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

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Introduction

- 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.
  - Reduces volume, removes water and renders 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]

Generated wastes

- 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

Generated wastes

- Many of the solid waste components are readily biodegradable organic materials such as food and human solid waste supporting the growth of microorganisms including potential human pathogens.
- Microbial metabolic by-products can also be generated causing unpleasant odors and accumulation of volatile organic compounds (VOCs).

Objectives

The project has three primary goals or tasks.

1. Microbiological analysis of HMC hardware surfaces before and after operation.
2. Microbiological and physical characterizations of heat melt tiles made from trash at different processing times and temperatures.
3. Long term storage and stability of HMC trash tiles or "Do the bugs grow back?"

Microbiological analysis of HMC hardware surfaces.

- 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.

Hardware surface samples

- 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**
- Total Direct Count (AODC).
- Cultivation based enumeration of bacteria and fungi.

Hardware surface samples. Results

- 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.
- Most of the bacterial species isolated are spore forming Bacillus species resistant to heat.
- 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.

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

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

Process time and temperature studies.

- Weight reduction after HMC could indicate a percentage of water removed in the process.
  - Loss ranged from 25% of pre-processing weight to 14%.

HMC for the sterilization of solid wastes- minimum temperature requirements

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

Preparation of inoculated waste

- **Ethylene oxide (ETO) sterilization tests.**
  - **Six approximately 525-gram samples of trash (including plastic packaging) were prepared for testing according to the waste formula.**
  - **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.**

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

Inoculum development

- 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.

- 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

Inoculum recovery

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

- Simulated, trash was used as the HMC feed to produce the tiles for this study.
- 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.

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	Grams 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**

Tile processing and sampling.

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

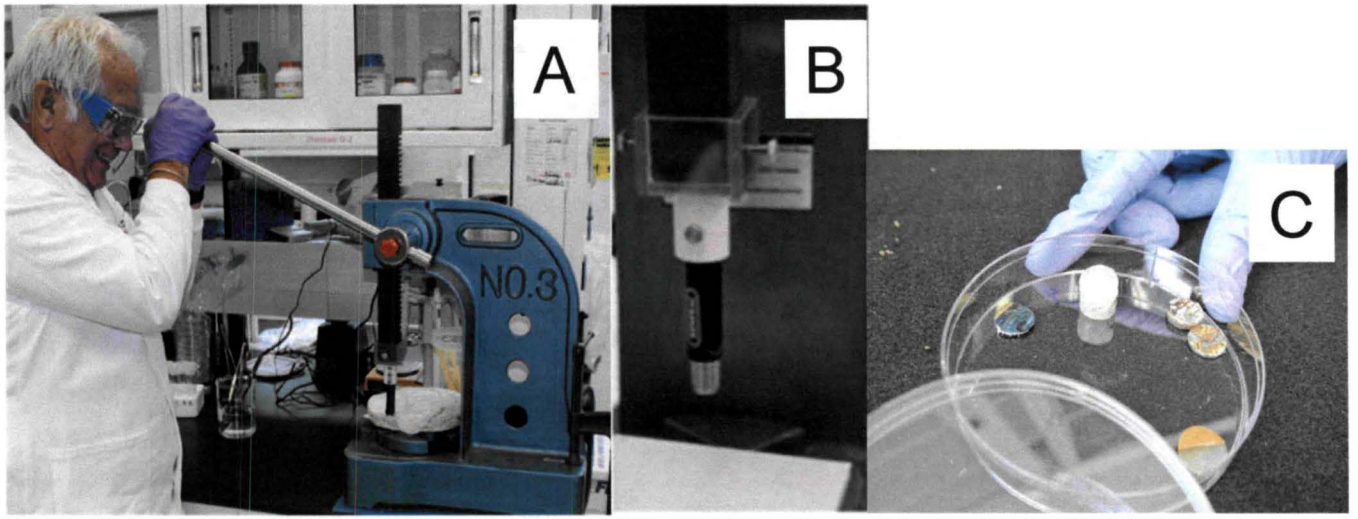

Figure 2. Picture A shows sample procedure using hand press with ½ inch hole punch (B) resulting in a core sample (C).

Process time and temperature studies. Results

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

Process time and temperature studies. Results-Microbiology

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)

Tile 10 130°C 2 hrs	Tile 11 140°C 2hrs	Tile 12 140°C 3hrs	Tile 13 130°C 2hrs	Tile 7m 140°C 2hrs	Tile 8m 140°C 2hrs
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*	*Penicillium chrysogenum* *Sphingomonas sanguinis*

Long term storage studies.

- 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°C, 50% RH and 3500 ppm CO_2,) were tested for the recovery of the bacterial/yeast inoculant, CO_2, and O_2.

Gas sampling and analysis of HMC tiles

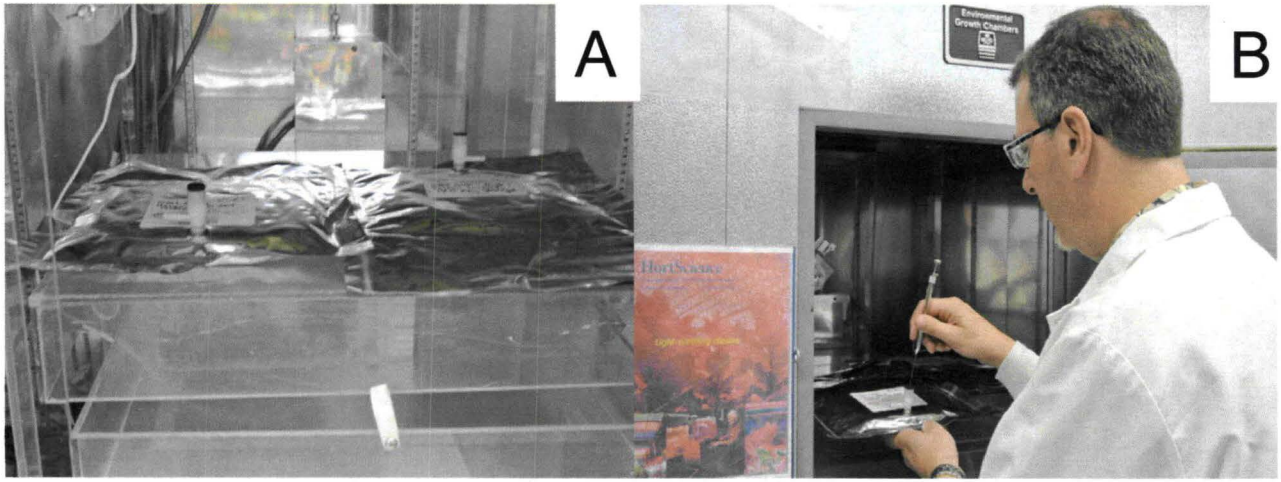

Figure 3. Storage bags used to store HMC prepared tiles. Picture A shows the bags inside the chamber. B shows samples being taken for gas analysis.

- 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.

Long term storage results
Microbiology

Table 6. Bacteria and fungi isolated and identified from tile core samples cut from HMC product tiles stored for different periods. (180 C , 2hrs, 40 mins).

Uninoc. Control, T=0 (1m)	Inoc. T=0 (3m)	Inoc.T=45 (4m)	Inoc. T=63 (5 m)	Inoc. T=65 (6m)
No IDs	*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* *R. mucilagenosa*[a] *P. chrysogenum*	*Strep. Salivarius* *Bacillus mojavensis*

[a]**Organisms used for inoculation.**

Long term storage

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

Questions?

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