

## Preliminary evaluation of in-vessel composting of inedible plant residue for long-term space missions

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### ABSTRACT

The development of bioregenerative systems for human life support during extended space missions will require effective methods for recycling the considerable quantities of inedible plant material produced as a result of food production. Preliminary studies were conducted to evaluate the feasibility of intermediate-scale (20 L) in-vessel composters for processing inedible wheat biomass produced in controlled environmental hydroponic systems. Reactor performance was very reproducible. Partial characterization of the microbiology of the system found consistent temporal shifts in bacterial density and community-level physiological profiles, although greater definition of community dynamics is necessary to define the microbial inoculum for a composter in a closed, extraterrestrial facility.

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### Introduction

Human survival on extended-duration space missions such as lunar or Mars bases will require reliable regenerative life support systems to supply food, oxygen, and water. The United States' National Aeronautic and Space Administration (NASA) is evaluating the feasibility of both physical/chemical and biological approaches as part of its Advanced Life Support (ALS) program. NASA's Kennedy Space Center (KSC) in Florida is the lead center for testing and development of biologically-based regenerative life support systems, including the use of higher plants to produce life support elements and biological reactors to process and recycle wastes.

*In situ* production of 100% of human food requirements will generate inedible plant material at an estimated rate of 0.3-0.6 kg person<sup>-1</sup> day<sup>-1</sup>, or over ten times the rate of human fecal production (6). This large "waste" stream is a valuable resource in a closed system, representing a sink of carbon and inorganic nutrients unless these elements are recovered using biological or physical/chemical methods. The inedible biomass grown in prototype ALS systems using hydroponic culture techniques contains especially high levels of inorganic nutrients due to luxuriant nutrient uptake (9). Hydroponic systems have been proposed for space missions due to reduced mass (i.e., no solid substrate) and easier harvest and clean-up; hydroponic culture in a closed system requires that nutrients be extracted from solid waste to effect recycling.

Composting may be more efficient for processing inedible plant material in extraterrestrial life support systems (12). Continuously-stirred tank reactors (CSTRs), for example, are a proven approach for extracting the majority of nutrients into an effluent

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compatible with long term plant growth, but requires significant 1) amounts of water (average solids loading rate of 20 g dry wt / 1 L water), 2) preprocessing of biomass (drying and grinding of biomass), and 3) postprocessing of reactor contents to separate solids (undigested plant residues, microbial cells) (11). Composting would require as much as 50 times less water (assuming a moisture content of 50% in the composter) and minimal preprocessing (i.e., chopping). However, the extraction of nutrients from the compost, and the compatibility of compost “leachate” with hydroponic plant growth is unproven. Current studies are evaluating the suitability of the compost leachate as nutrient source for hydroponic plant growth. Preliminary results indicate no significant effect of recycling compost leachate, but this topic will not be covered in this paper.

The goal of this research was to evaluate in-vessel composting of inedible plant waste as a means for waste processing. Specific objectives were to: 1) define the rate of degradation within the composting system; and 2) evaluate the reproducibility of the process during several replicated trials.

## **Materials and Methods**

### *Composter Design and Control*

The composting system consisted of a 18.5 L stainless steel cylinder (21 cm inner diameter, 51 cm height) with a thermally insulated water jacket. The water jacket temperature was set at 54°C to minimize conductive heat loss. The system was vertically oriented with air flow (7.5 L min<sup>-1</sup>) from the bottom. Five temperature probes spaced equidistantly along the cylinder measured temperature along the height of the composter at a distance of 3 cm from the center axis. Ventilation with room air at 22°C was used to maintain temperature within the composter using the following control criteria; 1) baseline ventilation of 1 min every 15 min; 2) additional ventilation when maximum temperature exceeded set point (54°C); and 3) additional ventilation “events” lasted 90 sec with a minimum 10 sec rest before next ventilation (3). Baseline aeration levels were defined by preliminary studies measuring O<sub>2</sub> concentrations in the exit gas.

### *Reactor Loading/Harvesting*

The reactor was loaded with the inedible portions of wheat (stems, leaves, and roots) grown in a controlled environmental chamber using recirculating nutrient film technique (NFT) hydroponic culture (13). Wheat plants were harvested from a 0.50 m<sup>2</sup> plot every 21 days, and the inedible material was immediately chopped into 1 cm to 2 cm lengths. A screen was placed on the bottom of the cylinder to hold the biomass. An additional screen was placed on top of the biomass and moved down on sampling days in response to volume reductions.

### *Analytical Methods*

The number of control ventilation events, a relative measure of microbial activity, was automatically collected. The contents of the composter were sampled at d 0, 4, 7, 14, and 21 of each of the six different trials and analyzed for volatile solids content and several microbiological parameters. Three 10 g (w.w.) samples were removed for dry (70°C overnight) and volatile (600°C for 6 h) solids analysis. Three 10 g (w.w.) samples were removed and blended in 100 ml of DI water to produce a suspension of microorganisms,

which was analyzed for 1) total cell density using acridine orange staining and epifluorescent microscopy (7), 2) culturable cell density using spread plating on tryptic soy agar incubated at either 25°C (mesophilic) for 48 h or 55°C (thermophilic) for 24 h, and 3) community-level physiological profiles (CLPP) based on direct inoculation into BIOLOG GN microtiter plates (4).

## **Results and Discussion**

### *Reactor Performance*

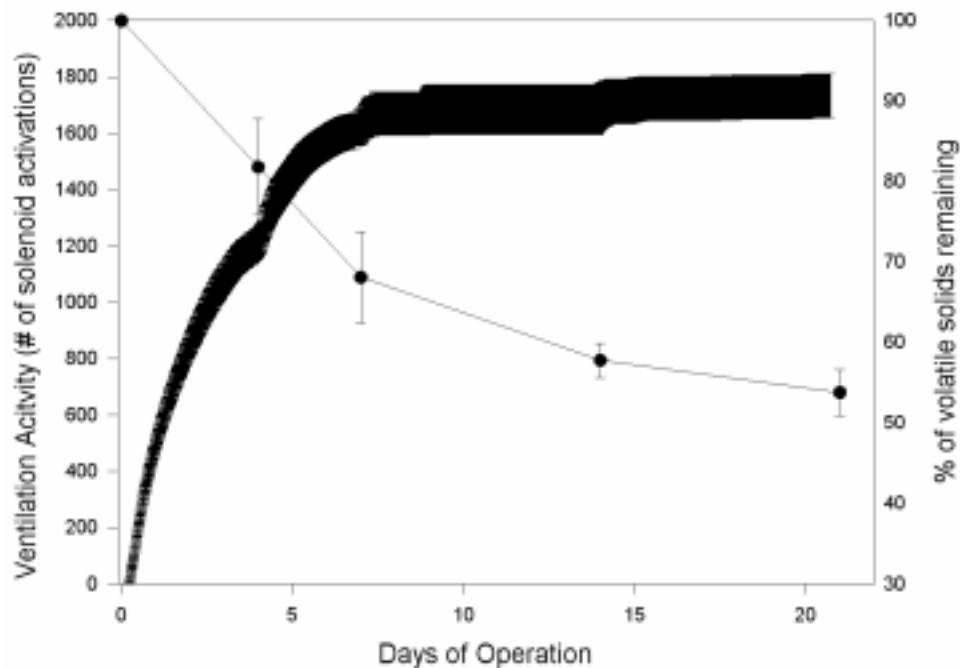
Ventilation activity was greatest during the first week of composting, with approximately 66% of the control ventilations occurring in the first 4 days, and over 90% within the first 7 days (Fig. 1). Slight increases in activity were observed following each sampling, probably as a result of substrate mixing during sampling. The coefficient of variation in ventilation activity among the six replicate tests was approximately 10%, indicating a high degree of reproducibility.

Overall, a 47% reduction in volatile solids occurred during the 21 d composting cycle (Fig. 1). Like ventilation activity, the rate of volatile solids loss was greatest during the first week. However, the rate of volatile solids loss was delayed relative to ventilation activity, with only 40% of the total volatile loss occurring within the first 4 d, and approximately 70% within the first week. The discrepancy between the two measures is most likely due to the fact that the ventilation activity is not a complete measure of microbial activity since baseline aeration events also provide oxygen and heat transfer to the system. Therefore, the lack of appreciable ventilation control events after d 7 does not indicate a lack of microbial activity, as reflected in the continuing loss of volatile solids in the system.

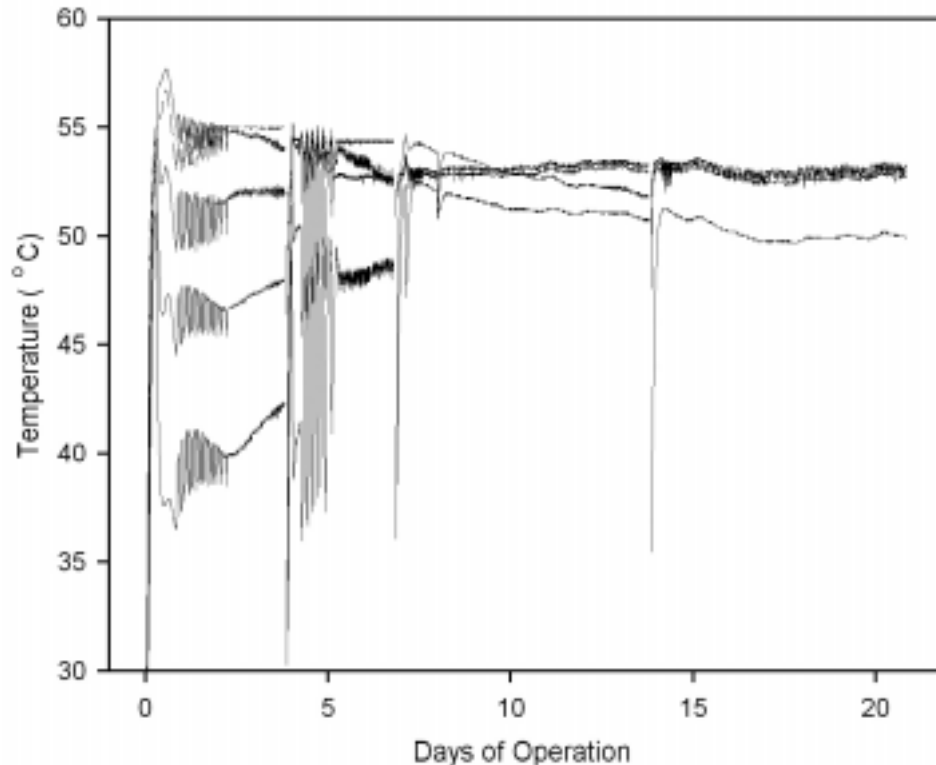
A significant spatial gradient in temperature (i.e., 10°-15°C) existed in the reactor during the first week in response to the high rate of ventilation and concomitant cooling of the lower layers (Fig. 2). After active ventilation control events decreased in frequency (i.e., after week) the temperature remained between 52-55°C throughout the vessel.

### *Microbiological Dynamics*

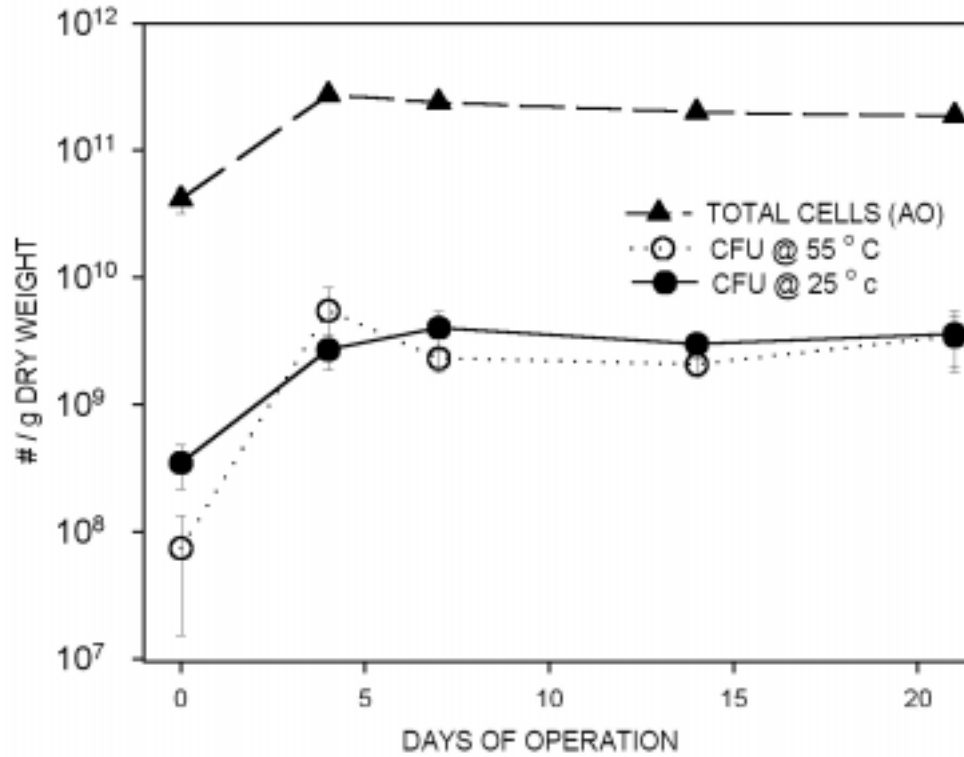
The total density of cells at time 0 was  $\sim 5 \times 10^{10}$  cells g<sup>-1</sup> d.w.. Rhizosphere microorganisms are the likely source of the majority of these organisms since roots comprise about 20% of the total mass added to the vessel, and the rhizosphere cell density of wheat grown in hydroponic systems is  $\sim 10^{11}$  cells g<sup>-1</sup> d.w. (5). The density of mesophilic and thermophilic culturable cells was 1 and 0.1% of the total cell density at time 0, respectively. The density of total, mesophilic, and thermophilic cells increased sharply between d 0 and d 4, then remained relatively stationary through d 21 (Fig. 3). Thermophilic cells showed the greatest relative change, increasing by over 10 times. A relatively equal proportion of organisms capable of growth at 25°C and 55°C were present from d 4 through d 21. The equivalent densities of mesophilic and thermophilic organisms at d 4 can be partially attributed to the large temperature gradient within the reactor at that time, but is less consistent with the relatively high temperature (>50°C) throughout the reactor from d 7-21. Campbell et al. (2) found that dominant type of culturable microorganisms in a small composting reactor were gram negative thermophiles. Atkinson et al. (1) found a higher proportion of mesophilic organisms in a bench-scale composter, but attributed it to the



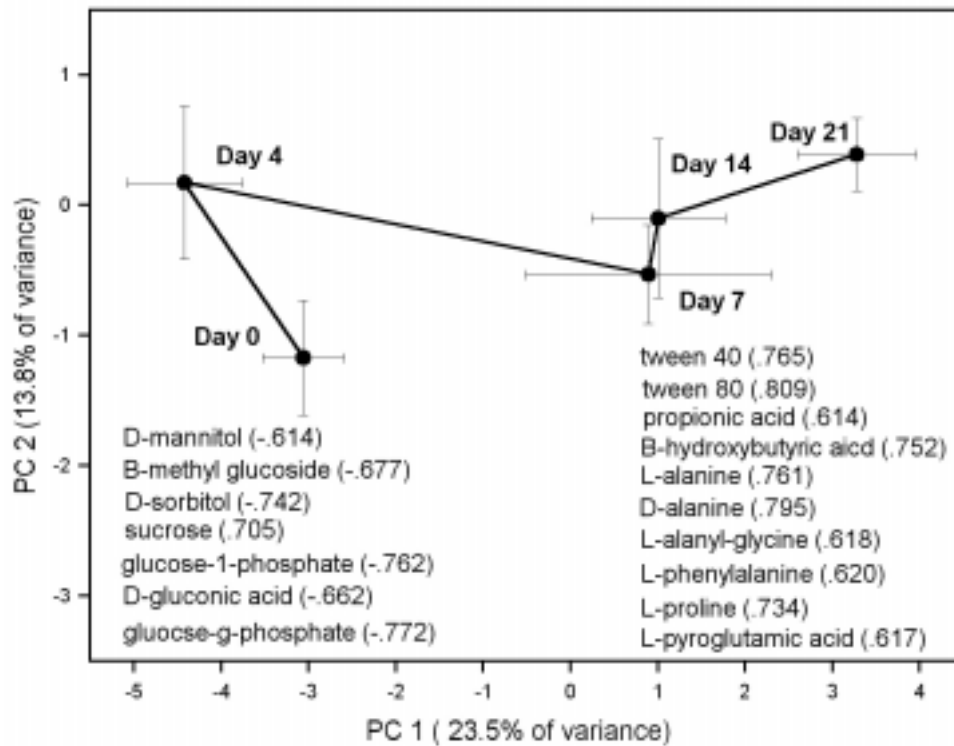
**Fig. 1.** Temporal change in microbial activity in the in-vessel composting reactor. Rising line represents mean and standard error in the cumulative number of ventilation control events for six replicate trials. Data was automatically collected at 10 min intervals. Decreasing line represents mean and standard error in the percent of volatile solids remaining in the reactor for the same six trials.



**Fig. 2.** Spatial temperature profile in the composter with time. Values represent automatically collected 10 minute data for one of the six trials.



**Fig. 3.** Temporal changes in total and culturable cell density in samples removed from the compost at 0, 4, 6, 14, and 21 days. Values represent mean and standard error of values for six replicate trials.



**Fig. 4.** Principal component analysis of community-level physiological profiles (CLPP) of samples removed from the compost at 0, 4, 6, 14, and 21 days. Data represent mean and standard error of six replicate trials. Carbon sources with highest degree of correlation to the axis are listed.

relatively short period (~5 days above 40°C) of elevated temperature in the reactor. Results of the present study suggest that mesophilic organisms can persist for extended periods (>2 weeks) at temperatures above 50°C, although further studies are needed to quantify the temperature-related growth limitations of isolates from the 25°C and 54°C plates.

Biolog plates incubated at 54°C showed no response (i.e., tetrazolium dye reduction). The cause(s) for this lack of response under thermophilic conditions are not clear, but warrants further research if the CLPP approach is to be further developed for composting systems. Potential explanations for the lack of response are that thermophiles can not actively grow in the Biolog plates or grow but do not reduce the tetrazolium dye. The second case is unlikely because no turbidity without color formation was observed. The inability to grow in the Biolog plates is surprising since TSA plates incubated concurrently showed rapid growth of a large number of colonies within 24 h. Biolog GP plates, developed for the identification of gram positive isolates, were tested since thermophilic *Bacillus* sp. are known to be common in compost (10), but also showed no response. Further tests are planned to: 1) test color response over a range of incubation temperatures; 2) evaluate the capacity of thermophilic isolates to respond in the Biolog plates; and 3) tests for community-level effects using other substrates, including tryptic soy broth.

Evaluation of the CLPP incubated at 25°C indicates a consistent temporal trend along the first principal component (PC), with the major difference occurring between d 4 and 7 (Fig. 3). The second PC, which explains only about half as much variance as the first component, primarily discriminated between the sample from d 0 and the other sampling days, although the effect was not consistent. These data indicate that composition and/or physiological state of the fraction of the community capable of growth in the Biolog plates undergo a significant shift between d 4 and 7. The fraction of the community which responds in the plates is not precisely defined, but is most likely aerobic mesophiles which can grow rapidly in response to high substrate concentrations. The timing of the shift in CLPP is not consistent with the numerical increase in total or culturable density observed between d 0 and 4. This discrepancy suggests that the aerobic, mesophilic copiotrophs show a general, non-selective increase during the initial few days of composting, then undergo a compositional or physiological shift between d 4 and 7 without any concomitant change in overall density.

The underlying functional basis for the shift between d 4 and 7 is a decrease in relative utilization in several carbohydrates and alcohols (carbon sources listed with negative correlations in Fig. 3) and increased relative utilization of several polymers, amino acids, and organic acids (carbon sources listed with positive correlations in Fig. 3). This change is consistent with the observation by Insam et al. (8), suggesting that a temporal shift from carbohydrate to amino acid use in CLPP may indicate a shift in use of labile plant material to microbial biomass (i.e., cell turnover). The maintenance of consistent cell density but continued microbial activity (as indicated by ventilation control and volatile solid loss) after d 4 supports this conclusion.

## **Conclusions**

The reproducible performance of the composter indicates that in-vessel composting appears to be a viable approach for processing inedible plant waste in closed bioregenerative

systems. The incomplete oxidation of the biomass (~50%) suggests that complete recovery of CO<sub>2</sub> from the biomass may require secondary physical/chemical (e.g., incineration) treatment. However, carbon storage may be beneficial in a closed system in which stored food is consumed since excess CO<sub>2</sub> will be produced through human metabolism; compost represents stabilized, low-volume storage product.

Further work is planned to optimize decomposition and nutrient recovery. More complete characterization of the microbial community within the composter is also required, particularly since the closed nature of an extraterrestrial bioregenerative systems raises unique questions related to the most effective and safe procedure for inoculating (and re-inoculating during long-term operation) a composter.

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