 1.25 cm diameter cores cut from HMC product disks. The core material on the left is from Disk 1, which was made from simulated space solid wastes. The core material on the right is from Disk 3, which was made from actual space solid waste from the Volume F compartment trash returned on STS 130.

separation can be seen. The core on right from Disk 3, which was made from STS 130 Volume F trash, shows some very solid fused material but the remainder of the core has separated into layers.

Cores were processed to obtain: Microbial load via acridine orange direct count (AODC), cultivatable total aerobic and anaerobic bacteria, cultivatable gram positive spore forming bacteria, selected cultivatable gram negative bacteria, cultivatable fungi. Microbial content of the cores were determined to assess the parameters for operation of the HMC procedure with regard to sterilizing the wastes. Results of these analyses on cores are shown in Table 2.

Table 2. Results of microbial analyses and some physical parameters for core samples cut from HMC product disks.

	Disk 1	Disk 2	Disk 3	Disk 4	Disk 5
Water wt (g)	ND	ND	64.0	43.1	25.3
Core samples showing growth	4	4	4	4	4
Mean Log ₁₀ CFU/core	1.72± 1.28	0.68± 0.95	0.62± 0.83	0.71± 0.98	0.78± 1.14
Mean Log ₁₀ cells /core	7.68 ± 0.35	7.78 ± 0.37	9.04± 0.36	8.35± 0.21	
CFU /cm ³	1.32E+03	1.37E+01	5.73E+00	1.46E+01	3.95E+01
Cells /cm ³	1.96E+07	2.48E+07	3.68E+08	7.83E+07	
<i>G. stearothermophilus</i>	ND	ND	Neg	Neg	Neg
<i>B. atrophaeus</i>	ND	ND	Neg	Neg	Neg

Water weight is shown for the three disks made from STS 130 trash. When taking core samples it was noted that liquid could be seen inside the disks. One goal of the HMC is to recover water from solid wastes, so the decision was made to obtain an estimate of the water that still remained in the disk. Fortunately, as described in section 4.4 the three disks had to be opened up to retrieve spore test strips from the interior of the disks to determine if the HMC operating parameters was sufficient to kill the spores. The disks were opened up layer by layer so it was thought that the entrained water could be evaporated off when determining dry weights. The first row in Table 2 shows the amount of water that was recovered from each of these disks. Refer to the FY11 EOY report by John Fisher entitled "Heat melt compactor in waste management" to see if this water found remaining in the disks is a significant fraction of the amount of water that was recovered during operation of the HMC.

Four of the 10 core samples obtained from each disk showed positive results for microbial growth. However, the number of viable / cultivatable bacteria per core were very low, between 0.62 and 1.72 log₁₀ cfu per core. A full mL of diluent had to be plated over several agar media plates to detect this number of cells, which averaged between 4.2 and 52.5 cfu per core. The standard deviations for these counts are very high because 6 of the 10 cores per disk had no cfus. That many more bacteria are present in the disks is evident by the direct counts (Mean Log₁₀ cells core-1 which ranged from 9.04 to 7.68 cells core-1 (4.8 x 10⁷ to 1.1 x 10⁹ cells core-1). Many cells were present, but almost all were not cultivatable, i.e., dead. Likely they were killed during operation of HMC, but viable counts of the feed material just before addition to the HMC were not made. The FY10 KSC project report indicated that many bacteria and fungi were originally in the STS 130 feed material prior to shipping it to ARC.

In comparison, in the KSC FY10 project, densities of cultivatable bacteria in the core samples ranged from 100 to 25000 colony-forming units (CFU) per core, while the direct counts indicated much higher numbers, 9.6 x 10⁶ to 3.2 X 10⁸ with a mean of 7.5 x 10⁷.

The cultivatable bacteria and fungi that grew on plate count media from HMC disk core samples were isolated and identified using the Microlog ID System (Biolog, Hayward, CA) with Gen III plates. Table 3 shows these results for each of the five HMC product disks. Disks 1 and 2, made with feed from simulated space wastes and which had been stored for over 2 years after production had a large number of identified isolates. Of note are the many from the *Bacillus* and *Paenibacillus* genera, spore-forming bacteria that can survive high temperatures and desiccation. The identified isolates from HMC product disks made from STS 130 trash had considerably fewer identified isolates, but these disks were analyzed much sooner after production. These IDs include one instance of *Staphylococcus aureus*, a pathogenic bacterium could be of concern if it can truly survive the HMC process.

3. Use of commercial spore test strips to test HMC treatment conditions with regard to sterilizing waste

The STS Volume F trash was of variable age when used as feed material for the HMC tests. Thus, the trash resident microbes had also undergone a variety of conditions prior to the tests, from incubating in the trash for the 1

Table 3. Identified isolates from core samples cut from HMC product disks

	Bacteria	Fungi
Disk 1*	<i>Lactococcus lactis</i> ss <i>lactis</i> , <i>B. licheniformis</i> , <i>Paenibacillus macerans</i> <i>acillus</i> <i>Neisseria flavescens</i> , <i>Rothia mucilaginosa</i> , <i>Streptococcus salivarius</i> <i>Sphingomonas aurantiaca</i> , <i>Bacillus circulans</i> , <i>Bacillus megaterium</i> <i>Bacillus azotoformans</i> , <i>Bacillus simplex</i> , <i>Neisseria macacae</i> <i>Neisseria flavescens</i> , <i>Sphingomonas abaci</i> , <i>Methylobacterium suomiense</i>	None
Disk 2	<i>Bacillus megaterium</i> , <i>Bacillus spp.</i> , <i>Paenibacillus agarexedens</i> <i>Staphylococcus simulans</i> , <i>Curtobacterium flaccumfaciens</i>	None
Disk 3	<i>Streptococcus salivarius</i> , <i>Rothia mucilaginosa</i> , <i>Staphylococcus aureus</i>	None
Disk 4	<i>Staphylococcus pasteurii</i> , <i>Staphylococcus sp</i>	<i>Penicillium chrysogenum</i> <i>Phyllosticta maydis</i> <i>Phoma terrestris</i>
Disk 5	<i>Brevibacillus choshinensis</i> , <i>Streptococcus salivarius</i> ATCC=7073	

– 16 days or so during the STS mission, to the days while being processed at KSC to the shipment and refrigerator storage at Arc before use. Therefore, consideration was given to the addition of a known ‘challenge’ microorganism to the trash just prior to putting it into the HMC. First, a well-defined pure culture of *Bacillus subtilis* was acquired from Dr. Wayne Nicholson, a University of Florida microbiology faculty member who occupies laboratories at KSC. This strain has had several antibiotic resistance markers added to its genome and it also has a reporter gene that fluoresces as the spores of this bacterium germinate. Unfortunately, when added to simulated food wastes, the recovery of the microbe on medium with the antibiotics added resulted in the growth of numerous other microorganisms also resident in the trash.

Next, the addition of commercial spore test strips to the trash just before adding it to the HMC was tried. Biological indicators are routinely used to monitor the effectiveness of various sterilization protocols such as steam sterilization (autoclaving) and dry heat. Different organisms are used to test different types of sterilization procedures. To test the lethal effect of the HMC process on microbes contained in the trash two types of test strips (www.namsa.com containing either spores of *Geobacillus stearothermophilus* (American Type Culture Collection culture # 7953), used to test steam sterilization, or *Bacillus atrophaeus* (ATCC 9372) used to test dry heat sterilization, were placed in the trash before compaction. The test strips were located so that at least one of each spore type was on the top and bottom surface of the disk and at least two of each type in the interior of the disk after compaction. The strips on the top and bottom would be closest to the heating sources and those placed in the interior the furthest away.

Fig. 7 shows Disk 3 as received at KSC with two spore test strips evident on the (upper/top) surface. Fig. 8 shows the aseptic removal of one of the top strips after cutting around it with a sterile scalpel and pulling it out with sterile tweezers.

Next, the addition of commercial spore test strips to the trash just before adding it to the HMC was tried. Biological indicators are routinely used to monitor the effectiveness of various sterilization protocols such as steam sterilization (autoclaving) and dry heat. Different organisms are used to test different types of sterilization procedures. To test the lethal effect of the heat melt compaction process on microbes contained in the trash, 2 types of test strips (www.namsa.com) containing either spores of *Geobacillus stearothermophilus* (ATCC 7953), used to test steam sterilization, or *Bacillus atrophaeus* (ATCC 9372), used to test dry heat sterilization, were placed in the trash before compaction. The test strips were located so that at least one of each spore type was on the top and bottom surface of the disk and at least two of each type in the interior of the disk after compaction. The strips on the top and bottom would be closest to the heating sources and those placed in the interior the furthest away.

Finding the spore test strips placed in the interior of the disks was a more difficult task because they could not be located until the disk was pulled open. Figure 9 shows a progression of photos when trying to locate the embedded test strips.

Results for the NAMS protocol for both *B. atrophaeus* and *G. stearothermophilus* are shown in the bottom two rows in Table 2. In short, all of the tests were negative. Apparently, the HMC operating conditions with regard to

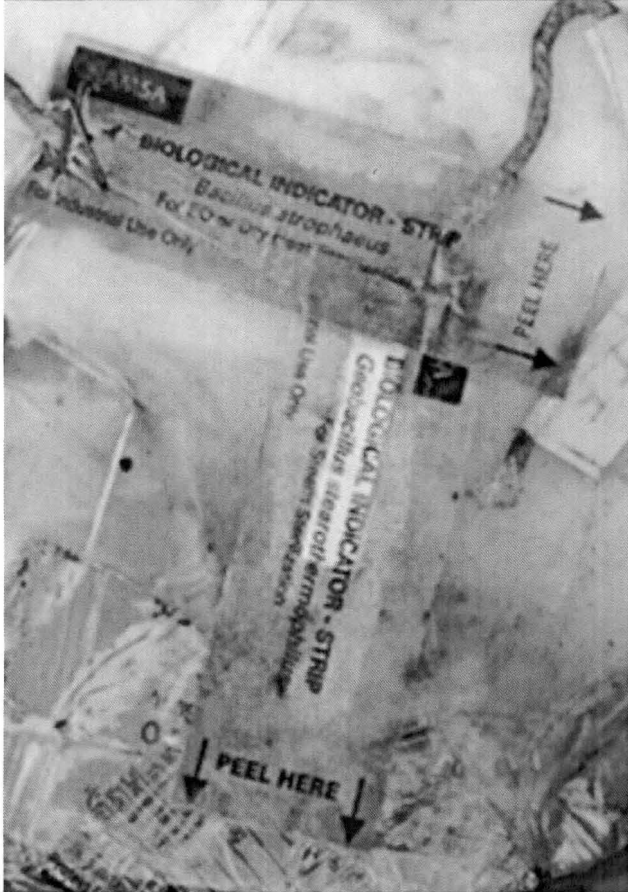
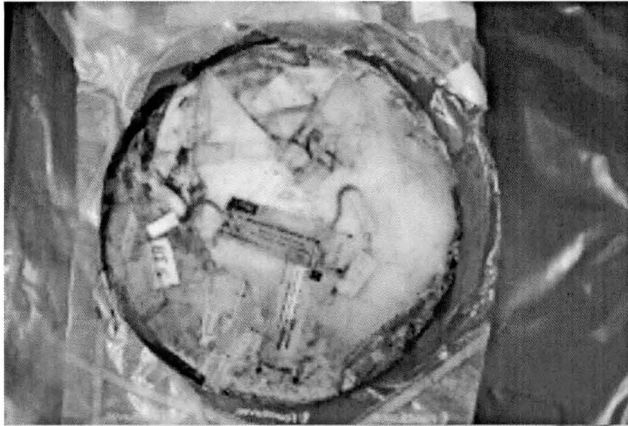


Figure 7. HMC product disk (Disk #3) with embedded spore test strips. The bottom photograph is a close-up of the strips showing the strip label indicating one contained spores of *Bacillus atrophaeus* (upper strip) and the other contained spores of *Geobacillus stearothermophilus*.



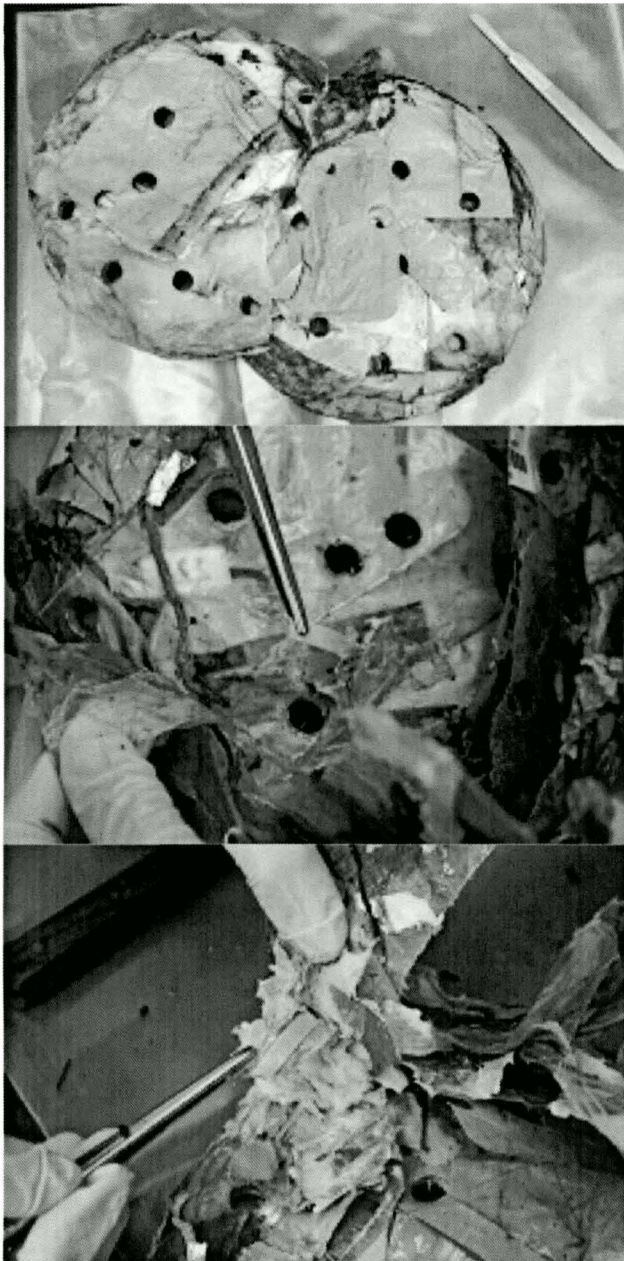
Figure 8. Aseptic removal of one of the top spore test strips after cutting around it with a sterile scalpel. The excised strip was pulled out with sterile tweezers.

temperature and duration were sufficient to kill the spores present in the embedded test strips. Further tests at less than optimal spore-killing conditions are recommended so positive results can be obtained to ensure that the testing procedures were done correctly. As a positive control, of sorts, spore strips that were not exposed to the HMC operating conditions, i.e., fresh out of the package, were run through the protocol and the numbers recovered were the same as specified by the manufacturer.

4. Microbial characterization of internal HMC hardware surfaces

Operation of the HMC could lead to contamination of the hardware interior surfaces if microbe-laden waste materials were squeezed out of the waste during piston operation and trash compaction. Surface swab samples (Sanicult), similar in technique to surface sampling of the HMC product disks (section 4.1), were taken twice when the HMC was operated in FY11, before production of Disk 3 from STS trash and before and after production of Disk 5. Figure 12 shows the results of the first hardware surface sampling. Count were below the lower detection limit for 3 of the 8 areas sampled. The highest counts, by several orders of magnitude, were obtained from the top piston front. Much lower counts of only several hundred per square centimeter were found on three other surfaces, the bottom piston center and two in the chamber itself. Nearly all of the isolates that were identified came back as members of the *Bacillus* genus, spore-forming bacteria that could possibly survive the heat and desiccation of the HMC surfaces.

HMC swab samples were also acquired from the top, back and bottom plate surfaces of the HMC before and after compaction of Disk 5 and sent to KSC for analysis. The back and bottom surfaces sampled after the production of the HMC disk yielded microbial growth. The bacterial isolates from the back surface were identified as *Bacillus* sp., *Bacillus atrophaeus*, *Strep. salivarius*, and *Strep. anginosus*. The *B. atrophaeus* was isolated after heat shocking the sample and aerobic incubation. Both *Streptococcal* species were isolated after heat shock and anaerobic incubation. Interestingly, the *B. atrophaeus*



isolated is the same species present on the biological indicator spore strips implanted in the disks. Whether this is the source of the organism on the compactor surface has not been determined. *Staphylococcus hominis novobiosepticus* ATCC=700236 was one of the organisms isolated from the bottom plate surface, the other isolate remains unidentified.

Figure 9. Finding and aseptically removing embedded spore test strips from the interior of HMC Disk 3. Top photo shows the top layers of the disk split apart. The middle photo shows the middle of the disk where spore strips were found and removed and the bottom photo shows a layer near the bottom of the disk with a spore strip being removed with sterile tweezers.

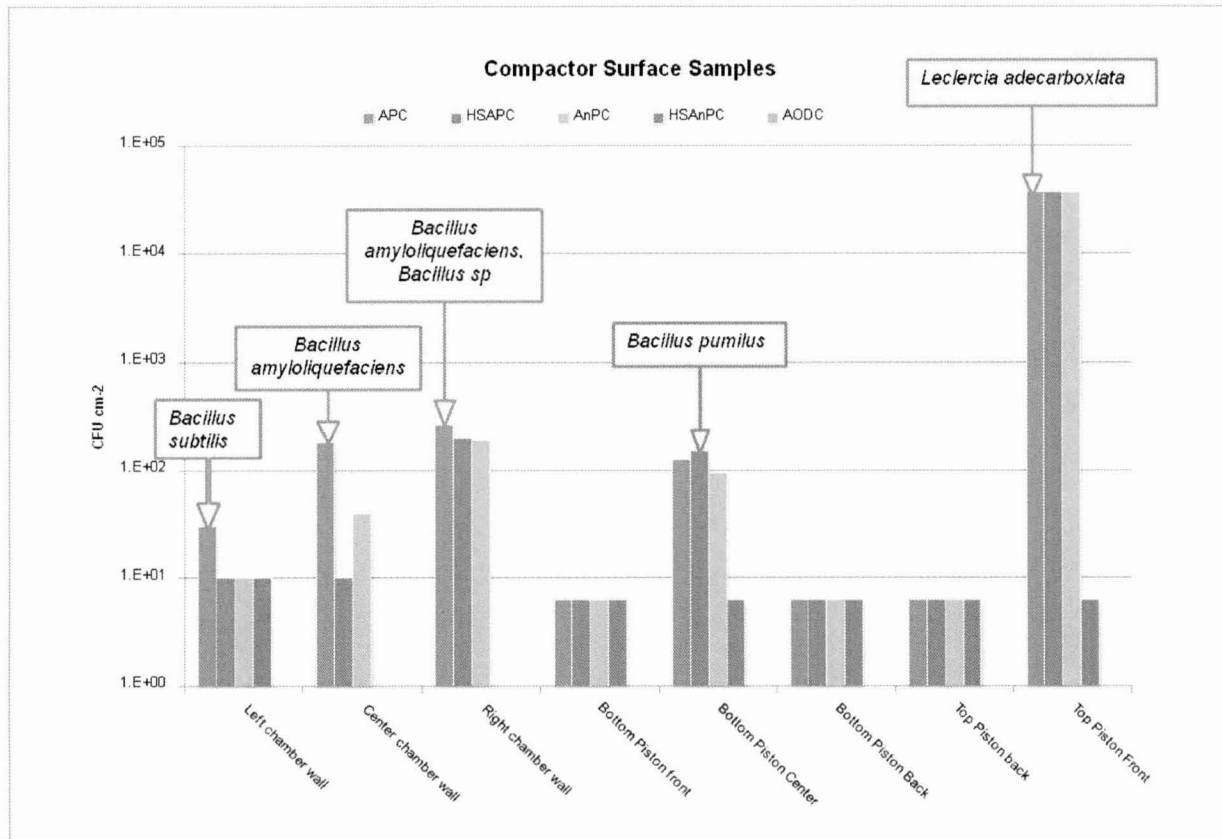


Figure 10. Bar chart of cultivatable counts of bacteria on HMC hardware surfaces prior to production of Disk 3. Also shown in the callout boxes are the names of isolates obtained from the various samples

IV. Conclusions & Recommendations

The on going purpose of the project efforts was to characterize and determine the fate of microorganisms in space-generated solid wastes before and after processing by candidate solid waste processing. For FY11, the candidate technology that was assessed was the Heat Melt Compactor (HMC). The scope included five HMC product disks produced at ARC from either simulated space-generated trash or from actual space trash, Volume F compartment wet waste, returned on STS 130. This project used conventional microbiological methods to detect and enumerate microorganisms in heat melt compaction (HMC) product disks as well as surface swab samples of the HMC hardware before and after operation. In addition, biological indicators were added to the STS trash prior to compaction in order to determine if these spore-forming bacteria could survive the HMC processing conditions, i.e., high temperature (160 C) over a long duration (3 hrs). To ensure that surface dwelling microbes did not contaminate HMC product disk interiors, the disk surfaces were sanitized with 70% alcohol. Microbiological assays were run before and after sanitization and found that sanitization greatly reduced the number of identified isolates but did not totally eliminate them. To characterize the interior of the disks, ten 1.25 cm diameter core samples were aseptically obtained for each disk. These were run through the microbial characterization analyses. Low counts of bacteria, on the order of 5 to 50 per core, were found, indicating that the HMC operating conditions might not be sufficient for waste sterilization. However, the direct counts were 6 to 8 orders of magnitude greater, indicating that the vast majority of microbes present in the wastes were dead or non-cultivable. An additional indication that the HMC was sterilizing the wastes was the results from the added commercial spore test strips to the wastes prior to HMC operation. Nearly all could be recovered from the HMC disks post-operation and all were showed negative growth when run through the manufacturer's protocol, meaning that the 106 or so spores impregnated into the strips were dead. Control test strips, i.e., not exposed to the HMC conditions were all strongly positive. One area of concern is that the identities of isolates from the cultivable counts included several human pathogens, namely *Staphylococcus*

aureus. The project reported here provides microbial characterization support to the Waste Management Systems element of the Life Support and Habitation Systems program.