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Heat Melt Compaction as an Effective Treatment for Eliminating Microorganisms from Solid Waste

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One of the technologies being tested at Ames Research Center as part of the logistics and repurposing project is heat melt compaction (HMC) of solid waste to reduce volume, remove water and render a biologically stable and safe product. Studies at Kennedy Space Center have focused on the efficacy of the heat melt compaction process for killing microorganisms in waste and specific compacter operation protocols, i.e., time and temperature required to achieve a sterile, stable product. The work reported here includes a controlled study to examine the survival and potential re-growth of specific microorganisms over a 6-month period of storage after heating and compaction. Before heating and compaction, ersatz solid wastes were inoculated with *Bacillus amyloliquefaciens* and *Rhodotorula mucilaginosa*, previously isolated from recovered space shuttle mission food and packaging waste. Compacted HMC tiles were sampled for microbiological analysis at time points between 0 and 180 days of storage in a controlled environment chamber. In addition, biological indicator strips containing spores of *Bacillus atrophaeus* and *Geobacillus stearothermophilus* were imbedded in trash to assess the efficacy of the HMC process to achieve sterilization. Analysis of several tiles compacted at 180°C for times of 40 minutes to over 2 hours detected organisms in all tile samples with the exception of one exposed to 180° C for approximately 2 hours. Neither of the inoculated organisms was recovered, and the biological indicator strips were negative for growth in all tiles indicating at least local sterilization of tile areas. The findings suggest that minimum time/temperature combination is required for complete sterilization. Microbial analysis of tiles processed at lower temperatures from 130°C-150°C at varying times will be discussed, as well as analysis of the bacteria and fungi present on the compactor hardware as a result of exposure to the waste and the surrounding environment. The two organisms inoculated into the waste were among those isolated and identified from the HMC surfaces indicating the possibility of cross contamination.

I. Introduction

ESTIMATES of wastes generated during space missions are approximately 1633 gm per crew member per day, 30-45% of that total being water. Almost half the weight of the solid waste considered is brine from urine processing and cloth items like towels. The third largest component by weight is food packaging. These estimates are based on the analysis of wastes collected from the ISS and shuttle missions as well as an examination and analysis of flight manifests and baseline human physiological data [1]. Many of the solid waste components are readily biodegradable organic materials i.e.food and human solid

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waste supporting the growth of microrganisms including potential human pathogens. Microbial metabolic by-products can also be generated causing unpleasant odors and accumulation of volatile organic compounds (VOCs).

Microbial characterization studies of space generated solid wastes have been done at Kennedy Space Center (KSC) since 2007. Earlier studies showed that simulated shuttle trash containing mostly food and packaging waste supported the growth of human associated pathogens over long periods of storage. Briefly, in a study done by Strayer et al[2], selected microorganisms, including pathogens, were added as challenge microbes into simulated food trash compartment wet waste. The list included those microbes that could be introduced as cross-contamination inocula from other solid waste sources: *Escherichia coli* and *Salmonella enterica serovar typhimurium* (enteric gram negative bacteria), *Staphylococcus aureus* (gram positive bacterium), *Aspergillus niger* (a common mold), *Pseudomonas aeruginosa* (gram negative bacterium), and *Bacillus pumilus* SAFR-032, a gram positive spore-forming bacterium which served as a positive control. Data showed that added challenge microbes, including pathogenic bacteria, could grow and proliferate during simulated spacecraft storage up to 6 weeks in food trash compartment wastes. Some added microbes, i.e., *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* increased between 3 and 5 orders of magnitude over the first 3 days of storage. After six weeks of storage, most added pathogens were still present at high levels with little die-off observed. These data illustrate the potential microbiological hazards present in untreated waste.

Studies were also done on the microbiological component of ISS and Shuttle mission (7 flights) generated trash[3]. The trash was returned to our labs at KSC for analysis and included the volume F compartment waste. This was a mixture of predominately meal and personal hygiene generated wastes. Human associated potentially pathogenic bacteria including *S. aureus* and *E. coli* were isolated from the waste. It should be noted that, while potentially pathogenic, these organisms are carried by humans as part of the normal flora of the skin and digestive tract so this is not an unexpected finding. Results from these studies demonstrate that potential problems regarding pathogens as cross-contaminants from other sources including other waste streams could develop during storage of space mission solid wastes especially without a safe storage regime or successful stabilizing treatment.

One of the technologies being tested at NASA Ames Research Center (ARC) for the Advance Exploration Systems program and as part of the logistics and repurposing project is heat melt compaction (HMC) of solid waste to reduce volume, remove water and render a biologically stable and safe product. The HMC compacts and reduces the trash volume as much as 90% greater then the current manual compaction used by the crew.[1]

Five HMC product tiles produced at ARC from either simulated space-generated trash or from actual space trash (Volume F compartment wet waste, returned on STS 130) underwent microbial characterization studies at KSC in 2011. Biological indicators (BI) were added to the STS trash prior to compaction in order to determine if imbedded spore-forming bacteria could survive the HMC processing conditions, i.e., high temperature (160 to 177 °C) over a long duration (1 to 3 hrs), i.e., dry heat sterilization conditions. No growth was evident from the biological indicator strips and some very low microbial counts were found in samples taken from the tiles. The BI showed that the HMC was an effective means of sterilizing the waste, however the survival of a very low number of microorganisms on some of the tile samples suggests that there may have been an incomplete sterilization throughout the tile under the specific operating conditons [3].

Further characterization of the tiles produced by heat melt compaction under specific time and temperature protocols is the focus of the work presented here. The project has three primary goals described below:

A. Microbiological analysis of HMC hardware surfaces before and after operation.

As with any solid waste processing technology, the crew might come into contact with hardware surfaces that have been contaminated by microorganisms during waste processing. The objective of this task was to determine the extent of microbial surface contamination of waste processing hardware. Hardware surface samples were analyzed for total bacterial and yeast counts and cultivable counts of aerobic and anaerobic bacteria, spore-forming bacteria, and fungi. Isolated microorganisms were identified.

B. Microbiological and physical characterizations of heat melt tiles made from trash at different processing times and temperatures.

Process parameters (time and temperature) were determined by ARC investigators, optimally to include a minimum time and temperature test point to achieve the processing goals of melting plastic and water removal and recovery of water, but not necessarily trash sterilization. The microbiological and physical characteristics of the completed tiles were examined to determine whether minimal processing treatments can achieve the reduction in microbial density and physical properties conducive for long-term storage of the HMC tiles, i.e. cohesive sealing properties of the exterior melted plastics to eliminate re-introduction of microorganisms into the interior of the tile from the exterior, and low moisture content.

C. Long term storage and stability of HMC trash tiles i.e. "Do the bugs grow back?"

Previous tests have shown that after treatment of trash by HMC, recovery of any cultivable microbes is minimal. BI test strips to test the efficacy of sterilization by dry heat and steam, *B. atrophaeus* and *G. stearothermophilis*, respectively were incorporated into the trash to test the efficacy of the HMC process in the reduction or elimination of microorganisms. The

test organisms were never recovered from the tiles after treatment indicating effective killing of these bacteria by the HMC process with the time and temperature protocols used. The question remains as to whether organisms that are not recovered by cultivable methods after treatment were injured but present and able to recover and grow within the matrix of the tile during long term storage. To determine the microbiological stability of the HMC tiles during storage up to 6 months, controlled experiments were started using a mixture of simulated trash that was sterilized and inoculated with a bacterial and yeast monoculture.

II. Materials and Methods

A. Microbiological analysis of HMC hardware surfaces before and after operation.

1. *Sample Collection and shipment.*
   Sterile Sanicult swabs were sent to Ames Research Center (ARC) to perform surface samples of the heat melt compactor (HMC) hardware before and after use. The sights sampled were the pistons, sidewall, and groove. After sampling, the swabs were shipped on ice, overnight to KSC for microbiological analyses on the day of receipt if possible or held at 4°C until processed. Samples were taken for tiles numbered 10-13 which included the four trash processed at 130°C for 2 hours and tiles 7m and 8m, processed at 140°C for 2 hrs 20 mins.
   Sanicult swabs are contained in a tube containing 5 mls buffer. Each tube was vortexed for 30 sec to remove microorganisms from the sampling swab for processing. The following analyses were performed on the buffer:
2. *Total Direct Count (AODC).*
   950 µl samples were fixed with formalin (50 µl) achieving a final fixative concentration of approximately 2%. Fixed samples were sonicated using a probe sonicator for 15 seconds at low power. 1 ml samples were stained with 100ul of acridine orange (AO) and filtered through a black polycarbonate filter with a .2 µm pore size. Filters were placed on a microscope slide and direct microscopic counts were done using a Ziess 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.
3. *Cultivation based enumeration of bacteria and fungi.*
   Buffer solution (see above) was directly plated onto agar growth media or diluted if necessary before plating. Different groups of bacteria listed below were selectively grown based on incubation atmosphere and/or heat treatment and enumerated following incubation (48 hrs-1 week):
   a. *Aerobic, mesophilic bacteria.* Sample was spread onto R2A and incubated aerobically at 25 °C.
   b. *Anaerobic, mesophilic bacteria.* Sample was spread onto R2A, incubated anaerobically, (plates were placed in an anaerobe box with an anaerobic atmosphere generating sachet) at 25 °C.
   c. *Aerobic sporeformers.* The liquid sample was heat treated by incubation for 15 minutes in a heat block set at 80 oC. The sample was allowed to cool to room temperature, plated onto R2A and incubated at 25 °C.
   d. *Anaerobic sporeformers.* Same method as above (aerobic) except the plates were incubated anerobically.
   e. *Fungi (yeasts and molds).* Samples were plated onto Inhibitory Mold Agar and incubated at room temperature for 48hours-1 week.
4. *Isolation and identification of recovered bacteria and fungi.*
   After colony enumeration, bacteria and fungi were streaked for isolation on a general growth media like TSA or R2A for bacteria and IMA or PDA for fungi and incubated for 24-48 hours. Isolates were identified first by the Biolog Micro-ID system following manufacturers protocols. If the Biolog micro-id (Biolog, Hayword, Ca) system failed to identify the isolate, then the Micro-Seq (Applied biosciences) PCR based method was used following manufacturers protocols.

D. The effectiveness of HMC for the sterilization of solid waste(s)-minimum temperature requirements

To perform studies on the survival of microoorganisms in waste treated by HMC, waste was prepared, sterilized and reinoculated with a know density of microorganisms that could be enumerated.

1. *Preparation of inoculated waste*
   Ethylene oxide (ETO) sterilization. Tests were done to confirm the effectiveness of the etyhylene oxide method of sterilization to achieve sterile aliquots of trash. Six approximately 525-gram samples of trash (including plastic packaging) were prepared for testing according to the waste formula in Appendix I. Items included in the waste were weighed and added to the mix. Three spore strips containing *Bacillus atropheus* (NAMSA, Northwood, Ohio) were placed in each of the six bags inside food or drink containers. Three ~525 g samples of trash were placed in individual sterilization pouches which were then placed in an ethylene oxide sterilizer (3M Steri-Vac Gas Sterilizer 4XL). The ETO-sterilizer cycle was run at 37° C for 2 hours. Sterilization pouches were left in the sterilizer for 5 days to off-gas

any residual ETO. Three 525 g trash samples not undergoing the ETO sterilization process were used as controls to determine baseline microbial counts of the unsterilized trash.

Microbiological analysis. Before addition of diluent, the spore strips were aseptically removed and placed into tryticase soy broth (TSB) and incubated at 37° C for 1 week following the manufacturers protocol, then examined for growth. To achieve adequate mixing of the trash contents and diluent, juice containers and bags containing food items were cut with a sterile scissors. Approximately 500 grams of trash was diluted in 1500 mL of sterile de-ionized water. Water and trash contained in double 1-gallon zip-lock bags was shaken for two minutes to obtain a homogeneous mixture. Care was taken to ensure contact of all items with the diluent. These sample plus diluent mixtures were then serially diluted and these were plated onto R2A and Inhibitory Mold Agar (IMA). Colony forming units (CFU) were counted after incubation at 28° C for 48 hours. Sterilization of the 525 g trash simulant with ETO was effective. The typticase soy broth (TSB) (BBL) test media inoculated with the ETO-sterilized B. atrophaeus strips or the R2A and IMA plates inoculated with serial dilutions of the ETO-sterilized simulated/ersatz trash showed microbial counts below the detection limit in the sterilized trash. Unsterilized trash contained an average bacterial count of 1.4×10^4 CFU $gram^{-1}$ and fungal counts of 5.2×10^1 CFU $gram^{-1}$.

Inoculum development: Inoculum density and recovery of microorganisms. Six approximately 525-gram samples of trash (including plastic packaging) were prepared and sterilized as previously described including the addition of *B. atrophaeus* test strips. Packaging containing food, drinks and wipes were cut in several locations to ensure mixing with diluent for microbial sampling. Three microorganisms were tested for use as an appropriate inoculm. *Bacillus amyloliquifaciens* a spore forming bacteria that has been recovered from shuttle trash, *Rhodotorula mucilagenosa,* a yeast also recovered from shuttle trash and Micrococcus luteus, a gram positive bacteria commonly found in the environment. Each were grown separately in TSB on a shaker incubator at 30°C for 18 hours. The absorbance of each culture was measured on a spectrophotometer at 590 nm to determine the approximate density of each organism, which was 10^9 cells ml^{-1}. A mixture of the three organisms was prepared by combining equal parts of each broth culture (15 ml of each 10^9 suspension). The mixed culture was thoroughly vortexed and serially diluted in sterile phosphate buffered saline, (PBS) to 10^8 and 10^7 cells ml^{-1}. Individual cultures were diluted and plated on tryptic soy agar (TSA) to determine the actual viable count of the bacteria and yeast before inoculation into the trash. The *B. atrophaeus* test strips place in the trash before sterilization were removed and placed into TSB before inoculation with test organisms. Bags were inoculated in duplicate with 15 ml of each culture density (10^9, 10^8, 10^7) in 1 ml amounts into 15 different food items in the simulated/ersatz trash. Inoculated bags were left at room temperature for 24 hr to simulate the eventual transit time from KSC to ARC for the HMC processing. Approximately 500 grams of trash was diluted in 1500 ml of sterile de-ionized water. Water diluent and trash was placed into double 1-gallon ziplock bags which were then manually shaken for two minutes. Care was taken to ensure contact of all items with the diluent. Mixed samples plus dilution water were serially diluted and plated onto R2A and TSA. These media were used to compare recovery of bacteria and yeast to determine which would be used in future assays. Table 1 shows the starting inoculum count (CFU ml-1) for all three organisms at three cell concentrations.

Table1. Inoculum colony counts (cfu/ml) of each test organism at 3 dilutions. *Estimation based on abs 590 nm.

Estimated* cell number	*B. amyloliquefaciens*	*M.luteus*	*R.mucilagenosa.*
1.00E+09	2.20E+08	4.00E+07	3.00E+07
1.00E+08	3.60E+07	6.60E+07	1.20E+07
1.00E+07	7.80E+06	3.00E+07	2.00E+07

The estimated recovery and actual counts after dilution with 1500 ml of diluent are listed in Table 2. *M. luteus* was below detection from simulated trash samples inoculated with the two lower cell concentrations. This lack of growth by *M. luteus* possibly indicates a bacteriocidal effect of one of the other added microbes. In fact, *B. amyloliquefaciens* is known to produce bacteriocins and *M. luteus* is in particular sensitive to these antimicrobial compounds.[4] For this reason we eliminated *M. luteus* from the inoculum mixture.

The calculated recovery estimate assumes no growth during the 24 hr incubation period. Actual counts show an increase in bacterial and yeast numbers after 24 hours incubation at room temperature. Growth of bacteria and yeast during shipping can, thus, be expected.

Table 2. Colony counts (cfu/g of wet trash) from trash samples. Actual recovery is after 24 hr incubation at room temperature.

	Estimated recovery with no growth.			Actual recovery		
Inoculum	*B. amyloliquefaciens*	*M.luteus*	*R.mucilaginosa.*	*B. amyloliquefaciens*	*M.luteus*	*R.mucilaginosa.*
1.00E+09	2.20E+06	4.00E+05	3.00E+05	5.30E+06	3.22E+05	9.65E+05
1.00E+08	3.60E+05	6.60E+05	1.20E+05	1.91E+06	<1.61E+04	1.21E+05
1.00E+07	7.80E+04	3.00E+05	2.00E+05	3.00E+05	<1.69E+04	5.57E+04

2. *Ersatz trash preparation and inoculation.*

Simulated, trash was used as the HMC feed to produce the tiles for this study. 500 gram units of trash were prepared at KSC according to the components in Appendix II. Trash was sterilized by ETO. The ETO sterilized trash was inoculated with *B. amyloliquifaciens and R. mucilaginosa*, if required. Controls and temperature studies were not inoculated. The inoculum procedures and densities were determined in the preliminary experiments described.

3. *Temperature tests.*

Process parameters (time and temperature) used in these experiments were 130°C for 2 hours, 140°C for 2 and 3 hours and 180°C for 2 hours as determined and processed by ARC investigators. The microbiological and physical characteristics of the completed tiles were examined to determine whether minimal processing treatments can achieve the reduction in microbial density and physical properties conducive for long-term storage of the HMC tiles i.e. cohesive sealing properties of the exterior melted plastics to eliminate reintroduction of microorganisms into the interior of the tile and low moisture content to prevent re-growth of microorganisms.

4. *Tile processing and sampling.*

BI test strips (NAMSA, Northwood, Ohio) were incorporated into the trash before compaction to test the efficacy of the HMC process in the reduction or elimination of microorganisms. The test strips are impregnated with spores of organisms to test the efficacy of sterilization by dry heat and steam, *Bacillus atrophaeus* (American Type Culture Collection (ATCC) #9372) and *Geobacillus stearothermophilus* (ATCC# 7953), respectively. The test strips were located so that at least one of each spore type was on the top and bottom surface of the HMC tile and at least two of each type in the interior of the tile 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. Spore strips close to the surface were excised from the tile (Fig. 1) before core sampling using a sterile scalpel and forceps. Strips were processed according to the manufacturer's protocol. Briefly, the strips were removed from the outer glassine envelope and placed into 10 ml of sterile trypticase soy broth (TSB) in a screw cap tube and incubated at 55-60oC and 30-35oC respectively for 7 days. After incubation tubes were examined for growth along with positive controls.

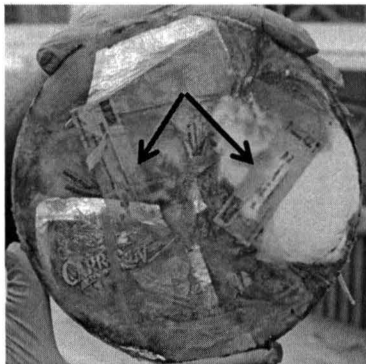

Figure 1. HMC tile showing excised spore strips (arrows).

Upon receipt, surface sample swabs and HMC tiles were placed into cold storage

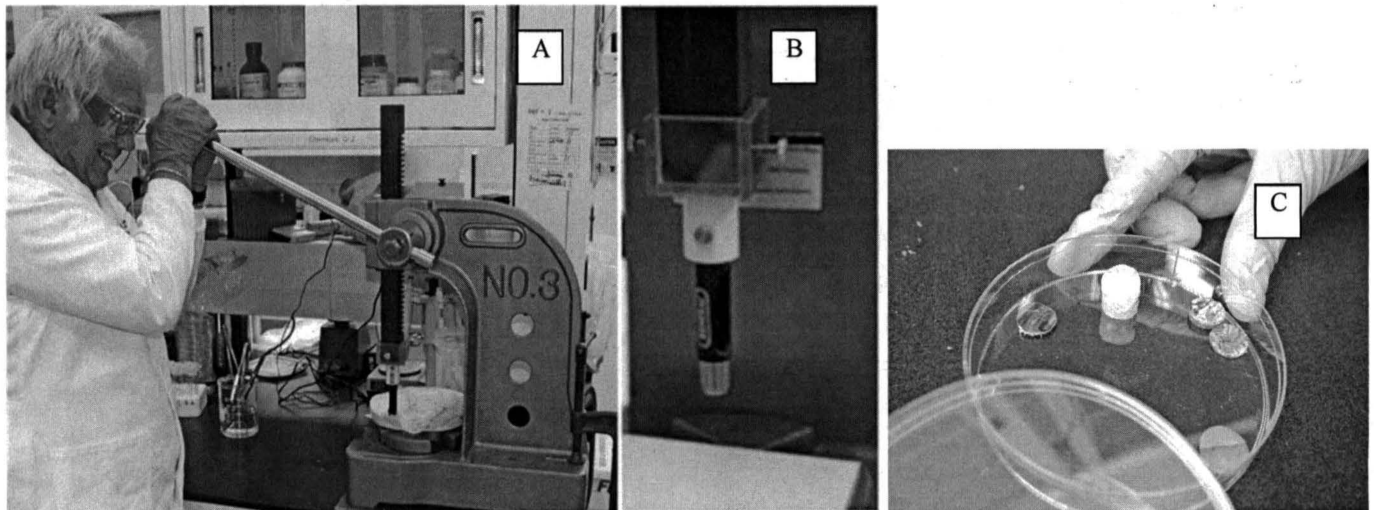

Figure 2. Picture A shows sample procedure using hand press with ½ inch hole punch (B) resulting in a core sample (C).

at 4°C until processing . Samples were removed from cold storage, equilibrated to room temperature for 2 hrs, then processed for microbiological characterization. Swab samples were analysed as described. The entire surface of the tile was swabbed before and after sanitizing the tile with 70% ethanol . This step was performed to minimize contamination of the core samples. Ten core samples, 1.25 cm in diameter were removed from the tile using a hand press with a sterile (autoclaved) sharpened hole punch (Fig. 2). Each core sample was then suspended in 10-20 ml sterile diluent (DI water) containing sterile glass beads and shaken vigorously for 2 minutes to remove microbes from the surfaces. All sample suspensions were processed for microbial characterization using the microbial characterization methods described in the surface sample section for total direct count of cells and the enumeration of aerobic and anaerobic bacteria, gram-positive spore forming bacteria and fungi.

E. Long Term storage studies.

1. *Ersatz trash preparation and inoculation.*

Simulated, trash was used as the HMC feed to produce the tiles for this study. We prepared 500 gram units of trash according to the components in Appendix II. Trash was sterilized by ETO before inoculation as described. The ETO sterilized trash was inoculated with a known bacterium and yeast previously isolated from shuttle trash and tiles, *B. amyloliquifaciens* and *R. mucilaginosa*, respectively. The inoculum procedures and densities were determined in the preliminary experiments described. HMC processing time and temperature used for the tiles prepared for this study was 180°C for 2 hours and 40 minutes. Four time points or storage durations, 0, 45 ,90 and180 days at ISS like storage conditions (25 oC, 50% RH and 3500 ppm CO2,) were tested for the recovery of the bacterial/yeast inoculant, CO2, and O2.

2. *Microbial and gas sampling and analysis of HMC tiles*

The tiles were removed from the outer covering and placed in gas sampling bags with sampling valves (Fig. 3). The valves were left open to allow airflow into the bag during incubation. (An airfilter microbial barrier was placed on the open valve to minimize contamination). All the bags were housed in a CEC set at ISS environmental conditions. Before the tiles were removed for microbiological sampling, the air valve was closed to allow accumulation of gases for gas sampling Gas Analyses. CO2 and O2 Gas samples were taken about 30 mins to 2 hours after closure of the sample port. Individual one ml samples were collected for each gas with a gas tight syringe (VICI Precision Sampling, Inc., Baton Rouge, LA USA) and injected into separate GCs for each gas coupled with a Thermal Conductivity Detector (TCD). For CO2 analysis, 1 ml of gas was injected into a GC containing a 30 m x 0.53 μm HPPLOT Q column (Agilent Technologies, Santa Clara, CA USA). Analysis method parameters are as follows: inlet temperature – 150 °C; column flow - 3.9 mL/min; oven temperature – 28 °C for 2 minutes then ramped to 35 °C at 1.8 °C/min; TCD detector - 250 °C. For O2 analysis, 1 ml of gas was injected into the GC containing a RT-Msieve 5A column (Restek, Bellefonte, PA USA) with the following parameters: inlet temperature – 35 °C; column flow – 3.9 mL/min; oven temperature – isocratic at 35 °C for 8 min; TCD detector – 200 °C.

Figure 3. Storage bags used to store HMC prepared tiles. Picture A shows the bags inside the chamber. B shows samples being taken for gas analysis.

The tiles were surface and core sampled and processed for microbial characterization as per the methods described previously for total direct count (AODC) and enumeration of aerobic and anaerobic bacteria, gram-positive spore forming bacteria and fungi. Quantitative culture and PCR methods were performed to enumerate *B. amyloliquefaciens* , *R. mucilaginosa* and other species recovered from the core samples. Identification of the microorganisms was confirmed by Biolog Micro ID and Microseq.

III. Results

A. HMC surface samples.

Varying degrees of microbial growth were found depending on the surface sampled. Generally, the piston surfaces exhibited much lower microbial counts then the groove surface. Bacteria and Fungi isolated and identified from the swab cultures are listed in Table 3. Most of the bacterial species isolated are spore forming bacteria i.e. Bacillus species resistant to heat. It is important to note that two of the organisms recovered from the surfaces of the compactor, *Bacillus amyloliquefaciens* and *Rhodotorula mucilaginosa* are the organisms used to inoculate the trash for the long term storage studies. Although possible, whether the presence of these microorganisms on the surfaces is a result of contamination from previous runs of inoculated trash or not cannot be determined without a more in depth comparison of the strains used and those recovered.

Table 3. Identities of microbial isolates from HMC surface samples.

Tile #	Surface	Bacteria	Fungi
10 DL	Comp.Piston	*Bacillus amyloliquefaciens*[a]	
	Rear Piston	*Bacillus subtilis subtilis ATCC=6051*	
	Groove	*A. amyloliquefaciens*	*R. mucilagenosa*[a]
		B. subtilis subtilis ATCC=6051	*Phyllosticta maydis*
11 DL	Rear Piston	*S. capitis capitis ATCC=27840*	*Cladosporium cladosporoides*
		S. epidermidis	
		S. lugdunensis	
		B. subtilis subtilis ATCC=6051	
		Strep. Salivarius	
	Groove	*B. subtilis subtilis ATCC=6051*	
		Bacillus atropheus[b]	
12 DL	Wall	*B. amyloliquefaciens*[a]	None
	Rear Piston	*B. amyloliquefaciens*[a]	
		B. subtilis subtilis ATCC=6051	
13 DL	None	*None*	None
7M	Comp. Piston	*B. amyloliquefaciens*[a]	None
		Bacillus pumilus	
	Wall	*B. amyloliquefaciens*[a]	
		B. pumilus	
	Rear Piston	*B. atropheus*[b]	
		Curtobacterium flaccumfaciens	
	Groove	*B. subtilis subtilis ATCC=6051*	
		Strep. cristatus	
8M	ID's pending	ID's pending	ID's pending

[a]Organism used for inoculation. [b]BI test strip organism

B. Process time and temperature studies.

Weight reduction after HMC could possibly indicate a percentage of water removed in the process. Weight loss was estimated by calculating the difference in the pre and post process weights. This loss ranged from 25% of pre-processing weight to 14%. (Table 4) There was no consistency in weight reduction with time and temperature treatment suggesting the

Table 4. Results of microbial analyses and some physical parameters for core samples cut from HMC product tiles treated at different time and temperature regimes (130° C or 140° C)

HMC tile number	10	11	12	13	7m	8m
HMC process temperature	130°C	140°C	140°C	130°C	140°C	140°C
HMC process duration	2 hrs	2hrs	3hrs	2hrs	2hrs	2hrs
Weight loss (%)	24	25	19	16	14	19
Core sample growth	4/10	1/10	6/10	3/10	3/10	5/10
G. stearothermophilus +	3/3	0/2	0	0	NA	NA
B. atrophaeus +	4/4	3/4	0	0	NA	NA
Sterilization time (hrs)	.49	.54	1.5	.54	.52	.52

possibility that liquids may be trapped in packaging in some instances, subsequently less readily removed by the heating and compaction process.

Sterilization, as indicated by biological indicator spore strips, was also inconsistent among the the different temperature treatments and bacteria and fungi isolated from a percentage of core samples (10) taken (Table 4) . From tile 10, however, both sets of test strips grew after treatment and core samples showed the most diverse growth with the isolation and identification of 9 different species (Table 5), more then any of the other samples indicating incomplete sterilization at this particular time and temperature regime (130°C, .49 hr sterilization time). Tile 10 was subjected to the lowest temperature and shortest sterilization time.

Bacterial and fungal species isolated from core samples of the tiles included in the time and temperasture studies are listed in Table 5. Six species of bacteria were isolated and identified from tile 10 core samples (130°C, 2hrs process time, .49 hrs sterilization time), *Brevibacillus agri ,Bacillus subtilis subtilis, Staphylococcus pasteuri, Kocuria kristinae, Staphylococcus epidermidis , Streptococcus salivarius.* Bacteria found in core samples from Tile 7 were identified as *Bacillus oleronius* and *Moraxella osloensis*. Two species of fungi were also identified, *Bipolaris micropus* and *Chaetomium atrobrunneum.* Fungi from tile 11 and 12 were identified as *Penicillium rubrum* and *Penicillium chrysogenum, Epicoccum nigrum,* respectively. None of these bacteria or fungi were present on the outer surface of the tile as determined by surface swabbing so the possibility that these bacteria were a result of surface contamination during processing is unlikely.

Table 5. Bacteria and fungi isolated and identified from tile core samples cut from HMC product tiles treated at different time and temperature regimes (130° C or 140° C)

10	11	12	13	7m	8m
Brevibacillus agri , *B. subtilis subtilis* *Staphylococcus pasteuri* *Kocuria kristinae* *S. epidermidis* *Streptococcus salivarius* *Bipolaris micropus* *Chaetomium atrobrunneum*	*Penicillium rubrum*	*Neisseria flavescens* *Penicillium chrysogenum,* *Epicoccum nigrum*	*Brachybacterium rhamnosum* *Streptococcus oralis* *Streptococcus mitis* *Streptococcus salivarius*	*Bacillus oleronius* *Moraxella osloensis*	IDs Pending

C. Long term storage studies.

1. *Gas analysis*

The concentrations of carbon dioxide and oxygen in the gas impermeable bags were determined for tiles 3-6 before sampling. Results showed O2 concentrations comparable to ambient atmosphere i.e. approximately 21% oxygen. CO_2 concentrations varied and reflected chamber concentration or lower, probably due to inadequate diffusion of chamber CO_2 into the bag. No biological activity as indicated by an increase in CO_2 could be detected by these results.

2. *Microbiological analysis*

Results from the analysis of the inoculated tile at day zero showed microbial growth from all 10 core samples. The bacteria isolated were identified as 6 different Bacillus species none of which was the inoculum species, *B. amyloliquefaciens*. The tile stored for 45 days was sampled and bacterial and fungal identifications are shown in Table 6. These results show microbial growth in Day 0 tiles (1m and 3m) where no growth was expected. As the storage test for this task depended on sterile tiles at the start, the decision was made to alter the time and duration of 180 0C HMC

Table 6. Bacteria and fungi isolated and identified from tile core samples cut from HMC product tiles stored for different periods.

Uninoc. Control, T=0 (1m)	Inoc. T=0 (3m)	Inoc.T=45 (4m)	Inoc. T=63 (5 m)	Inoc. T=65 (6m)
IDs pending	*Bacillus soli* *B.thuringiensis* *B. alkalitelluris* *P. agaridevorans* *B. megaterium* *B. niacini*	*B.thuringiensis* *Strep. mitis* *Cladosporium cladosporoides* *Penicillium chrysogenum*	*B. amyloliqufaciens*[a] *Strep. mitis* *Strep. salivarius* *Veillonella dispar* *Strep. Parasanguinis* *Neisseria flavescens*	*Strep. Salivarius* *Bacillus mojavensis*

processing and to restart the storage study after these new processing conditions have been shown to produce sterile tiles.

As a result, the tiles that were already in storage and meant for 90 and 180 days were sacrificed and tested, as shown in Table 7, at days 63 and 65 instead.

Table 7. Results of microbial analyses and some physical parameters for core samples cut from HMC product tiles (180 C , 2hrs, 40 mins.).

Storage duration (days) and tile number	Uninoc. Control, T=0 (1m)	Inoc. T=0 (3 m)	Inoc.T=45 (4 m)	Inoc. T=63 (5 m)	Inoc. T=65 (6 m)
Weight loss (%)	23	25	30	28	29
Core samples showing growth	7/10	10/10	3/10	4/10	5/10
R. mucilaginosa recovery	Not inoculated	NEG	NEG	POS	NEG
B. amyloliquifaciens recovery	Not inoculated	NEG	NEG	POS	NEG

IV. Conclusion

The product of heat melt compaction of ersatz solid waste was tested for it's ability to sterilize the waste and render a biologically stable tile . Our analysis showed that under certain time and temperature conditions this could be achieved but these conditions must be further defined and tightly controlled to ensure adequate sterilization. Studies are being done to complete the long term storge study of HMC tiles under conditions that achieved sterilization.

Appendix I

Average weights (n=6) of items composing trash for ETO testing

	Food	Package
Dried Apricots	6.20 ± .04	2.50 ± 00
Sausage Pattie x 2	26.57 ± .05	2.50 ± 00
Scrambled Eggs	12.43 ± .04	2.50 ± 00
Orange-Pineapple Drink	26.90 ± .00	5.78 ± .36
Frankfurter	12.67 ± .05	2.50 ± 00
Macaroni & Cheese	15.83 ± .04	2.50 ± 00
Tortilla	7.70 ± 00	2.50 ± 00
Peaches	14.26 ± .09	8.07 ± .18
Macadamia Nuts	8.98 ± .04	2.50 ± 00
Apple Cider	26.54 ± .05	5.35 ± .12
Sweet 'n Sour Pork	25.25 ± .16	2.50 ± 00
Rice w/ Butter	13.90 ± .78	2.50 ± 00
Creamed Spinach	7.78 ± .06	2.50 ± 00
Tortilla	7.72 ± .01	2.50 ± 00
Vanilla Pudding	9.98 ± .13	6.50 ± .22
Pineapple Drink	27.40 ± .00	5.42 ± .29
Total	**250.12 ± .86**	**58.62 ± .71**
Dry wipes		9.00
1 gallon ziplock		10.60
Wet wipes		116.00
Additional plastic		80.78
Calculated total		**525.12**
Micro sample weight (-Bags)		484.52

Appendix II

Formulation of simulated space trash used by Logistics Reduction and Repurposing grant for HMC and other tasks.

HMC Batch constituents	**Grams in 500g batch**	**Food Item**	**Gram in 500g batch**
Cotton T-shirt	72.7	Sausage patty	6.55
Towels	36.3	Dried apricots	3.05
Computer paper	4.0	Scrambled eggs	6.11
Dry lab chem wipe	13.0	Orange-pineapple drink	13.25
Huggies wipes	37.0	Frankfurter	6.25
Nitrile gloves	14.0	Macaroni & cheese	7.78
Shampoo	4.3	Tortilla	3.79
Toothepaste	2.2	Peaches	6.99
Plastic-PET	2.2	Macadamia nuts	4.43
Chewing gum	4.3	Apple cider	13.14
Duct tape	2.0	Sweet&Sour chicken	12.3
Vecro	0.0	Rice	6.99
Disinfectant wipes	3.0	Creamed spinach	3.79
All food (see food columns)	117.0	Tortilla	3.79
Poluetylene	32	Strawberries	.44
PET	129.0	Vanilla pudding	4.88
Aluminum foil	4.0	Pineapple drink	13.49
Polyethylene	8.0		
Salt-NaCl	11.0		
TOTAL	**500.00**		**117.0**

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