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ABSTRACT

The coupling of plant growth and waste recycling systems is an important step toward the development of bioregenerative life support systems. This research examined the effectiveness of two alternative methods for recycling nutrients from the inedible fraction (residue) of candidate crops in a bioregenerative system; 1) extraction in water, or leaching, and 2) combustion at 550 °C, with subsequent reconstitution of the ash in acid. The effectiveness of the different methods was evaluated by 1) comparing the percent recovery of nutrients, and 2) measuring short- and long-term plant growth in hydroponic solutions, based on recycled nutrients.

INTRODUCTION

Typical management practices in modern agroecosystems tend to upset the normally conservative nutrient cycles of natural ecosystems, resulting in systems which have relatively high influx and efflux of nutrients (1). Agricultural crop-based bioregenerative systems proposed for life support on long-term space missions will require internal nutrient cycles in order to reduce reliance on external supplies. The inedible fraction (residues) of agricultural crops represents a pool of both organic and inorganic matter which will have to be recycled to CO_2 and soluble nutrients, respectively, in order to reduce resupply requirements.

Two separate approaches to recycling crop residues in a bioregenerative system have been proposed; 1) physical-chemical oxidation, and 2) biological oxidation. Potential physical-chemical methods include wet oxidation (2, 3), dry incineration (4), and low temperature plasma reaction (5). In the biological oxidation approach, microorganisms would enzymatically convert the organic matter to CO_2, releasing or solublizing inorganic constituents of the biomass in the process. Selection of the optimal resource recovery method for bioregenerative systems will depend on evaluation of several factors: 1) resource requirements (e.g., mass, energy, volume), 2) alternative food production from the biological oxidation pathway, 3) extent of organic matter conversion to CO_2, 4) recovery of inorganic nutrients in a form suitable for plant growth systems (i.e. hydroponic systems), and 5) identification of possible contaminants (chemical and biological) deleterious to plant or human health.

Preliminary research associated with the Controlled Ecological Life Support System (CELSS) Breadboard project at Kennedy Space Center (KSC) indicated that the water soluble extract of inedible wheat may be suitable for use as a plant nutrient source in hydroponic systems (6). Water extraction, or leaching, can be viewed as one step in an overall biological oxidation scheme (7), or as a pretreatment of biomass to remove nutrients prior to physical-chemical oxidation (8). Further discussion of how the leaching process can be integrated into potential resource recovery pathways is presented elsewhere (9).

This paper details our efforts in quantitatively comparing different nutrient recycling systems within a CELSS. Our goals were to 1) determine the percent recovery of inorganic nutrients via leaching for various candidate CELSS crops, and 2) evaluate the effects of leachate-based nutrient solutions on short- and long-term plant growth. In addition, we report our preliminary evaluation of long-term plant growth in nutrient solutions based on reconstituted ash from high temperature incineration of crop residue.

METHODS

LEACHATE AND ASH CHARACTERIZATION - Plant biomass - The inedible fractions of mature wheat (*Triticum aestivum* L. cv. Yecora rojo), white potato (*Solanum tuberosum* L. cv. Norland) and soybean (*Glycine max* L. Merr. cv. McCall), grown in the Biomass Production Chamber (BPC) at KSC, were used in these studies. Wheat biomass included chaff, leaves, stems, and roots. Potato biomass included leaves, stems, and roots. Soybean biomass included leaves, stems, roots, and pods. Crop biomass was oven-dried at 70 °C and ground through a 40-mesh stainless steel screen prior to use.

Leaching - The specific protocol for leaching of inedible plant biomass is elsewhere (9). An overview of relevant conditions were as follows: 1) loading rate of 50 g biomass L^{-1} deionized water, 2) duration of 2 hr, 3) aeration rate of 20 L min^{-1}, and 4) rinsing with an equal volume of deionized water in five separate washings.

Dry ashing - A large quantity of inedible biomass was needed for plant growth studies involving ash-based nutrient solutions (approximately 250 g ash per wheat grow-out). Since the laboratory-scale muffle furnace was too small to handle the entire sample, the standard dry ashing technique (10) was modified. Instead of bringing samples from room temperaure to 500 °C within 2 hours, samples were placed into a 500 °C preheated oven. This caused "flaming" of the samples which may have resulted in some loss of elemental content, but the technique decreased the processing time by 60%. Porcelain crucibles were filled to a greater depth than recommended, and the oven was filled with crucibles (even near the door), in order to increase the amount of biomass combusted per run. These steps are known to cause incomplete combustion of samples, and residual "charcoal-like" residue was visible in our ashed samples. The resulting ash was then solubilized in 2.0% (v/v) HNO_3 prior to being used in studies.

Nutrient analysis - Untreated, leached, and combusted plant biomass was analyzed for all plant nutrients (excluding nitrogen) by ICP emission spectrometry (Wade Berry, Biomedical and Environmental Sciences Laboratory UCLA, Los Angeles, CA). Ash was reconstituted in either water or 5% HNO_3 prior to analysis. Nutrient content in leached, combusted, and untreated biomass was used to determine percent recovery of nutrients from leaching and combustion, respectively. Leachate samples were also analyzed with ICP emission spectrometry (Teresa Englert, Bionetics Corp., Kennedy Space Center, Fla.). NO_3-N and PO_4-P content of aqueous samples were analyzed using a Technicon Autoanalyzer. Total organic carbon (TOC) content of leachates was measured using ultraviolet-assisted persulfate oxidation.

SEEDLING BIOASSAYS - Solution preparation - Growth of wheat, potato, soybean (cvs. noted above), and lettuce, (*Lactuca sativa* L. cv. Waldmann's Green) seedlings were compared in leachate-based solutions versus an inorganic salt-based solution, namely, a modified, half-strength Hoagland's (MH) (11). LB solutions were prepared as follows: 1) wheat, potato, and soybean leachates were diluted ten-fold with deionized water to reduce nutrient concentrations to workable i.e. non-toxic, levels. Inorganic salts were supplemented where nutrient concentrations were less than 100% of the MH treatment.

Plant culture - Wheat, lettuce, and soybean seeds were germinated for 3 days on moistened filter paper in sealed petri plates prior to use in the experiments. Nodal explants of potato were grown for 3 weeks in sterile culture tubes containing modified Murashige and Skoog medium, 2% sucrose, and 0.7% agar, prior to use in the experiment. All plants were transferred to acid-washed, wide-mouth glass vessels (baby-food jars) containing 50 ml of nutrient solution. Seedlings were placed into slits of autoclaved, foam plugs, which were inserted into the mouths of the jars. Nylon strips were used to wick up nutrient solution to the plants until roots reached the solution. Air was delivered to the nutrient solutions via an aquarium pump and Tygon™ tubing that terminated at a syringe needle located at the bottom of each jar (Figure 1). The aeration maintained dissolved oxygen levels in the jars above 6 ppm. Jars were placed in a controlled environment chamber with a 12h/12h photoperiod, using HPS lamps at 300 μmol m^{-2} s^{-1} PPF, 23 °C continuous temperature, and 65% relative humidity. At 7 days the plants were harvested and shoot and root lengths, shoot fresh mass (FM), and shoot and root dry mass (DM) (48 h at 70 °C) were determined.

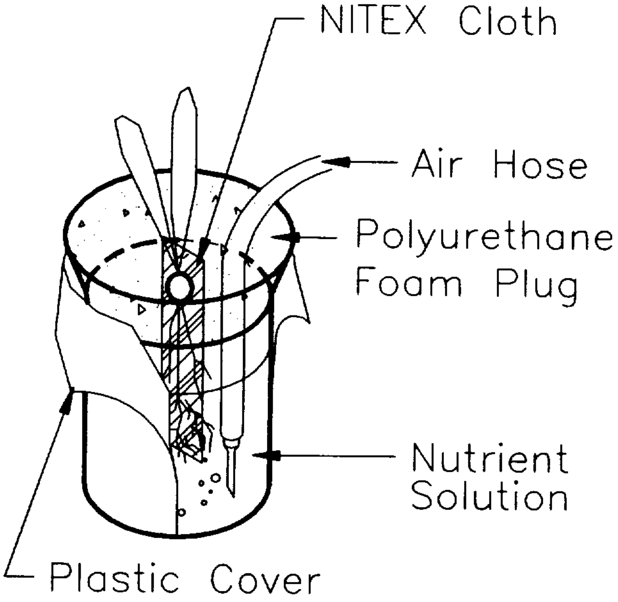

Figure 1. Plant growth system used in seedling bioassays.

Preatreatment study - Leachate was biologically pretreated for a subsequent seedling study. A small inoculum of microorganisms (0.125 ml) from a benchtop plant growth system containing leachate was added to 50 ml of crop leachate in 250 ml Erlenmeyer flasks. The composition of the microbial inoculum was not characterized; it was assumed that the conditions within the plant growth system (i.e. presence of leachate) had selectively enriched for organisms capable of degrading organic molecules within leachate. Cultures were shaken at 150 rpm for 72 h. Leachate was clarified by centrifugation at 10000 rpm for 15 min prior to use in nutrient solutions. Leachates were diluted and admended as in the first study. Only wheat plants were used in this study. Plants were grown as discussed above.

FULL TERM PLANT STUDIES - Benchtop plant growth study - Wheat growth and development over the entire life cycle was compared in LB and MH solutions using recirculating nutrient film technique (NFT). Specific methods are presented elsewhere (12). Solution pH (5.8 units), solution liquid level, and relative humidity (63%) were automatically monitored and controlled. Photoperiod was 20 h light/4 h dark period, using HPS lamps at 500-700 μmol m^{-2} s^{-1} PPF. Air temperature fluctuated from 23-27 °C during the light period and 18-21 °C during the dark period, but temperature variations were consistent between the two treatments. Four plant growth trays were used to test the two nutrient solution treatments. Each tray was separated into nine distinct channels (67.6 cm^2), using PVC inserts (Figure 2). This facilitated time-course sampling of the individual trays. Wheat was grown from seed through maturity three times.

Electrical conductivity (EC) of the solutions was controlled manually by daily additions of concentrated nutrient replenishment solutions in order to maintain original nutrient concentrations throughout the study. The majority of the total

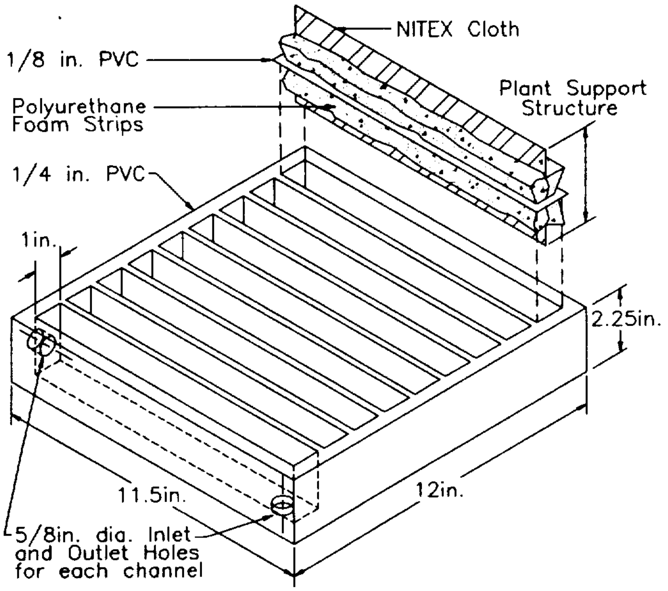

Figure 2. Plant growth trays used in benchtop plant growth studies.

plant nutrient demand was supplied by these replenishments. The percentage of each nutrient within the replenishment solution provided by the leachate is listed in Table 1.

Dissolved organic carbon (DOC) content (organic material < 0.22 µm) of the nutrient solution was monitored. Solution samples were filtered through PTFE membrane syringe filters (0.22 µm pore size) prior to analysis using UV-assisted persulfate oxidation.

Controlled environment study - This study compared wheat growth in wheat MH solution ,wheat LB, and wheat ash-based (AB) nutrient solutions, using recirculating NFT. The culture trays were similar to those used in past studies at KSC (13). The photoperiod was 24 h continuous light using HPS lamps at 800 µmol m^{-2} s^{-1} PPF. The temperature was maintained at 23 °C and 65% relative humidity. Atmospheric CO_2 was maintained at 1000 µmol mol^{-1} using an infrared gas analyzer. Nutrient solution pH was automatically controlled at 5.8 units with 0.39 M HNO_3 and EC was controlled manually with daily additions of concentrated stocks to maintain original nutrient concentrations. Composition of the stock solutions was calculated from values obtained from inorganic analysis of both the leachate and ash. Percentages of the nutrient concentrations within the replenishment solutions supplied by either leachate or ash are presented in Table 1.

Total organic carbon (TOC) and nutrient content of hydroponic solutions were measured as descibed above. Nutrient content of the harvested biomass was analyzed for all plant nutrients (excluding nitrogen) by ICP emission spectrometry.

RESULTS AND DISCUSSION

LEACHATE AND ASH CHARCTERIZATION - Leaching was effective at removing the majority of nutrients from the inedible biomass. Recovery of nutrients through leaching was greater than 60% for K, P, Mg, Mn, Zn, and Cu (Table 2). Only Ca, Fe, B and Mo were recovered at levels less than 50%.

Table 1 - solution from recycled inedible biomass (benchtop and controlled environment studies).

	Experiment		
	Benchtop	**Controlled Environment**	
Mineral*	Leachate	Leachate	Ash
	%	%	%
NO_3-N	77	100	100
PO_4-P	33	100	100
K	85	100	100
Ca	20	100	100
Mg	33	100	100
Fe	0	0	0
Mn	15	40	30
Zn	100	80	100
Cu	100	100	100
B	100	30	0
Mo	100	0	0

*Values are based on a modified half-strength Hoagland's nutrient solution (see Table 4).

The percent recovery of N could not be calculated since the biomass was not analyzed for N content, but it was assumed to have been high, given the high nitrate concentrations in the leachate (i.e. 40-100 mg NO_3 per g inedible biomass, depending on the species. The total weight of soluble macronutrients in the leachates (NO_3, PO_4, K, Ca, Mg) represented 25, 10, and 17% of the dry mass of potato, soybean, and wheat residues, respectively. Based on these nutrient levels and the rate of production of inedible biomass in the BPC, the total weight of nutrients associated with inedible biomass per growout of potato, soybean, and wheat was 315, 190, and 248 g m^{-2}, respectively.

The data indicate that nutrients in crop residues represent a significant nutrient reservoir for a CELSS which, if not recycled, would result in an appreciable nutrient resupply requirement. For a 4-person lunar base scenario having 100 m^2 of growing area and 3 - 4 growouts per year for each crop type, approximately 320 kg of leachable nutrients would be incorporated into inedible biomass each year. Recycling these nutrients could reduce estimated resupply requirements by 15% (14).

Recovery of nutrients via combustion was highly dependent on the reconstitution media; water resulted in very low recovery of most nutrients while 5% HNO_3 yielded nutrient recoveries similar to leaching (Table 2). Nitrogen recovery was negligible, regardless of reconstitution, although use of HNO_3 for resolubilizing the ash replaced the "missing" N neccessary for AB solutions. Our reconstitution method involved mixing 25 g ash per L of 2% HNO_3. Using a more concentrated acid would have probably increased the solublization of elements in the ash, but this would have required subsequent neutralization with a base. Clearly, the combustion process and reconsitution of ash can be optimized to enhance nutrient recovery. The degree of optimazation may be limited in a CELSS by the availability of acids and bases.

Table 2 - Percent recovery of minerals from potato, soybean and wheat biomass via leaching and combustion.

		Mineral recovery (%)							
Crop	**Treatment**	**P**	**K**	**Ca**	**Mg**	**Mn**	**Fe**	**Cu**	**Zn**
Potato	Leached	80 (1)	98 (0)	24 (5)	81 (1)	66 (2)	36 (2)	84 (2)	90 (2)
	Combusted (water)*	3 (0)	43 (4)	3 (0)	31 (2)	0 (0)	0 (0)	1 (1)	0 (0)
	Combusted (acid)**	105 (1)	44 (5)	87 (6)	85 (3)	61 (5)	56 (4)	44 (8)	28 (4)
Soybean	Leaching	55 (1)	98 (2)	44 (6)	81 (1)	52 (4)	15 (4)	90 (6)	99 (1)
	Combusted (water)	2 (0)	54 (7)	2 (0)	29 (0)	0 (0)	0 (0)	35 (34)	2 (1)
	Combusted (acid)	91 (4)	54 (7)	71 (3)	87 (2)	73 (7)	52 (3)	48 (3)	41 (8)
Wheat	Leached	59 (3)	97 (1)	71 (2)	94 (0)	62 (4)	26 (8)	80 (1)	88 (0)
	Combusted (water)	9 (1)	60 (10)	2 (0)	21 (3)	0 (0)	0 (0)	1 (1)	1 (1)
	Combusted (acid)	100 (9)	58 (9)	64 (5)	80 (6)	76 (18)	47 (7)	47 (4)	18 (3)

*The combusted material (ash) was soaked in deionized water prior to analysis.
**The ash was soaked in nitric acid (5% v/v) prior to analysis.
Data represents the means and standard deviations of 3 replicates.

Whether nutrient recovery is by physical-chemical oxidation or biological oxidation, the amount of nutrients that can be recycled from inedible material will depend on the crop residues. An estimate of total plant nutrient requirements for BPC grown crops was derived from nutrient budget data and compared to total nutrient recoveries from crop residues (9). Focusing on leached material, results indicated that the water soluble component of inedible crop residues could potentially provide the majority of macronutrient requirements for wheat, but less than 50% for soybean and potato (Figure 3). In other words, the majority of the total nutrient uptake by soybean and potato was located in the edible fraction of the plant. Nutrients from the edible portion of the crops would have to be recycled to plant nutrient systems via the processing of human wastes.

SEEDLING BIOASSAYS - Root length was the most sensitive response variable of those measured (see Materials and Methods--Seedling Bioassays), which made it the indicator of choice for testing leachate effects on seedling development. Root length was also the method of choice when testing allelopathic substances on alfalfa (15). Mean root length of the tested crops tended to react to the different leachates as follows; MH > soybean leachate > wheat leachate > potato leachate (Figure 4). However, Duncan's multiple range test showed no differences among leachates. Evenso, analysis of variance (ANOVA) of each of the individual test crops indicated that differences among leachate types significantly ($p < 0.5$) affected wheat and soybean seedlings, but not potato and lettuce seedlings. Based on Duncan's multiple range test, root length of soybean seedlings was less in all three leachate types compared to MH, while root length of wheat seedlings was less in soybean and potato leachate (but not in wheat leachate) relative to MH. From these results it is concluded that further replication of this study is needed to see whether the observed trends are real.

In a subsequent study using wheat seedlings, the effect of biological pretreatment of leachates as a means of eliminating phytotoxic compounds was tested. For all three leachate types (i.e. wheat, soybean, potato), wheat root length was greatest in the biologically pretreated leachate solutions (Figure 4). Results from ANOVA indicated a significant pretreatment effect ($p < 0.05$) across all leachate types. Average root length in the treated solutions ranged from 21-24 cm; values similar to the average root length of wheat grown in MH solution (21 cm). Similar effects had been observed in 21-day studies where wheat was grown in treated and untreated leachate (6). These data suggest that phytotoxic compound (s) exist in each leachate type but the compounds may be readily degraded by microbial activity.

Previous research has documented inhibitory effects of wheat and soybean crop residues on crop growth (16, 17, 18). Inhibitory effects of potato residue has, to the best of our knowledge, not been reported. Some water-soluble phytotoxins contained in plants and derived from the microbial decay of plant material (particularly anaerobic decay) have been identified (19, 20). We have not, at present, identified specific phytotoxic compounds in any of the leachates. Determining the source of phytotoxic compounds in crop leachates needs further study. In regards to our systems, the leachates were well aerated, so it was less likely that phytotoxins were derived from anaerobic decay than from aerobic decay of from the residues directly.

LONG-TERM PLANT GROWTH STUDIES - Benchtop plant study - Wheat yield was not significantly different in LB and MH solutions. Total DM per channel (see Materials and Methods--Benchtop Plant Growth Study) was approximately 8 g seed, 8 g shoot, and 2 g root for both treatments (Table 3). Total plant DM on an area basis (channel size of 67.6 cm^2) was approximately 2700 $g\ m^{-2}$, or 43 $g\ m^{-2}\ d^{-1}$ (plants harvested at

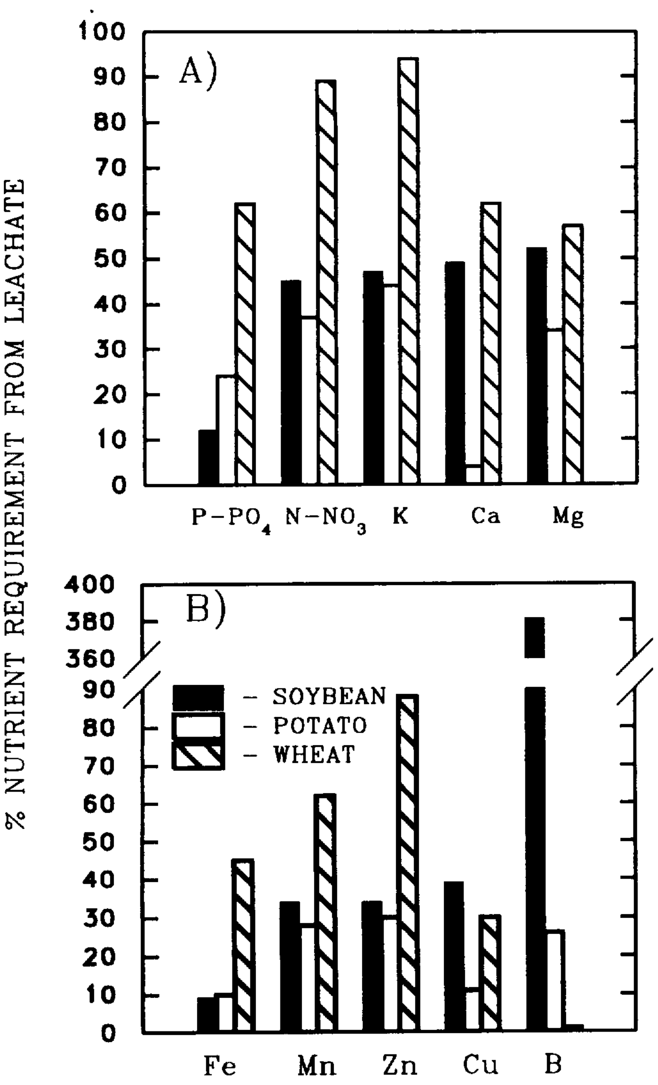

Figure 3 - Percent of total nutrient requirements of different crops which could be supplied from the leachate. Values for A) macronutrients and B) micronutrients are based on both biomass production and nutrient utilization from Biomass Production Chamber (BPC) studies.

Table 3 - Harvest data from wheat grown in two different nutrient solutions.

Nutrient Solution	Shoot DM	Seed DM	Root DM	HI*
	g	g	g	%
Modified Hoagland's	7.77 (1.93)**	8.25 (2.02)	1.90 (1.02)	38 (4)
Wheat Leachate	8.76 (2.20)	8.84 (2.75)	1.83 (0.63)	38 (4)

* HI = harvest index = Total plant DM/Seed DM

** Mean values for 3 replicates, with standard deviations in parentheses

63 d). Seed production on an area basis was approximately 1200 g m^{-2}, or 19 g m^{-2} d^{-1}. Growth rate in this study was greater than 80% of the optimal predicted for the given PPF (21).

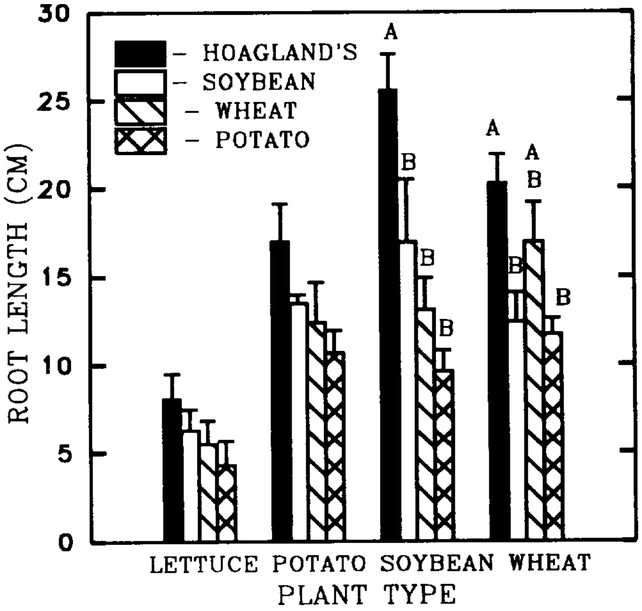

Figure 4- Effect of several crop leachates on average root length of soybean, wheat, and potato seedlings. Mean values for four replicates with vertical bars equal to standard error. Letters denote significantlly different levels of solution treatments as determined by Duncan's Multiple Range test.

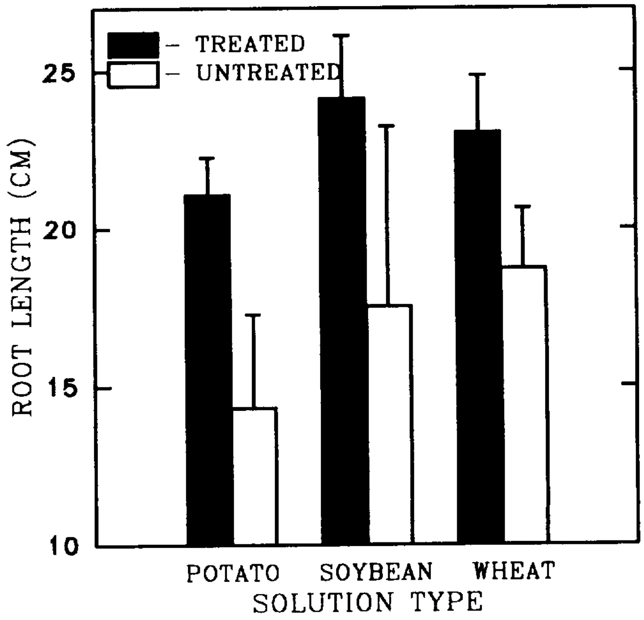

Figure 5 - Effect of microbial pretreatment of leachate on average root length of wheat seedlings. Mean values for four replicates with vertical bars equal to standard error.

Dissolved organic carbon (DOC) in the LB solution (240 ppm) was almost five times higher than that in the MH solution

(50 ppm) at day 0, but rapidly declined to 60-95 ppm by day 7 (Figure 6). This decline likely reflected mineralization of organic material contained in the leachate. In fact, bacterial cell densities in the nutrient solution were 1-2 orders of magnitude greater during the first week of plant growth in LB systems (12). DOC concentrations in LB solutions increased slightly during the period of high plant nutrient demand (and concomitant additions of leachate). DOC subsequently decreased towards the end of the study, when plant nutrient demand declined. Based on the harvest results and DOC values, the microbial community associated with the plant growth systems effectively degraded the organic supplementation associated with the LB solutions.

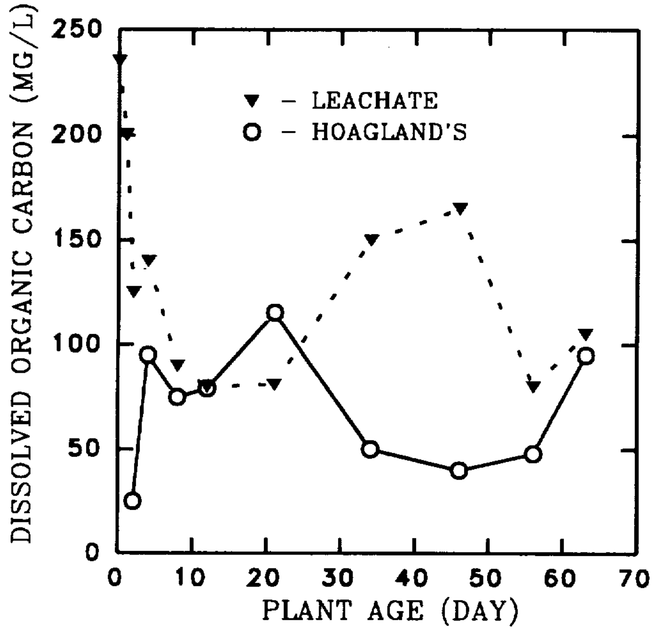

Figure 6 - Comparison of the dissolved organic carbon concentration of wheat leachate nutrient solution with modified Hoagland's nutrient solution. Data points represent mean values from three separate studies.

Controlled Environment study - Wheat grown in larger populations (0.25 m^{-2}) and in an environment containing a high PPF (800-1000 μmol m^{-2} s^{-1}) and CO_2 enrichment (1000 mmol mol^{-1}) seemed to have responded less favorably to the nutrient recycling treatments. Total plant DM production was similar between MH and AB solution treatments (Figure 7). However, total biomass for the LB solution was approximately 80% of the MH treatment (Figure 7). Harvest Indices [HI--(DM seed/DM total biomass) x 100] for MH, AB, and LB were 42, 32, and 27%, respectively. Spikelet counts on subsamples from the treatments were equivalent but there were 34% fewer heads in the LB treatment and 8% fewer heads in the AB treatment, compared to MH. It appeared that the AB treatment affected seed set, with minor effects on head production and no effect on vegetative production. However, the LB treatment had moderate effects on vegetative production and quite a large effect on head production and possibly seed set.

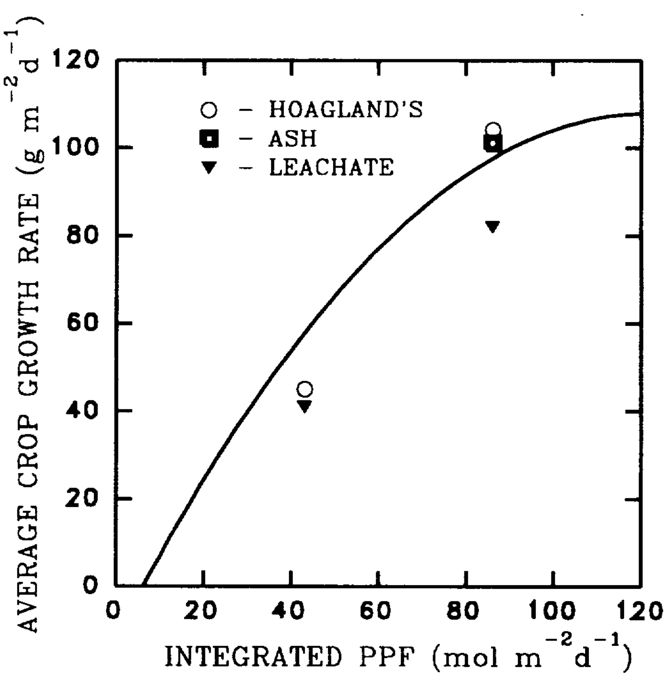

Figure 7 - Harvest results of the benchtop and controlled environment studies normalized for PPF. The solid line represents the theoretical yield of hydroponically grown wheat, which was taken from Bugbee and Salisbry (1988).

Contrary to the results of the previous study, organics accumulated in the LB system over time (Figure 8). The rate and extent of leachate additions were much greater in the present study for two reasons: 1) Higher light levels and CO2 enrichment, increased crop growth rate and thus nutrient demand, and 2) Leachate provided a greater percentage of the total plant nutrient requirement than in the benchtop study (Table 1). The total amount of leachate added per liter of nutrient solution was approximately 25 times greater this study. The concomitant increase in carbon-loading rate appeared to have exceeded the capacity for microbiological degradation as indicated by the accumulation of organics within the nutrient solution (Figure 8). It is important to note that the percentage of total nutrient demand supplied by leachate in this study (near 100% for most nutrients) was greater than the potential for recycling, based on the reservoir of nutrients within inedible biomass (see Results and Discussion--Leachate and Ash Characterization). Current studies are evaluating intermediate levels of leachate additions to plant growth systems which more accurately reflect what would be supplied by available biomass.

Reduced seed yield in the AB treatment may have been related to the composition and availability of nutrients from the nutrient solution. Relative to the MH solution, Mg, Mn, and Cu levels were lower in the AB treatment (Table 4). Based on published values (21), the AB treatment had low Mn, Zn, and Cu concentrations in the harvested straw as well (Table 4). Deficiency symptomology as it relates to more than one element is difficult (23). To minimize potential nutrient deficiencies, we will supply more Mg, Mn, Zn, and Cu in subsequent studies. If these nutrients continue to have low tissue elemental concentrations, attention will be focused on nutrient availability in the solution. It is worth noting that the LB treatment had some of the same deficiencies detected in its biomass, which may have compounded the negative results seen with that treatment. The knowledge gained from elemental analysis of the nutrient solutions and plant tissue in this study will make it possible to more accurately control LB and AB solutions to reflect MH solutions in future studies.

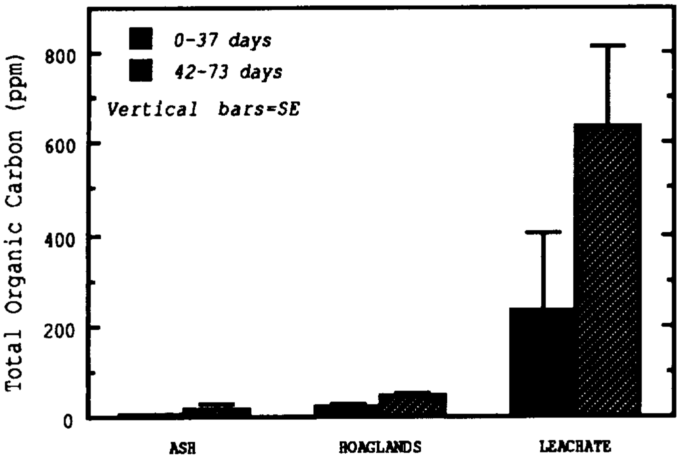

Figure 8 - Comparison of the total organic carbon concentration of the nutrient solutions used in growth chamber study. Values represent mean and standard error of sampling dates from the first half (0 37 days) and second half (42 - 72 days) of the study.

Table 4 - Nutrient solutions and wheat straw elemental compositions as related to nutrient recycling method.

	NUTRIENT SOLUTION				STRAW AT HARVEST (73 days)			
Mineral	**Set point**	**MH**	**AB**	**LB**	**Acceptable range***	**MH**	**AB**	**LB**
	mM	mM	mM	mM	%	%	%	%
N	7.5	5.7	8.7	7.6	**	**	**	**
P	0.5	0.1	0.1	0.1	0.1 - ?	0.14	0.15	0.15
K	3.0	1.6	3.9	6.0	1.0 - ?	5.5	7.6	12.5
Ca	2.5	3.3	2.4	1.2	0.3 - ?	0.9	0.6	0.5
Mg	1.0	1.6	0.8	0.8	0.2- ?	0.3	0.2	0.2
	µM	µM	µM	µM	ppm	ppm	ppm	ppm
Fe	100	143	72	108	50-250	120	244	253
Mn	3.70	1.82	0.91	0.91	16-1000	20	15	16
Zn	0.64	0.61	0.46	0.31	20-150	21	14	19
Cu	0.52	1.42	0.52	1.26	3-33	4	4	5
B	4.80	6.48	48.00	48.00	14-25	14	30	31
Mo	0.01	<0.10	<0.10	<0.10	0.1-135	0.5	4.7	5.6

*Acceptable range of agronomic crops in general. Macronutrientts upper limits were not reported. Data taken from Berry (1992).

**Nitrogen was not determined in the biomass.

SUMMARY

This series of experiments indicates both the large potential and significant challenge to nutrient recyling in bioregenerative systems. Inedible biomass from CELSS candidate crops contained a large reservoir of nutrients that was readily recovered by simple water extraction or (with somewhat greater difficulty) by high temperature incineration. However, long-term plant growth in both LB and AB solutions was less than optimal. A potential cause of plant growth inhibition in the LB solution was the presence and/or build-up of organic compounds. Seedling studies indicated the presence of phytotoxic compounds in leachate, but also indicated that these compounds were readily degraded. Long-term plant growth in LB solutions was inhibited, where plant nutrient demand and concomitant leachate additions were higher than in past studies. It is unclear, at present, if the plant growth reduction in the LB solution was caused by a direct interaction between organic material and the plants, an indirect interaction between organic material and nutrients resulting in inhibited plant nutrient uptake, anaerobic conditions in the root zone, and/or any other undefined factor (s). Seed production was inhibited in the long-term plant study using reconstituted ash. Some micronutrients were at deficient levels in the nutrient solution, which may have been related to the relatively poor yields, however, total biomass was equivalent to plants grown in MH solution.

The long-term plant growth studies indicated that nutrient recycling could be accomplished with limited success, and given time for refinements, future results will likely become comparable to inorganic salt-based systems. Supplementation of recovered "waste" streams with reagent-type salts may be used to a larger degree than what we have done in these studies. Although this would not be a complete nutrient recycling process, it would reduce the use of waste streams in the hydroponic system, which may alleviate some of the observed yield losses. Concerns with organic load and composition of LB solutions (or other waste streams) are difficult to address, but preliminary results suggest that success depends on insuring the carbon loading rate of the system does not exceed the microbial degradation rate. Biological or physical-chemical pretreatment of leachate or other waste streams to remove organics may be necessary, although these results indicate that recirculating hydropoonic systems have a significant capacity for in situ organic degradation.

Perhaps more important than the method of nutrient recycling is the ability to use continuously recirculating nutrient delivery systems for extended periods of time (years) without discarding the solution between crops. Not only is this a challenge when considering hydroponic systems based on recycling, but also for systems where nutrients will be completely resupplied. The ability to monitor and control the nutrient solution (i.e. - pH, elemental composition, DOC, and microbial populations) for continuous operation will be quite important in a CELSS.

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