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USE OF BIOLOGICALLY RECLAIMED MINERALS FOR CONTINUOUS HYDROPONIC POTATO PRODUCTION IN A CELSS

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ABSTRACT

Plant-derived nutrients were successfully recycled in a Controlled Ecological Life Support System (CELSS) using biological methods. The majority of the essential nutrients were recovered by microbiologically treating the plant biomass in an aerobic bioreactor. Liquid effluent containing the nutrients was then returned to the biomass production component via a recirculating hydroponic system. Potato (*Solanum tuberosum* L.) cv. Norland plants were grown on those nutrients in either a batch production mode (same age plants on a nutrient solution) or a staggered production mode (4 different ages of plants on a nutrient solution). The study continued over a period of 418 days, within NASA Breadboard Project's Biomass Production Chamber at the Kennedy Space Center. During this period, four consecutive batch cycles (104-day harvests) and 13 consecutive staggered cycles (26-day harvests) were completed using reclaimed minerals and compared to plants grown with standard nutrient solutions. All nutrient solutions were continually recirculated during the entire 418 day study. In general, tuber yields with reclaimed minerals were within 10% of control solutions. Contaminants, such as sodium and recalcitrant organics tended to increase over time in solutions containing reclaimed minerals, however tuber composition was comparable to tubers grown in the control solutions.

INTRODUCTION

Ash (inorganic nutrients) can comprise over 10% of inedible plant dry mass when the plants are grown in recirculating hydroponics (McKeehen *et al.*, 1996; Wheeler *et al.*, 1994). A majority of these nutrients are easily recovered by solubilizing the biomass in water (leaching) or treating the biomass in an aerobic bioreactor (Garland *et al.*, 1993; Finger and Strayer, 1994). In a single potato production study, recycling nutrients from bioreactor processing resulted in crop yields equal to the control treatment composed of reagent-grade salts (Mackowiak *et al.*, 1996).

When the nutrients are solubilized quickly, as in the case of leaching, a large amount of organic compounds are also solubilized, which directly or indirectly impair plant growth (Garland *et al.*, 1993; Mackowiak *et al.*, 1996). The majority of soluble organics are labile, resulting in rapid degradation in an aerobic bioreactor. Some of the organics are oxidized to recalcitrant phenolic compounds which give the nutrient solution a (tea-colored) appearance (Kumada, 1965). The concentration of these organic compounds can be monitored using spectrophotometric methods (Schnitzer, M., 1978; Mackowiak *et al.*, 1994). In addition to monitoring the spectral composition of the aging nutrient solutions, elemental analyses of the solution and the plant biomass from successive generations provided data on the potential for build-up of elemental contaminants.

MATERIALS AND METHODS

The study was conducted in NASA's Biomass Production Chamber (BPC), located at Kennedy Space Center (Wheeler *et al.*, 1996). The BPC is one component of the Breadboard Project's test of bioregenerative systems for advanced life support (Prince and Knott, 1989). Potato (*Solanum tuberosum* L.) cv. Norland were started from in vitro nodal explants and thinned to four plants per culture tray at 10 days-after-planting (DAP). The upper chamber compartment (10 m^2) had plants grown in a batch culture system (all plants grown to maturity and then immediately replanted), whereas the lower chamber compartment had plants grown in staggered procession (with plantings made at 26-day intervals resulting in four different age groups). Each chamber was comprised of two levels for growing plants. Each chamber had nutrient recycling tested on one level to compare with a modified half-strength Hoagland's control, thus providing four different treatments (two culture systems and two nutrient formulations) and each treatment had 16 culture trays (5 m^2 growing area).

Details on bioreactor engineering requirements and effluent production methods using the Breadboard-scale aerobic bioreactor (B-SAB) have been described by Finger and Alazraki, (1995) and Strayer and Cook, (1995). Details on nutrient solution recipes and replenishment methods can be found in Mackowiak *et al.* (1996). The starting nutrient solution was a modified half-strength Hoagland's solution (Mackowiak *et al.*, 1996). A concentrated replenishment solution, used to replace nutrients removed by the plants, is presented in column 2 of Table 1. Since our goal was to recycle 50% (by mass) of the crop's nutrient requirement, we had to add back some nutrients as reagent grade chemicals, mainly Ca, P, Mg, and Fe (Table 1). Micronutrient composition (using ICP analysis) varied quite a bit over time in the effluent, so in most cases over 80% of the micronutrients were amended with reagent-grade chemicals to assure adequate amounts in the replenishment solution. The effluent was analyzed for elemental content whenever changes in the bioreactor processing occurred (Table 1). The analytical information let us know what nutrients needed to be amended with reagent-grade chemicals. Weekly samples from the nutrient delivery systems also were analyzed for elemental composition, using colorimetric methods for N and P and inductively coupled plasma spectrometer (ICP) for the other nutrients. Bioreactor effluent and nutrient solution were analyzed periodically for total organic carbon (TOC) using a high temperature furnace analyzer.

All plants received the same environmental conditions, with lighting provided by high-pressure sodium (HPS) lamps as a 12-h light / 12-h dark photoperiod and PPF levels averaged 814 $\pm$ 15 $\mu mol\ m^{-2}\ s^{-1}$. Temperature was set at 20/16 °C (light/dark) and relative humidity controlled at 70 $\pm$ 10%. Carbon dioxide was added during the light phase to maintain 1200 $\mu mol\ mol^{-1}$ (0.12 kPa). Plants were harvested at 104 DAP (except for the first harvest at 105 DAP). It took 105 days to get the staggered planting system to steady-state (four plant ages on a single system). Tuber, root, and shoot biomass were freeze-dried for dry mass determinations. Tissue samples were analyzed with a DC-arc spectroscopy multielement analysis system.

RESULTS AND DISCUSSION

At 418 days the entire BPC was harvested and all the dry mass values that had been collected during the course of the study tallied to obtain overall biomass production rates. Using recycled minerals for potato production resulted in biomass yields as good as yields from using only reagent-grade chemicals (Figure 1). The staggered planting system resulted in greater biomass than the batch planting, which may have been a result of improved light interception by the plants grown on that system (Stutte *et al.*, this issue). The average total biomass production rates from this study were within 10% of single batch values from previous BPC potato studies, while tuber production was within 18% (Wheeler *et al.*, 1996). The somewhat lower average production values in this study may be related to a buildup of biogenic compounds in the nutrient solutions over time (Stutte *et al.*, 1995). These compounds tend to hasten tuberization and provide an extremely strong induction signal, resulting in reduced biomass (Engels and Marschner, 1986; Wheeler *et al.*, 1995). Nonetheless, it does not appear that plants grown on recycled nutrients were affected more than those on the control solutions (Table 2).

Table 1. Composition of effluent from bioreactor harvests over the course of the study.

Nutrient	Inorganic Stock	Effluent Composition* 0 DOE**	41 DOE	73 DOE	105 DOE	217 DOE	350 DOE	C.V.***
	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(%)
NO_3-N	62	56	73	138	56	49	53	48
PO_4-P	9	2.4	2.2	2.6	2.4	3.7	2.1	23
K	48	51	40	50	52	49	43	10
Ca	9	1.8	3.4	2.6	2.1	4.0	2.9	29
Mg	10	5.0	4.6	3.8	3.8	4.2	8.5	36
	(µM)	(µM)	(µM)	(µM)	(µM)	(µM)	(µM)	
Fe	134	0	0	0	0	89	0	
Mn	74	0	18	4	3.2	4.0	15	
Zn	9.6	0	54	0	2.9	4.6	0.8	
Cu	10.4	0	0	0	25	0	1.3	
B	95	0	0	0	4.8	0	6.4	
Mo	0.1	0	0	0	0	0	0	

*Data normalized to a bioreactor loading rate of 20 g L^{-1}. If required (41, 73 DOE), effluent was diluted with chamber condensate water to provide 50% of the nutrient delivery system's potassium requirements.
**DOE = Day of experiment.
*** C.V. = coefficient of variation of reactor harvests.

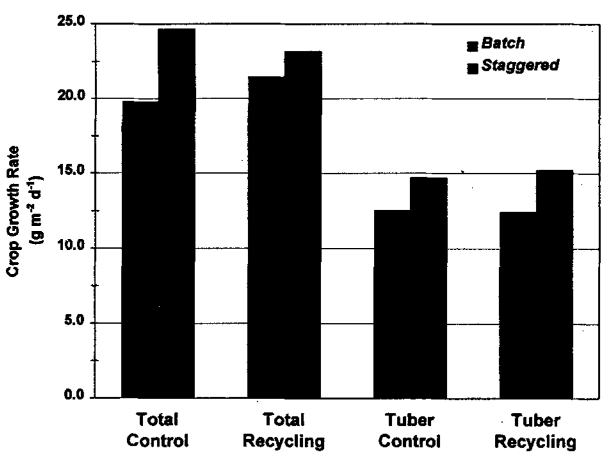

Fig 1. Culture system and nutrient recycling effects on potato tuber and total dry mass production rates. Production rates were calculated from the biomass summations for the entire 418 day study.

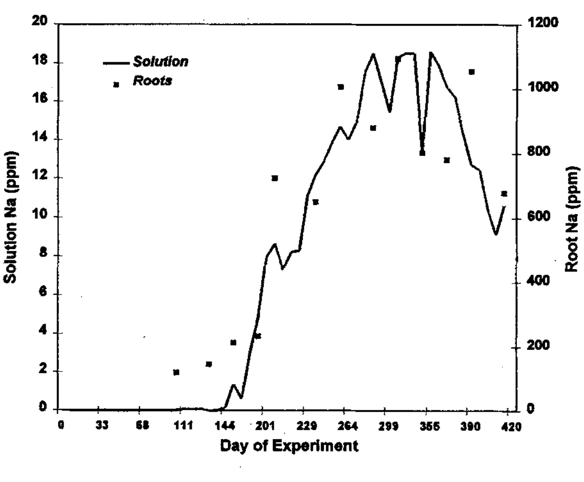

Fig. 2. Na concentration of nutrient solution and root tissue in the recycling treatment of the staggered culture system. There were no changes in the control treatment.

The coefficient of variation for macronutrients suggest that the effluent's potassium content was the most uniform over successive bioreactor harvests (Table 1). In most cases there were single instances where a nutrient value would be quite different from surrounding values, as was the case with NO_3-N at 73 DOE and PO_4-P at 217 DOE (Table 1). In the case of Ca and Mg, there was variation throughout the study. Robinson *et al.* (1993) reported that P, Ca, and Mg release by the fungal degradation of wheat varied by fungal species. It may be in our own case that as the bioreactor microbial population adjusted, release of these elements was affected. In the case of micronutrients, the low concentrations made accurate analysis difficult. A study which covered several U.S. analytical labs found a wide variation in the levels of Ca, Fe, Cu, and B reported for the same plant tissue (Sterret et. al., 1987). Minor adjustments were made in the quantities of reagent-grade nutrients needed (approximately every 8 weeks), which was based on effluent inorganic analysis. This kept the recycled nutrient replenishment solution comparable to the control replenishment.

Weekly spectral absorbency scans of hydroponic solutions from the four treatments were taken in the range of 220 - 420 nm to track organic loading of the nutrient solutions over time. This range was selected because light absorption of humic substances becomes greater with decreasing wavelengths (Kumada, 1965; Baes and Bloom, 1990). Absorbency values were unchanged in the control treatment but values increased in the nutrient recycling treatment of the staggered culture system by a factor of 4.5. Similar trends were seen with the batch culture systems, until activated carbon was added to both the control and recycling treatments at the beginning of the last 104 d cycle, which was done to test the removal of some potato exudates (Wheeler *et al.*, 1995). The two day carbon treatment reduced absorbency levels approximately 10%. Nutrient solution total organic carbon (TOC) was also measured. The bioreactor effluents varied from 150 - 230 ppm, and the nutrient recycling treatments increased over time (from 22 to 150 ppm), but the control treatments had only a small increase (5 to 15 ppm). The recalcitrant organics accumulating in treatments with recycled nutrients may be humic substances, viz., humic acid and fulvic acid. Humic substances have measured half-lives of 200 - 2000 years (Martin and Haider, 1986), and make up over 70% of the organic matter in most soils. At pH 6, humic substances can form water-soluble complexes with many organics and inorganics, as in the case of fulvic acid, or adsorb to organics and inorganics, as in the case of humic acid (Schnitzer, 1986). Due to their nature, humic substances provide some buffering capabilities. Acid use efficiency (biomass per amount of acid added) was greater in the effluent treatments than the controls, where the staggered planting effluent > batch effluent > staggered planting control > batch control (1.67, 1.25, 1.13, and 1.02 g biomass $mmol^{-1}$ HNO_3, respectively). Although we did not see a plateau in TOC or in spectral absorbency of organics, it may have eventually reached a plateau as organic degradation in the nutrient solutions reached a steady-state. If soluble organics become unacceptable in the solution, it is easily removed by using activated carbon (Stutte *et al.*, 1995), however, the organics did not appear deleterious in our study (Figure 1).

There was no build-up of heavy metal contaminants in the biomass, especially in the root tissue over time. Mature plants from the recycling and control treatments of the staggered culture system were analyzed for plant nutrients and heavy metals, such as Al, Co, Cr, Ni, Pb, and Ti, to name a few. Even Si, a good indicator of soil contamination from shoes, etc., remained stable over time. Sodium was the only contaminant that tended to increase over time, but only in the recycling treatment (Figure 2). Tissue from the control solution never exceeded 280 ppm Na. The effluent originated from successive potato harvests, where people handled the biomass during harvesting and processing. Sodium added to the biomass from human handling would be easily solubilized in the bioreactor. The potato roots could take up the Na in the next planting and thus continue the cycle. Although there was increased Na in the root tissue of plants receiving bioreactor effluent, the Na levels in the tubers were comparable to that found in the control (< 90 ppm).

CONCLUSIONS

Recycling 50% of the nutritional mass requirements (based on K needs) for potato was successful in recirculating hydroponics with either batch or staggered production, as compared to using only reagent-grade salts. The K concentrations in bioreactor effluent were most consistent over successive bioreactor harvests, whereas Ca and Mg were much more variable. The changes in recovery may be related to changes in microbial community composition of the bioreactor over time and/or variation caused by nutrient analytic equipment. Recalcitrant soluble organics increased over time in the nutrient recycling treatments but not in the control solutions. Although continued build-up may be a problem in longer studies, it did not hinder production in our 418 day study and may even improve nutrient solution buffering capacity, resulting in a lower acid requirement for pH control. It was notable that heavy metals did not build up in plant tissues over successive harvests during this study, however Na did increase in nutrient solution and plant roots of the recycling treatments. The Na probably came from human handling of the harvested biomass prior to its being recycled and thus could be alleviated by automating the harvesting process.

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