Recovery of Nutrients from Inedible Biomass of Tomato and Pepper to Recycle Fertilizer

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We explored different approaches to recover nutrients from inedible biomass (leaves and stems) of pepper and tomato plants grown in controlled environments. As with previous studies in the literature, nutrients could be solubilized and recovered from dry, ground biomass through physical leaching-- approximately 70% of the plant essential nutrients were recovered from the inedible biomass with two 10-min leachings in water at a ratio of 50 g dried, ground biomass L^{-1}. Increasing the duration to 24 h or including a biological pretreatment did not significantly increase the nutrient recovery. However, digesting the tissue for 10 min in 0.01 M HCl increased nutrient recovery to over 85%, and a concentration of 0.1 M HCl resulted in nearly 95% nutrient recovery from the tissue. Although processing the biomass in small bioreactors did not improve nutrient recovery in our tests, the microbes helped break down soluble organics (measured as total organic carbon). Previous studies using leachates or bioreactor effluent in hydroponic systems have shown these organics to be harmful to plants. Based on these and prior findings, we used data from hydroponically grown crops in NASA's Biomass Production Chamber to estimate total fertilizer required to provide food (calories) for one human. Assuming a requirement of 2500 kcal $person^{-1}$ day^{-1}, 93 kg of fertilizer salts would be needed to grow food for 1 person per year. The hydroponic approaches for these studies were deliberately maintained at nutrient-rich levels to avoid any stress, so less fertilizer should be possible. But these estimates point out the importance of recycling nutrients to support development of bioregenerative life support technologies.

Nomenclature

CSTR	=	continuous stirred tank reactor
ICP	=	inductively coupled plasma (spectrometer)
PBS	=	phosphate-buffered saline
PPF	=	photosynthetic photon flux
TOC	=	total organic carbon
VOA	=	volatile organic analysis

I. Introduction

The importance of recycling resources from wastes during space exploration missions was recognized very early in discussions of regenerative life support concepts[1]. For example, if plants are used to generate food and oxygen for life support, they will require nutrients (fertilizer) to sustain their production. If all the fertilizer has to be imported from Earth, this would impose a large mass penalty on the mission. On the other hand, if wastes from the habitat or spacecraft can be processed to recycle nutrients for the plants, this could reduce the costs for plant growth and bioregenerative life-support approaches. Depending on the scale of the crop production systems, inedible biomass from the plants themselves can become a significant component of a mission's solid waste, and hence a particularly large resource for nutrient recovery and recycling[2,3].

Various types of bioreactors have been studied for processing both liquid and solid wastes as a means to purify water, degrade organic compounds, and recover nutrients[2,4-6]. Results from bioreactor studies with inedible plant biomass showed a relatively rapid release of inorganic nutrients from the biomass, depending on the plant materials and the processes used. For example, continuous stirred tank (bio) reactors (CSTRs) can release (solubilize) many of the nutrients from inedible plant biomass in less than 24 h[7,8]. Breakdown of more recalcitrant compounds, such as cellulose, hemicellulose, and lignin, requires longer retention times, e.g., 5 days for 30%–40% breakdown, and even longer times for greater breakdown[7]. The actual release of soluble nutrients from the inedible biomass appears to occur largely by physical leaching with water and can occur in a matter of hours [2,9,10]. This would make a case for simply leaching or soaking the biomass in water, but this leaching process also releases soluble organics, which can have harmful effects on hydroponically grown plants [2,3,7,11]. This could include direct toxic effects on the plants, increased biofilm growth on hydroponic hardware, reduced dissolved oxygen levels in hydroponic solutions, and the risk of denitrification of dissolved nitrate[12]. Testing showed that treatment in bioreactors for times as short as 6 h to 24 h can degrade about 75% of the soluble organics and eliminate the negative effects on plants [7,13].

Early testing with water leaching (soaking in deionized water for 2 h) of dried wheat, potato, and soybean biomass (50 g L^{-1}) showed high recovery of elements such as K (97%), Mg (85%), Zn (91%), and Cu (84%), but lower recovery of Ca (47%) and Fe (26%)[2,10]. In addition, about 25% of the organic matter in the biomass was released as soluble organic compounds. Use of CSTRs yielded similar results for elemental recovery, depending on the biomass, and sometimes showed reduced recovery for some elements, such as Mg and Ca, which potentially were getting bound to the recalcitrant biomass[14]. By rinsing residual solids from the bioreactors following effluent removal, the recovery of K, Mg, and Ca could be improved[14]. These observations of losing some elements from residual biomass prompted the exploration of treating filtered leachate (with solids) directly with trickling bed bioreactors for strictly reducing the dissolved organics[15]. These findings suggested that elemental recovery could still be improved, especially for elements such as Ca, Fe, and possibly for P.

Full-term hydroponic production tests have been performed using bioreactor leachate from inedible plant biomass to grow wheat[2] and potato[16]. In each case, the effluent was amended with missing nutrients to generate a complete nutrient solution. These tests demonstrated the ability to retrieve and recycle nutrients from inedible plant biomass to grow subsequent generations of plants. Recycling these nutrients from inedible plant biomass could reduce the need for resupplying fertilizer from outside a life support system. Studies with potatoes showed that approximately 50% of the nutrients required to grow subsequent plantings could be recycled from inedible biomass from previous plantings[3]. The amount of fertilizer required for growing crops on space missions will depend on the total planted areas and harvest index of the crops, i.e., the edible-to-total biomass ratios[17], where for lower harvest index crops like grains and legumes, larger amounts of nutrients would be partitioned to the inedible biomass. Garland et al. estimated that for a 4-person crew with 100 m^2 plant area with 3–4 plantings per year[2], up to 320 kg per year of leachable nutrients would be incorporated in inedible biomass, and that recycling these nutrients could reduce nutrient resupply requirements by 15%. For smaller systems that might provide just salad crops, Wignarajah et al. estimated a requirement of 1 to 2 g N, 1 to 2 g K, and 0.1 g to 0.2 g P to produce 250 g fresh weight of salad per day per person[18]. Using common hydroponic salts with waters of hydration, this would be about 15 g fertilizer per person per day; if acid was included for pH control, this would add 3.75 g HNO_3 (40% in solution) per person day. But, aside from these few papers, no careful estimates have been made of the mass of fertilizer that might be needed to grow a range of crops to support human life.

We wanted to explore the use of leaching and bioreactor treatments of inedible biomass from two “salad crops”, tomato and pepper, to see we could attain similar recovery of nutrients from inedible biomass, and hopefully improved recovery of elements such as Ca, Fe, and P. We also sought to use these findings and published literature to more carefully estimate the amount of fertilizer mass needed to sustain plant growth systems for future life support systems.

II. Materials and Methods

Plant material

Six cultivars of tomato (*Solanum lycopersicum*) – ”Red Robin”, ”Scarlet Sweet ‘N’ Neat”, ”Tiny Tim”, ”Mohamed”, ”Patio Princess Hybrid”, and ”Tumble” – were grown in a controlled environment chamber (M36, Environment Growth Chambers, Chagrin Falls, OH) in the Space Life Sciences Laboratory (SLSL), Kennedy Space Center, FL, US. All cultivars were grown under a 16 h light/8 h dark photoperiod for 88 days under triphosphor T-8 fluorescent lamps (Sylvania FP541/841/HO, Danvers, MA) with an average photosynthetically photon flux (PPF) of

310 µmol m^{-2} s^{-1} (18 mol m^{-2} d^{-1}). Average temperature, relative humidity, and CO_2 concentrations were 22.0 °C, 61%, and 1449 µmol mol^{-1}, respectively.

Four plants of each cultivar were planted in 4-inch (10 cm) pots with custom potting media consisting of 7:3 Fafard #2B (Conrad Fafard, Agawam, MA):unsieved turface [arcillite]) (Profile Products, LLC, Buffalo Grove, IL) with Nutricote controlled-release fertilizer (18-6-8 type 180) (Florikan, Sarasota, IL) mixed at a ratio of 10 grams fertilizer per liter of dry media. The media was premixed with water until damp and the pots were filled. Four seeds were planted per pot with plants thinned to one per pot after germination. An automatic drip-irrigation system was used, and adjusted to provide adequate water.

Six cultivars of pepper (*Capsicum annuum*) – "Red Skin", "Fruit Basket", "Cajun Belle", "Chablis", "Sweet Pickle", and "Pompeii" – were grown in controlled-environment chambers under conditions similar to those described above. All cultivars were grown under a 16 h light/8 h dark photoperiod for 109 days under triphosphor T-8 fluorescent lamps (Sylvania FP541/841/HO, Danvers, MA) with an average light reading of 316 µmol m^{2} s^{-1} (19 mol m^{-2} d^{-1}) PAR. Average temperature, relative humidity, and CO_2 concentrations were 22.2 °C, 56 %, and 1506 µmol mol^{-1}, respectively. Planting density, potting media, and irrigation regime was the same as described for the tomatoes.

Following harvest, the leaves and stems of the tomatoes and peppers were oven-dried at 70 °C in a forced-air oven for determination of dry mass. Once dry, the inedible biomass from each of the six tomato cultivars was ground using a Wiley mill (Model 3383-L60, Thomas Scientific, Waltham, MA) to pass through a 40-mesh (0.42 mm) screen and combined into a composite sample. Following grinding, the composite tomato samples were mixed thoroughly, and total elemental content of the combined tomato biomass was determined following tissue digestion using inductively coupled plasma (ICP) analysis (iCap 6500, Thermo Scientific, Waltham, MA). The bulk of the tissue was saved for various treatments to test recovery of nutrients (below). Dried inedible biomass of the six pepper cultivars were ground, combined, and analyzed in a similar fashion.

Baseline elemental content

The inedible biomass was digested prior to inductively coupled plasma (ICP) analysis using standard laboratory protocols. Tissue was digested in 70% nitric acid at 95°C for 2 hours, cooled to room temperature, incubated with 2.5 mL of 30% hydrogen peroxide, heated at 95°C for 50 minutes, cooled, diluted to 50 mL with nanopure (18 MΩ) water, and filtered through a 0.45 µm syringe filter just prior to analysis. The concentration of the inedible biomass macro- and micronutrients is summarized in Table 1.

Table 1. Elemental content of combined samples of inedible pepper and tomato tissue.

	Macronutrients					Micronutrients			
	(mg/g dry mass)					(µg/g dry mass)			
	Ca	K	Mg	P	S	Cu	Mn	Na	Zn
Tomato	6.8	14.0	3.4	0.81	1.23	10.28	260	519	130.5
Pepper*	11.8	26.3	4.0	1.45	5.00	2.75	350	320	54.6

*Mean of two samples from composite sample

Preliminary testing

A series of exploratory tests were conducted to assess various pretreatments that might increase the release of useful nutrients or elements from the inedible biomass. These included testing with different levels of acid for digesting the biomass, testing mixed heterotrophic bacterial communities (obtained from urine/wastewater-processing bioreactors), and using cellulose degrading fungi (*Trichoderma sp.*).

Acid digestion treatment

These initial experiments were conducted to optimize the concentration and duration of tissue leaching with acid in order to maximize nutrient recovery. An unreplicated factorial of nine treatments, consisting of final acid concentrations (0.0 M, 0.01 M, 0.10 M HCl) and digestion times (10 min, 2 h, and 24 h), were evaluated. Using 20 mL plastic volatile organic analysis (VOA) vials, 1 g dried, ground (Wiley mill Model 3383-L60, Thomas Scientific) pepper biomass was mixed with 9 mL of nanopure water (18 MΩ). Following this, 1 mL of either 0.0 (water), 0.1 M HCl or 1.0 M HCl was added to the vials to reduce the pH of the solution. A small magnetic stir bar was placed into each vial and vials transferred to a stir plate and stirred at 1200 rpm for either 10 min, 2 h, or 24 h.

Following the digestion, samples were transferred to a 15 mL centrifuge tube, the VOA vial rinsed with 5 mL of 18 MΩ water, and the solution then transferred to the centrifuge tube. The sample was centrifuged at 4000 rpm for 10 min (Allegra X-14R Centrifuge, Beckman Coulter). This produced an organic solid-waste pellet and an aqueous leachate layer. The resulting supernatant was decanted into a 50 mL volumetric flask and then diluted to 50 mL using

GenPure water (18.2 MΩ). The supernatant was then filtered through a 22 µm syringe filter attachment into a 50 mL centrifuge tube and the filtrate analyzed for elemental concentration using ICP spectrometry.

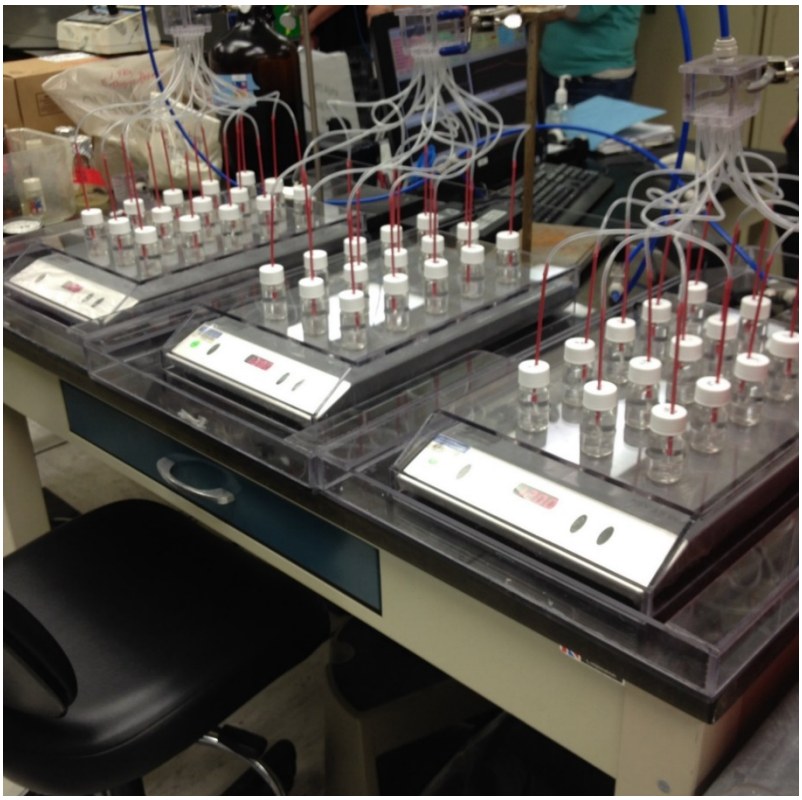

Figure 1. Vial minireactors, referred to as VOA (volatile organic analysis) vials, used for various treatments of inedible plant biomass. For some treatments, vials were continuously aerated at 60 mL/min and volumes were adjusted as needed to offset evaporation. The small vials allowed simultaneous testing of multiple treatments.

Leaching treatments

For testing water or combined microbial/water leaching approaches for the plant biomass, the biomass-to-water ratio was reduced from 1 g dry mass/9 mL liquid to 0.5 g dry mass/9 mL liquid to reduce sample viscosity and improve recovery following homogenization. Samples were processed for various incubation times (6, 24, and 168 hours) using aerated (Fig. 1) or non-aerated vials and the sample was leached two times. Exploratory tests that included acid used 0.1 M HCl for digestion.

For combined biological/leaching tests, initial tests used mixed heterotrophic bacterial communities obtained from urine/wastewater-treatment bioreactors available in the laboratory at KSC, and all treatments were carried out using aerated, 20 mL plastic VOA-vials containing 10 mL of solution. Air was bubbled through each vial at a rate of ~60 mL/min (Fig. 1). Other samples were treated with a *Trichoderma* fungus inoculum to assess whether a cellulose-degrading organisms might improve nutrient recovery.

Concentrations of elements in the final solution were analyzed by ICP for these and other treatments and are reported in µg/mL (e.g., X µg Ca/mL) in the sample. This value was adjusted for sample dilution (e.g., assuming 50 mL) and dry mass of tissue being extracted. The relative effectiveness of a treatment for extracting nutrients from inedible biomass was determined based on elemental content of the biomass and the amount recovered.

Based on the preliminary experiments, a number of modifications to the assays were made for follow-on tests, including a reduction of the ratio of inedible biomass from 1 g/10 mL to 0.5 g/10 mL, limiting treatment times to10 min or 24 h, and performing two extractions of the tissue. A comparison of the biological (mixed heterotrophic community) for 24 h prior to acid digestion for 10 min or 24 h were undertaken for both tomato and pepper. As with preliminary studies, mixed heterotrophic communities for the VOA vials (minireactors) were obtained from urine/wastewater-processing bioreactors used for related studies.

Soluble organics from biomass leaching

For most of the factorial tests, soluble organics were not tracked because of the small sample volumes. But a separate test was conducted with inedible tomato biomass to track microbial degradation of soluble organics. Microbial inoculum obtained from a home compost pile (L. Koss, Cocoa, FL) was placed in a 3-L stirred reactor with DI water and fed some ground dried inedible tomato biomass to establish a microbial community. The flask reactor was operated for approximately 1 week with continuous stirring at room temperature. Flask contents were then filtered through a paint strainer, followed by a second filtration through a coffee filter using a Buchner funnel and vacuum flask. Filtrate was divided into 50 mL centrifuge tubes and spun at 4000 rpm for 5 min. The supernatant was discarded, and the pellets were resuspended in sterile PBS (phosphate-buffered saline) and centrifuged again to regenerate the pellets. This was carried out to "wash" the inoculum culture. Pellets from this process were then resuspended in 10 mL of water and stored as inoculum starter cultures. The 3 L vessel was cleaned and 2 L of DI water were added,

along with a 20 mL of starter culture inoculum. Ground, dried, tomato biomass (50 g total) was added to the stirred flask and samples were taken at regular intervals over a 160 h period for total organic carbon (TOC) analysis. TOC includes all suspended or dissolved organics, and provides a good estimate of how quickly the soluble organics are degraded[2,13].

III. Results and Discussion

A. Preliminary tests

Biological pretreatment: There was no apparent benefit from pretreating the dried pepper biomass with either the mixed heterotrophic community (non-aerated) or *Trichoderma* inocula for the recovery of nutrients compared to leaching with water (control) (Fig. 2). This was independent of the incubation period, with incubation periods of up to 1 week (168 hours) having no appreciable increase in fractional recovery of either macroelements or microelements over a 6 h leaching in water. Leaching the tissue in water alone resulted in >90% recovery of P and S, and 60% recovery of K from the dried tissue, and 30% to 40% recovery of Cu, Mg, Mn, Na, and Zn. There was minimal recovery of Ca from the tissue with leaching or biological pretreatment (Fig. 2).

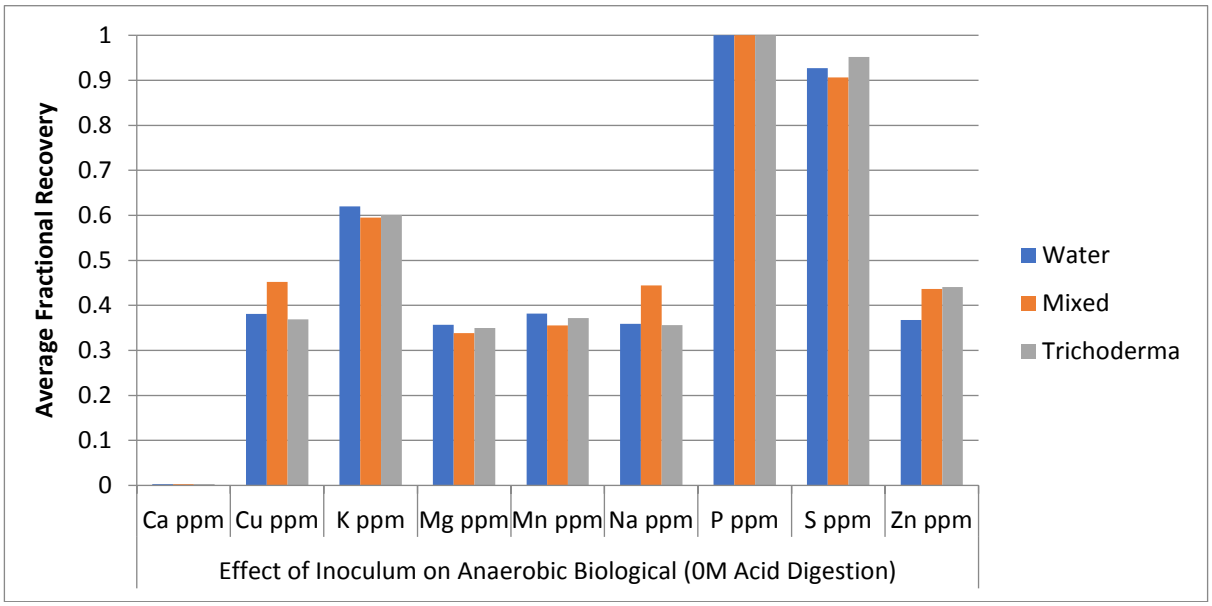

Figure 2. Effect of biological inocula on nutrient recovery from dried inedible pepper biomass. Inocula showed no effect on nutrient recovery in an anaerobic environment. Anaerobic refers to the use of nonaerated vials.

Digesting the inedible biomass with 0.1 M HCl following incubation with mixed heterotrophic community or *Trichoderma* significantly improved the recovery of nutrients from inedible pepper biomass, with >90% recovery of Cu, P, and Zn being achieved. Recovery of S was reduced from >90% with no acid digestion to between 70% and 80% with 0.1 M HCl. This would be consistent with reduction of S compounds to volatiles, which would be lost to the atmosphere. Recovery of K and Mn were increased to 70 - 80%, Mg and Na to about 40% - 50%, and Ca to about 30 - 40% (Fig. 3). There was no appreciable benefit to a biological pretreatment for increasing nutrient recovery from the tissue. When all the samples were combined to assess whether the longer-duration treatments could improve recovery, we found that increasing the incubation with biological pretreatment of 6 h to up to 1 week had only minimal effect (<10% improvement) on nutrient recovery with no acid treatment or 0.1 M HCl treatment (data not shown). Digesting the tissue with 0.1 M HCl increased recovery of all nutrients except S.

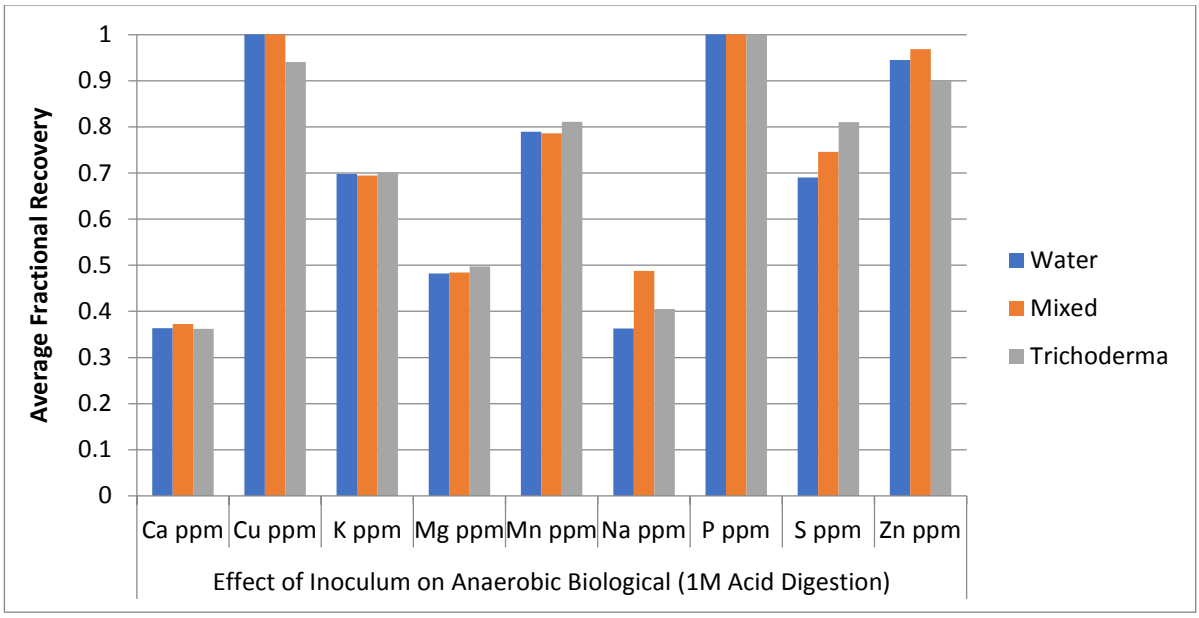

Figure 3. Effect of biological inocula on nutrient recovery from dried inedible pepper biomass followed by a 0.1 M HCl acid digestion. Anaerobic refers to the use of nonaerated vials.

B. Primary Tests

The fractions Ca, Cu, K, Mg, Mn, P, S, and Zn remaining in inedible pepper biomass and inedible tomato biomass that were either biological pretreated (in aerated vials reactors, see Fig. 1) or untreated, followed by two 10-min or 24-h leachings using water, with or without acid are shown in Tables 2 and 3. Simple leaching with water for 10 min with no follow on acid treatment removed > 90% of the K and about 50-75% of the Mg, Mn, P, and S for both pepper and tomato biomass (Tables 2 and 3). There were no consistent effects of using a microbial or just water pretreatment for recovery of nutrients. In comparison to other elements, recovery of Ca with water or microbial inoculum was only 20-30% for pepper and 30-40% for tomato. This poor recovery of Ca is consistent with the findings of others using leaching or bioreactor treatments with wheat, soybean, and potato biomass[2,3,8,10,12,16].

Treating (leaching) the tissue in 0.01 M HCl for 10 min recovered >90% of Mg and Mn, and about 50-60% of P in pepper, and about 70% of P in tomato, with recovery slightly less in biologically pretreated tissue. For S, recovery was about 70-80% for 0.01 M HCl for 10 min, while Ca was about 50% for pepper and about 80% for tomato biomass. Treatment with 0.1 M HCl for 10 min recovered > 95% of the Ca, K, Mg, and Mn for both types of biomass, but only about 50-70% of the P for pepper (Table 2) and 60-80% for tomato, and a slight increase in recovery of S (Table 3). Extending the 0.1 HCl treatment to 24 had only marginal benefit for most elements, but generally improved P recovery for both types of tissue. Interestingly, extending the 0.1 HCl treatment from 10 min to 24 h showed little or no improvement on S recovery, which again might suggest loss of volatile S compounds following acid treatment. Recovery of nutrients from the leaching treatments are consistent with previous, published findings using stirred tank reactors [2,3,5,8]. Acid treatments were required to significantly improve the recoveries, particularly for elements like Ca, Mg, and Mn using pepper and tomato biomass.

Table 2. Fraction of nutrients removed from inedible pepper biomass following two extractions with and without 24 h microbial (mixed heterotrophic) pretreatment followed by 10 min or 24 h digestion in 0 M, 0.01 M, or 0.1 M HCl. All values referenced against elemental content of composite sample of tissue following total digestion (Table 1).

	HCl digest		Elemental Recovery (fraction of total in composite sample)							
Pretreat	time	(M)	Ca	Cu	K	Mg	Mn	P	S	Zn
Mixed Heterotroph Microbial Community (24 h)	24 h	0	0.22	ND	**0.95**	0.72	0.56	0.58	0.68	ND
		0.01	0.48	0.70	**0.98**	**0.98**	**0.97**	0.65	0.72	ND
		0.1	**0.97**	0.76	**0.98**	**0.98**	**0.98**	**0.88**	0.77	ND
	10 min	0	0.27	ND	**0.94**	0.69	0.57	0.67	0.69	ND
		0.01	0.46	0.58	**0.98**	**0.98**	**0.96**	0.62	0.69	ND
		0.1	**0.97**	0.74	**0.98**	**0.98**	**0.98**	0.69	0.74	ND
Control (no microbes)	24 h	0	0.18	0.13	**0.94**	0.67	0.53	0.68	0.65	ND
		0.01	0.56	**0.83**	**0.98**	**0.98**	**0.96**	0.50	0.73	ND
		0.1	**0.97**	**0.82**	**0.98**	**0.98**	**0.98**	**0.80**	0.75	ND
	10 min	0	0.19	0.00	**0.94**	0.69	0.55	0.51	0.63	ND
		0.01	0.49	0.74	**0.98**	**0.98**	**0.96**	0.47	0.73	ND
		0.1	**0.96**	**0.81**	**0.98**	**0.98**	**0.98**	0.54	0.77	ND

Recovery > 80% are highlighted. ND = not detected for Cu and no data for Zn.

Table 3. Fraction of nutrients removed from inedible tomato biomass following two extractions with and without 24 h microbial (mixed heterotrophic) pretreatment followed by 10 min or 24 h digestion in 0 M, 0.1 M, or 1.0 M HCl. All values referenced against elemental content of composite sample of tissue following total digestion (Table1).

	HCl digest		Elemental Recovery (fraction of total nutrient recovered)							
Pretreat	time	(M)	Ca	Cu	K	Mg	Mn	P	S	Zn
Mixed Heterotroph Microbial Community (24 h)	24 h	0	0.41	0.38	**0.95**	**0.83**	0.68	0.53	**0.71**	0.46
		0.01	**0.83**	**0.88**	**0.98**	**0.98**	**0.98**	0.46	0.67	**0.82**
		0.1	**0.97**	**0.94**	**0.97**	**0.97**	**0.97**	**0.89**	**0.70**	**0.80**
	10 min	0	0.31	0.40	**0.93**	**0.75**	0.53	0.57	0.64	0.37
		0.01	**0.78**	0.65	**0.98**	**0.94**	**0.91**	0.67	**0.78**	0.68
		0.1	**0.96**	**0.85**	**0.98**	**0.97**	**0.97**	**0.76**	**0.80**	**0.81**
Control (no microbes)	24 h	0	0.32	0.34	**0.93**	**0.75**	0.63	**0.70**	0.64	0.41
		0.01	**0.80**	**0.88**	**0.97**	**0.97**	**0.97**	**0.71**	**0.70**	**0.82**
		0.1	**0.96**	**0.93**	**0.97**	**0.97**	**0.97**	**0.89**	**0.75**	**0.81**
	10 min	0	0.31	0.64	**0.95**	**0.76**	0.59	**0.75**	0.65	0.39
		0.01	**0.76**	**0.87**	**0.98**	**0.96**	**0.96**	0.68	**0.70**	**0.73**
		0.1	**0.96**	**0.91**	**0.97**	**0.97**	**0.97**	**0.78**	**0.72**	**0.75**

Recoveries greater than 70% are highlighted.

CSTR for soluble organic analysis

The 3-L continuously-stirred tank reactor used to test organic degradation is shown in Fig. 4. TOC measurements following the addition of 50 g of dried tomato biomass to 2 L of solution (25 g L^{-1}) are shown in Fig. 5. Results showed the expected spike in TOC following addition of the dried plant biomass, followed by a gradual decline in TOC over time. After 24 h, TOC levels dropped to approximately 40% of the starting values, and about 12% of the peak TOC after 732 h. The decline in TOC is typical for stirred-tank reactor tests with dried plant biomass and reflects the microbial degradation of soluble organics released from the biomass[13]. Previous studies indicate the use of raw

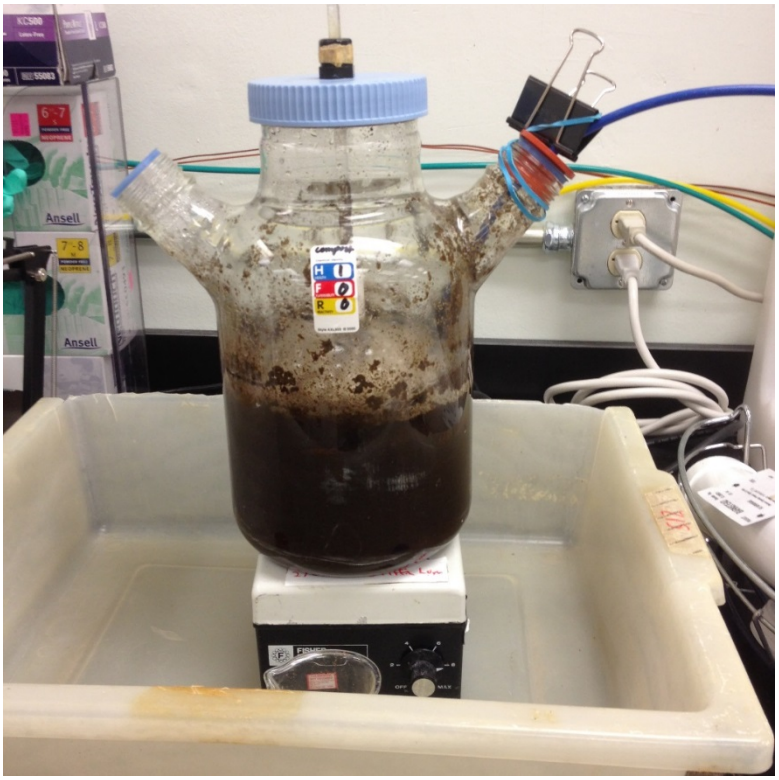

Figure 4. Stirred-flask reactor used to track TOC concentration degradation over time. Testing used a working volume of 2 L of solution to which 50 g of dried ground tomato biomass was added, along with a microbial inoculum originally obtained from a home compost system for yard waste.

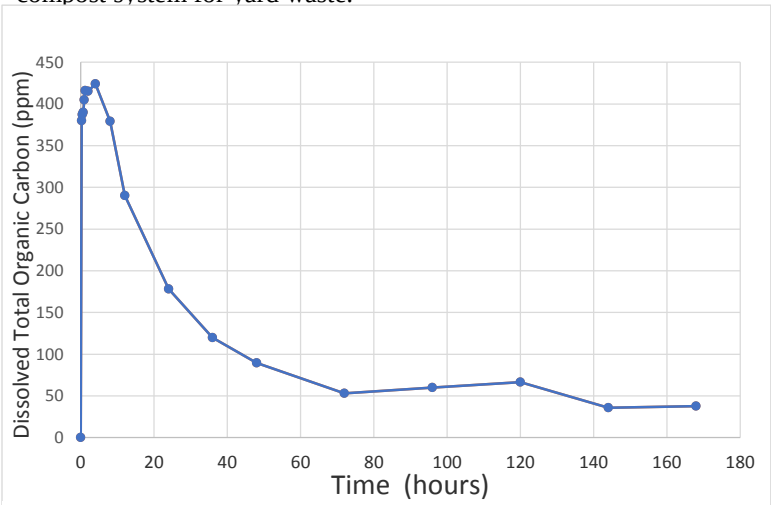

Figure 5. TOC concentration in a stirred flask reactor following addition of 25 g L^{-1} of dried ground tomato biomass (inedible leaves and stems), and a microbial inoculum from a home yard-waste compost pile. Solution volume for the reactor was 2 L**.**

leachate from dried plant biomass (wheat, soybeans, and potatoes) can be toxic to hydroponically grown plants, and this toxicity has been attributed to soluble organic compounds that can leach out of the biomass; microbes rapidly degrade many soluble organics, rendering the effluent nontoxic to plants[2,3,8,13]. Our findings showed that TOC in a bioreactor using ground tomato biomass followed a very similar trend of degradation with time (Fig. 5).

C. Fertilizer Required to Support One Human—A case for nutrient recycling

The use of crops for life support systems could span scenarios ranging from small, supplemental food production systems[18,19], through partial food and O_2 production, to near full dietary calorie production (2500 kcal $person^{-1}$ day^{-1}) with complete O_2 regeneration[17,20,21]. Sustaining these outputs will require a continuous supply of fertilizer. If the system uses recirculating hydroponics, then acid and/or base might also be needed for pH control[22]. Possible sources of fertilizer include: 1) stowage and resupply from Earth, 2) extracted from in situ materials such as surface regolith [23,24], and 3) recycled from wastes like inedible biomass or urine[25-27]. It is unlikely that *in situ* regolith in a setting like Mars could supply most of the plants' nutrient needs. On the other hand, wastes such as human urine [18,28,29], or inedible biomass from previous plantings could be a significant source of N, P, K, Ca, Mg, and several other elements [2,3,26,27]. For life support systems with large bioregenerative components, a mix of plant species grown under moderate to high light conditions could generate up to of 30 g m^{-2} day^{-1} of dry biomass[17]. For a 200 m^2 of plant growing area (50 m^2 per person for four people), this would amount to 30 g m^{-2} d^{-1} X 200 m^2 = 6 kg of dry mass per day, or 6 kg d^{-1} X 365 d yr^{-1} ≈ 2.20 MT yr^{-1}.

Tests in NASA's 20 m^2 Biomass Production Chamber at Kennedy Space Center carefully tracked nutrient (fertilizer) uptake and water use by multiple crops throughout growth and development[30]. Total nutrient additions were then expressed as mmol of cations (Ca, Mg, and K) added per m^2 of crop area per day[30]. Nutrient uptake showed a near linear relationship to light and overall biomass yields. The three major cations Ca, Mg, and K represent most of the cation mass in fertilizer, and an equivalent molar

rate of major anions such as nitrate, phosphate, and sulfate would also be required. These essential anions are typically provided with the same salts as the cations. Thus, by using these hydroponic data, the weights of typical salts for fertilizer to grow the crops can be calculated.

The following equations were fit to the data from the hydroponic studies that included two tests with lettuce, three tests with soybean, two tests with potato, and two tests with wheat[30]:

Nutrient Use: $y = 0.42 + 0.84\ x$, where y = mmol m^{-2} d^{-1} (K, Ca, Mg), and x = mol m^{-2} d^{-1} of light (PAR*)
Biomass Production: $y = -2.71 + 0.60\ x$, where y = g dry mass m^{-2} d^{-1}, and x = mol m^{-2} d^{-1} light (PAR*)
*PAR = Photosynthetically Active Radiation or Light in the 400-700 nm band width

The major fertilizer salts used for these studies are shown in Table 4[30]. The starting concentrations and refill "stock" solution concentrations are also shown. The refill stock solution was about 10 × the concentration of the starting (working) solution concentration. Assuming most of the nutrients were provided from the refill solutions, each liter of stock solution contained 78 mmol of cations or 9160 mg L^{-1} of salts. The average weight of the salts required for this input was 117.4 g mol^{-1} using anhydrous weights for $Ca(NO_3)_2$ and $MgSO_4$.

Table 4. Nutrient solution for crops grown in recirculating nutrient film technique (NFT) hydroponic systems at NASA's Biomass Production Chamber, Kennedy Space Center.

	N	P	K	Ca	Mg	S
Salt			(mmol L^{-1})			
KNO_3 (101 g mol^{-1})	2.5		2.5			
$Ca(NO_3)_2$ (164 g mol^{-1})	5.0			2.5		
$MgSO_4$ (120 g mol^{-1})					1.0	1.0
KH_2PO_4 (136 g mol^{-1})		0.5	0.5			
Starting Concentration	7.5	0.5	3.0	2.5	1.0	1.0
Refill Stock Concentration	70	10	56	12	10	10

Salts for micronutrients Fe, Mn, Zn, Cu, B, and Mo were also included but were low in total mass.

Using equations relating biomass production and nutrient uptake, dry biomass productivity for a range of crops at different light levels is estimated (Table 5, Row 1). Assuming a daily requirement of 2500 kcal person, the areas of crops needed to support one person were then determined (Table 5, Row 2). Note that the area required per person decreases as light increases, because of the increased crop yield per unit area. Nutrient salts required to sustain crop growth at each of the daily light integrals was then calculated in mmol m^{-2} d^{-1} (Table 5, Row 3) along with the mass of nutrients (Table 5, Row 4). Biomass and required nutrients increased in a near-linear fashion with light.

Multiplying the crop area required per person and the nutrients per unit area allows an estimate of the mmol (Table 5, Row 5) and mass (Table 5, Row 6) of salts required per person per day. One would expect these to be relatively close across all the different light values, since the required crop areas per person are greater at lower light. The results show that about 260 g of fertilizer salt are required per day (mean value Table 5, Row 6). Since the data were generated from a linear equations fit to the findings from multiple studies, it is difficult to know if crops are genuinely more efficient with nutrients across the different light levels or if this is just an artifact of the equation fit. Multiplying daily fertilizer requirements (Table 5, Row 6) by 365 days per year gives the fertilizer requirement to support a person for a year (Table 5, Row 7). At 40 mol m^{-2} d^{-1}, a moderately high light level, the predictions show that about 93 kg of fertilizer salts (nonhydrated) would be required to support one person for one year (Table 5, Row 7, shaded cell). Because these nutrient solutions were maintained at a constant electrical conductivity, it is likely that these estimates are representative of a nutrient rich production system.

Table 5. Calculation of nutrient (fertilizer) salts needs for crop growth for life support using different light levels.

	Daily Light Integral (mol m^{-2} d^{-1})	20	30	40	50	60
	Units					
1) Total Biomass vs. Daily Light *	g DW m^{-2} d^{-1}	9.29	15.29	21.29	27.29	33.29
2) Crop area per Person†	m^2 $person^{-1}$	135	82	59	46	38
3) Nutrient Use vs. Daily Light ‡	mmol m^{-2} d^{-1}	16.38	24.78	33.18	41.58	49.98
4) Mass of Fertilizer Salts	mg m^{-2} d^{-1}	2022	3008	3994	4980	5966
5) Fertilizer per Person (molar)	mmol $person^{-1}$ d^{-1}	2204	2026	1948	1905	1877
6) Fertilizer per Person (mass)	g $person^{-1}$ d^{-1}	288.5	265.2	255.0	249.3	245.7
7) Fertilizer per Person (365 days)	kg $person^{-1}$ $year^{-1}$	105.3	96.8	93.08	91.0	89.7

*Conversion formulas from Wheeler et al. 1999[30].

†Assumes 2500 kcal $person^{-1}$ d^{-1} to provide dietary energy and a harvest index of 50%, i.e., 50% of the dry biomass is edible.

‡Essential anions such as N, P, S, and Cl get added as part of Ca, K, or Mg salts (Table 4).

This analysis reveals that a total mass of about 93 kg of fertilizer would be required per person per year using the hydroponic approaches tested by NASA. This would be nearly 1.3 times the weight (mass) of a 70 kg human to provide their hydroponic fertilizer for growing food. There are ways to reduce this value, such as lowering the concentrations of nutrient solutions during later growth phases for determinate crops like wheat, soybeans, and many potato cultivars. In addition, our assumption of 2500 kcal $person^{-1}$ day^{-1} for food production may be high and hence the total amount of fertilizer would be less for lower caloric requirements. Nonetheless these mass numbers are significant.

These values highlight the importance of nutrient management and recycling as many nutrients as possible from waste streams and materials (e.g., human urine, inedible plant biomass, and other wastes). Mackowiak et al. [3, 16] showed that over 50% of many required nutrients for growing wheat and potatoes could be recycled from the inedible biomass from previous plantings. Assuming this would hold for a wider range of crops, this could reduce the fertilizer requirements predicted from our analysis from 93 kg down to 46.5 kg $person^{-1}$ yr^{-1}. This could be reduced further by recycling useful nutrients from urine and other wastes [18, 20, 25-27].

During a 2-month test in the Russian BIOS-3 facility, two phytotrons (41 m^2 total growing area) produced about 117 kg of plant dry mass, with 37.4 kg of it being edible. This required 20.6 kg of fertilizer salts and acid (along with about 5 kg of water of hydration in the salts) to be added to the nutrient solution[33]. Assuming an average energy value of 4 kcal g^{-1} dry edible biomass, this would equate to 37,400 g X 4 kcal g^{-1} = 149,600 kcal from edible biomass in 60 days, or about 2500 kcal day^{-1}, which would support one human. Using non-hydrated fertilizer and acid mass for their 60-day test, this would equate to 20.6 kg x (365 d yr^{-1} / 60 d) = 125 kg of fertilizer and acid per person per year. We did not include acid required for pH control in our studies, since acid requirements can vary by the type of nitrogen fertilizer used [31.32], plant species, and other factors. But our estimate for acid use in the BPC tests for the studies reported here was about 28.5 kg $person^{-1}$ yr^{-1} anhydrous nitric acid (data not shown), for a total mass of 122 kg of fertilizer and acid per person per year, which is very close to the Russian data for BIOS-3[33].

A key challenge for recycling nutrients from urine would be the ability to remove or reduce the amount of sodium, since too much sodium can stress the crops and eventually make nutrient solutions too saline for healthy growth. This has been the focus of various strategies, such as the use of moderately salt-tolerant crops like amaranth, beet, and chard [29, 34, 35] or even using halophytic (highly salt-tolerant) plants such as *Salicornia* [36].

The startup and expansion of a crop-production system in space would require the import of fertilizer salts to "prime" the system and manage the first crops. During these first crops, nutrients could be recycled from urine to

offset some fertilizer needs. Following this, inedible biomass could be processed (along with urine) to recycle more nutrients and further close the mass loop. For planetary surface settings such as Mars, some additional nutrients might be obtained from local regolith. These processes will require additional capabilities but could ultimately reduce the cost for *in situ* food production by reducing the need for imported fertilizer, and provide a higher level of autonomy for space exploration.

IV. Conclusion

Plants can be used for both near-term and future space missions to provide fresh foods to the diet. As mission durations and distances increase, plants could provide more of the bulk foods (carbohydrate, protein, and fat) and contribute to air and water recycling for life support. To do this will require a continuous supply of fertilizer. The fertilizer could be stowed and resupplied from Earth but this would become increasingly costly over time. Our calculations suggest that about 93 kg of fertilizer salts would be needed to grow enough plants to provide all the dietary calories (energy) for one person per year. Depending on how the plants were grown, about 28 kg of anhydrous acid would also be needed, for a total mass of 122 kg to grow the plants to support one person per year. A more sustainable approach would be to recycle nutrients from wastes, such as the inedible plant biomass. We could recover approximately 70% of the plant essential nutrients from tomato and pepper leaves and stems with two 10-min leachings in water, which was consistent with published results with wheat, soybean, and potato inedible biomass. If acid was added for leaching, the recovery could be increased to 85-95% of the nutrients from the inedible biomass. By combining this approach with recovery of nutrients from other waste streams (e.g., urine, solid wastes), and the possibility of using in situ minerals, the need for importing fertilizer could be greatly reduced making the system much more sustainable.

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