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Controlled Ecological Life Support Systems: CELSS '89 Workshop

Robert D. MacElroy

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Robert D. MacElroy, Editor, Ames Research Center, Moffett Field, California

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PREFACE

In February 1989, NASA's Controlled Ecological Life Support Systems (CELSS) program assembled a diverse group of investigators in Orlando, Florida, to discuss a variety of topics which must be addressed in order to develop bioregenerative systems for use during piloted space missions early in the next century. The meeting was attended by investigators from several NASA centers (Ames, Johnson, JPL, and Kennedy), as well as scientists from universities and private industries from around the United States. These proceedings contain the 25 papers presented during the two days of the conference. Topics concerning the production of edible biomass range from studies on the efficiency of plant growth, to the conversion of inedible plant material to edible food, to the use of plant tissue culture techniques. Models of plant growth, and of whole CELSS systems, are also included. The use of algae to supplement and improve dietary requirements is addressed. Several papers discuss the development of CELSS technology, both ground-based and flight-qualified. Work at Kennedy Space Center's Breadboard Facility and Ames Research Center's Crop Growth Research Chamber is described. Flight experimentation is covered in topics ranging from a Salad Machine for use on Space Station *Freedom* to conceptual designs for a Lunar Base CELSS. Control issues are reviewed and recommendations made concerning a strategy for development of a robust control system. Processing of waste materials is covered, including analysis of physical/chemical, biological, and hybrid systems, and how source characterization affects design criteria.

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EXPLORING THE LIMITS OF CROP PRODUCTIVITY:
A MODEL TO EVALUATE PROGRESS

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ABSTRACT

The productivity of higher plants is determined by the photosynthetic photon flux (PPF) and the efficiency of the following four physiological processes: PPF absorption by photosynthetic tissue, carbon fixation (photosynthesis), carbon use (respiration), and carbon partitioning (harvest index). These constituent processes can be integrated to establish potentially achievable productivity, which is estimated to be 1.64 g mol^{-1} of photons. We obtained $1.3 \text{ g biomass mol}^{-1}$ ($0.56 \text{ g seed mol}^{-1}$) at a PPF of $50 \text{ mol m}^{-2} \text{ d}^{-1}$, but this decreased to $0.8 \text{ g biomass mol}^{-1}$ at a PPF of $150 \text{ mol m}^{-2} \text{ d}^{-1}$. Photosynthetic and morphological measurements of wheat suggest that source strength (leaf area) greatly exceeds sink capacity (grain number) at high PPF levels. High plant densities improve sink strength, but result in excessive leaf area. Gradually decreasing temperature during the life cycle from 23°C to 17°C appears to improve yield by reducing maintenance respiration of the biomass. We are evaluating cultivars with reduced leaf size and number to decrease leaf area index at high plant densities. These cultivars may also have an improved harvest index. Hydroponic studies indicate that 1 mM nitrate in solution is adequate to support maximum growth in our systems, provided iron nutrition is adequate. Wheat does not accumulate nitrate in leaves even when the solution nitrate concentration is 15 mM . Long-term photosynthetic efficiency (g mol^{-1} of photons) and harvest index were not altered by photoperiod (16, 20, or 24-h). Wheat does not need, nor benefit from, a diurnal dark period.

INTRODUCTION

Our goal has been to determine the limits of crop productivity when all environmental constraints are removed. We define productivity as food output per unit of input, and are quantifying the output/input (efficiency) ratio for two of the most fundamentally limiting inputs to a CELSS: energy and volume.

Energy efficiency can be expressed as g of food per mole of photosynthetic photons or as percent (kJ food per kJ of photons).

Volume efficiency is best expressed as $\text{g m}^{-3} \text{d}^{-1}$, but the final volume of a production system depends on design factors that are difficult to estimate so we have measured volume efficiency as g m^{-2} (surface area) d^{-1} . These numbers can then be used to determine system volume. It appears that productivity m^{-2} could also be expressed m^{-3} because the production system could be about 1-m high (plants, lights, and roots).

Achieving high productivity and efficiency has required the development of unique apparatus to optimize environments, unusual cultural techniques and considerable genetic selection. Our studies have indicated that higher plant photosynthetic efficiencies can be similar to efficiencies obtainable with algae.

The following aspects of our approach are particularly important:

1. We have studied communities of wheat plants, rather than single plants, and can thus directly extrapolate to a larger scale from our small research plots (0.2-m). We were forced into this approach at an early stage because the morphology of a wheat plant is very different when it is grown without competition from neighboring plants. Findings based on individual plants are very useful for some types of studies, but they can be highly misleading when used to predict community productivity.
2. We have not tried to simulate field conditions. Part of yield optimization results from a significant departure from "normal" environmental conditions. The changes include elevated CO_2 , 24-h photoperiod, high

photosynthetic photon flux (PPF), carefully managed hydroponic culture, and very high planting densities.

3. Because of the vast genetic diversity of wheat we have been able to study genetic/environment interactions and then use this data to select and develop appropriate lines for controlled environments. We now have a large collection of wheat genotypes.
4. We have studied closure of the root-zone environment by using recirculating hydroponic culture. Water and nutrients are added to replace what the plants remove but nothing is discarded.

MAXIMUM PRODUCTIVITY

Our accomplishments to date are best summarized as a maximum productivity curve (Figure 1). It appears that productivity is limited by PPF at even the highest PPF level. Potential productivity and field productivity are included in this Figure for comparison. The assumptions that are necessary to determine potential productivity are discussed in detail in two recent papers (1, 2). World record field yields are also reviewed in these papers.

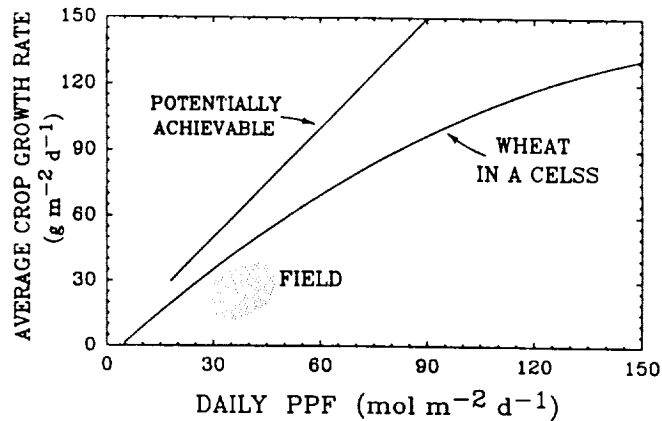


Figure 1. A comparison of measured growth rates (total biomass) in a CELSS with potentially achievable growth rates. The shaded area represents the range of record yields in the field. Note that the CELSS growth rate approaches the potentially achievable growth rate at low PPF levels and that the growth rate does not saturate at high PPF levels.

ENERGY EFFICIENCY

Figure 2 includes the same data for crop growth rate as Figure 1, but indicates the energy efficiency associated with different PPF levels. Efficiency is measured as percent by assuming 217 kJ per mole of photosynthetic photons and 17.8 kJ per g of dry biomass (average of seeds and stems). Efficiency does not reach a maximum until about 30 mol m⁻² d⁻¹ and then gradually decreases.

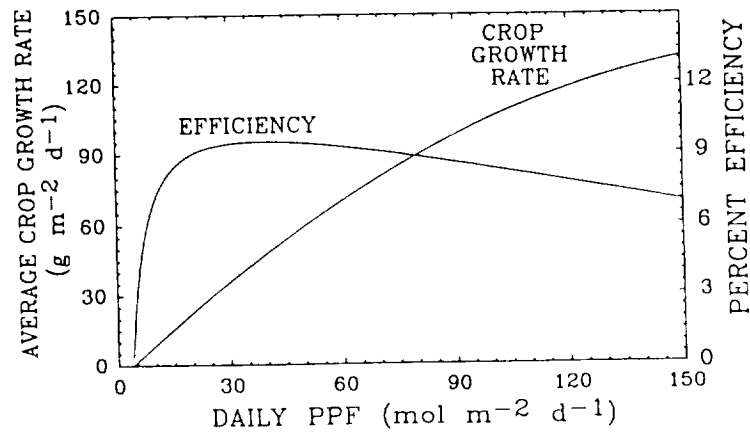


Figure 2. The effect of daily PPF on PPF utilization efficiency. The crop growth rate curve is the same as in Figure 1.

REPRODUCIBILITY

How reproducible is the yield curve in Figures 1 and 2? Figure 3 shows the overall mean from eight separate studies and compares this mean with the yield from our best single study. The 8 studies include different environmental conditions (photoperiod, temperature); different cultivars (Yecora Rojo and Veery 10); and different cultural conditions (planting densities, etc.). The studies were also conducted in different types of growth chambers. About half of the scatter in the data is the result of parameters other than PPF, but much of the scatter is from unidentified causes (experimental error). Reproducibility is critical in a CELSS, but variability is inherent in biology.

As we identify the causes of low yields, we improve our ability to accurately predict yields.

Figure 3 also indicates the lack of data at low and high PPF levels. Additional research at low PPF levels would help to identify the peak energy efficiency. Studies at higher PPF levels would help to determine the peak efficiency per unit volume. Studies at all PPF levels are important because PPF interacts with other environmental, cultural and genetic factors.

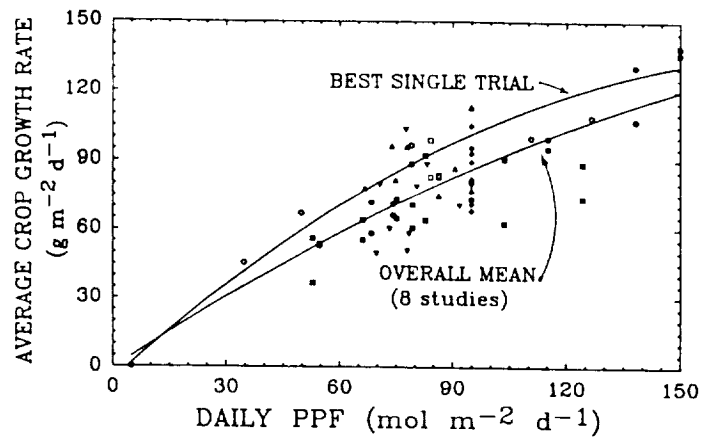


Figure 3. Reproducibility in a CELSS: A comparison of our best single trial with the overall mean of 8 different trials. Symbols represent different studies.

A MODEL TO EVALUATE PROGRESS

Crop physiologists have used correlation analysis to identify factors associated with high yields, but as we learn more about crop plant communities it has become useful to identify and separately analyze the constituent processes that determine yield. The model outlined here consists of the PPF input and the four, primary plant processes that determine yield.

This type of analysis is not unique and has been used by several other investigators to analyze field productivity (3, 6, 7, and 8). The four constituent plant processes are:

1. Percent PPF absorption by photosynthetic tissue
2. Photosynthetic efficiency (moles of CO₂ fixed per mole of photons absorbed).
3. Respiratory efficiency (net carbon fixed in biomass per unit carbon fixed in photosynthesis).
4. Harvest Index (edible biomass / total biomass).

Considerable research has been done on each of these processes so it is possible to determine theoretical maximum, and potentially achievable values for each factor (Table 1). A detailed analysis of the derivation of each of these values is presented in Bugbee and Salisbury (2).

	<u>THEORETICAL</u>	<u>POTENTIALLY ACHIEVABLE</u>	<u>WHEAT IN A CELSS</u>
ABSORPTION	100 %	98	90
PHOTOSYNTHETIC EFFICIENCY	34	18	16
RESPIRATION EFFICIENCY	82	75	70
HARVEST INDEX	<u>100</u>	<u>90</u>	<u>44</u>
TOTAL	27.5	11.9	4.4

Table 1. Values for the four constituent physiological processes that determine yield. All values are in percent. The total at the bottom is the result of successive multiplication. Values for wheat in a CELSS are average values over the life cycle. Higher instantaneous values have been measured, but cannot be sustained.

PERCENT PPF ABSORPTION

Measurements of PPF absorption over the life cycle are shown in Figure 4. A maximum absorption of 98% is possible about 15 days after emergence (emergence occurs 48 to 72 hours after germination). The high plant densities necessary to obtain high yields in a CELSS cause very rapid PPF absorption. Senescence during the last part of the life cycle reduces absorption. High density maize from the field is included as a comparison.

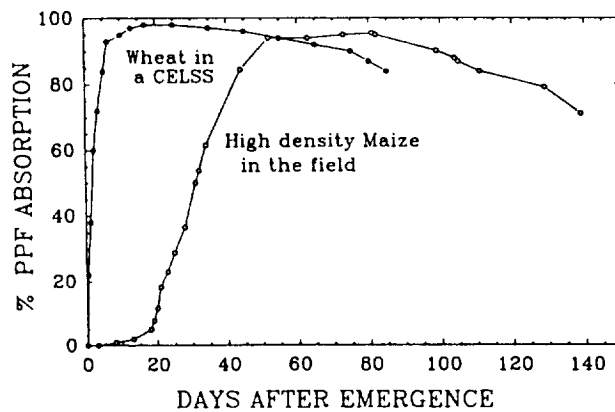


Figure 4. A comparison of PPF absorption of wheat in a CELSS with maize in the field. Complete data were not available for field grown wheat, but field wheat absorbs significant amounts of PPF up to 5 days sooner during early growth than maize.

PHOTOSYNTHETIC AND RESPIRATION EFFICIENCY

We have used a sealed growth chamber as a cuvette to measure canopy photosynthesis and a smaller cuvette to measure photosynthesis of individual leaves in the canopy. All of the measurements in the following figures were made with CO₂ enrichment. Figure 5 indicates the photosynthetic capacity of

single leaves at two different temperatures. The response of these wheat leaves is considerably different than typical field curves. The assimilation rate of $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ is very high, but these leaves had ample nitrogen and high CO_2 . Single leaves at ambient CO_2 typically reach a maximum photosynthetic rate at a PPF of about $600 \mu\text{mol m}^{-2} \text{s}^{-1}$, at 25°C these leaves did not reach a maximum at a PPF of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. A temperature of 17°C is too low for maximum photosynthesis, but note that dark respiration at 17°C was slightly lower than at 25°C .

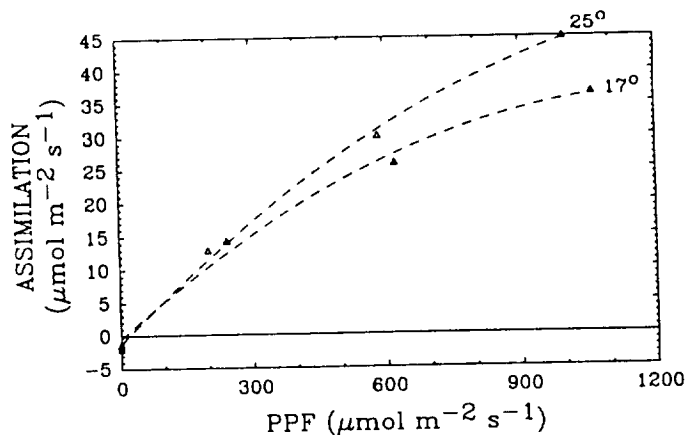


Figure 5. Single leaf photosynthesis a function of PPF level at 17 and 25°C . These leaves were part of a canopy, grown in a CO_2 enriched CELSS environment.

Figure 6 compares gross assimilation of single leaves and a canopy. The gross assimilation data in this figure do not include dark respiration. They indicate only the photosynthetic response to PPF. The response of the canopy to PPF is almost perfectly linear. The single leaf data indicate that the top leaf layer ($\text{LAI}=1$) is responsible for all of the canopy photosynthesis up to a PPF of about $400 \mu\text{mol m}^{-2} \text{s}^{-1}$.

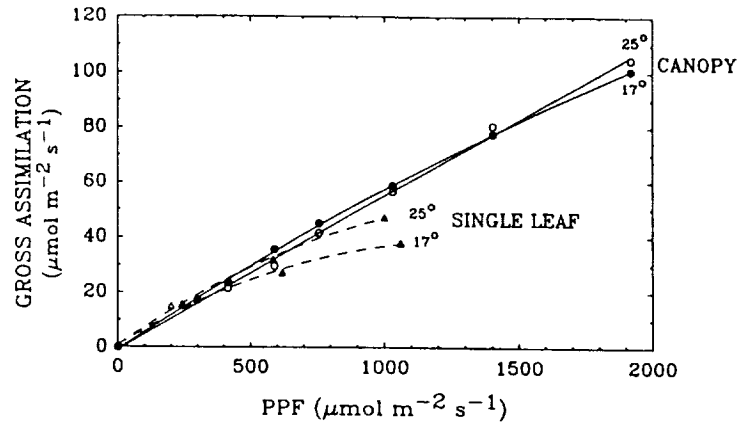


Figure 6. The effect of PPF level on gross assimilation at two temperatures.

QUANTUM REQUIREMENT

The dimensionless ratio of moles of photons absorbed to moles of CO_2 fixed is called the quantum requirement. (The inverse of this ratio, moles of CO_2 fixed per mole of photons absorbed, is sometimes used and is called the quantum yield). The best quantum requirement that has been measured in a single leaf has been about 12.5, and this was with 2% oxygen, which almost completely eliminated photorespiration (4). Figure 7 indicates the apparent quantum requirement for single leaves and canopies at different PPF levels. This figure is developed directly from the data in Figure 6. It is necessary to use the term "apparent" quantum requirement because we measured incident PPF and not absorbed PPF. The difference between apparent and actual quantum requirements is about 10% for single leaves and 2 to 4% for the canopy. If we had made these measurements with 2% oxygen and measured absorbed photons, it is likely that the quantum requirement would have approached 13 (below $200 \mu\text{mol m}^{-2}$

s⁻¹ PPF) in both single leaves and the canopy.

The most striking aspect of Figure 7 is that the quantum requirement for canopies remains very low as the PPF increases to full sunlight!

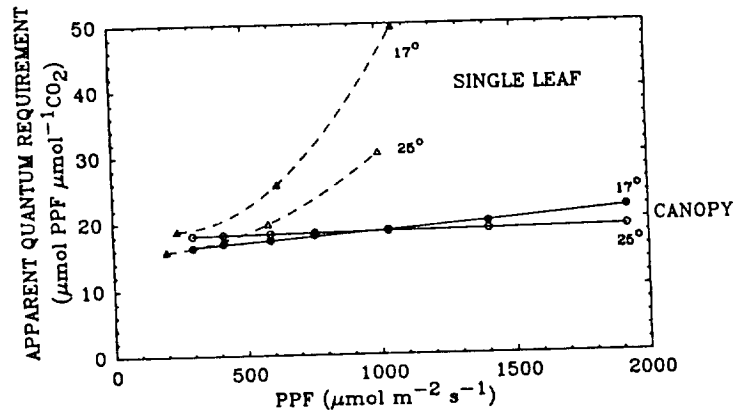


Figure 7. The apparent quantum requirement for single leaves and a canopy at two different temperatures.

NET PHOTOSYNTHESIS

Photosynthesis cannot be measured in the absence of dark respiration, but the standard assumption is that dark respiration occurs at the same rate in the light as in the dark. This assumption may not be perfectly accurate (see discussion in 2), but it is necessary to estimate gross photosynthesis. Figure 8 shows what was actually measured to get the data in Figure 6. Although 25°C is optimum for photosynthesis, far less dark respiration occurs at 17°C resulting in a higher rate of net photosynthesis at all PPF levels. The high respiration rates in the canopy are the result of an excessively high leaf area index (about LAI=30). Most of these leaf layers are in a very low PPF environment and contribute almost nothing to photosynthesis. The

top layer of leaves, as indicated by the single leaf measurements, is responsible for almost all of the photosynthesis at the lower PPF levels. The high LAI results in a high respiration rate, which causes the respiration efficiency to be low. Note that the PPF compensation point (point at which assimilation is zero) is $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 17°C and $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 25°C .

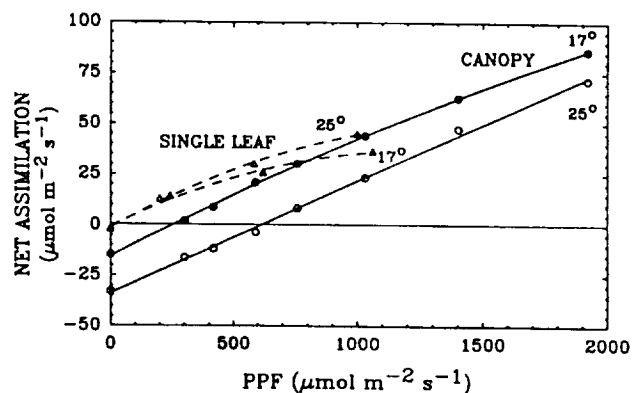


Figure 8. Net assimilation (photosynthesis) in single leaves and a canopy at 17 and 25°C .

THE RELATIONSHIP BETWEEN LAI AND PPF ABSORPTION

An LAI of about 10 is adequate to absorb 95% of the PPF (Figure 9). Canopies, grown in a CELSS environment, reach this LAI at 10 to 15 days after emergence. The LAI continues to rapidly increase to a maximum of about 40 and then begins to decrease because the lower leaves senesce. LAI continues to decrease until it reaches an LAI of about 10 at harvest. This would seem to be a strong argument against the use of high plant densities, but tillering causes even plants grown at low densities to reach the same high LAI's.

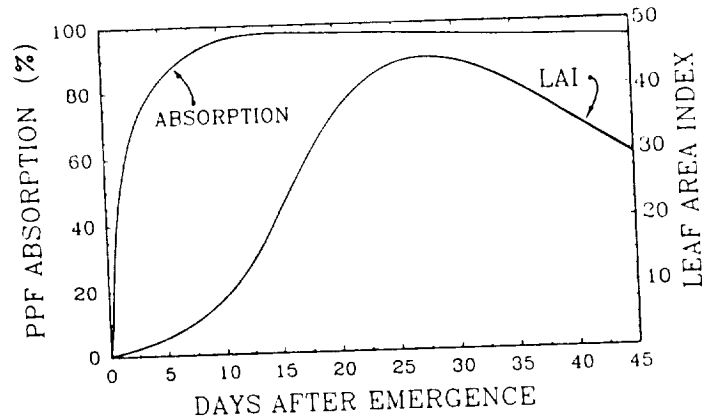


Figure 9. The relationship between leaf area index and PPF absorption. Head emergence is at about day 40. An LAI of 10 is sufficient to absorb over 95% of the PPF.

EXCESSIVE TILLERING

Wheat plants form more tillers in response to favorable environmental conditions. Tillering is usually beneficial in the field, but optimal conditions in a CELSS cause excessive tillering and result in an excessive LAI. Figure 10 indicates culm formation at two planting densities. More culms are formed than can be supported by the PPF levels. Late forming culms are at lower levels in the canopy and do not have sufficient PPF for maintenance respiration after the canopy fills in. After about day 15 they begin to senesce until the number of culms is reduced to a level that can be maintained. This represents a large waste of resources that cannot be alleviated by reducing the planting density. High density planting results in slightly more culms (heads) per unit area on day 45 than the low density planting. This increase in head number is typically associated with increased grain yields at harvest.

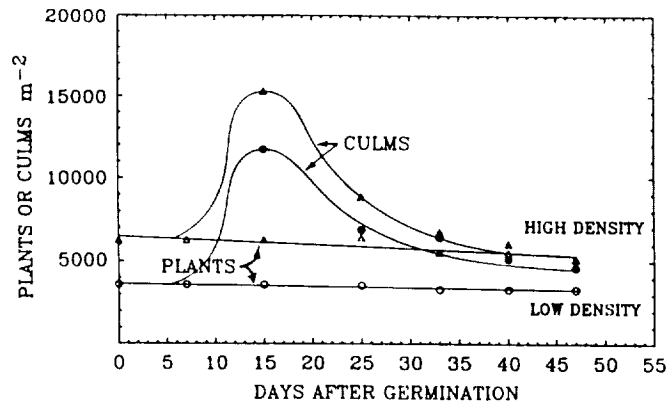


Figure 10. The effect of plant density on culm number from germination to 45 days old. Excessive culm formation (tillering) occurs, followed by senescence.

HARVEST INDEX

Unlike the other components of yield, our harvest indexes (seed mass divided by total plant mass) have not exceeded those in the field (40 to 55%). In fact, many of our early studies resulted in harvest indexes of 25 to 35%. Recent refinements have helped us achieve harvest indexes of over 50% and we are confident harvest indexes of 55% or even 60% will ultimately become routine. Harvest index appears to be particularly sensitive to environmental conditions in the final two weeks before harvest.

Harvest index is reduced considerably by late forming tillers. Figure 11 indicates the effect of primary, secondary, and tertiary tillers on harvest index (data from 1). Note that the mean harvest index was 40 to 43% in this study, but that the harvest index of primary and secondary tillers was 47 to 52%. This is another important reason to try to eliminate late forming

tillers.

The elimination of tillering has long been a goal of this project. We developed 20 wheat lines that do not tiller in the field but in optimal controlled environments form 2 to 4 tillers per plant. Genetic alterations do not appear to provide an easy solution, but altering the red/far-red radiation ratio might be very effective. This ratio directly alters the phytochrome equilibria in plant tissue, which in turn regulates tillering (see discussion in 2). We hypothesize that a high level of far-red radiation during the first 10 to 20 days of growth may be sufficient to eliminate late forming tillers. The red/far-red ratio appears to be sensed at the base of the wheat plant so the canopy itself becomes a biological far-red filter after canopy closure. As indicated in Figure 10, culms are initiated a few days after emergence and reach a peak at day 15.

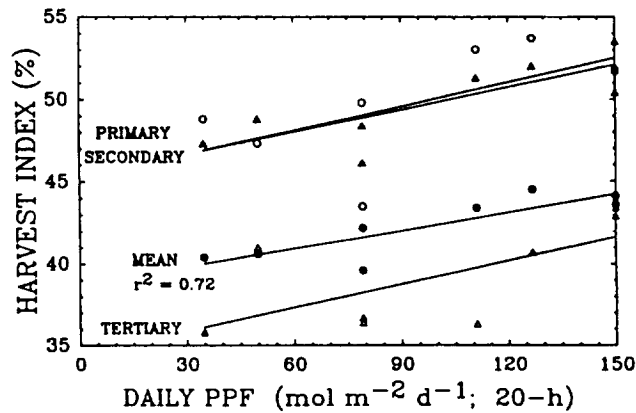


Figure 11. The harvest index of three different tiller categories as affected by PPF level. The photoperiod in this study was 20-h.

PHOTOPERIOD

When daily PPF is the same, low PPF levels and long photoperiods usually result in better growth than high PPF levels and short photoperiods. Wheat is a long-day plant for reproductive initiation and thus does not have an obligate need for a daily dark period. Wheat plants yield well and appear healthy in continuous light, but continuous light might reduce efficiency per photon. Three recent studies with 16, 20, and 24-h photoperiods have indicated that wheat plants do not need, or benefit from, a daily dark period. Crop growth rate and yield per photon were nearly identical in all photoperiods. Photoperiod has large effects on plant height and length of the life cycle, however. Compared to a 16-h photoperiod, continuous light shortened the life cycle by 30% and shortened plant height by 25%. Both of these effects would be beneficial in a CELSS.

CARBON DIOXIDE CONCENTRATION

Based on an extensive literature of CO₂ research we have used CO₂ enrichment in all of our studies. Some of our early studies on optimum CO₂ levels indicated a possible detrimental effect of very high CO₂ concentrations on growth and yield. Other studies have also found toxic effects of CO₂ concentrations above about 1500 $\mu\text{mol mol}^{-1}$ (5). Because of the evidence for CO₂ toxicity, we have elevated CO₂ levels only to 1200 $\mu\text{mol mol}^{-1}$. We have recently begun to investigate the effects of higher levels. High CO₂ levels increase photosynthesis on a short term (hours) basis in single leaves and we have also found that they increase short-term canopy photosynthesis (Figure 12).

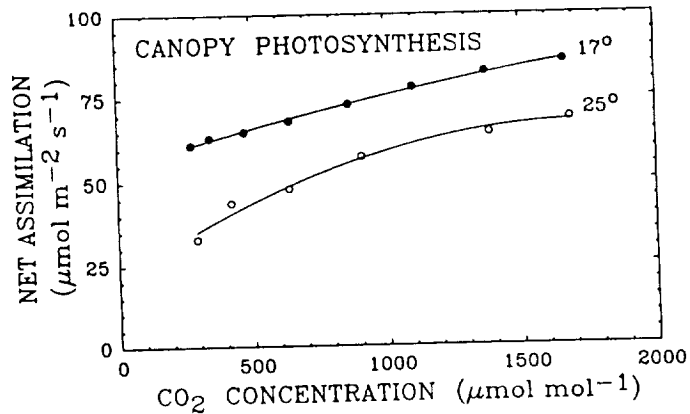
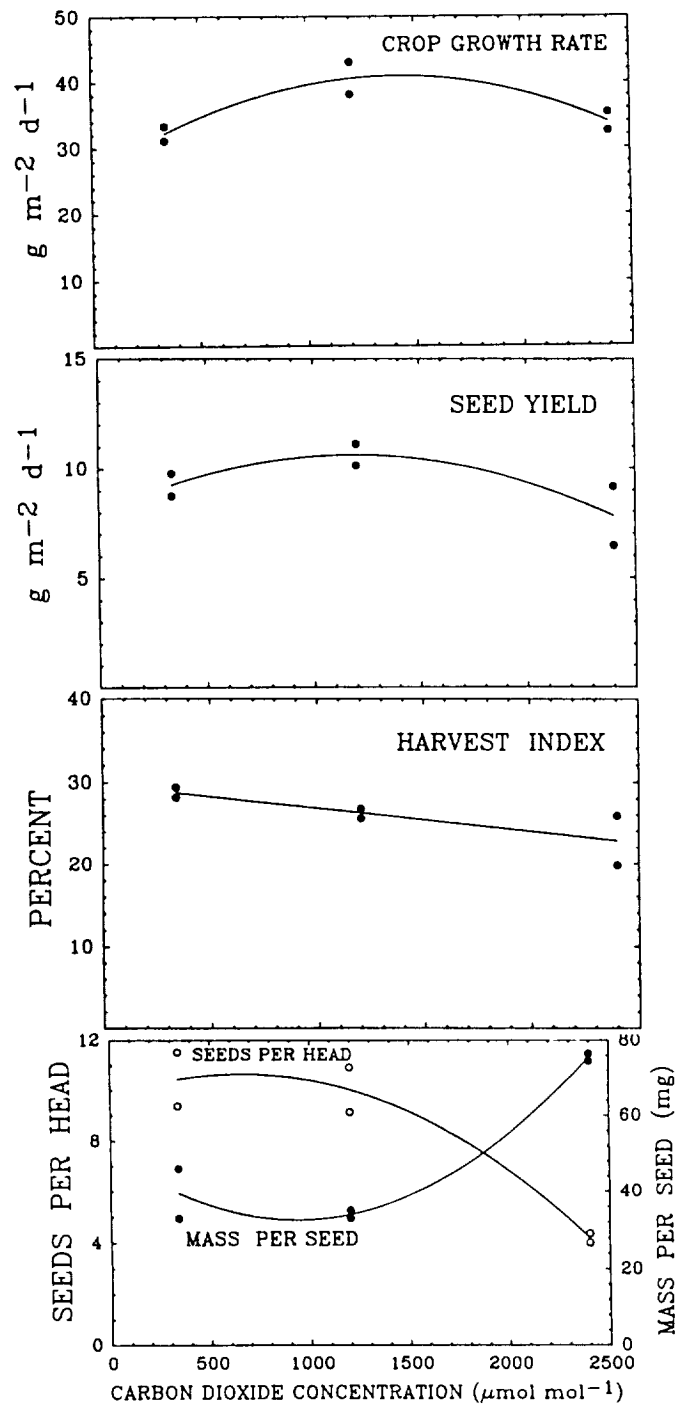


Figure 12. The effect of CO₂ concentration on canopy photosynthesis at 17 and 25°C.

We have modified six, plexiglass cylinders to allow the evaluation of different foliar environments in a common hydroponic root-zone environment. A preliminary study was conducted with two replicate cylinders at each of three CO₂ concentrations (340, 1200, and 2400 μmol mol⁻¹). Carbon dioxide enrichment to 1200 μmol mol⁻¹ resulted in a 20% increase in total biomass (crop growth rate; Figure 13) and a 15% increase in seed yield (Figure 14). Enrichment to 2400 μmol mol⁻¹ resulted in decreased growth and a larger decrease in yield. Elevated CO₂ levels appear to have an inhibitory effect on harvest index (Figure 15). This reduction in harvest index may be caused by a decrease in seed set (Figure 16). The seeds that were set in the highest CO₂ treatment were exceptionally large (75 mg per seed) but this increase did not overcome the effects of poor seed set. A replicate trial is in progress. If inhibition of seed set is reproducible we need to examine the casual factors. One

hypothesis is low boron concentrations in the emerging heads. High CO₂ levels close stomates and dramatically reduce transpiration. Boron (and calcium) are passively absorbed and delivered to the top of plants in the transpiration stream. Elevated CO₂ reduces these elements in foliar plant parts and boron is essential for good pollen formation. Low boron levels in emerging wheat heads might be ameliorated by increasing the concentration of boron in the nutrient solution or with the application of foliar sprays of boron.



Figures 13, 14, 15, and 16. Crop growth rate, seed yield, harvest index, seeds per head and mass per seed as affected by CO₂ concentration.

NITRATE CONCENTRATION IN HYDROPONIC SOLUTION

Nitrate concentrations of about 0.5 mM in nutrient solutions are sufficient to allow maximum nitrogen uptake by small seedlings and isolated root pieces. Concentrations of up to 30 times higher than necessary (15 mM) are often used in hydroponic solutions to insure adequate nitrogen nutrition. If the nitrate concentration is maintained above 0.5 mM and if the solution flow rates are sufficiently rapid to deliver the nitrogen to all parts of the root-zone, then low nitrogen concentrations should result in maximum nitrogen uptake and growth rates. We tested this hypothesis in two recent studies. Nitrate concentrations were maintained at 1, 5, and 15 mM in each of three, identical hydroponic systems. Each hydroponic system delivered solution to four, 0.2-m plots arranged in a completely random design (12 total plots). An initial trial indicated that there may be an interaction between nitrate concentration and iron nutrition of wheat plants.

In a second trial, the iron deficiencies were alleviated by changing the iron chelate in solution. Results of the second trial are shown in Figure 17. There was no statistical difference in growth rates among any of the three treatments at any time. One mM nitrate resulted in a slightly higher growth rate at the end of the life cycle, but there was insufficient replication to associate this increase with statistical significance.

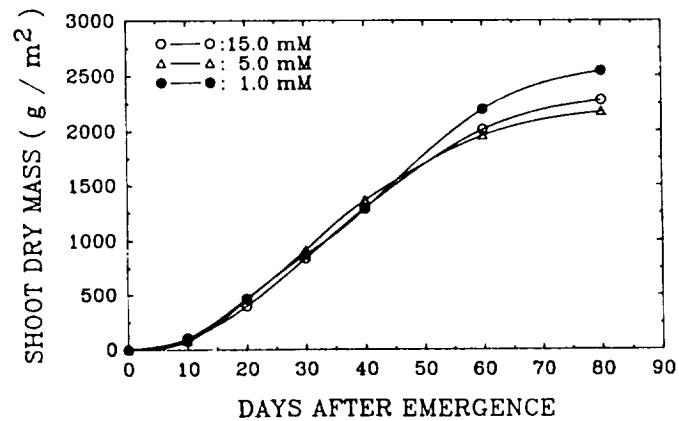


Figure 17. The effect of solution nitrate concentration on crop growth rate.

There were no differences in seed or leaf protein among the treatments. There were also no significant differences in tissue nitrate concentration, indicating that wheat may be able to regulate the translocation of nitrate to foliar plant parts even at high nitrate levels in solution.

A small amount of the nitrate that reaches foliar plant parts is effluxed through stomata as ammonium gas. This concentration is usually less than 5% of the total nitrogen in the plant. Figure 18 indicates the nitrate removal from two of the 3 systems. The 5 mM treatment was in between the 1 and 15 mM treatments and has been left out of this figure for clarity. About 10% more nitrate disappeared from the 15 mM treatment than the 1 mM treatment. If this went into the plant, it should have resulted in a higher nitrogen concentration in the plant tissue.

If it was lost to the atmosphere, it suggests an important incentive to use low nitrogen concentrations in a CELSS. We are currently replicating this study to more accurately measure the fate of nitrogen added to the solution.

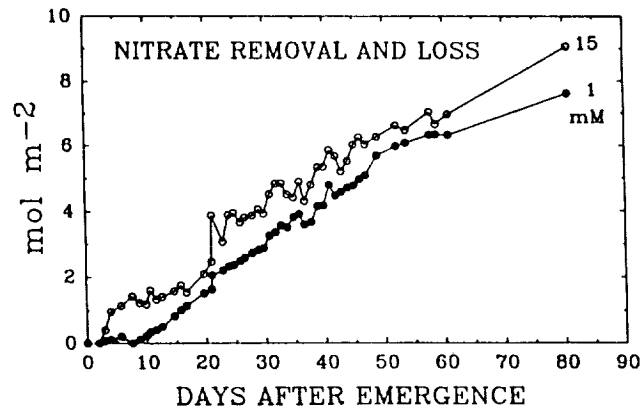


Figure 18. Nitrate removal from hydroponic solution as a function of two nitrate concentrations in hydroponic solution.

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ENVIRONMENTAL MODIFICATION OF YIELD AND FOOD COMPOSITION OF
COWPEA AND LEAF LETTUCE

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) and leaf lettuce (*Lactuca sativa* L.) are candidate species to provide legume protein and starch or serve as a salad base, respectively, for a nutritionally balanced and psychologically satisfying vegetarian diet in CELSS. Greenhouse-grown cowpeas (cv. IT84E-124) were harvested according to several different strategies. Total edible yield (34 gDW plant⁻¹) was equal for vegetative and reproductive harvest strategies, but the vegetative product could be harvested 47% sooner and from smaller plants. Yield efficiency was 2.9 to 4.4 times greater for the vegetative than for a reproductive or mixed harvest strategy. Leaf carbohydrate content increased with leaf age (32-43% of DW), but was greatest in the seed (56%). Protein content of older leaves was similar to that of seeds (31%), while that of young leaves was greatest (43%). Fat content of cowpea leaves (5%) and seeds (1%) was quite low, allowing great flexibility for cowpea in formulating healthy diets. Hydroponic leaf lettuce grew best under CO₂ enrichment and PPF enhancement. High CO₂ (1500 μ l l⁻¹) enhanced leaf number 69% relative to ambient CO₂. Leaf protein content reached 36% with NH₄⁺ + NO₃⁻ nutrition, and starch and free sugar content were as high as 7 and 8.4% of DW, respectively, for high PPF/CO₂ enriched environments.

Research supported by NASA Ames Cooperative Agreement NCC 2-100.

As members of the CELSS Food Production group, we have been interested in candidate species selection since the beginning of the CELSS program. First and foremost in selecting plants for CELSS is the question of how they contribute to human nutrition (Fig. 1). Energy content and nutritional composition of the harvestable part, as well as processing requirements, are the most important nutritional use criteria. Other nutritional criteria are listed in Figure 2.

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Cultural criteria also are important, especially proportion and yield of edible biomass. Long juvenile periods and dormancy requirements would be very undesirable, but suitability for soilless culture would be very desirable. Given these selection criteria and a scoring system with weighting factors, we evaluated and ranked 115 world food crops for candidacy in the CELSS program. The 36 species listed in Figure 3 are part of a "generous" diet scenario selected from the original list. They tend to be fast-growing herbaceous annuals which, in appropriate proportions, provide a nutritionally balanced vegetarian diet with some variety. The more species used to compose a vegetarian diet, the less risk of deficiency or toxicity. Legume, root and tuber, salad, sugar, cereal, leaf and flower, fruit, and stimulant crops all were part of the generous diet scenario. However, early emphasis in the CELSS program of defining conditions for optimum productivity precluded the opportunity for initially working with a variety of species. With only a handful of candidate species presently under development, nutritional criteria take on exaggerated importance (Fig. 4). In this "modest" diet scenario, only 5 categories of crops are represented: legumes and cereals for complementary protein, tuber crops mainly for complex carbohydrate and calories, cooked vegetables, and raw salad vegetables. The first category of legumes has higher fat content, the second category lower fat content. The low-fat legumes and cereal grains also provide substantial complex carbohydrate. One can argue whether the vitamins, minerals, and fiber provided by the vegetables alternatively could be provided by stored supplements, but fresh vegetables and salad greens definitely are preferred for a psychologically satisfying, nutritionally balanced diet. These analyses and interpretations are contained in NASA Contractor Report 166324, entitled "Nutritional and Cultural Aspects of

Plant Species Selection for a Controlled Ecological Life Support System"

(Fig. 5).

NIH has set an eventual goal for a prudent diet for Americans that would consist of no more than 15% of total Calories as protein, 65% as complex carbohydrate, and 20% as fat (Fig. 6). Presently, about 50% of our daily Calories are from fat, but only a few percent fat (as particular fatty acids) is essential for normal growth, development, and maintenance of the human body. The prudent diet will involve 4-5 times more starch foods than protein, and 7 times more starch than fat. On a dry weight basis, much of the carbohydrate in the present candidate species is in a digestible form, except for that in lettuce (Fig. 7). Soybean presently is the main protein source, but since it contains 2-3 times more protein than the NIH desired amount, soybean would have to be mixed with other species to lower overall protein content, raise carbohydrate, and keep fat about where it is in the formulated diet. Water content, of course, determines the absolute food content of the parts that are consumed. This is not a limitation for soybean or wheat, but is somewhat for the other three species. Water content is not a serious limitation for potato and sweet potato because the solid parts are so rich in edible carbohydrate. The main nutritional value of lettuce, besides providing vitamins, minerals, and fiber, appears to be as a water source, plus the fact that it is pleasant to eat, and is a dietary enhancement food. Leaf lettuce is the species we have emphasized in our environmental optimization program for CELSS at Purdue University, primarily because the senior author is interested in its use as a model crop to maximize photosynthetic productivity of leafy vegetable crops, and also because we initially were operating under the assumption of a generous diet scenario. That is gradually developing, and with the recent grass roots demand

for a "Salad Machine" on Space Station, lettuce will play an important role in a "modest" diet scenario (Fig. 8).

Leaf lettuce has a short production cycle, a promising yield rate to build upon, an excellent harvest index, a minor nutritional contribution, air revitalization capacity throughout production, adaptability for many forms of hydroponics, and tolerance for NH_4^+ nitrogen during vegetative development (Fig. 9). Actually, the biggest selling point of lettuce is its positive psychological impact. Being around something green in an otherwise austere institutional environment is pleasing to humans (Fig. 10). Furthermore, lettuce has ornamental value. It can be bolted by long photoperiods, gibberellic acid, or heat stress; it even can be decorated for Christmas, and then eaten after the holiday season. We have decorated edible Christmas trees with *Zea mays* (popcorn), *Vaccinium* (cranberry), and *Carambola* (Starfruit), all candidate edible ornaments (Fig. 11).

Conditions found conducive to productivity rates of at least 60 gDW of edible biomass $\text{m}^{-2} \text{day}^{-1}$ for a responsive cultivar of leaf lettuce are summarized in Fig. 12. All of these conditions are important, but CO_2 enrichment, nitrogen (level and form), and radiation enhancement during critical periods of exponential growth are drivers. We intend to double this figure with judicious canopy management and use of growth-stimulating agents during the early lag phase of seedling development.

We have been successful in growing this salad vegetable with high rates of productivity, but recently we have asked whether optimizing conditions alter the quality of the product. Lettuce is not nutritionally rich, but as a model leafy vegetable crop, we want to know if environmental modification affects the levels of important nutritional components of leaves in a favorable way.

We generate the edible leaf biomass in a 100 ft² walk-in growth room equipped with fluorescent and incandescent lighting fixtures (Fig. 13). Within the chamber is a table supporting recirculating hydroponics systems. Troughs mounted on the table are constructed from vinyl downspouts of rain guttering (Fig. 14). These slotted units are nursery troughs into which seeds are sown. Cloth wicks lining the slots keep the seedlings moist. Lids over the nursery troughs keep seedlings dimly illuminated and humid for the first 2 days of germination (Fig. 15). After uncovering, the seedlings are left in the nursery troughs for an additional 4 days until they are transplanted (Fig. 16). The cloth wicks are taken out of the nursery, pulled apart, and polyester wicks are prepared for individual seedlings. The exposed seedling roots are kept moist with Shur-Wipes misted with water. A forceps is used to gently lift a hanging seedling, and it is carefully placed within a slitted Ethafoam plug along with the wick (Fig. 17). The seedlings and wicks mounted in plugs are then floated in a tray containing dilute nutrient solution until they are all transplanted to holes in the troughs at once. The transplanted seedlings have only the cotyledons and rudimentary true leaves on the day of transplant (Fig. 18). From then (day 6) until day 12, when environmental optimization treatments are initiated, the plants develop one leaf per day. On day 12, light treatments begin, CO₂ enrichment is initiated, and various N treatments are applied in nutrient solution (Fig. 19). By day 18, the plants are in rapid exponential growth (Fig. 20), and they are harvested on day 21 (Fig. 21). At this time the entire foliar canopy is closed. Until day 21 when plants are harvested, the plants are in rapid exponential growth (Fig. 22). As the plants are harvested, they are quick-killed with microwave radiation just prior to oven drying.

Proximate and growth analyses were performed on lettuce leaves grown under various optimizing environments. Figure 23 shows the combined effect of light

level, CO₂ concentration, and different species and levels of N on leaf number after 21 days of growth. CO₂ enrichment clearly stimulates leaf development, and for all combinations of light and CO₂, NH₄⁺ and NO₃⁻ together tend to enhance leaf number. As far as photosynthetic productivity is concerned, leaf dry weight of lettuce was lowest when light of 330 μmol m⁻² s⁻¹ and ambient CO₂ were used, and was highest when light of 800 μmol m⁻² s⁻¹ plus 1500 ppm CO₂ were used (Fig. 24). High CO₂ alone was more effective than high light alone in stimulating photosynthetic productivity. In terms of the quality of the product, as a point of reference, field-grown, loose-leaf, dark-green lettuce of the 'Grand Rapids' type has 22% protein, 58% carbohydrate distributed between cellulose and starch, 5% fat, including chlorophyll, and 15% ash (Fig. 25). For chamber-grown, hydroponic lettuce, protein content of the leaves in all cases was greater than 22% (Fig. 26). Its content relative to total dry weight tended to be greater for ambient CO₂, regardless of light level. That seems logical because high CO₂ would favor accumulation of carbohydrate *per se*, rather than protein. It also makes sense that protein tends to accumulate when NH₄⁺ is included in the nutrient solution, especially double-strength NH₄⁺. If total carbohydrate of leaf lettuce is about 58%, then most of it is in the form of non-digestible cellulose, because lettuce normally makes no more than a few percent starch, as opposed to alfalfa leaf, which might be 45% starch. As you would expect, conditions favoring high photosynthetic rates resulted in the greatest accumulation of leaf starch, and in all cases NH₄⁺ nitrogen reduced starch content, by siphoning off carbon skeletons to support protein synthesis at the expense of carbohydrate (Fig. 27). The highest starch content we achieved was about 7%. One really interesting finding was that free sugar content was consistently as high or higher than starch content regardless of environmental regime (Fig. 28). Sugar contents of 8-10% were common in freshly

harvested hydroponic lettuce, whereas leaf lettuce off the shelf of the grocery store or out of the garden last summer had essentially zero free sugar. Leaf sugar evidently represents a very labile carbon pool that is easily respired away. Microwave quick-kill of enzymes immediately upon harvest apparently preserved free sugar as well as starch in our experimental material. The only fresh tissues that tasted sweet, however, were young leaves of plants that had been grown with single-strength nitrogen (i.e., 15 mM) as $\text{NH}_4^+ + \text{NO}_3^-$. Incidentally, NH_4^+ -treated plants also had the least bitter principle. Sugar and starch together brought total edible carbohydrate to about 15%, still leaving more than 40% as cellulose and other wall polysaccharides. Fat content of controlled environment lettuce was consistently lower than the 5% average of field-grown lettuce (Fig. 29). Components of the solar spectrum may stimulate membrane lipid and chlorophyll synthesis more than do fluorescent and incandescent radiation. Field-grown lettuce has about 15% ash. Most of our controlled-environment lettuce was in that range or a little higher (Fig. 30). 15 to 20% inorganic content seems like a rich source of minerals. Perhaps lettuce is a good source of mineral water!

The results of these proximate analyses demonstrate that the protein and bioavailable carbohydrate composition of leaf lettuce can indeed be modified by environmental and nutritional manipulation. Lettuce will not be a rich Calorie source for CELSS, but as a model leafy vegetable crop the results of this study demonstrate that nutritional value can be improved by certain optimizing environments. The principles we have learned with lettuce in this regard should transfer readily to other, more nutritious crops with edible foliage.

One such promising new candidate species is used as a staple food along with sorghum in certain drought and heat-stricken countries of Central Africa. It is cowpea, or black-eyed pea (Fig. 31). The pods themselves are edible when

the seeds are immature, but if the seeds mature you have a dry bean with considerable shelf life. Furthermore, the leaves of this legume are edible either as a raw salad green or as a cooked vegetable. The foliar canopy is "aggressive", and when we have grown it hydroponically along with other legumes in the growth chamber, it tends to choke out all competitors (Fig. 32). Can the same plant produce both seeds and leaves for human consumption? We have obtained additional cowpea lines from a breeder in Niger and continue the screen. Using a promising determinate line, we compared biomass distribution among plant parts as a function of various harvest scenarios (Fig. 33). All parts of a cowpea plant are edible, although not necessarily at the same time. Seeds were harvested either once at 75 days in the greenhouse, or leaves were stripped at 15-day intervals. We compared these with a mixed-harvest scenario of young leaves stripped periodically and the seeds still harvested at 75 days. Total biomass was greatest for the seed-harvest scenario, and least for the mixed scenario. However, at 75 days it is not a good assumption that all leaf, stem, and pod tissues are edible, or even palatable, without sophisticated food processing procedures. The mixed harvest was lowest because the leaves were harvested when they were still expanding, and had not yet contributed to the photosynthetic productivity of the plant. But this is the way subsistence cultures in Africa do it. They are concerned about immediate nutrition and palatability, and don't think about photosynthetic productivity. This shows up in total edible yield, which is equal for seed and vegetative harvests but is reduced 30% for the mixed harvest (Fig. 34). Given other important yield considerations such as harvest index and time to harvest, yield rates and efficiencies were found to be greatest for the vegetative harvest, by far. We think the mixed harvest strategy can be made competitive with the vegetative strategy by allowing leaves to expand fully and contribute something to plant

biomass production before harvest, and we plan to test this. We also have subjected cowpea leaves and seeds to proximate analysis, and find that protein content of mature leaves is the same as that of seed protein on a dry weight basis (Fig. 35). Protein content of expanding leaves is 43% greater. The amino acid composition of cowpea seed protein is known and is comparable to that of soybean protein. I am not aware of the quality of cowpea leaf protein, however, but we plan to have amino acid composition done on leaf samples once we get cowpea into hydroponics and CO₂ enrichment in the growth chamber. We also don't have starch analyses on cowpea leaves or seeds yet, but presumably much more total seed carbohydrate (e.g., starch) is potentially bioavailable than is leaf carbohydrate (e.g., cellulose). However, cowpea leaves are much less succulent than lettuce leaves, and it is likely that starch makes up a much higher proportion of cowpea leaf biomass. Fat and ash are about what one would expect for distribution between leaves and seeds.

Future efforts with cowpea will emphasize effects of modified controlled environments on the quality as well as quantity of vegetative and reproductive parts.

CANDIDATE SPECIES SELECTION CRITERIA

<u>Criterion Number</u>	<u>Nutritional use Criteria</u>
1	Energy concentration
2	Nutritional composition
3	Palatability
4	Serving size and frequency
5	Processing requirements
6	Use flexibility
7	Storage stability
8	Toxicity
9	Human use experience

CANDIDATE SPECIES SELECTION CRITERIA

<u>Criterion Number</u>	<u>Cultural Criteria</u>
10	Proportion of edible biomass
11	Yield of edible biomass
12	Continuous vs. determinate harvestability
13	Growth habit & morphology
14	Environmental Tolerance
15	Photoperiodic & temperature requirements
16	Symbiotic requirements & restrictions
17	Carbon dioxide-light intensity response
18	Suitability for soilless culture
19	Disease resistance
20	Familiarity with species
21	Pollination & propagation

PLANT SPECIES RECOMMENDED FOR THE "GENEROUS"
DIET SCENARIO

LEGUMINOUS CROPS:

- DRY BEAN
- SNAP BEAN
- CHICK PEA
- SHELL PEA
- SUGAR PEA
- PEANUT
- SOYBEAN

ROOT & TUBER CROPS:

- GARDEN BEET
- CARROT
- POTATO
- SWEET POTATO
- TARO

SALAD CROPS:

- CELERY
- LEAF LETTUCE
- ONION
- TOMATO

SUGAR CROPS:

- SUGAR BEET
- SUGAR CANE

CEREAL GRAIN CROPS:

- BARLEY
- CORN
- OATS
- RICE
- RYE
- WHEAT

LEAF AND FLOWER CROPS:

- BROCCOLI
- CHINESE CABBAGE
- HEAD CABBAGE
- CAULIFLOWER
- CHARD
- KALE
- SPINACH

FRUIT CROPS:

- BANANA
- GRAPE
- STRAWBERRY
- CANTALOUPE

STIMULANT CROP:

- TEA

CROP SPECIES TO SATISFY A "MODEST"
(MINIMUM) DIET SCENARIO

SOYBEAN AND/OR PEANUT
DRY BEAN OR COWPEA OR GARDEN PEA
WHEAT AND/OR RICE
POTATO
CHARD AND/OR CABBAGE
TOMATO AND LETTUCE

HOFF, J.E., J.M. HOWE, AND C.A. MITCHELL. 1982.
NUTRITIONAL AND CULTURAL ASPECTS OF PLANT
SPECIES SELECTION FOR A CONTROLLED
ECOLOGICAL LIFE SUPPORT SYSTEM. NASA
CONTRACTOR REPORT 166324, 122 PP.

DAILY ALLOWANCE FOR A 180-LB MALE FOLLOWING A PRUDENT DIET.

DIETARY CONSTITUENT	CALORIC DISTRIBUTION (%)	G·LB DESIRABLE BODY WT ⁻¹ ·DAY ⁻¹	G·DAY ⁻¹ ·PERSON ⁻¹
PROTEIN	15	0.66	118
CARBOHYDRATE	65	2.85	513
FAT	20	0.39	70

FOOD COMPOSITION OF PRESENT CANDIDATE CROP SPECIES FOR CELSS.

SPECIES	<u>COMPOSITION (% OF DRY WEIGHT)</u>			WATER CONTENT (%)
	CARBOHYDRATE	PROTEIN	LIPID	
WHEAT	82	14	2	13
SOYBEAN	38	38	20	10
POTATO	85	10	0.5	80
SWEET POTATO	89	6	1.4	71
LEAF LETTUCE	58	22	5	94

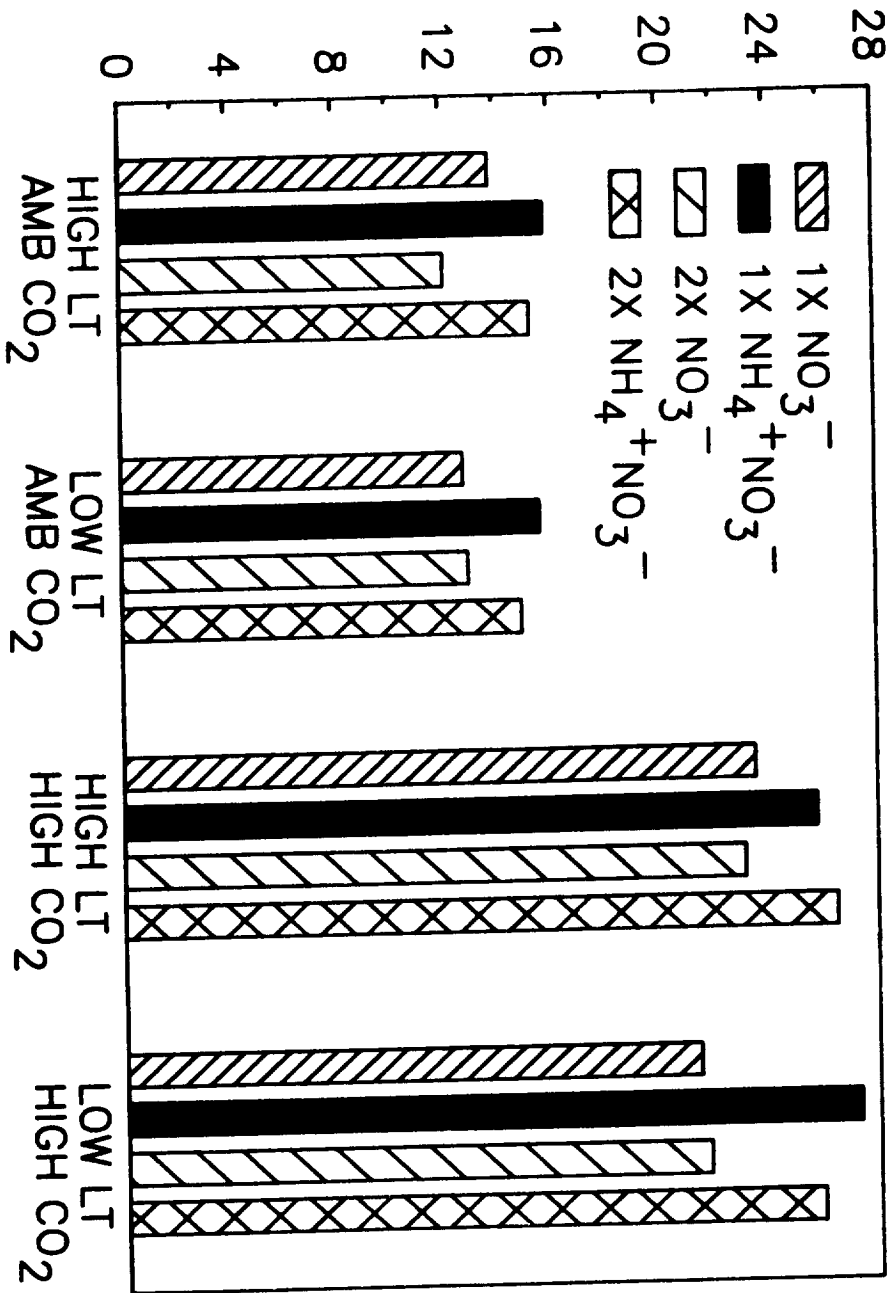
JUSTIFICATION FOR OPTIMIZING PRODUCTIVITY OF LEAF LETTUCE
(Lactuca sativa L.) IN THE CELSS PROGRAM:

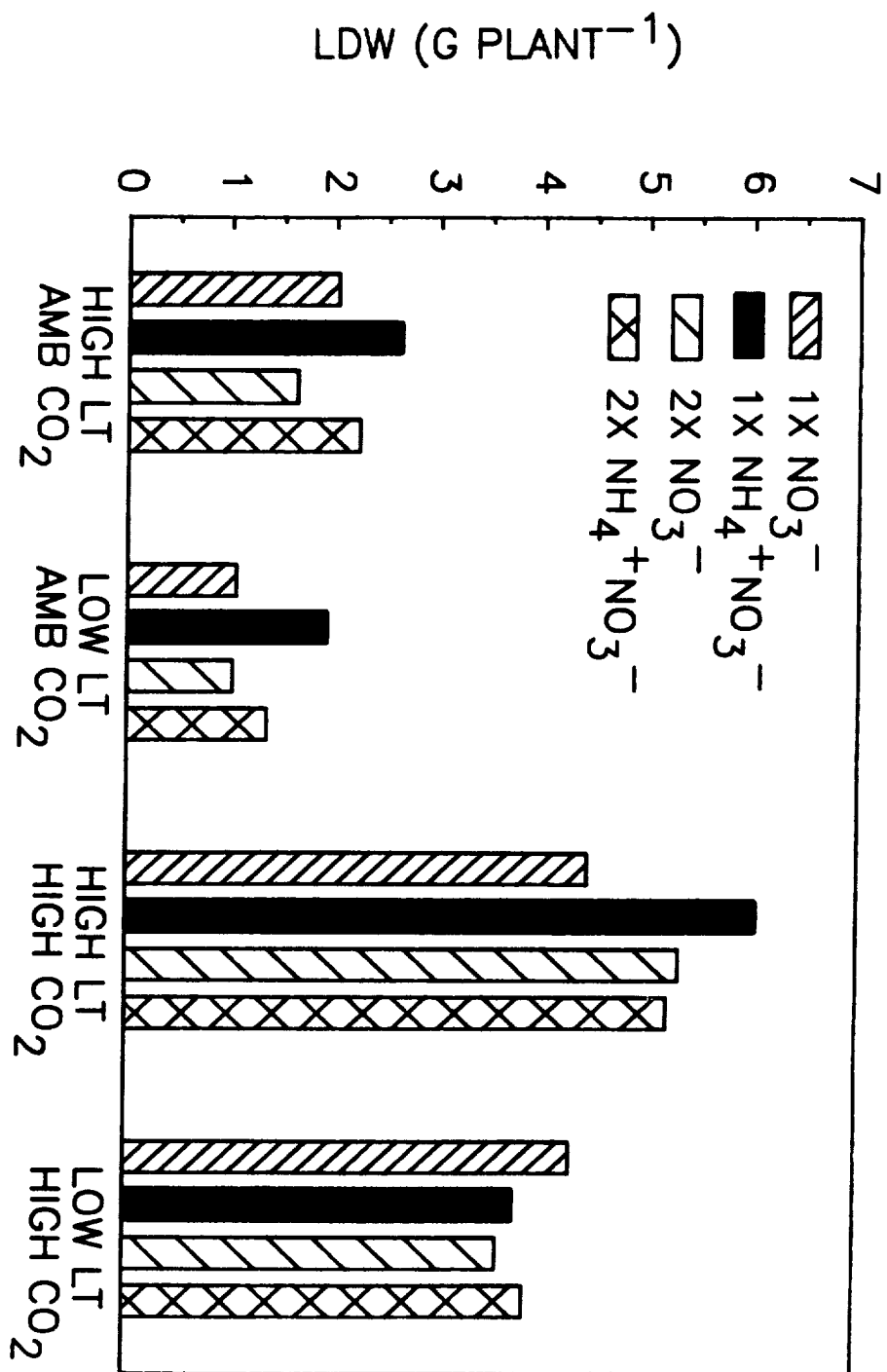
- Short production cycle (≤ 22 days by CEA vs. 55-70 days by OFA).
- Promising yield rate (2.6 g DW edible biomass $m^{-2} day^{-1}$ from OFA vs. 16.4 g $m^{-2} day^{-1}$ from unoptimized CEA vs. ≥ 60 g $m^{-2} day^{-1}$ from "optimizing" CEA vs. ? g $m^{-2} day^{-1}$ from optimum CEA).
- Favorable harvest index ($\geq 80\%$ of total DW).
Excellent dietary enhancement food for the psychologically satisfying diet (is the traditional salad "base" in our culture).
- Provides some vitamins, minerals, and fiber for a nutritionally balanced diet.
- Sustains high level of Pn ($O_2 \uparrow$ and $CO_2 \downarrow$) throughout production as a leafy salad crop.
- Suitable for all forms of soilless culture (hydroponics, aeroponics, NFT, tubular membrane system, etc.).
- Excellent tolerance of NH_4^+ beyond the seed-germination stage, especially in the presence of NO_3^- and radiation enhancement.
- Ideal model system for maximization of vegetative growth, photosynthesis, and productivity without complications arising from source/sink movement and monocarpic senescence.
- Diminutive stem in vegetative stage to pose few gravitropism problems in hypogravity.
- Extensive data base on culture to build upon.

Cultural conditions to give $\geq 60 \text{ gDW m}^{-2} \text{ day}^{-1}$ of edible biomass for 19-day-old 'Waldmann's Green' leaf lettuce:

- 13.5-cm spacing of plants
- continuous 25°C air temperature
- 85% relative humidity
- 20-h photoperiod
- 900 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ of PAR from 84% incandescent + 16% fluorescent radiation from days 11-19
- single-strength Hoagland's nutrient solutions. pH 6.0 ± 0.2 , containing 5 mM NH_4^+ + 25 mM NO_3^-
- 1500 $\mu\text{l l}^{-1}$ CO_2 from days 11-19

LEAVES PLANT - 1



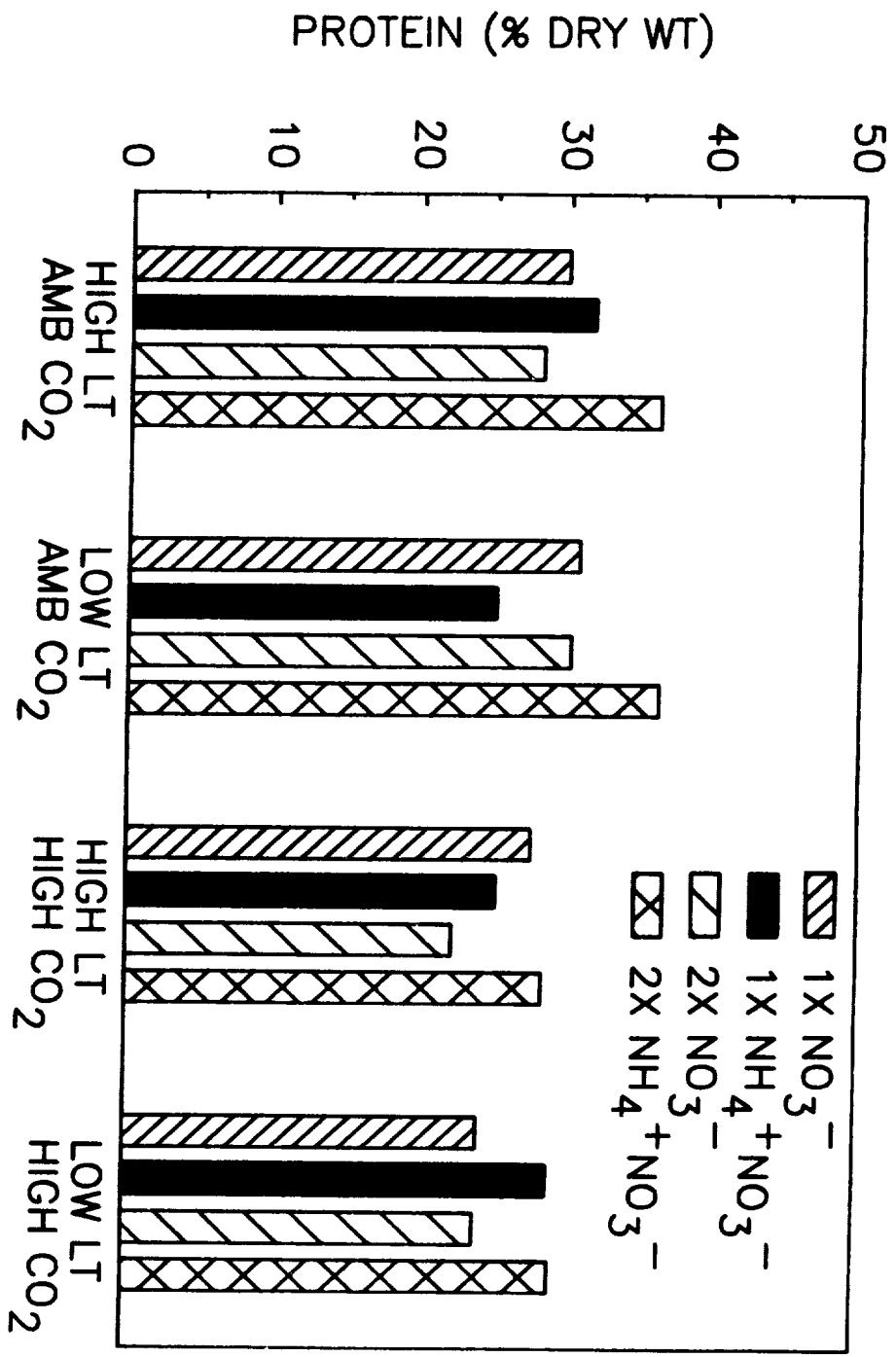


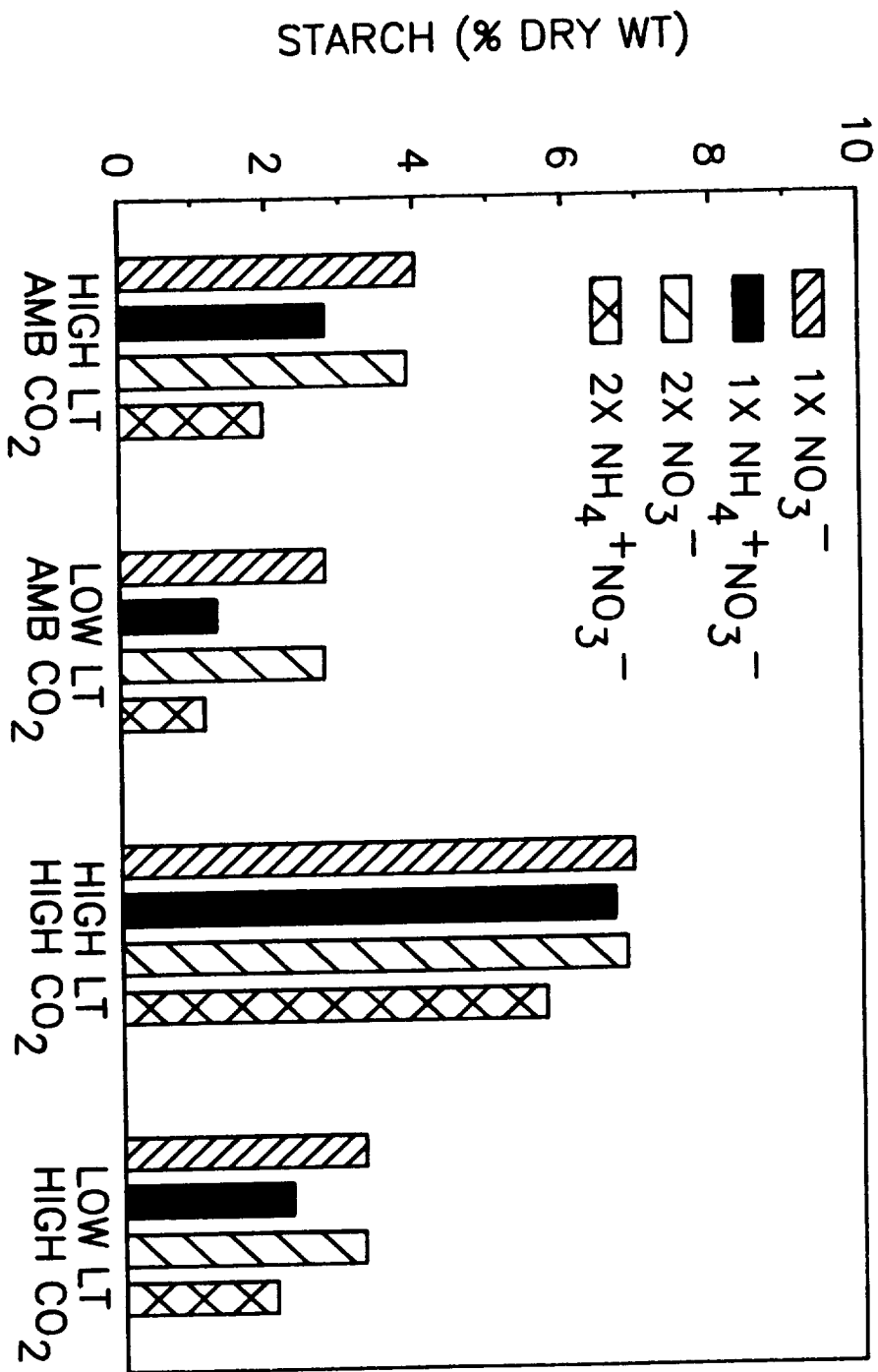
Proximate composition¹ of field-grown
loose-leaf lettuce.

Component	Composition (% of DW)
Protein	22
Carbohydrate ²	58
Fat	5
Ash	15

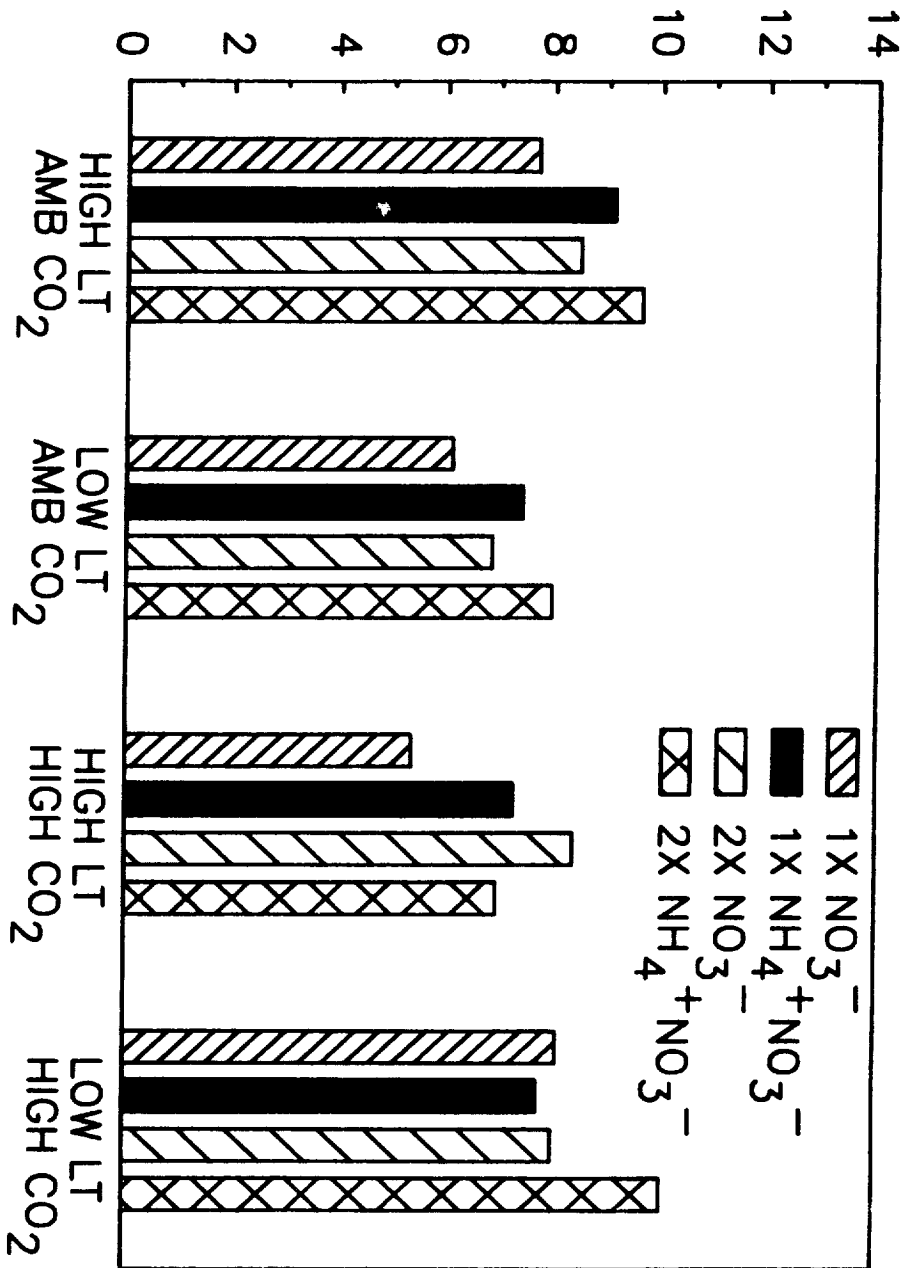
¹Composition of Foods. Agriculture
Handbook No. 8. USDA, ARS.

²Includes total structural and non-
structural carbohydrate.

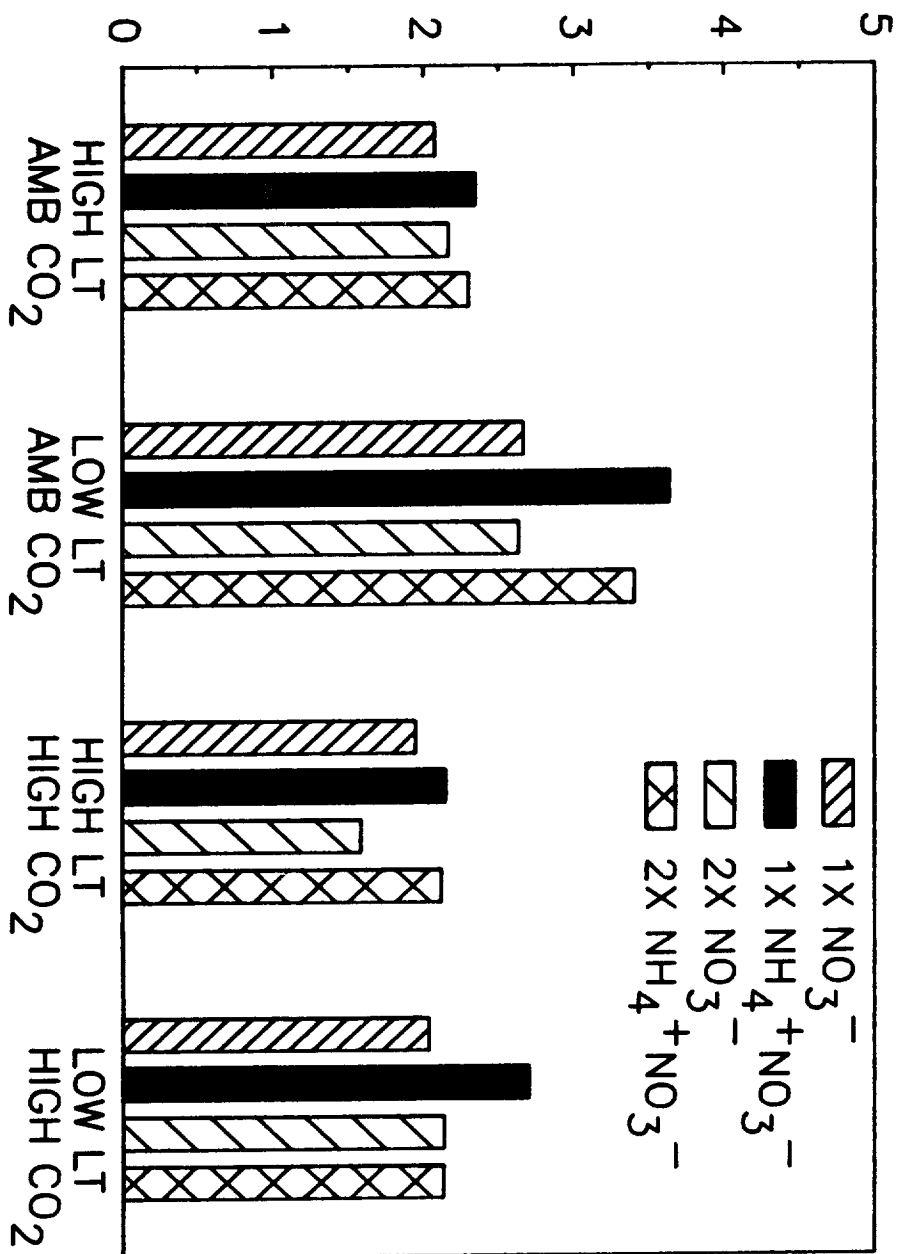


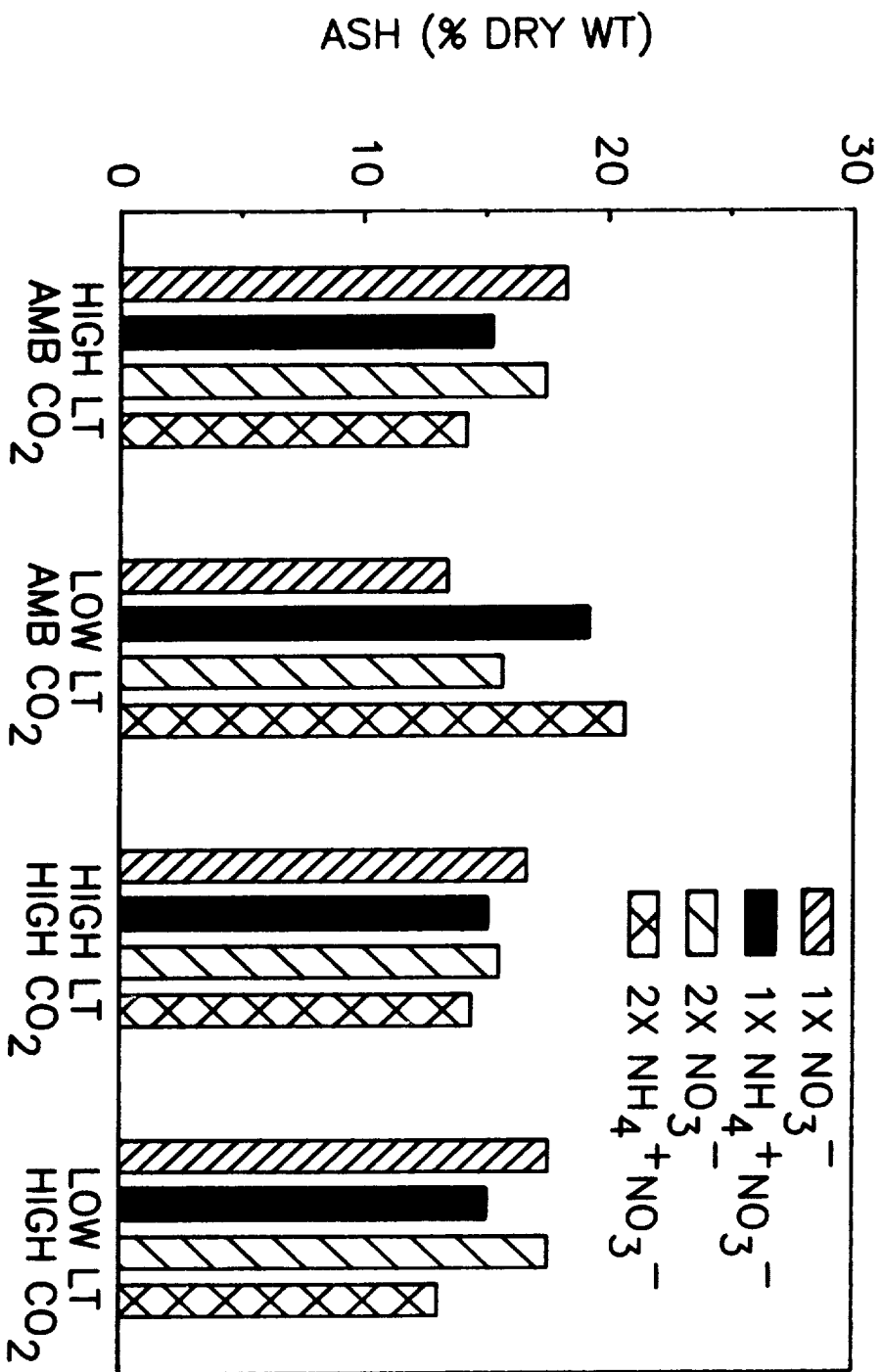


SUGARS (% DRY WT)



FAT (% DRY WT)





Biomass distribution in Vigna unguiculata cv. IT84E-124 as a function of traditional seed harvest (75 days), vegetative harvest (40 days), or mixed seed/vegetative harvest.

Plant part	Seed harvest (g)	Vegetative harvest (g)	Mixed harvest (g)
Leaves	24	34a	7c
Stem	28	20b	12c
Pod	46	-	13
Seed	35	-	10
Total	117	60b	44

Yield characteristics of *Vigna unguiculata* cv. IT84E-124 as influenced by harvest strategy.

Harvest Parameter	Seed harvest		Mixed harvest		Vegetative harvest
Seed yield (g plant ⁻¹)	35	**	10		-
Edible leaves (g plant ⁻¹)	-		15	**	34
Total edible yield (g plant ⁻¹)	35a		25b		34a
Harvest index (%)	30c		72a		58b
Time to harvest (days)	75a		75a		40b
Daily yield (g plant ⁻¹ day ⁻¹)	0.46b		0.33c		0.85a
Yield/area (g m ⁻² canopy)	69b		46c		105a
Yield efficiency (g m ⁻² day ⁻¹)	0.92b		0.60c		2.64a

Proximate composition of expanding (7-10 day old) and fully expanded leaves (22-25 days old) as well as seeds of Vigna unguiculata cv. IT84E-124.

Component	Expanding leaves (%)	Fully expanded leaves (%)	Seed (%)
Carbohydrate	32c	43b	56a
Protein	43a	30b	31b
Fat	5a	5a	1b
Ash	14a	15a	4b

EFFICIENCY OF N USE BY WHEAT AS A FUNCTION OF

INFLUX AND EFFLUX OF NO_3^-

R.C. Huffaker, M. Aslam, M.R. Ward, University of California, Davis, CA

ABSTRACT

Since N assimilation is one of the most costly functions of a plant, its efflux before assimilation results in a serious energy cost and loss in efficiency which could decrease yields. Efficient crop production is critical to CELSS. Our objective is to determine the extent of efflux of the N species NO_3^- , NH_4^+ , NO_2^- , and urea after uptake, and possible means of regulation. We found that NO_3^- efflux became serious as its substrate level increased. Efflux/Influx (E/I) of NO_3^- was greater in darkness (35%) than in light (14%) and the ratio greatly increased with increased substrate NO_3^- , (up to 45% at 10 mM). It seems advantageous to use the lowest possible nutrient concentration of NO_3^- . The feasibility of using ClO_3^- as a trapping agent (competitive inhibitor of NO_3^- uptake) for effluxed NO_3^- was assessed and its toxicity determined.

INTRODUCTION

Crop production during extended space flights requires the development of procedures leading to the optimum use of the available energy. Optimizing the utilization of N by crop plants represents an area where significant progress can be made in the CELSS program.

It is well recognized that ion uptake requires ATP and electron flow. The cost of NO_3^- assimilation is particularly high not only due to the energy requirement of uptake but also because of the need for 10 electrons to reduce it to the level of glutamate. In addition are the costs of maintaining pH balance, since each reduction of NO_3^- to the level of NH_4^+ produces an OH^- . Thus it is not surprising that estimates of respiratory costs of ion absorption range up to 50% of total root respiration and 20% of total plant respiration (1).

Recent reports in the literature indicate that NO_3^- efflux can begin very rapidly after its influx (2,3,4,5,6). In addition, prolonged leakage of NO_3^- occurs from root storage pools dependent upon the N-status of the roots

(7,8,9,10,11). Lee and Clarkson (6) estimated that efflux, at external concentrations above 1 mM, could account for up to 40% of the influxed NO_3^- . Thus NO_3^- efflux could represent a significant additional cost to an already energy costly assimilatory pathway. Little is known concerning the regulation of NO_3^- efflux. Recent work of Briskin (12) showed evidence that efflux of NO_3^- utilizes ATP, which could greatly add to the energy cost of NO_3^- assimilation.

Measurement of NO_3^- efflux has been hampered by the lack of easy, rapid, and less costly analytical techniques for detecting the very low concentrations involved in a process with a half-life ($t_{1/2}$) of minutes. The use of ^{15}N as a tracer is laborious and has problems of sensitivity. ^{13}N has only a 10 min half-life, must be used at the site of generation (cyclotron), and is very costly to use. We developed an HPLC method which has the sensitivity, ease and low cost required. In addition, we evaluated ClO_3^- as a trapping agent for NO_3^- .

This report presents results estimating NO_3^- influx, efflux, and net uptake across several mechanisms of NO_3^- uptake.

MATERIALS AND METHODS

Plant growth. Wheat seedlings were grown hydroponically in a 1/4 strength Hoagland's solution for 8 days in an environmentally controlled growth chamber at $400 \mu\text{E}/\text{m}^2\text{sec}$, at 18°C , and 80% relative humidity (13,14). On the 7th day they were transferred into 1/4 strength Hoagland's (loading solution) containing NO_3^- at the concentration to be used in the efflux study (specified in each experiment).

Measurement of NO_3^- efflux. After removal from the loading solution, 10 seedlings were placed in 300 ml of the efflux solution for various periods of time (specified below) containing 0.06 mM Pi at pH 5.8, 0.2 mM CaSO_4 , and with or without ClO_3^- at the specified concentrations. The seedlings were rinsed

for 2 sec in 300 ml of efflux solution, then placed in 60 ml of efflux solutions for the following times: 10 sec, 30 sec, 1, 2, 5, 10, 15, and 20 min and the amount of NO_3^- released at each time from the roots was determined.

Measurement of NO_3^- and ClO_3^- . These compounds were measured by HPLC as described previously for NO_3^- (13,14). ClO_3^- was also measured at 210 nm with a UV monitor.

Uptake rates of NO_3^- and ClO_3^- . Uptake rates were determined as previously described by determining rates of depletion of NO_3^- from substrate solutions, then fitting the rate curves to best fit curves by polynomial analysis using a computer (13).

RESULTS

Mechanisms of NO_3^- uptake. The results in Figures 1 and 2 show several mechanisms for NO_3^- uptake. Uptake as a function of NO_3^- concentration can be determined by either step up or step down, on continuous depletion experiments; Figures 1 and 2 are the results of continuous depletion experiments. In Figure 1, one mechanism is readily seen between about 0.2 and 0.7 mM. This is commonly referred to as Mechanism I in the literature (14). The rates above 0.7 mM are largely undefined but are referred to in the literature as Mechanism II. Another mechanism is indicated in Figure 1 at concentrations below 0.1 mM and it is readily seen when the data are plotted between 0 and 0.1 mM (Fig. 2).

Comparison of uptake of NO_3^- and ClO_3^- . The comparative uptake of NO_3^- and ClO_3^- is shown in Figures 3 and 4 at initial concentrations of 0.5 and 1 mM. Wheat plants deplete the NO_3^- concentration very efficiently to near zero (Fig. 1), whereas depletion of ClO_3^- is not straight forward. The ability to take up ClO_3^- is continuously lost with time (Fig. 4).

Toxic effects of ClO_3^- on NO_3^- uptake. The increasingly toxic effects of ClO_3^- on NO_3^- uptake with time is seen in Table 1.

Comparative effects of pretreatments of ClO_3^- and NO_3^- on their uptakes.
The comparative effects of pretreatments of ClO_3^- and NO_3^- on their uptakes are shown in Table 2. Pretreatments varying in time had little effect on subsequent uptake of NO_3^- , whereas increasing time of pretreatments greatly decreased ClO_3^- uptake.

ClO_3^- as a competitive inhibitor of NO_3^- uptake. Double reciprocal plots of rates vs concentrations show evidence that ClO_3^- is a competitive inhibitor of NO_3^- uptake (Fig. 5).

NO_3^- efflux. Figure 6 shows a typical example of a determination of NO_3^- efflux. Our results matched quite closely those reported in the literature showing two different early losses of NO_3^- , one with a $t_{1/2}$ of less than 10 sec and another with a $t_{1/2}$ of minutes. After one min, the second set of rates approximate an apparent first order reaction. Extrapolation to t_0 gives an estimate of the rate of NO_3^- efflux. At a concentration of 1 mM NO_3^- , efflux varied consistently between 2.0 and 2.5 $\mu\text{mol/gxh}$.

Effect of increasing concentrations of NO_3^- and ClO_3^- on NO_3^- efflux. Efflux in the presence of increasing concentrations of NO_3^- and ClO_3^- is shown in Figure 7. As expected, efflux increased with increasing concentrations of NO_3^- and ClO_3^- .

Influx, efflux, and net uptake of NO_3^- . Comparative rates of the three kinetic components of NO_3^- absorption are shown in Table 3. Efflux and influx greatly increased between 0.2 and 10 mM external NO_3^- , whereas net uptake remained about the same. Efflux/influx increased from 15 to 45% with increasing concentration of NO_3^- .

Effect of light and dark on influx, efflux, and net uptake of NO_3^- . NO_3^- efflux was similar in plants in darkness and in light; however, influx and net uptake were much greater in light (Table 4). Thus, in darkness 35% of the influxed NO_3^- was effluxed, while in light the proportion effluxed was reduced to 14%.

DISCUSSION

It is important to continue to develop information concerning the mechanisms of uptake of nutrient ions both to understand the reactions of the plants to changing concentrations and also for planning optimum concentrations of nutrient solutions for maximum efficiency.

Mechanisms of NO_3^- uptake. At least three mechanisms of NO_3^- uptake are present, one between 0 and about 0.05 to 0.08 mM with a K_m of ca 0.012 to 0.018 mM, and another between 0.1 and about 0.7 mM with a k_m of ca 0.025 to 0.04 mM (Figs. 1 and 2). The latter is the typical mechanism reported in the literature (14). At concentrations above 1 mM, the mechanisms are largely undefined and are difficult to determine because efflux becomes such an important component (discussed below).

Comparison of uptake of NO_3^- and ClO_3^- . In a determination of NO_3^- efflux, very low concentrations of NO_3^- are present in the external solution. At these low levels of NO_3^- (see Fig. 3), the wheat plant can very efficiently absorb the NO_3^- as it is effluxed into the external solution. Hence, ClO_3^- has been used as a trapping agent for the effluxed NO_3^- .

Although much work has been reported on the effects of ClO_3^- on NO_3^- uptake, the analytical procedures reported to separate ClO_3^- from NO_3^- in the solutions were in some cases non specific, i.e., ion electrodes (3). Radioactive ClO_3^- was also used (3) which presents problems of low specific activity and sticking to glassware. We developed an HPLC method which effectively separates NO_3^- from ClO_3^- and both can be measured simultaneously. In addition, much of the reported literature did not discriminate between kinetic effects and toxic effects of ClO_3^- on NO_3^- uptake (15).

Toxic effects of ClO_3^- . The results showed that toxicity symptoms, as shown by decreased rates of uptake of NO_3^- and ClO_3^- , were apparent after 1 h (Fig. 4, Tables 1 and 2). Toxic effects of ClO_3^- were greater toward its own

uptake than towards NO_3^- uptake. We found that little toxicity occurred during the 20 min period of the efflux experiments.

ClO_3^- as a competitive inhibitor of NO_3^- uptake. The results verified that ClO_3^- was a competitive inhibitor of NO_3^- uptake as earlier reported (3) (Fig. 5). Apparently the NO_3^- transporter discriminated effectively between NO_3^- and ClO_3^- since proportionately much larger concentrations of ClO_3^- were required to inhibit NO_3^- uptake. In summary, ClO_3^- could be used as a trapping agent for effluxed NO_3^- for short time experiments.

NO_3^- efflux. The method for determining efflux was based on some excellent work done by Lee and Clarkson (6) and Shone and Flood (16) who used cereal roots to determine the kinetic parameters required to measure efflux of NO_3^- . They found that the combined $t_{1/2}$ of release of NO_3^- in the surface film attached to the roots from the external solution and from the root free space was ca 7 sec. In addition, their results showed that the $t_{1/2}$ for cytoplasmic release of NO_3^- was ca 4 min. A semilog plot of efflux rate vs time resulted in a linear regression line after 1 min, since at that time the first two kinetic parameters had passed through ca 9 half-lives. In our results, the relative contributions of these parameters would be very small in relation to the amount of NO_3^- efflux. The rates of efflux we measured are similar to those reported for cereals by workers using $^{13}\text{N}\text{-NO}_3^-$ at NO_3^- concentrations of 1.5 to 5 mM (3,4,6).

Effect of increasing concentrations NO_3^- and ClO_3^- on NO_3^- efflux. As expected, efflux increased with increasing concentrations of NO_3^- (Fig. 7). In the region of Mechanism II of uptake (0.2 mM), efflux was a significant deterrent to N use efficiency. Here 15% of the NO_3^- influxed was effluxed. Net NO_3^- uptake remained quite constant as external NO_3^- increased beyond 0.2 mM. This occurred because, although influx increased, efflux correspondingly increased. The ratio of efflux/influx, expressed as a percentage, increased up to 45% at 10 mM NO_3^- . These results help explain results from Raper's

laboratory which showed that net uptake changed little as NO_3^- concentrations increased to high levels (11). These workers also presented evidence for significant levels of efflux at increasing concentrations of NO_3^- .

Effect of light and dark on influx, efflux, and net uptake of NO_3^- . Since efflux is a function of the concentration of NO_3^- in the cytoplasm, light and dark periods had a profound effect on the ratio of efflux/influx (Table 4). Although efflux was the same in light or dark, influx was greatly increased in light along with the rate of NO_3^- reduction (17,18). Therefore, the relative percentage of NO_3^- effluxed is much less in light.

Effect of increasing concentrations ClO_3^- on NO_3^- efflux. Higher rates of NO_3^- efflux were detected as ClO_3^- increased in the efflux solution (Fig. 7). This could be because of ClO_3^- serving as a trapping agent or because of an unknown effect. The increase in efflux rate with 5 and 10 mM ClO_3^- was sufficiently large to be somewhat uncertain. We are currently evaluating this result.

Effect of NH_4^+ on NO_3^- efflux. The literature is confusing on this issue with results of little if any effect of NH_4^+ on NO_3^- efflux (5,6), and of large effect (4). We have not yet examined the interactions of the different N species on NO_3^- efflux.

Ramifications. As the concentration of NO_3^- increases in the external solution, efflux becomes an increasingly important energy cost to the plant. Not only is energy needed for NO_3^- influx, it now appears that NO_3^- efflux may utilize ATP (12). This results in an almost doubling of the cost of absorption at higher concentrations of NO_3^- . On this basis, it would seem that a nutrient solution for crop growth in CELSS should be optimized at the lowest concentrations possible. In addition, it is increasingly critical to determine the regulation of efflux of N compounds since it is not known how NO_3^- , NH_4^+ , NO_2^- , and urea influence each others efflux. Will total efflux

decrease if the total concentration of N in the nutrient solution is made up of the four species instead of only NO_3^- and NH_4^+ ?

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Table 1. Effect of pretreatment of ClO_3^- on NO_3^- uptake. The initial concentration of substrate NO_3^- was 0.6 mM.

Hours Pre-treatment	[ClO_3^-]	
	1 mM	5 mM
0	7.3	7.3
1	6.2	5.3
2	5.3	3.0
3	4.9	2.3

Table 2. Effect of pretreatment of ClO_3^- or NO_3^- on their uptakes.

Hours Pre-treatment	Pretreatment (1 mM)	
	NO_3^-	ClO_3^-
	Uptake (at 1 mM) ($\mu\text{mol/gxh}$)	
0	6.3	3.5
3		1.1
5	6.8	0.5

Table 3. Efflux, net uptake and influx of NO_3^- . See Materials and Methods for procedures.

EFFLUX, INFLUX, AND NET UPTAKE OF NO_3^-				
	Efflux	Net Uptake	Influx	Efflux Influx
$[\text{NO}_3^-]$ (mM)		$\mu\text{mol/gxh}$		%
0.2	1.1	6.5	7.6	15
1.0	2.5	8.5	11.0	23
10.0	7.7	9.5	17.2	45

Table 4. Effect of light and dark treatments (24 h) on NO_3^- efflux, net uptake and influx. See Materials and Methods for procedures.

Treatment	Efflux	Net Uptake	Influx	Efflux
	(μmol/gxh)			‡
Light*	1.3	7.9	9.2	14
Dark	1.0	1.8	2.8	35

*plants induced in 0.2 mM NO_3^- for 24 h light or dark.

FIGURE LEGENDS

Fig. 1. NO_3^- uptake from 0 to 1000 μm . See Materials and Methods for procedures.

Fig. 2. NO_3^- uptake from 0 to 100 μm . See Materials and Methods for procedures.

Fig. 3. Depletion of NO_3^- from 0.5 and 1.0 mM substrate solutions. See Materials and Methods for procedures.

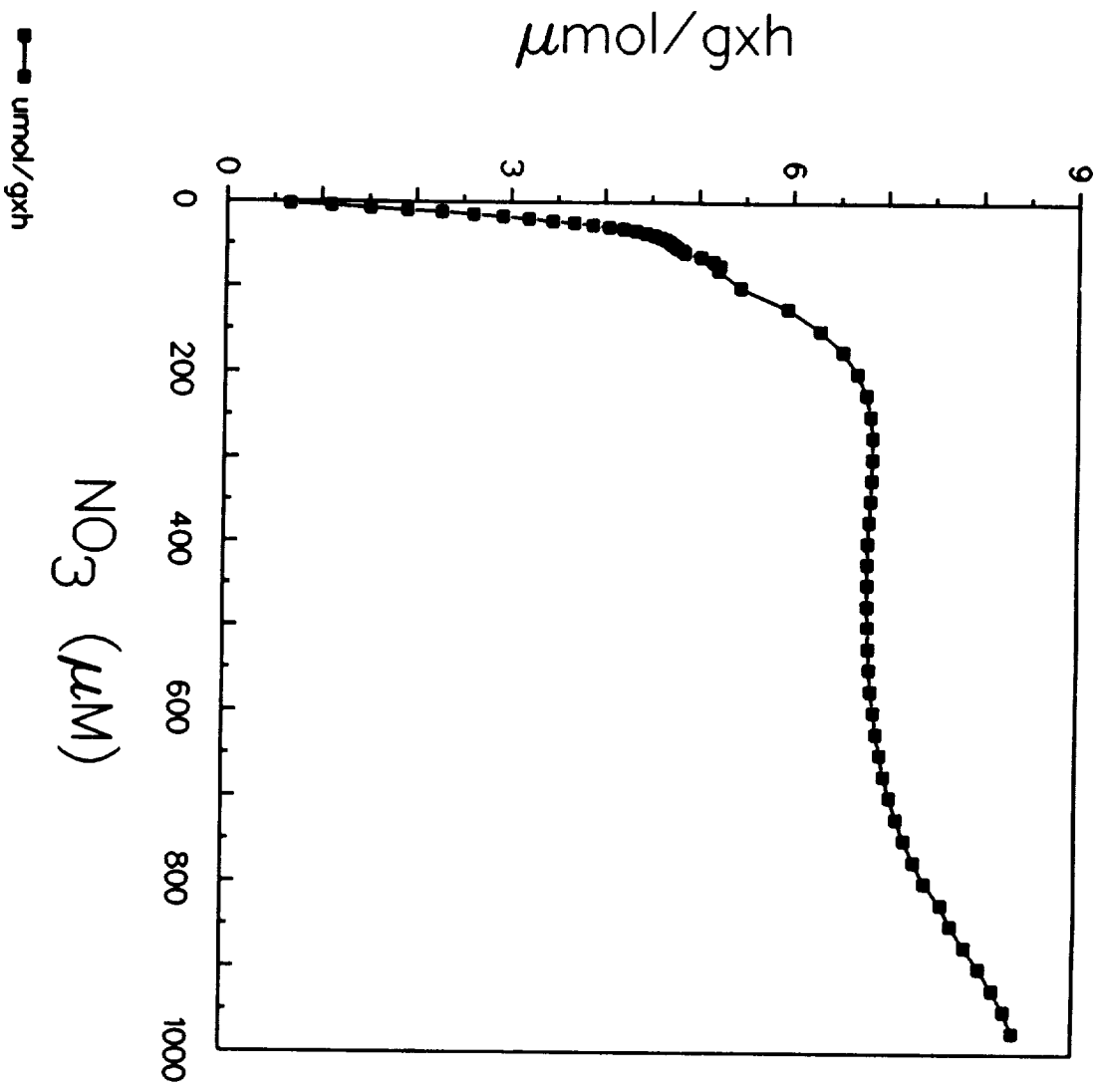
Fig. 4. Depletion of ClO_3^- from 0.5 and 1.0 mM substrate solutions. See Materials and Methods for procedures.

Fig. 5. ClO_3^- as a competitive inhibitor of NO_3^- uptake. See Materials and Methods for procedures.

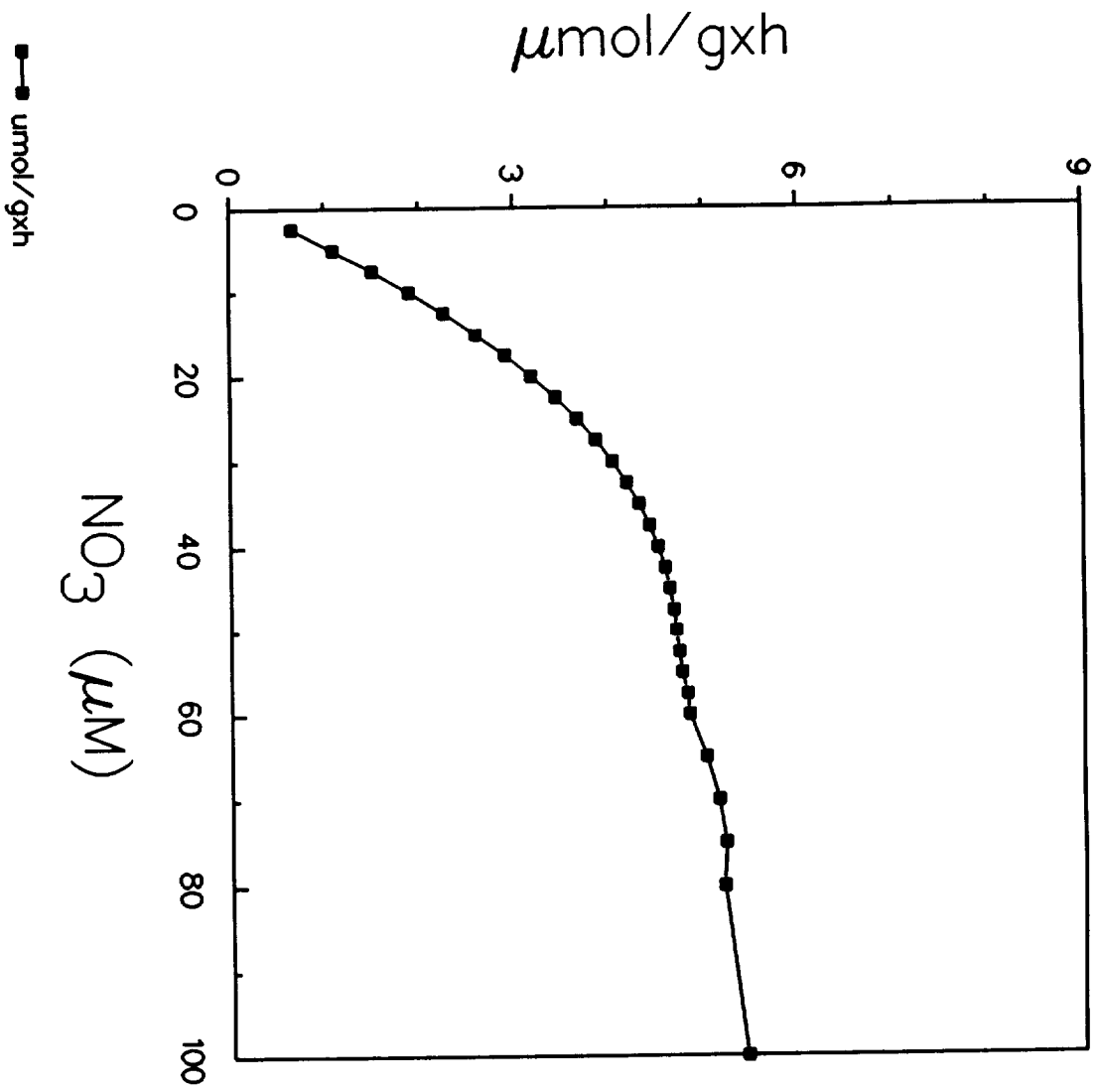
Fig. 6. Semilog plot of NO_3^- efflux vs time. See Materials and Methods for procedures.

Fig. 7. Effect of ClO_3^- on NO_3^- efflux. See Materials and Methods for procedures.

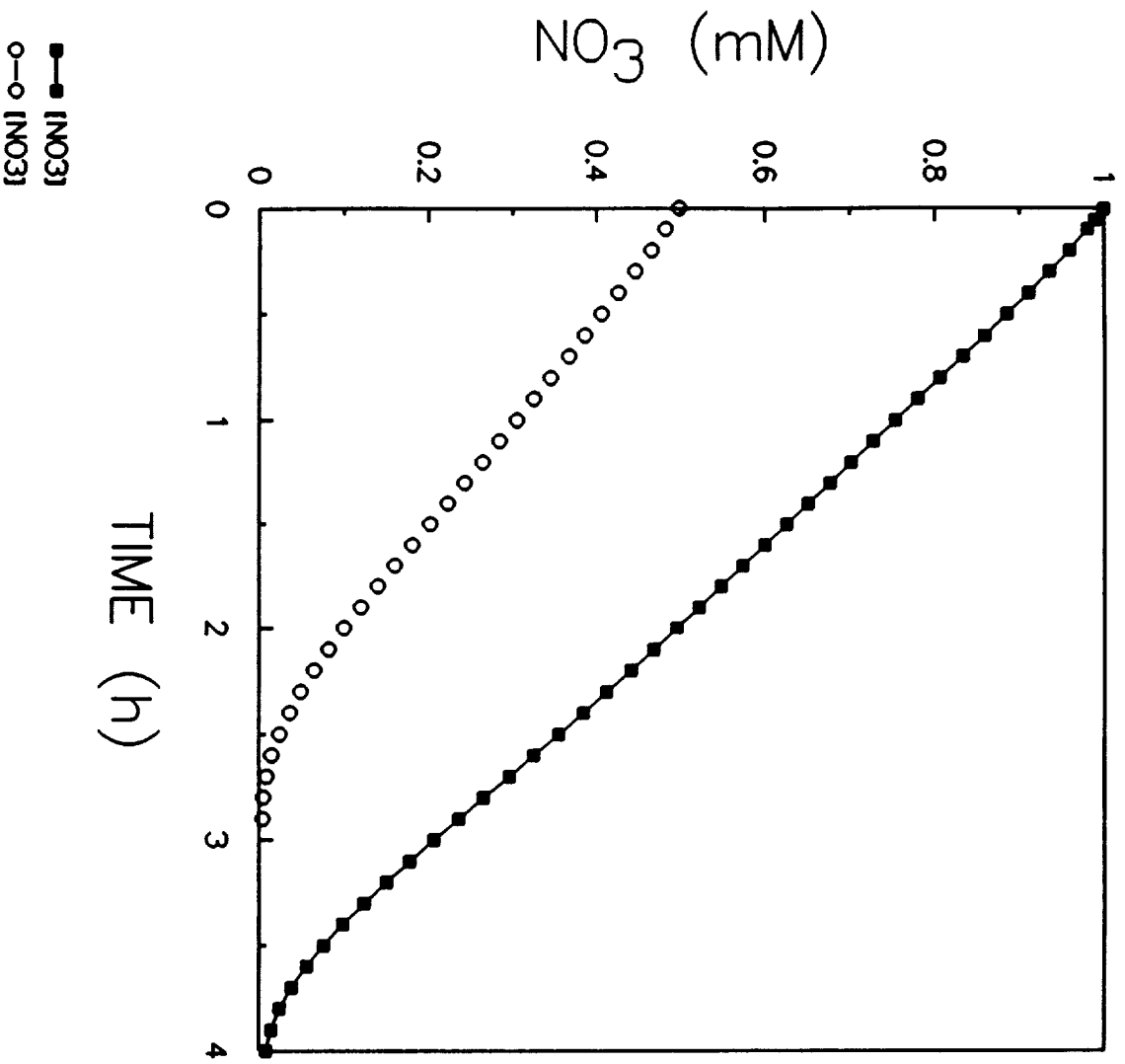
NO₃ UPTAKE



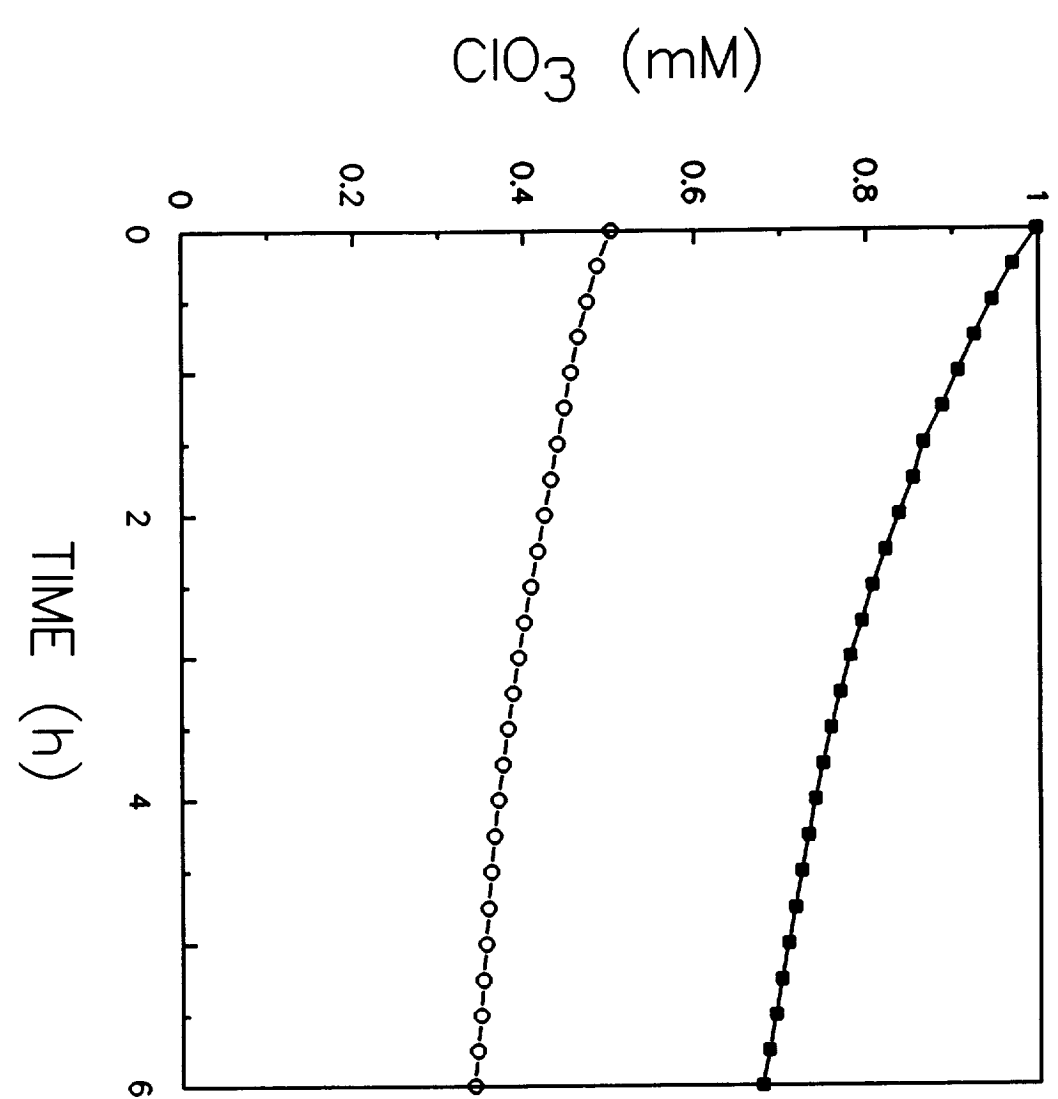
NO₃ UPTAKE



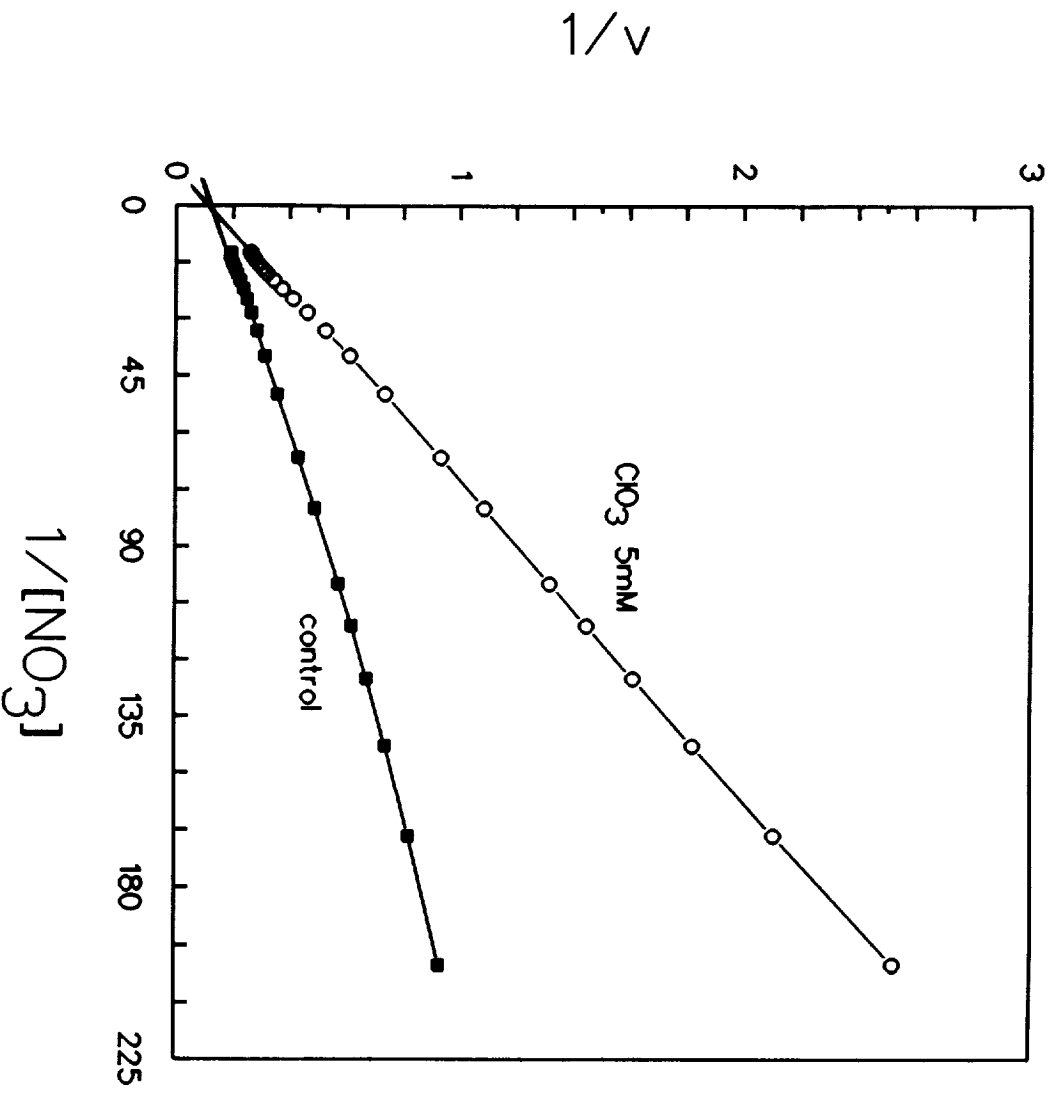
NO₃ DEPLETION

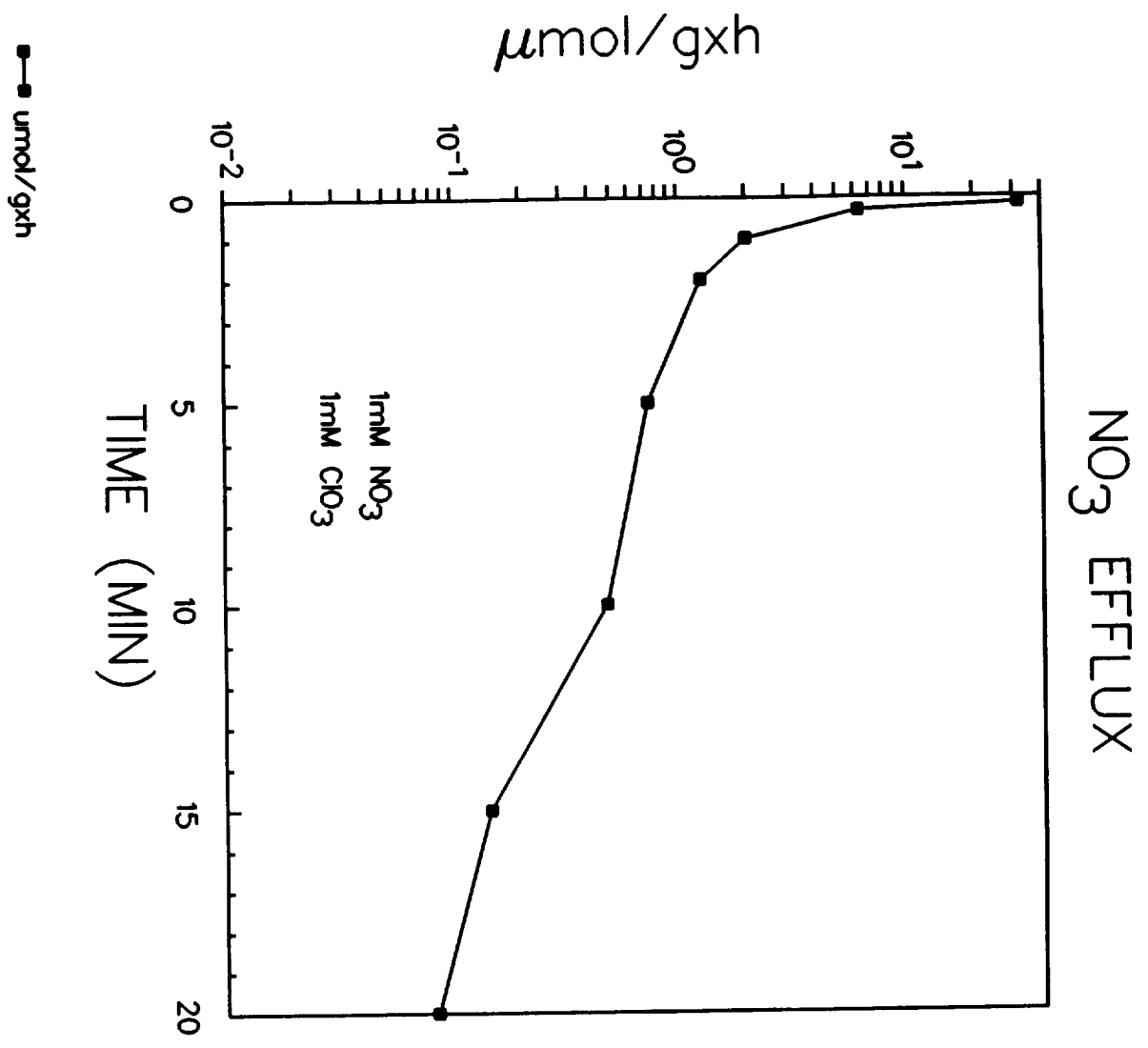


ClO₃ DEPLETION

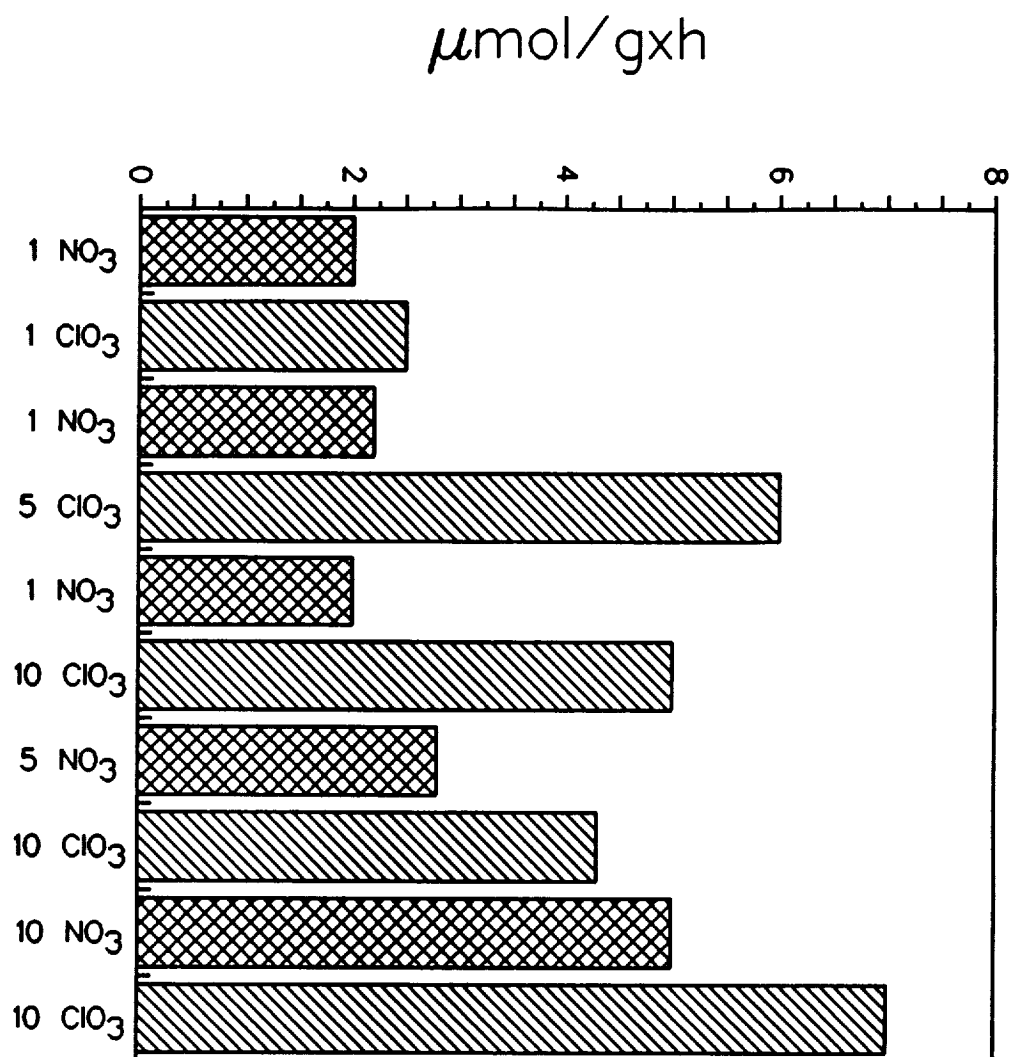


NO₃ UPTAKE





▨ EFFLUX
▩ EFFLUX



ClO₃ ON NO₃ EFFLUX

ENVIRONMENTAL AND CULTURAL CONSIDERATIONS
FOR GROWTH OF POTATOES IN CELSS

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The white potato (Solanum tuberosum) is being evaluated for use in CELSS because of its high ratio of edible to inedible biomass (80%) and highly nutritious tubers that consist of readily digestible carbohydrates (82%) and proteins (11%) (1). This edible portion can be easily prepared in many different ways to provide a wide variety to meals. The research with potatoes at Wisconsin has been underway for four years and directed toward both determining the range of yield response under different environmental conditions and establishing procedures for growing the crop effectively in space bases.

A major effort has been directed toward finding the combination of environmental conditions that maximizes the tuber production per unit area and unit time. The conditions, as shown in Figure 1, indicate the levels derived from the experiments conducted to this date that will produce the highest yield. This has been obtained using the Denali cultivar that was developed under long days in Alaska. The study of environmental conditions has been undertaken in 19 l or 38 l plastic containers filled with peat-vermiculite (50/50 v/v) and watered automatically 4 times daily with a balanced nutrient solution.

Temperature: The temperature of 16 C was derived from experiments undertaken at temperatures from 12 to 28 C as shown in Table 1. These data was taken from studies with Russet Burbank cv, grown for a period of 56 days at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux (PPF) with continuous irradiation and 70% RH (3). Tuber production was maximum at 16 C with shoot production

maximum at 24 C. Study has been made of the advantages of alternating temperatures over the 24 h period with cvs grown with $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 70% RH. It has been found that there is no advantage from an alternation of temperatures when the irradiation is continuous. A temperature change between 22 and 14 C after each 12 h period produced the same tuber weight as a constant 18 C temperature (Table 2). However that same temperature change with plants grown under 12 h irradiance, did produce a significant yield gain with Denali cv although only a small gain for Norland cv (Table 2). Of significant interest is that the alternation of temperature under the continuous light did essentially eliminate the continuous light injury that had been evident on certain cvs as Kennebec and Superior (4). This response is shown in the total plant weight data of Table 3. Effort has also been directed toward screening cultivars for effective tuberization under elevated temperatures. The 24 cultivars shown in Table 4 were grown for 56 days at 26 C under 70% RH and 12 h irradiance at $700 \mu\text{mol m}^{-2}\text{s}^{-1}$. Superior, Rutt, Troll, Haig, and Bake King cvs exhibited the highest number of initiated tubers and greatest tuber enlargement with no apparent plant stunting or chlorosis.

Irradiance: An irradiance level of $600 \mu\text{mol m}^{-2}\text{s}^{-1}$ with continuous irradiance is indicated in Figure 1, for research indicates that tuber production will not be increased significantly with higher levels or irradiance. A comparison of tuber production at 400 and $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ with both 12 h and 24 h of irradiance is shown in Table 5. Denali cv was grown at 18 C and 70% RH for this study. Extending the photoperiod and increasing photon levels increased tuber yield. Note that plants at $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ for 24 h produced the same yield as plants grown at $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ for 12 h. These two treatments had the same total irradiance in each 24 hours. In contrast however, there as a significantly less shoot growth with the 12 h irradiance thus indicating that there was greater allocation of

photosynthates to tubers with the 12 h photoperiod. The yield gain with plants grown with 800 μmol for 24 h over plants grown with 400 μmol for 24 h varied with cultivar. Denali had a large increase in tuber weight but Norland and Russet Burbank had little or no increase (Table 6).

Study was made of the effect of light duration changes during a 16 week growing period as depicted in Figure 2, using both Denali and Norland cvs. Plants were grown at 16 C and 70% RH. Tuber production was not increased with any change of 12 h to 24 h or 24 h to 12 h (for four week periods) compared to the production obtained with continuous 24 h irradiation. However, the data in this figure does show that, when changes were made, the tuber production was greater if the early growth was under 12 h irradiance than if early growth was under 24 h irradiance.

Studies undertaken under low earth orbital light dark periods of 60 min light and 30 min dark were compared to plants grown under 16 h light and 8 h dark. Plants were grown at 18 C and 70% RH for 8 weeks. The tuber and total plant dry weight was reduced about 50% under the orbital light cycle (Table 7). Large reductions in stomatal opening and leaf photosynthetic rates were found in plants grown under the orbital light cycle (2).

Carbon Dioxide: Supplementation of the ambient carbon dioxide concentration to maintain 1000 $\mu\text{mol mol}^{-1}$ levels has increased tuber weight when potatoes have been grown under 12 h photoperiods but not when grown under continuous irradiation (Table 8) (5, 7). Potatoes, Denali cv, were grown at 16 C and 70% RH. The percentage gain in yield was greater with supplementation at 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$ than at 800 $\mu\text{mol m}^{-2}\text{s}^{-1}$ however a similar gain in total plant weight at both irradiance levels was obtained. Additional study should be made of supplementation at higher irradiance levels with 12 h photoperiods and at low irradiance levels at continuous irradiance. Study of supplementation at elevated humidity levels, indicated no added benefit of supplementa-

tion at 80% as compared to 50% relative humidity level. Screening of the cultivars listed in Table 4 with elevated carbon dioxide, under high irradiance, using continuous irradiance at $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, has indicated that the cultivars, Desiree, Ottar, Haig, Denali, and Rutt have the highest capabilities for photosynthetic dry matter accumulation.

Humidity: Increasing the humidity level from 50 to 80% has produced greater than 50% increase in yield of tubers for Denali cv but less than 10% increase for Norland cv (Table 9) (6). Potatoes were grown at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ of continuous irradiation and 18 C temperature for 8 weeks. The total plant dry weight was similar under 50 and 80% RH, indicating that the elevated humidity encouraged a greater percentage allocation of photosynthates to the tubers. A similar response with increasing RH has been found with plants grown under 12 h irradiation periods.

Cultural Studies for Growth of Potatoes in Space Bases

Root Environment: Research with nutrient film procedures, with and without media, has demonstrated that tuber production can be obtained either without any, or with only a shallow layer of media. High production has been obtained without the deep soil layers utilized in field plantings. However it has been found that a shallow layer of media is apparently essential for high production of tubers, for without the media, tubers form under the root mat feeding the plant and lift the roots out of the nutrient solution and cause injury to the root system. This is avoided if a 1 or 2 cm layer of media is utilized. Baked montmorillinitic clay (arcillite) has been found to be particularly effective because the roots can be easily separated from the media and the material can withstand high temperature sterilization. The root compartment must be kept dark to avoid light contacting the tubers and encouraging the accumulation of toxic glycoalkaloids in the tubers. The root compartment must also be closed to maintain a near saturated environment so

that there is no significant evaporation from the surface of the exposed tubers. Evaporation of the film of nutrient solution on the surface of the tubers causes an accumulation of salts and produces an injurious burn on the enlarging tubers.

Plant Spacing: The production of potatoes, cv Denali, was studied at different spacings in plastic trays 54 cm wide, 83 cm long and 11 cm deep under an environment of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 16 C and 70% RH for 87 days. Wire fencing was placed around each tray to contain the shoots to the tray area. The trays were filled with peat vermiculite medium and planted with two, four or eight potato plants which provided .224, .112 and .056 m^2 , respectively, of growing area for each plant. The shoot and dry weight per unit area increased with decreasing space per plant (increasing number of plants per tray) as shown in Table 10. It is suspected that the increasing yield with close spacing was a result of the more rapid development of a solid canopy covering the surface of the tray area.

Root and Stolon Containment: The effect of root and stolon containment was studied in trays constructed of 0.3 thick polyvinyl chloride sheeting that were 96 cm by 96 cm and 20 cm high. Two trays were constructed with dividers to partition the tray area into 9 separate compartments, each 32 cm by 32 cm. Two trays were left with no compartmentalization. The trays were filled with peat-vermiculite media. Nine potato plants (cv Denali) were planted in each tray with a single plant positioned in the center of each compartment or in similar locations in the open tray. An automatic watering system was installed with four drip tubes positioned beside each plant and nutrient solutions waterings were made to excess four times each day. Plants were grown with $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiation at 16 C and 70% RH for 59 days. No difference in tuber or total plant dry weight was found with the two types of trays.

Continuous Harvesting: Potatoes, cv Denali, were grown in the trays described in the spacing study but filled with only 2 cm of arcillite and covered with first a sheet of black plastic and then a sheet of white plastic. Two plants were grown in each tray at an irradiation of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF of 12 h duration, 16 C and 70% RH. When plants had been grown for 8 weeks, all tubers over ≈ 5 cm in diameter were removed from two trays by reaching under the cover with the room dark and separating the tubers from the stolons. This harvest was repeated at weekly intervals to the 16th week. The total weight of tubers harvested from trays over this 8 week period was 541 grams per plant compared to 572 grams per plant from trays that were undisturbed for 16 weeks. Thus no real difference in yield was evident. Tuber removal disturbed the source-sink balance in the plants as evidenced by the collapse of small areas of tissue on exposed leaflets within 24 h after the removal of the tubers at the first harvest.

Within-Canopy Lighting: Four light pipes from TRI Industries of Burnaby, B.C. have been obtained and installed between plants just above the container level. These pipes are fabricated with flexible prismatic film that promotes propagation of radiation along the length of the pipe. The pipes are 250 cm in length and 15 cm in diameter and constructed so that light is propagated down the pipe and emitted quite uniformly out of the two sides of the pipe. Each pipe is fitted with a 250 W metal halide lamp at each end. The construction and uniformity in irradiation from the pipes is shown in Figure 3. Irradiation was elevated about 40% over the first 40 cm and then was quite uniform over the center area of the pipes. The lamps have been installed in a controlled environment room, 18 inches apart and just above the level of the plant pots. Three rows of potato plants, 6 in each row, are being grown between the rows of pipes. The irradiation from overhead CWF lamps is $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF and maintained continuously along with the continuous lighting from the pipes. The room is maintained at 18 C and 70%

RH. Potato growth has been very vigorous, with no evidence of leaf epinasty. The pipes have a cool surface temperature so that leaves are not stressed by longwave radiation and leaves commonly abut on the pipe. It was hoped that the increased irradiance within the canopy would decrease branching but this has not been found. Branching is very vigorous and appears similar to plants irradiated only from overhead lighting.

Research efforts in the coming year will be directed toward the following efforts.

a) Study will be made of the wavelengths controlling branching in the plant. It is suspected that the ultraviolet or infrared wavelengths may have controlling effects on branching. Reduction of branching has significant implications for use of potatoes to reduce the amount of space required and to insure maximum allocation of photosynthates to the tubers.

b) Extended investigations will be undertaken to establish the optimum nutrient concentrations and balance for potatoes being grown under different environmental conditions. Of particular concern will be study of the form of nitrogen, level of phosphorus, and balance between the different cations.

c) Continued study will be made with cultivars shown to have superior qualities in the screening tests described previously. Production studies, involving growth for 20 weeks, will be undertaken with Haig and Trogg cvs to determine their relative productivity compared to the previously studied cvs Denali, Norland, and Russet Burbank.

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Table 1. Effect of temperature on tuber and shoot dry weight of Russet Burbank cv grown for 56 days at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF of 24 h duration and 70% RH.

Temperature ($^{\circ}\text{C}$)	Tuber Dry weight per plant (gm)	Shoot Dry weight per plant (gm)
12	73 ± 3	50 ± 3
16	123 ± 9	86 ± 3
20	95 ± 19	91 ± 5
24	2 ± 2	150 ± 10
28	---	116 ± 3

Table 2. Effect of alternating temperature every 12 hours upon tuber dry weight of two potato cultivars and 12 hour irradiation at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 18°C , and 70% RH.

Cultivar	Irradiation duration (h)	Tuber dry weight (g per plant)	
		22/14 C (12 h/12 h)	18 C (continuous)
Denali	24	378 ± 74	351 ± 51
Norland	24	288 ± 114	282 ± 19
Denali	12	$405 \pm$	$263 \pm$
Norland	12	$332 \pm$	$309 \pm$

Table 3. Effect of alternating temperatures each 12 hours upon dry weight of four cultivars grown under continuous irradiance at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF and 0.6 kPa atmospheric moisture deficit.

Cultivar	Plant dry weight (g)	
	22/14 C (12 h/12 h)	18 C (continuous)
Denali	115.2 ± 7.1	133.5 ± 12.0
Norland	111.5 ± 7.5	117.1 ± 7.5
Kennebec	89.4 ± 6.4	7.5 ± 3.6
Superior	91.9 ± 8.1	12.9 ± 2.2

Table 4. Cultivars evaluated under a growing temperature of 26°C for capability to form tubers.

Cultivar	Country of origin	Cultivar	Country of origin
Alaska 114	US (Alaska)	New York 81	US
Alpha	Holland	Norland	US
Atlantic	US	North Dakota	US
Bake King	US	Ottar	Norway
Bintje	Holland	Russet Burbank	US
Denali	US (Alaska)	Rutt	Norway
Desiree	Holland	Snogg	Norway
Gualauge	Norway	Snow Chip	US (Alaska)
Haig	US	Spunta	Holland
Kennebec	US	Stately	US (Alaska)
La Rouge	US	Superior	US
New York 72	US	Troll	Norway

Table 5. Effect of irradiance level and duration on tuber and shoot weight of Denali cvs.

Photon Level ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Duration (h)	Dry weight per plant (g)	
		Tubers	Shoots
400	12	253 \pm 19	97 \pm 7
800	12	334 \pm 11	117 \pm 8
400	24	334 \pm 45	217 \pm 12
800	24	488 \pm 51	185 \pm 14

Table 6. Effect of photon level on tuber weight of different cultivars grown under continuous irradiation.

Photon level* ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Tuber dry weight per plant (g)		
	Denali	Norland	R. Burbank
400	334 \pm 45	304 \pm 41	339 \pm 91
800	488 \pm 51	317 \pm 32	311 \pm 78

*24 h duration

Table 7. Comparison of potato growth with low earth orbital light:dark cycles of 60:30 minutes to growth with 16:8 hour light:dark cycle.

Duration		Dry weight per plant (g)	
Light	Dark	Tubers	Total Plant
60 min	30 min	168.8 ± 7.6	529.0 ± 32.7
16 hrs	8 hrs	373.9 ± 57.2	1030.0 ± 125.8

Table 8. Effect of elevated carbon dioxide level in combination with different irradiance amounts on weight of potato plots.*

Photon level ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Duration (h)	Carbon dioxide level ($\mu\text{mol mol}^{-1}$)	
		<u>350</u>	<u>1000</u>
		(g dry weight per plant)	
400	12	253 ± 19	363 ± 53
800	12	334 ± 11	456 ± 46
400	24	334 ± 45	366 ± 40
800	24	488 ± 51	469 ± 44

*cv Denali

Table 9. Effect of humidity level on tuber production of two different cultivars of potatoes.

Humidity level (% RH)	Tuber dry weight per plant (g)	
	Denali	Norland
50	94 ± 13	209 ± 33
80	156 ± 36	227 ± 29

Table 10. Effect of plant spacing on tuber and shoot weight per unit area.

Area per plant (m ²)	Tuber dry weight (g m ⁻²)	Shoot dry weight (g m ⁻²)
.224	907 ±	830 ±
.112	1010 ±	882 ±
.056	1285 ±	952 ±

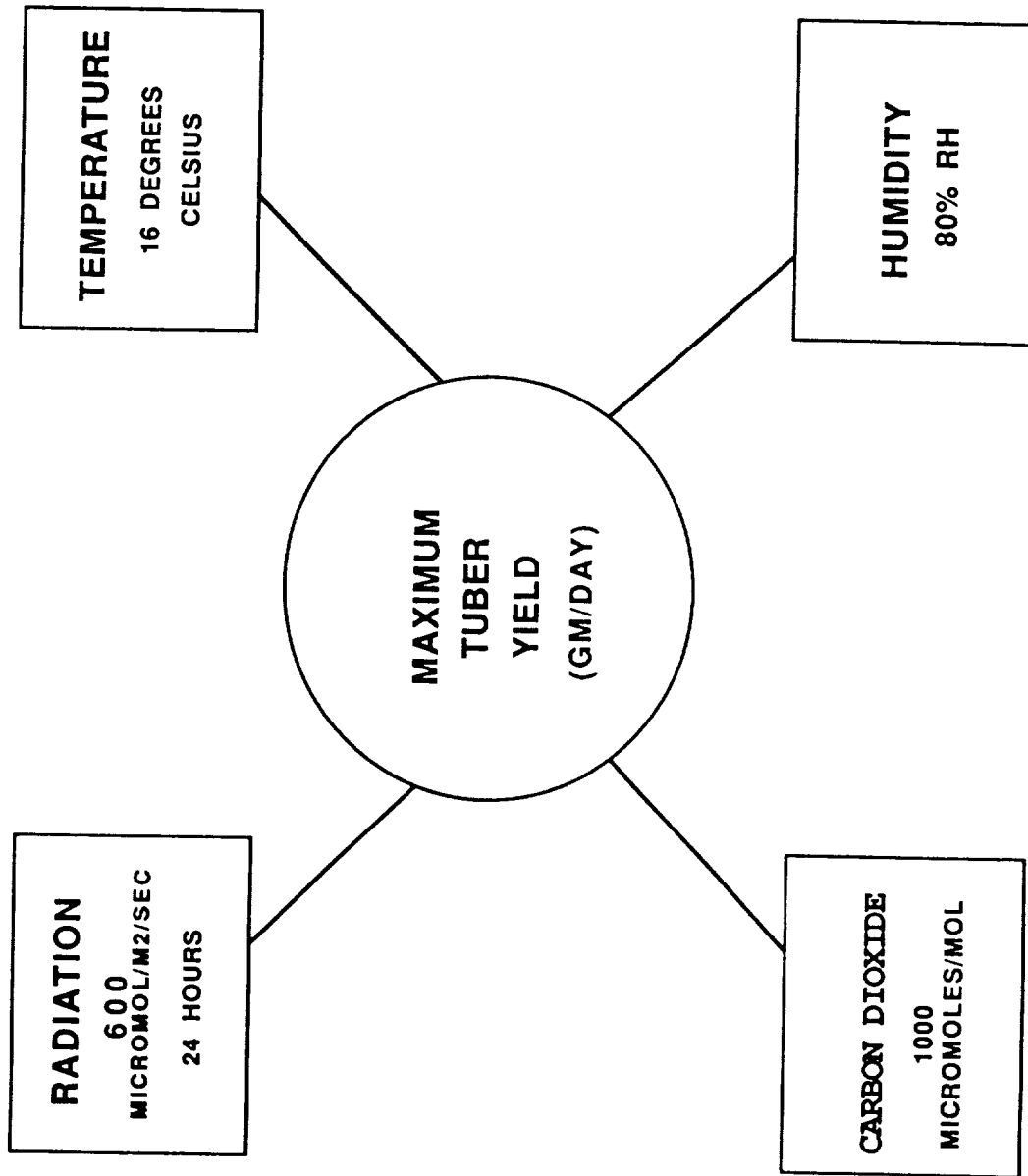


Figure 1. Conditions established for maximum tuber production per unit area and unit time.

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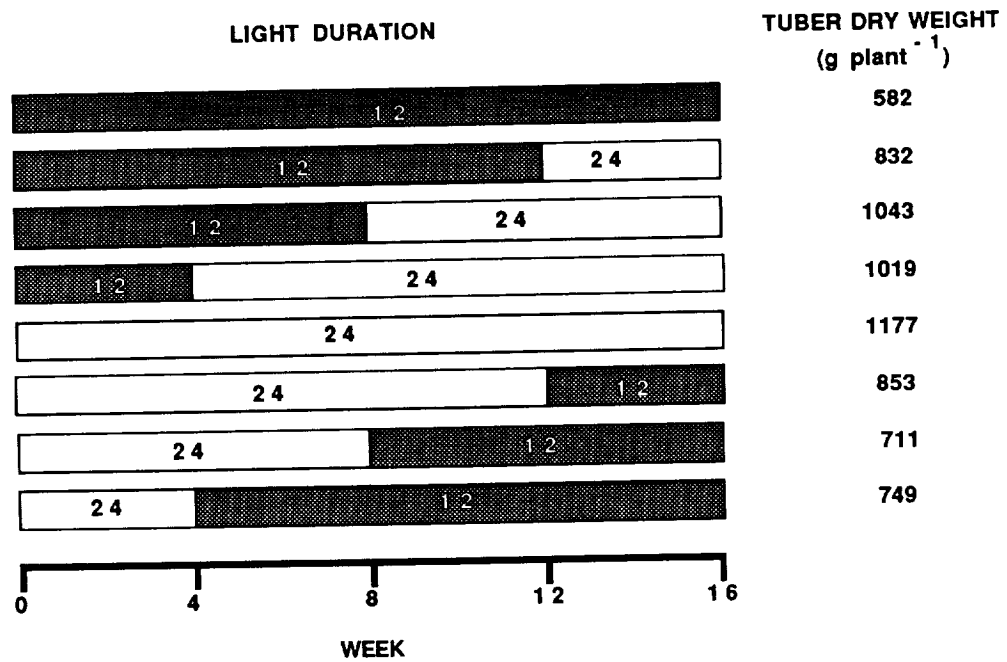


Figure 2. Effect of light duration changes at different times during a 16 week growth period on yield of potato tubers.

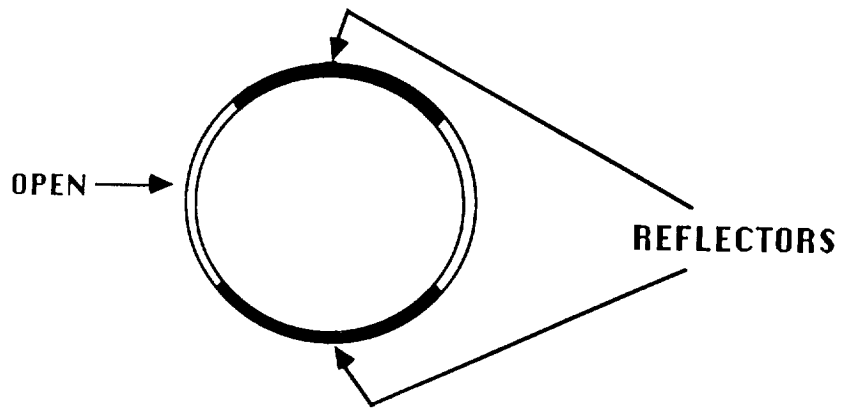
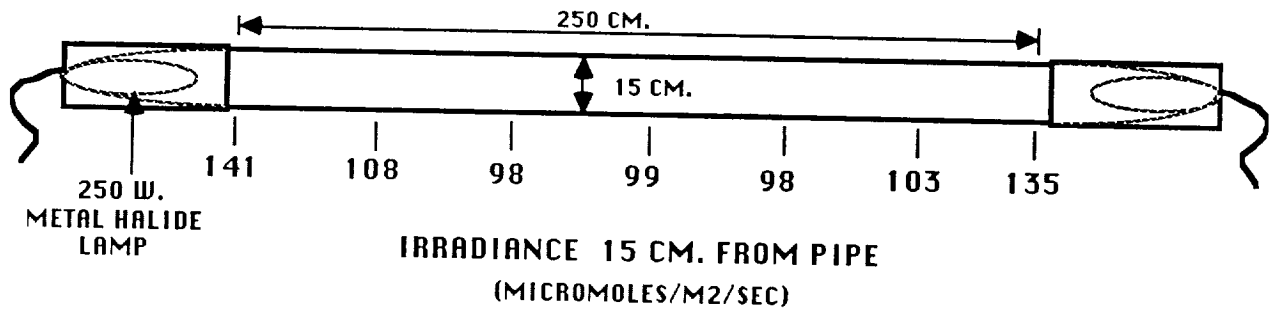


Figure 3. Distribution of irradiance from light pipes utilized for within canopy irradiance.

EFFECTS OF ATMOSPHERIC CO₂ ON PHOTOSYNTHETIC
CHARACTERISTICS OF SOYBEAN LEAVES

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ABSTRACT

Soybean (Glycine max cv. McCall) plants were grown at 500, 1000, and 2000 $\mu\text{mol mol}^{-1}$ CO₂ for 35 days and a photosynthetic photon flux (PPF) of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Individual leaves were then exposed to step changes of CO₂ concentration and PPF to study CO₂ assimilation rates (CAR), i.e., leaf net photosynthesis. In general CAR increased when CO₂ increased from 500 to 1000 $\mu\text{mol mol}^{-1}$, but not from 1000 to 2000 $\mu\text{mol mol}^{-1}$. Regardless of the previous CO₂ level, all leaves showed similar CAR at similar CO₂ and PPF. This observation contrasts with reports that plants tend to become "lazy" at elevated CO₂ levels over time. Although leaf stomatal conductance (to water vapor) showed diurnal rhythms entrained to the photoperiod, leaf CAR did not show these rhythms and remained constant across the light period, indicating that stomatal conductance had little effect on CAR. Such measurements suggest that short-term changes in CO₂ exchange dynamics for a Controlled Ecological Life Support System (CELSS) can be closely predicted for an actively growing soybean crop.

INTRODUCTION

Soybean (Glycine max L.) is among the candidate crops currently under study for use in a Controlled Ecological Life Support System (CELSS; 1) and is tentatively scheduled for testing in the Biomass Production Chamber (BPC) at Kennedy Space Center in 1990. Preliminary tests with soybeans are currently underway in growth chambers at Kennedy Space Center in preparation for BPC studies. A major focus of these studies has been the effects of elevated CO₂ on plant development and biomass production. During all of these tests, leaf gas exchange (i.e. photosynthesis and transpiration) measurements were taken from plants grown under the different atmospheric CO₂ levels. In addition, the leaves were exposed to temporary changes in

irradiance and atmospheric CO₂ to determine whether the growing environment had any effects on inherent photosynthetic capabilities of the leaves. The results should provide an indication of the effects of transient changes in either CO₂ or irradiance on the rate of CO₂ uptake by plants within a closed system. A set of follow-up measurements will be conducted during the BPC grow-outs to directly compare events at the leaf level with events at the plant canopy or community level.

METHODS AND MATERIALS

Soybean (Glycine max cv McCall) plants were grown in 0.25 m² plastic trays in a walk-in growth chamber using nutrient film technique and a complete nutrient solution (2). A photosynthetic photon flux (PPF) of $300 \pm 30 \text{ umol m}^{-2} \text{ s}^{-1}$ was provided by 30 VHO Vita Lite fluorescent lamps with a 12-hr light / 12-hr dark photoperiod. Temperatures were maintained at $26 \pm 0.5 \text{ C}$ during the light cycle and $20 \pm 0.5 \text{ C}$ during the dark; relative humidity was kept constant at $65\% \pm 5\%$. A series of three separate studies was conducted during which chamber CO₂ levels were maintained 500, 1000, and 2000 umol mol^{-1} (ppm) (set points held to within approximately $\pm 2\%$ full scale). Carbon dioxide levels were monitored and controlled using an infrared gas analyzer (Anarad, Santa Barbara, CA) with a dedicated computer control system. Analyzer zero and span points were taken automatically each day to update the regression used for CO₂ determination, while manual adjustments for instrument drift were made as necessary.

At 36 days after planting, single fully-expanded leaves at the top of the canopy were selected for gas exchange measurements. Carbon dioxide assimilation rates (CAR) of the leaves were determined using an LCA2 portable photosynthesis system with a PLC model B leaf chamber (ADC Co., Hoddesdon, England). The incoming gas stream to the cuvette was provided from a CO₂-enriched (3510 $\mu\text{mol mol}^{-1}$) compressed air supply. Different CO₂ concentrations were obtained from this air stream using an ADC GD600 gas diluter to selectively shunt portions of the flow through a soda lime column to remove CO₂. This system was used to provide gas supplies of 0, 255, 440, 695, 1040, 1290, 1480, and 2030 $\mu\text{mol mol}^{-1}$ CO₂. Higher levels were not used because of the inability to span the infrared analyzer unit beyond 2100 $\mu\text{mol mol}^{-1}$. Different PPF levels were obtained by using the existing fluorescent radiation with neutral (metal) screening for levels less than 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (63, 40, 28%), or with fluorescent plus supplemental radiation from a rheostat-controlled incandescent lamp with dichroic reflector and focused with a fiber optic guide. This supplemental radiation was filtered through a glass petri dish to reduce the long wave component. Cuvette temperatures could thus be kept within ± 0.3 C of the initial temperature. In addition to the radiation sensor on the ADC leaf cuvette unit, PPF levels were checked with a Li-Cor quantum sensor (Li-Cor Inc, Lincoln, NE).

Each single leaf was exposed to the entire set of CO₂ and PPF regimes, with a set of measurements lasting approximately 4 hours. This was done to expedite measurements during the middle of the photoperiod and to avoid leaf to leaf variability. This

approach risked disturbing the leaf (e.g. closing leaf stomata) from the physical contact and/or altered environment of the cuvette. To avoid drying the leaf, the air stream desiccant loop of the gas supply system was bypassed thereby keeping cuvette relative humidities between 60 and 80%. To determine whether the measurements were themselves having any disruptive effects, measurements at the ambient CO₂ and PPF levels were taken before, in the middle, and at the end of each set of measurements. In all cases, initial photosynthetic rates were consistently repeatable even after 4 hours, indicating minimal effects of the physical measurements on leaf photosynthetic rates.

Carbon assimilation rates were calculated as the difference between incoming and outgoing CO₂ concentrations (on a molar basis) multiplied by the air stream flow rate (approx. 300 ml min⁻¹) and divided by the leaf area (6.25 cm²) (3). No corrections were made for water interference in the readings.

RESULTS

Prior to testing leaf photosynthetic response to changing CO₂ and PPF levels, CAR was measured across the 12-hr photoperiod to determine whether any diurnal differences existed. As shown in Figs. 1 and 2, CAR measurements tended to remain constant across the light period, but stomatal conductance to water vapor showed a distinct diurnal rhythm, peaking prior to the middle of the light period and then decreasing with the onset of the dark period. Interestingly, changes in stomatal conductance had little effect on leaf photosynthetic rates (CAR) (Figs 1 and 2). However, to avoid any possible diurnal effects, all gas exchange data were taken within 2 hours of the middle of the photoperiod.

The effect of increasing CO₂ concentration at different PPF levels on leaf CAR for plants grown at 500 umol mol⁻¹ CO₂ is shown in Fig. 3. Leaf CAR at the lower PPF levels tended to plateau at relatively low CO₂ levels, i.e. PPF was limiting and the CO₂ response was saturated. But at a PPF of 510 or 840 umol m⁻² s⁻¹, no CO₂ saturation occurred, i.e. maximum rates were not achieved, even up to 1040 umol mol⁻¹ CO₂. Leaves from plants grown at 1000 umol mol⁻¹ CO₂ showed a similar trend of CO₂ saturation at lower PPF levels (Fig. 4). At a PPF of 510 or 840, CAR increased up to 1040 umol mol⁻¹ CO₂, but did not increase when CO₂ was increased to 1290 umol mol⁻¹. Leaves from plants grown at 2000 umol mol⁻¹ CO₂ also showed this trend, with peak CAR occurring at the highest PPF level near 1040 umol mol⁻¹ CO₂ (Fig 5); raising the CO₂ higher than 1040 had no positive effect and tended to decrease leaf photosynthetic rates. A comparison of data from leaves taken from plants grown at the different CO₂ levels indicates that CAR was similar for similar combinations of CO₂ and PPF (Fig. 6).

DISCUSSION

The results suggest that regardless of the CO₂ concentration in the "native" environment, transient changes in the atmospheric CO₂ and irradiance have similar effects on carbon assimilation rates of healthy soybean leaves (Fig. 6). This contrasts with findings from other species in which CO₂ enrichment tends to reduce photosynthetic capacity with time (4). But recent field studies with soybeans have shown that long-term CO₂ enrichment had no adverse effects, and even increased photosynthetic capacity (5). From a CELSS perspective, it is noteworthy that

the effects of transient changes on soybean CAR can be predicted independent of the crop's prior history. Thus, leaf systems may serve as useful models for testing transient changes in a closed life support module. However, this presumes that single-leaf gas exchange measurements closely reflect community gas exchange, which remains to be tested.

A comparison of CAR curves from Fig. 5 indicates that there is no advantage to raising the CO₂ much above 1000 umol mol⁻¹ and that levels greater than this (e.g. 2000 umol mol⁻¹) may be supraoptimal. The drop in photosynthetic rates by increasing CO₂ from 1000 to 2000 umol mol⁻¹ may be a result of some feedback inhibition, e.g. excessive starch accumulation in leaves (6,7). Aside from determining the optimum environment for photosynthesis, such data will be useful for the purposes of a CELSS, where plants may be subjected to transient changes in CO₂ levels, or levels much higher than have been traditionally studied (e.g. >1000 umol mol⁻¹).

Because the plants were all grown at a PPF of 300 umol m⁻² s⁻¹, we can only speculate on the effects that a native lighting environment might have on photosynthetic capacities. It is likely that the lighting history would affect leaves differently than the CO₂ history because of irradiance effects on leaf chlorophyll content and chloroplast structure (8). However results from this study did show that when CO₂ levels were 440 umol mol⁻¹ or greater, a PPF of 840 umol m⁻² s⁻¹ was still below the light saturation point for soybean leaves.

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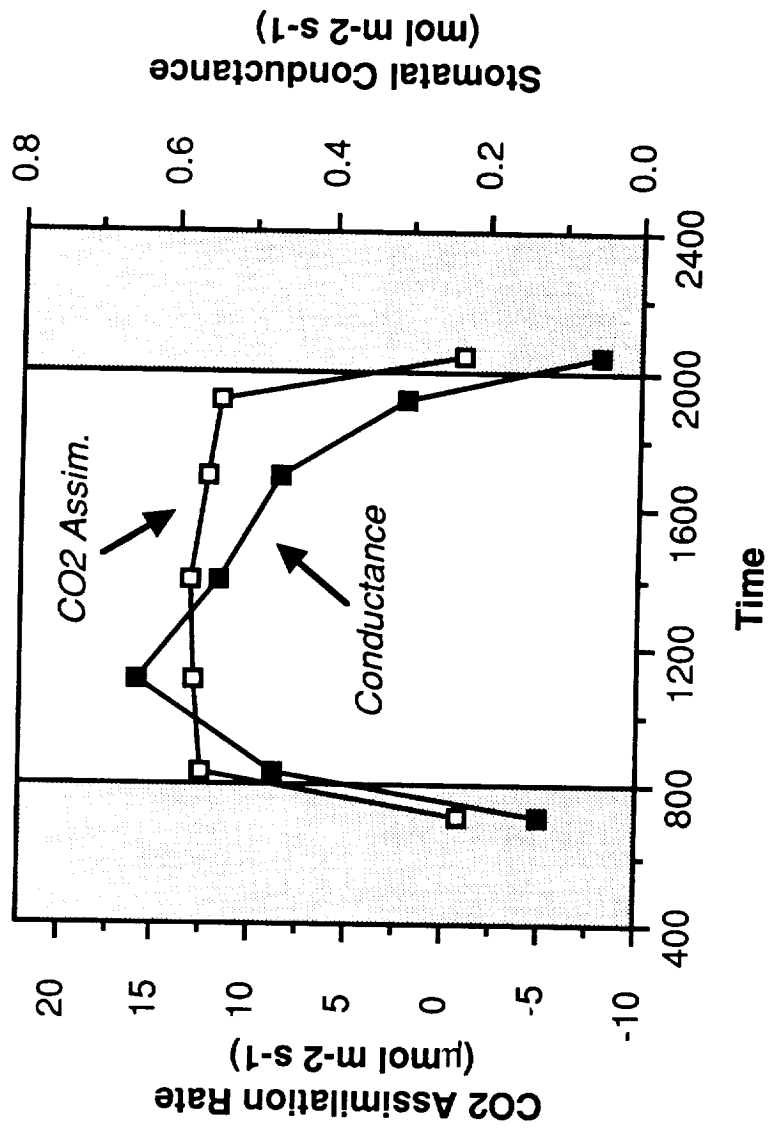


Figure 1. Diurnal trend of CO₂ assimilation (photosynthesis) rate and stomatal conductance of soybean leaves at 500 $\mu\text{mol mol}^{-1}$ CO₂. Photoperiod began at 0800 and ended at 2000 each day.

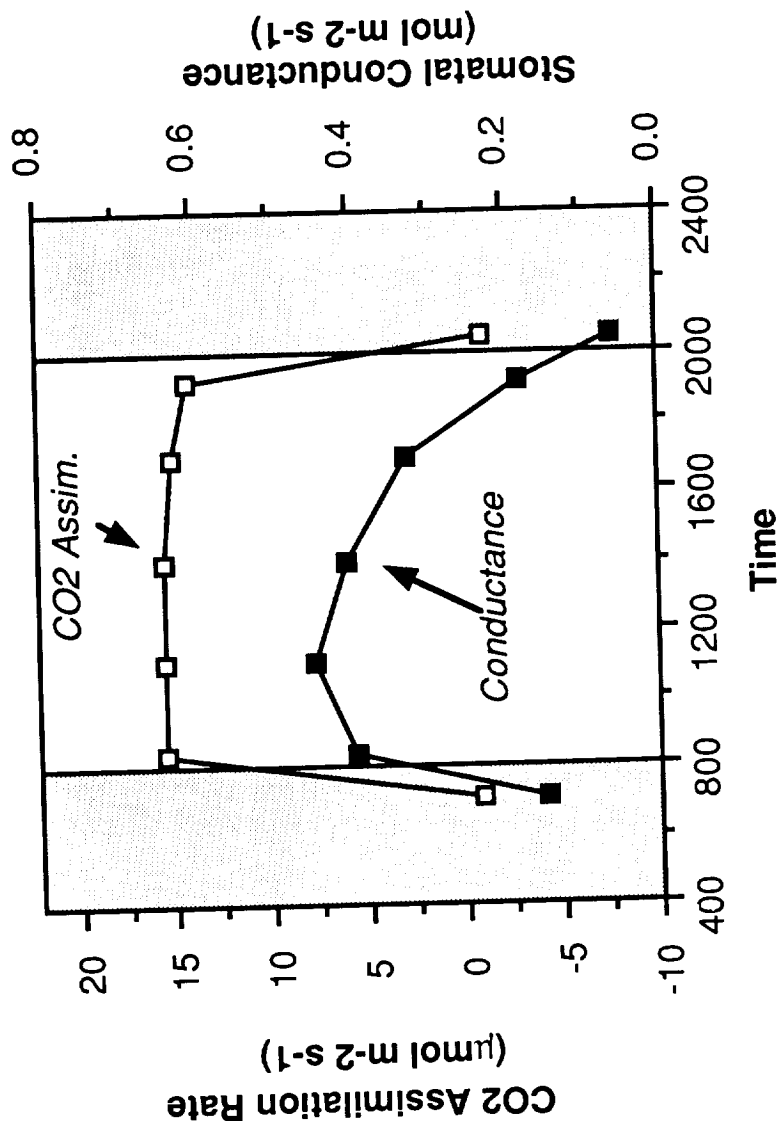


Figure 2. Diurnal trend of CO₂ assimilation (photosynthesis) rate and stomatal conductance of soybean leaves at 1000 $\mu\text{mol mol}^{-1}$ CO₂. Photoperiod began at 0800 and ended at 2000 each day.

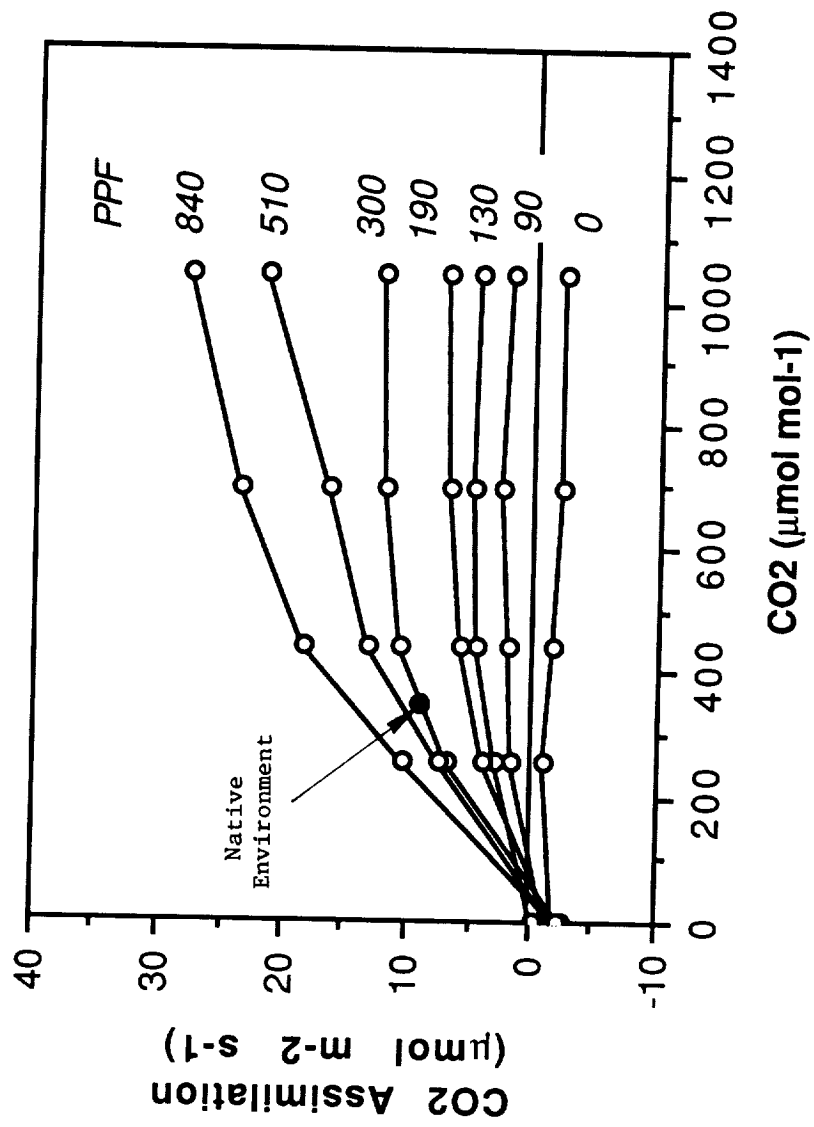


Figure 3. CO₂ assimilation (photosynthesis) rates of soybean leaves at different CO₂ and photosynthetic photon flux (PPF) levels. Plants were grown at 500 μmol mol⁻¹ CO₂.

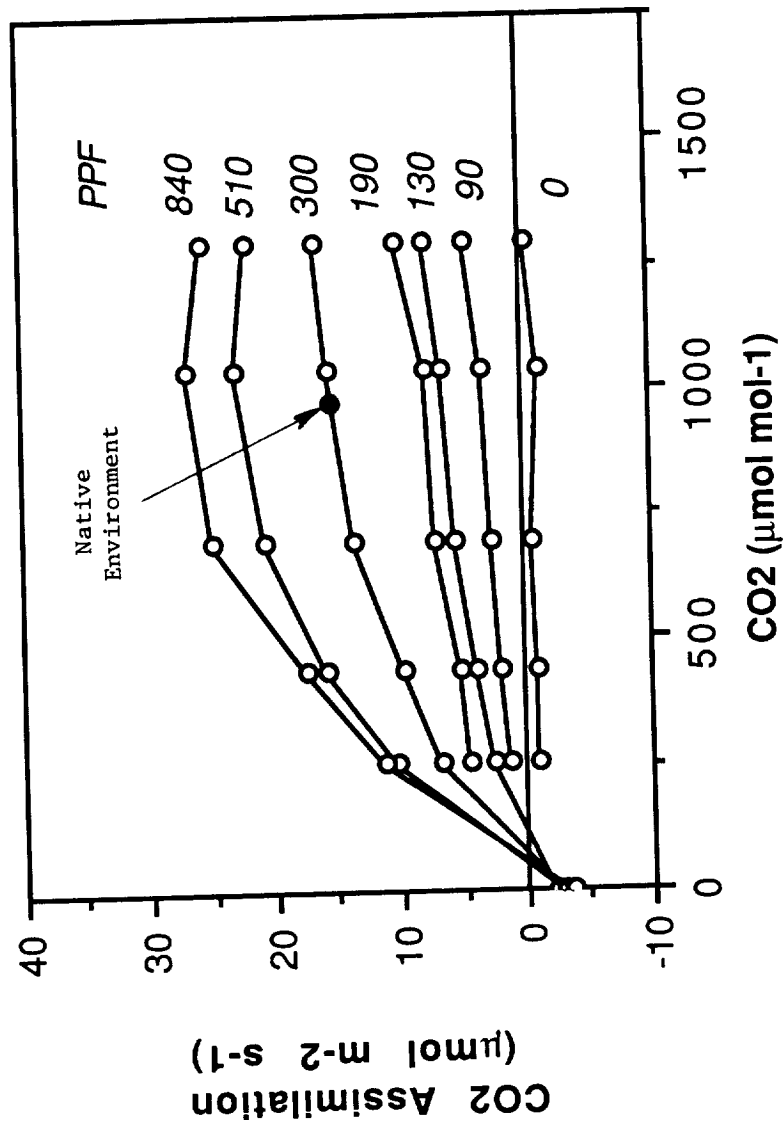


Figure 4. CO₂ assimilation (photosynthesis) rates of soybean leaves at different CO₂ and photosynthetic photon flux (PPF) levels. Plants were grown at 1000 $\mu\text{mol mol}^{-1}$ CO₂.

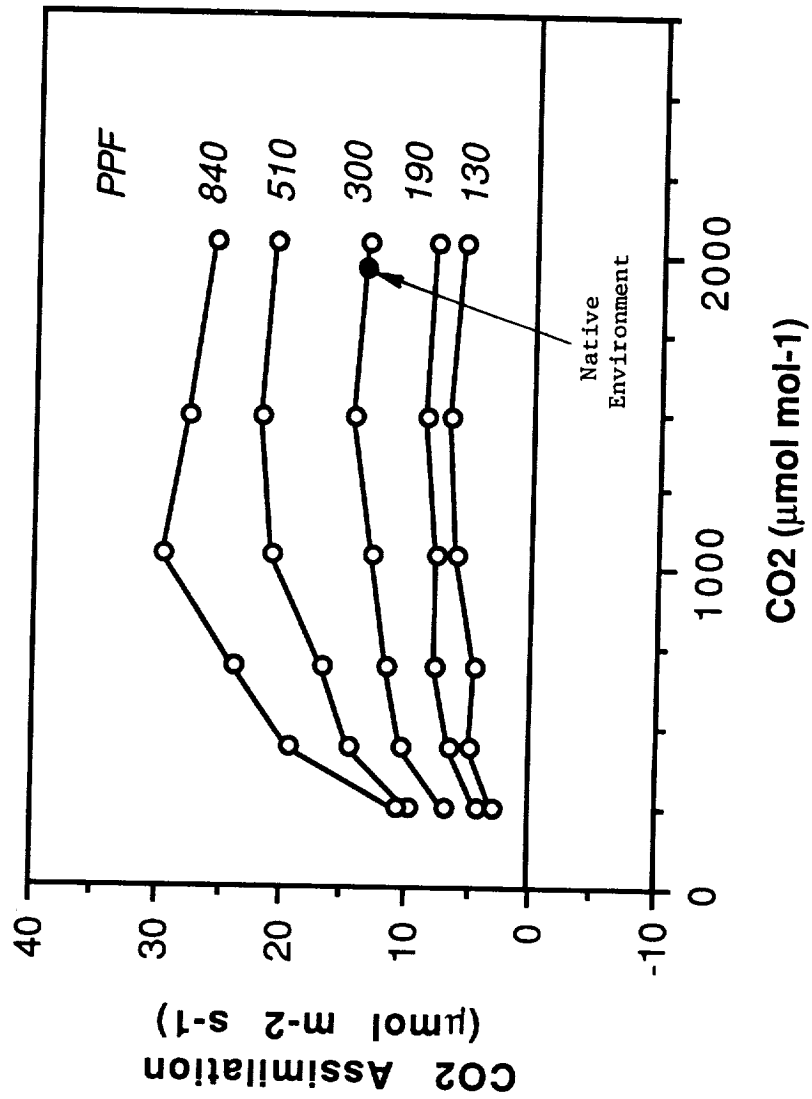


Figure 5. CO₂ assimilation (photosynthesis) rates of soybean leaves at different CO₂ and photosynthetic photon flux (PPF) levels. Plants were grown at 2000 μmol mol⁻¹ CO₂.

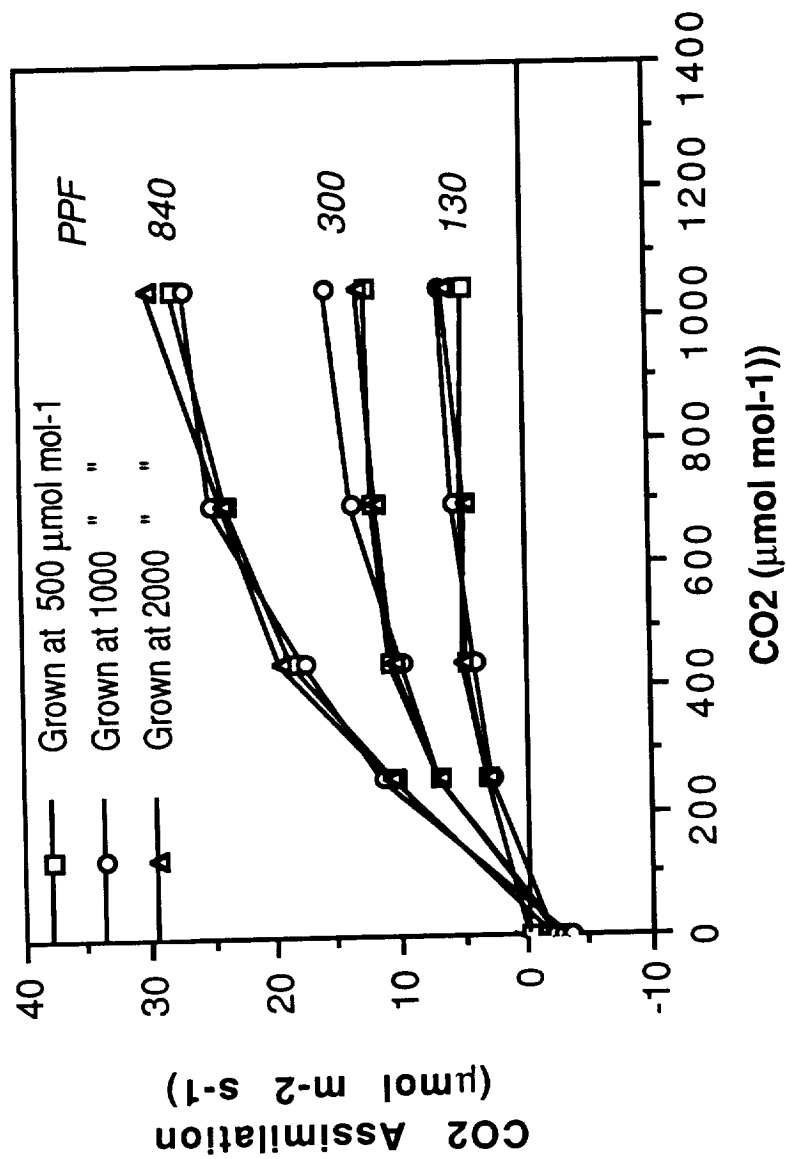


Figure 6. Comparison of CO₂ assimilation (photosynthesis) rates of soybean leaves at different CO₂ and photosynthetic photon flux (PPF) levels. Leaf measurements were taken from plants grown at 500, 1000, and 2000 µmol mol⁻¹ CO₂.

EFFECTS OF ELEVATED ATMOSPHERIC CARBON DIOXIDE CONCENTRATIONS
ON WATER AND ACID REQUIREMENTS OF SOYBEANS
GROWN IN A RECIRCULATING HYDROPONIC SYSTEM

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ABSTRACT

Establishing mass budgets of various crop needs, i.e. water and nutrients, in different environments is essential for a Controlled Ecological Life Support System (CELSS). The effects of $[\text{CO}_2]$, 500 and 1000 umol mol^{-1} , on water and acid use (for pH control) by soybeans in a recirculating hydroponic system were examined. Plants of cvs. McCall and Pixie were grown for 90 days using nutrient film technique (NFT) and a nitrate-based nutrient solution. System acid use for both CO_2 levels peaked near 4 weeks, during a phase of rapid vegetative growth, but acid use decreased more rapidly under 500 compared to 1000 $\text{umol mol}^{-1} \text{CO}_2$. Total system water use by 500 and 1000 umol mol^{-1} plants was similar, leveling off at 5 weeks and declining as plants senesced (ca. 9 weeks). However, single-leaf transpiration rates were consistently lower at 1000 umol mol^{-1} . The data suggest that high CO_2 concentrations increase system acid (and nutrient) use because of increased vegetative growth, which in turn negates the benefit of reduced water use (lower transpiration rates) per unit leaf area.

INTRODUCTION

Establishing mass budgets of plant needs is essential for a Controlled Ecological Life Support System (CELSS), especially when focusing on system efficiency. Measuring the amount of water, minerals, and carbon dioxide a particular crop needs when grown in various environments will provide important data for the modeling and development of future CELSS. Work is under way at

NASA and various universities to model and emulate a CELSS with data collected from field studies and short term (vegetative) studies in controlled environments. Obtaining accurate information on system budgets is best done with data from production studies in controlled environments rather than field studies since results can differ (1,2). As part of a series of studies involving the effect of CO₂ on soybean production (two cultivars at two planting densities), the mass budgets of water and acid use of the recirculating hydroponic system were monitored.

MATERIALS AND METHODS

Each study was carried out in a 1.8 m x 2.4 m walk-in growth chamber (model M48, EGC Inc, Chagrin Falls, OH). Plants were grown in eight trapezoidal-shaped PVC plastic trays, 0.25 m² (Fig 1) which is the same as those used in the Biomass Production Chamber (BPC) at NASA's Kennedy Space Center (3).

A complete nutrient solution was continuously pumped from the same reservoir to each tray at a flow rate of approximately 1 L min⁻¹. The back (wide) end of each tray was elevated 2 cm to allow passive (gravity) flow of nutrient solution to the front (narrow) end, where the solution returned to the reservoir (Fig 2).

The nutrient solution was a modified 1/2 strength Hoagland's solution. Since only nitrate-nitrogen was used in the nutrient solution, the pH tended to increase during periods of rapid growth and heavy nitrate uptake (4). To balance this, an

automatic pH controller was used to add dilute HNO_3 (2.5% v/v) to continuously maintain the pH 5.7 ± 0.5 units. Each day deionized water was added to the reservoir to replenish water taken up (transpired) by the plants. Twice each week nutrients were replenished by adding stock solutions of nutrients that were removed from the solution. Weekly samples of the nutrient solution were collected for inorganic nutrient analysis.

Seeds of cvs McCall and Pixie were planted onto each tray top and covered by a Plexiglas cover for 4 days (Fig 1), after which the cover was removed and plants were thinned to either three or six per tray. Both studies used a 12-hr photoperiod with a canopy photosynthetic photon flux (PPF) of $294 \pm 33 \text{ umol m}^{-2} \text{ s}^{-1}$ for the 500- CO_2 treatment, and $318 \pm 44 \text{ umol m}^{-2} \text{ s}^{-1}$ for the 1000- CO_2 treatment. Daily light-period temperatures averaged $25.6 \pm 0.6^\circ\text{C}$ and $25.8 \pm 0.1^\circ\text{C}$ for the 500- and 1000-treatment, respectively, while dark-period temperatures averaged $20.1 \pm 0.6^\circ\text{C}$ and $20.2 \pm 0.1^\circ\text{C}$. Relative humidities were kept constant and averaged $62 \pm 2\%$ and $64 \pm 3\%$ for the 500 and 1000-treatments. The CO_2 was monitored and controlled at either 500 or $1000 \pm 50 \text{ umol mol}^{-1}$ using an infrared analyzer. Four weeks after planting white, vinyl-plastic coated cages (60 cm high) were placed around the perimeter of each tray for support and to confine each tray to 0.25 m^2 of growing area. Single-leaf transpiration measurements of the abaxial (lower) leaf surface were taken at regular intervals throughout the studies using a steady state porometer (LI-1600, LI-COR Inc, Lincoln, NE). All

plants were harvested at 90 days. The results represent total system (chamber) averages and are not segregated by cultivar or density.

RESULTS AND DISCUSSION

Based on individual leaves, transpiration rates ($\mu\text{g cm}^{-2} \text{s}^{-1}$) were lower in the study having $1000 \mu\text{mol mol}^{-1} \text{CO}_2$ versus $500 \mu\text{mol mol}^{-1}$ (Fig 3). Similar findings for soybean were recorded by Rogers et al. (5). Under both CO_2 concentrations leaf transpiration rates were relatively stable until 10 weeks, when rates declined rapidly as the crop senesced. Although the single-leaf transpiration rates differed between CO_2 treatments, total system (i.e. canopy) water use was equivalent over time (Fig 4). However more biomass was produced at $1000 \mu\text{mol mol}^{-1}$ treatment and thus water use efficiency (WUE), based on total biomass/total water (g L^{-1}), was greater at $1000 \mu\text{mol mol}^{-1} \text{CO}_2$ (Table). Valle et al. (6) have found that 94% of the WUE increase was from increased photosynthetic rates (not decreased transpiration) at elevated CO_2 levels.

The amount of HNO_3 needed for controlling pH was much greater for plants grown at 1000 than $500 \mu\text{mol mol}^{-1} \text{CO}_2$ (Fig 5). As plants are grown with NO_3^- as the N source, the pH tends to increase over time, whereas NH_4^+ causes solution pH to decrease (4). Work has been done to maintain pH homeostasis in hydroponically-grown soybeans by using a 3:1 ($\text{KNO}_3:\text{NH}_4$) ratio (7). This may be a practical alternative to controlling pH in a CELSS, where acids and bases could be costly.

Creating budgets of acid and water use for soybean growth led to some interesting results. In doubling the CO_2 from 500 to 1000 $\mu\text{mol mol}^{-1}$, biomass increased 18%, HNO_3 use increased 48%, and water use remained constant on a per area basis (Table 1). Although the high- CO_2 -grown plants have a greater WUE, they are less efficient in acid use per unit dry weight. The acid and water requirements can be calculated per hectare (Table 1), an area approximately twice the size of a farm needed to support a large lunar colony (8). Approximately 45,000 L of water per day would be needed, and depending on the CO_2 treatment, between 5 and 10 L of 15.7 M HNO_3 would be needed for nutrient solution pH control. Similar NFT production studies with potatoes at cooler temperatures and ambient CO_2 (350-450 $\mu\text{mol mol}^{-1}$) used half the amount of water per day and similar quantities of acid when compared to the 500 $\mu\text{mol mol}^{-1}$ soybean treatment (9). Although such numbers initially appear staggering, it is interesting to note that typical water consumption for greenhouse hydroponic systems range from 21,500-134,000 $\text{L ha}^{-1}\text{d}^{-1}$, depending on the crop (10). Unlike greenhouse situations, where transpired water is lost, the transpired water in a CELSS will be recycled as high purity (potable) water. This translates into a system where condensing and recycling equipment must handle at least 45,000 $\text{L ha}^{-1}\text{d}^{-1}$ water, based on the data. The orchestration of various crops with the other components of a CELSS will require more information of the type shown in order to create the most productive and efficient system possible.

Table 1. Effects of carbon dioxide based on the growing area

CO ₂ TREATMENT ($\mu\text{mol}\cdot\text{mol}^{-1}$)	EXTRAPOLATED WATER USE		CONCENTRATED		TOTAL		SEED	
	AREA	(L·day)	HNO ₃ USE (L·day)	DRY MATTER (Kg)	DRY MATTER (Kg)	DRY MATTER (Kg)	DRY MATTER (Kg)	
500	m ²	4.67	5.0×10^{-4}	1.02	0.451			
500	hectare	46700	5.0	10200	4510			
1000	m ²	4.55	9.6×10^{-4}	1.244	0.516			
1000	hectare	45000	9.6	12440	5160			

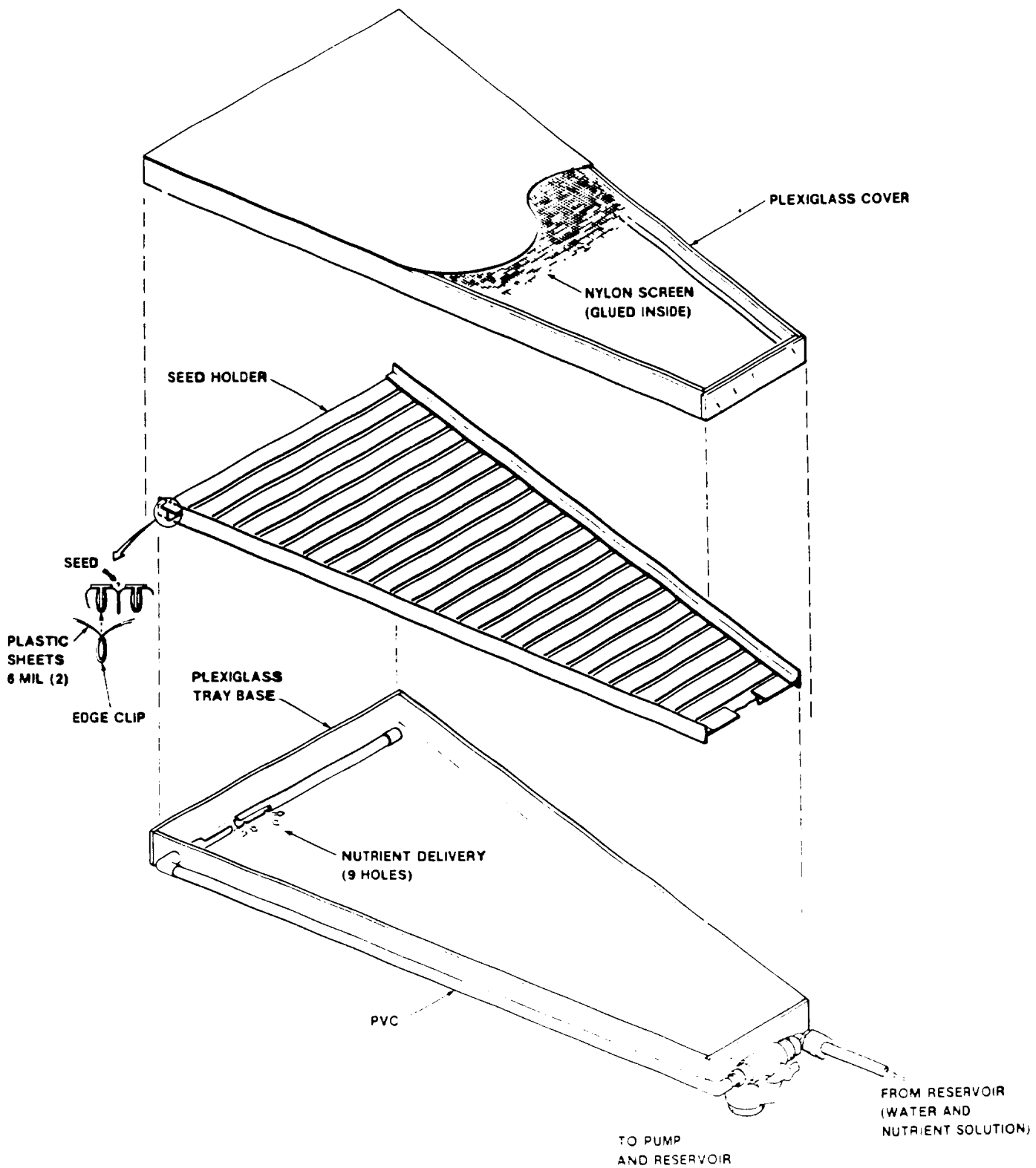


Fig 1. Hydroponic growing tray bottom, top, and germination cover.

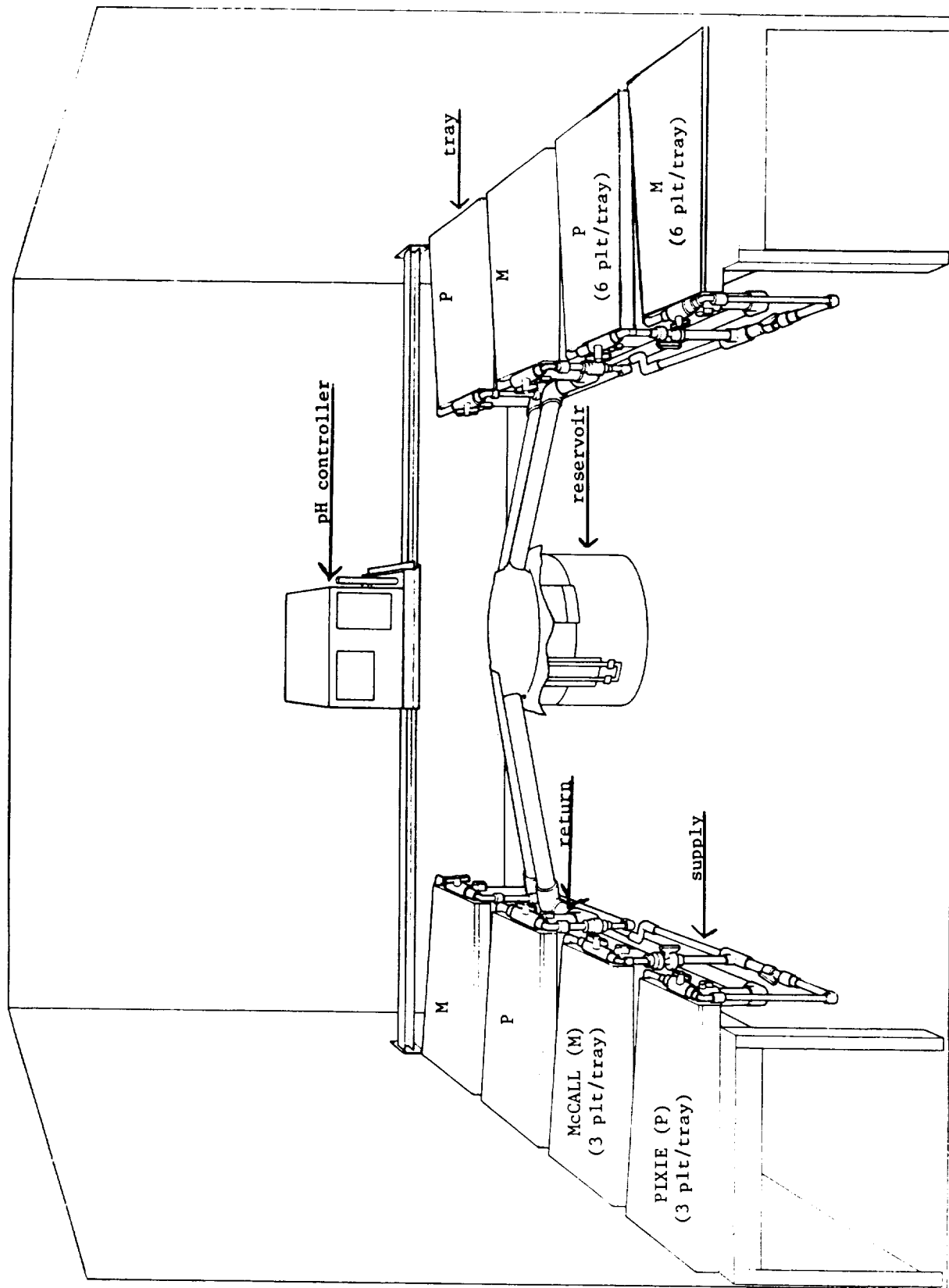


Fig 2. Growth chamber (4.4 m²) layout showing growing trays and nutrient delivery system.

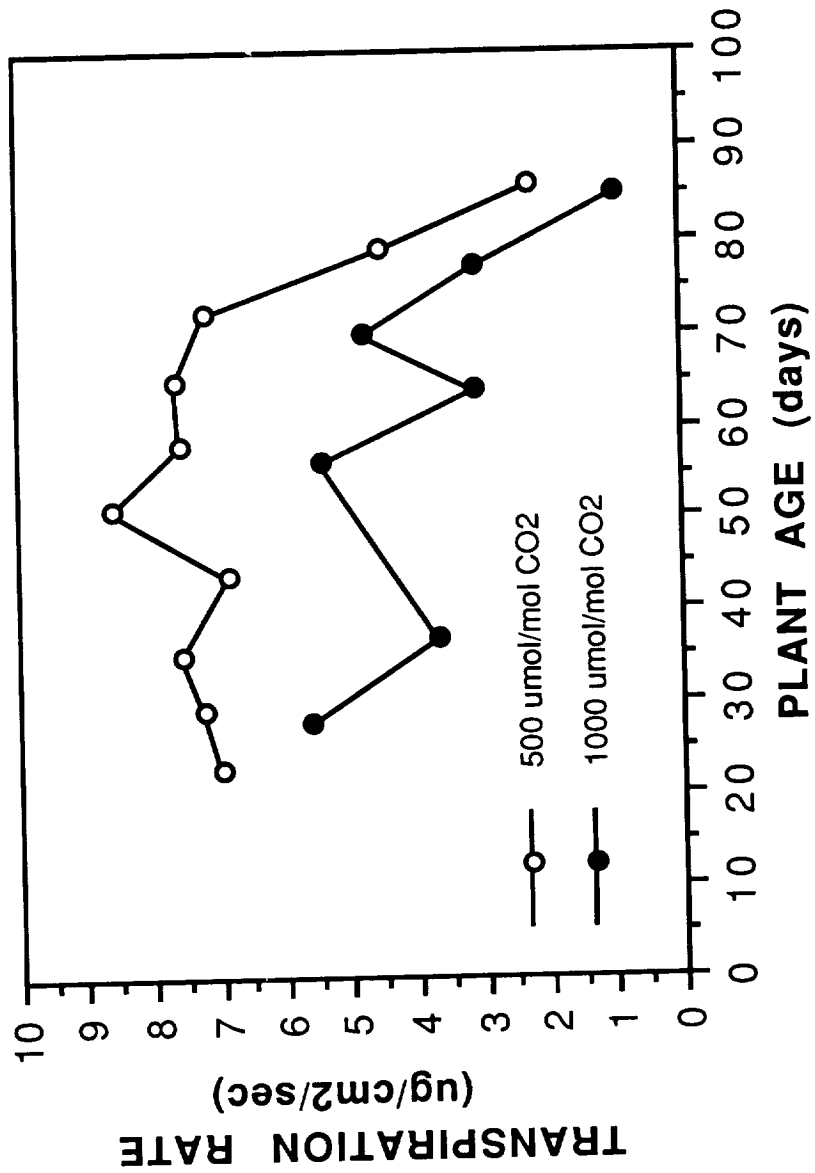


Fig 3. The effect of carbon dioxide on soybean leaf transpiration rates.

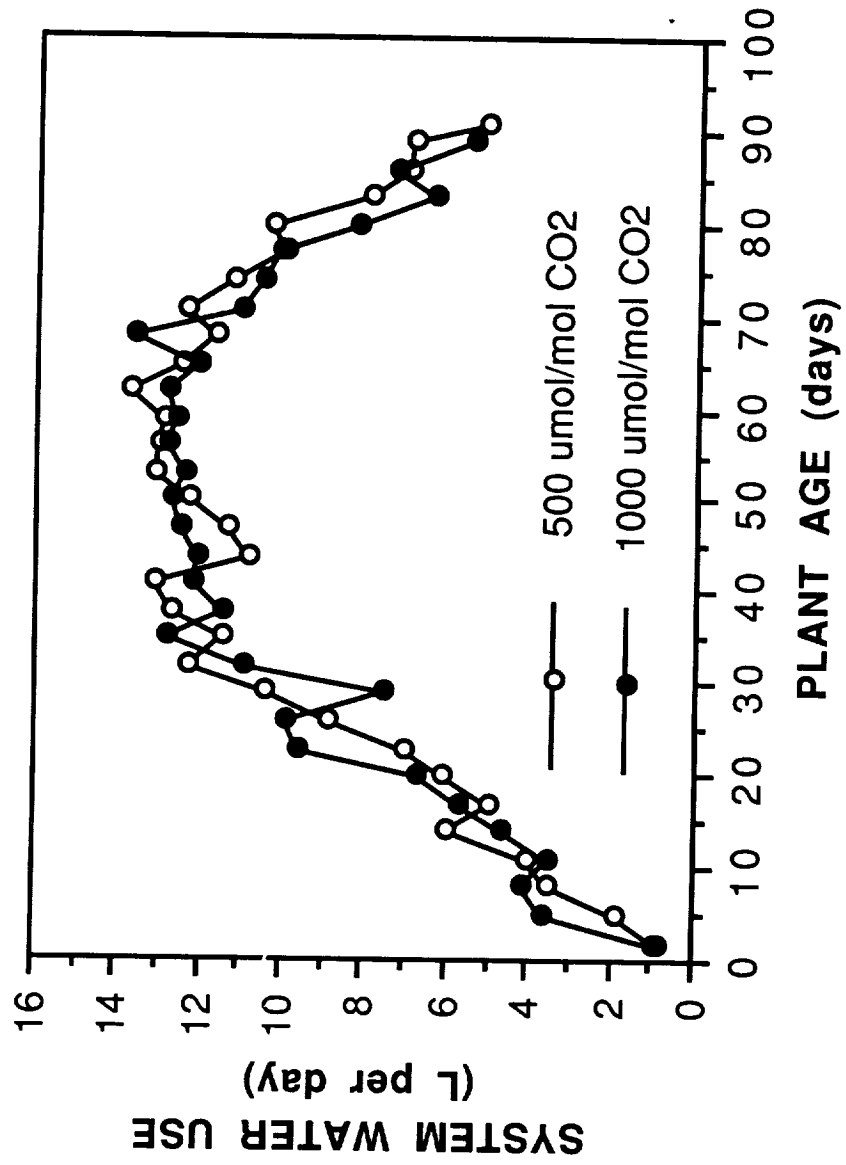


Fig 4. The effect of carbon dioxide on water use by soybean crops.

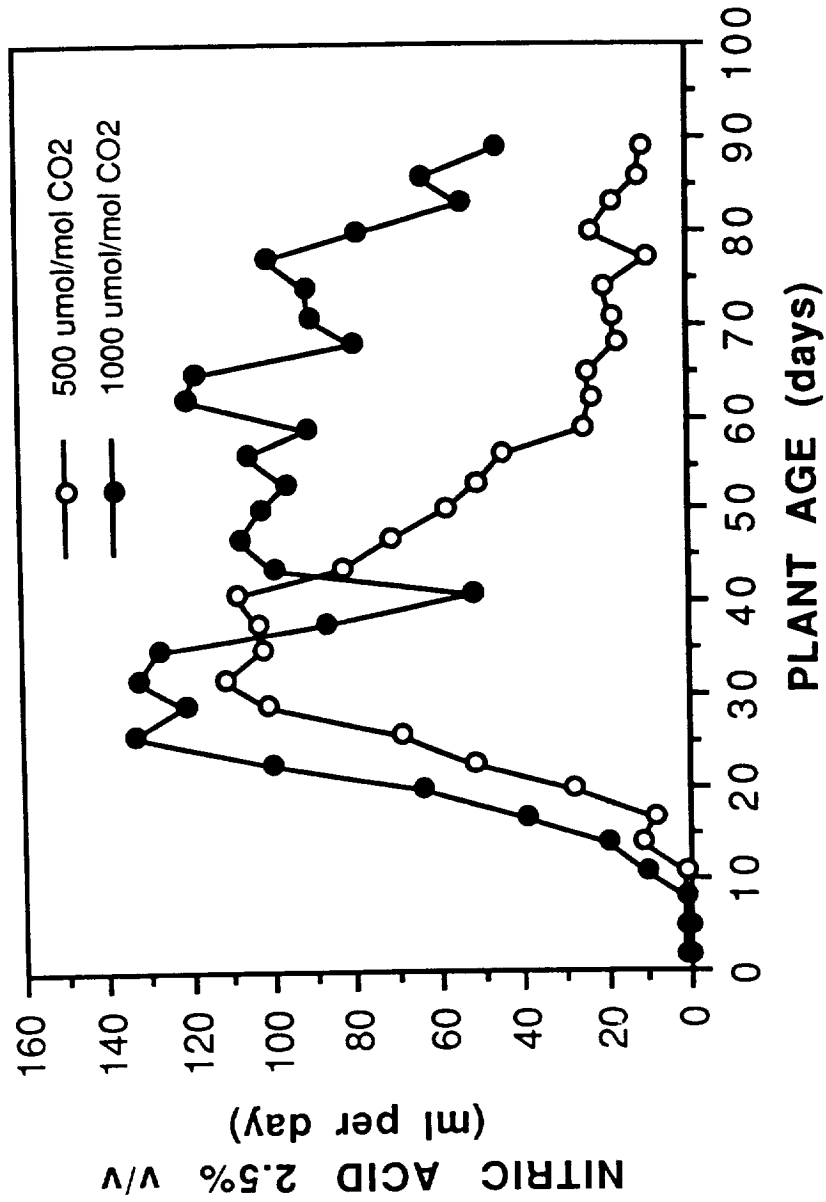


Fig 5. The effect of carbon dioxide on acid use by soybean crops.

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SWEET POTATO FOR CLOSED ECOLOGICAL LIFE SUPPORT SYSTEMS
 USING THE NUTRIENT FILM TECHNIQUE

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ABSTRACT

Sweet potatoes have been grown hydroponically using the nutrient film technique (NFT) in support of the CELSS program. Experiments in the greenhouse with the 'TI-155' sweet potato cultivar produced up to 1790 g/plant of fresh storage roots. Studies with both 'TI-155' and 'Georgia Jet' cultivars have resulted in an edible biomass index of approximately 60.0% with edible biomass linear growth rates of 12.1-66.0 g m⁻² d⁻¹ in 0.05-0.13 m² in 105 to 130 days. Proximate analysis of the storage roots indicated nutritive components similar to field grown sweet potatoes. Varietal selection studies showed the potential of other cultivars for this system. 'Georgia Jet' sweet potatoes grown in environmental chambers produced 545 g/plant in 90 days with a light intensity of 960 umol m⁻² s⁻¹. With 480 umol m⁻² s⁻¹ only 304 g/plant were produced. Experiments with various cultivars, photoperiods, light intensities and nutrient solution compositions are in progress. All studies indicate good potential for sweet potatoes in CELSS.

INTRODUCTION

Tuskegee University (TU) has been conducting research on sweet potatoes for CELSS since 1986. The various aspects of growing sweet potatoes for CELSS were reviewed by Hill et al. (1). Initial basic research needs were identified as: (a) the actual production of sweet potatoes in soilless media and (b) the optimization of parameters leading to optimal production. This presentation will briefly review some of the work conducted to address these needs.

GROWING SYSTEM

Preliminary investigations into hydroponic production of sweet potatoes involved aggregate studies (2). Such an aggregate system--sand in 10 L pots--was used to study the influence of

nutrient solutions with different N:K ratios (1:1.1 and 1:2.4) on the growth of 'Georgia Jet' and 'Jewel' sweet potatoes in 90 and 120 days. In each case, nutrient solutions with an N:K ratio of 1:2.4 tended to produce a higher storage root weight in this open hydroponic system (3). However, considering the mass and volume limitations of a space-based plant production system, the TU team fairly early designed a system making use of the nutrient film technique (NFT). Very little work had ever been done on such a hydroponic system for root crops.

The TU NFT system designed is described by Hill et al. (4) and includes three growth channels to which nutrient solution is supplied from a nutrient reservoir by a pump. The 15 cm sweet potato vine cuttings are spaced at 25 cm both within the channel and between channels. A black-white vinyl film encloses the channel preventing light from entering the root zone. As the foliage grows, it is supported by and trained onto a string from above the channel. Good storage root enlargement has resulted in this system.

REVIEW OF SELECTED EXPERIMENTS

Photoperiod Experiment. Since little is known about the effect of photoperiod on sweet potatoes, experiments were set up to study the effects of two photoperiods and temperature regimes on sweet potato production using reach-in growth chambers. Vine cuttings of three cultivars--'Georgia Jet', 'TI-155' and 'Georgia 120'--were placed in 4 L pots with a 1:1.1 mixture of sterilized sand and soil. The plants were subjected to a 24 h photoperiod or to a 12:12 h light/dark photoperiod, a constant temperature of 28°C or light/dark temperature regime of 28/22°C. A light irradiance of 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy level and 70% RH were

maintained throughout the growing period. Results (Table 1) of the study with 'Georgia Jet' sweet potato provide a sample of the cultivar-specific data presented previously by Mortley et al. (5) and showed that the 24 h photoperiod increased storage root number, yield and fibrous root dry weights of the sweet potato. Some evidence of the positive effect of thermoperiodicity is reflected in storage root number and fibrous root dry weight. This study is now being conducted with 'TI-155' and 'Georgia Jet' cultivars using NFT.

Light Intensity--The effect of light intensity on the growth of 'Georgia Jet' sweet potato has been studied using the TU NFT system. The experiment was conducted using two environmental growth chambers and has been reported by Bonsi et al. (6). Sweet potatoes were grown under two light intensities for 85 days with a 14 h photoperiod, 70% RH and a 28:22°C diurnal temperature. Of the two light intensities, 480 and 960 $\mu\text{mol m}^{-2}\text{s}^{-1}$, plants grown with the latter produced the highest fresh and dry storage root weight: 545.3 and 104.6 g/plant, respectively (Table 2). There was no difference in number of storage roots or fresh or dry foliage weight caused by light intensity. The experiment is being repeated with the 'TI-155' cultivar.

Cultivar Studies. A number of experiments have been carried out in which the growth of different sweet potato cultivars has been monitored in both sand and NFT. The focus was on storage root yield. Hill et al. (4) reported on yields (Table 3) for three different cultivars--'Georgia Jet', 'Jewel' and 'TI-155.' 'Georgia Jet' grown in sand in pots outproduced 'Jewel' in 120 days. It has also produced up to 1308 g/plant in NFT in 105 days compared to 1790 g/plant produced with 'TI-155' in 130 days. In

these studies, the edible biomass index ranged from 60.0 - 89.2 % while the edible biomass linear growth rate ranged from 9.4 to 66 $\text{g m}^{-2}\text{d}^{-1}$ with a plant spacing of 0.05 - 0.13 m^2 . Preliminary screening of other sweet potato cultivars (Table 4) using NFT and a 120-day growing period identified both orange- and white-flesh cultivars for nutritional variety that have potential for CELSS (7).

NUTRITIVE PROXIMATE ANALYSIS OF SWEET POTATOES

Proximate analysis of 'Georgia Jet' sweet potato storage roots (Table 5) grown in NFT as compared to that for field-grown storage roots has been reported by Hill et al. (4). The data indicate that hydroponically-grown storage roots provide comparable nutrition to field-grown plants (8). Apparent differences in values can be attributed to the fact that the roots from the NFT system were analyzed immediately after harvest while the field-grown sweet potatoes were cured and stored prior to analysis. Carbohydrates and vitamins A and C are the major nutritional components from the 'Georgia Jet' roots which have an orange flesh. Preliminary research with white-flesh varieties such as 'TU-52' indicates they have virtually no vitamin A but are comparable in other nutrients.

CONCLUSIONS

Sweet potato appears to have good potential for CELSS. Experiments with sweet potatoes on the effect of various photoperiods, light intensities and cultivars have helped to indicate the range of parameters required for their good growth and storage root yield in NFT.

ACKNOWLEDGEMENTS

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Table 1. Effects of two photoperiod and temperature regimes on growth responses^z of 'Georgia Jet' sweet potato cultivar.

Photoperiod (Light/Dark) (h)	Temp. (12/12) (C)	Storage Roots No.	Roots Wt. (g)	Fibrous Roots Dry Wt. (g)	Foliage Wt.	
					Fresh (g)	Dry (g)
12/12	28/28	0.7 b ^y	19.5 b	0.2 c	276.2 a	34.9 b
	28/22	1.0 b	11.0 b	4.3 b	242.5 a	25.8 c
24/0	28/28	2.3 b	187.9 a	0.4 c	306.6 a	47.4 a
	28/22	5.3 a	202.8 a	7.5 a	276.8 a	31.7 bc

^zMean of six plants.

^yMean separation in columns by DMRT (5% level). Means followed by the same letter are not significantly different.

Table 2. Growth of 'Georgia Jet' sweet potato under different light intensities in environmental growth chambers using the nutrient film technique (NFT).

Irradiance PPF ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	No.	Storage Root		Foliage	
		Fresh Wt. (g/plant)	Dry Wt. (g/plant)	Fresh Wt. (g/plant)	Dry Wt. (g/plant)
480	3.9 a*	304.1 b	58.7 b	396.7 a	51.7 a
960	4.2 a	545.3 a	104.6 a	342.0 a	57.5 a

*Mean of 12 plants grown in 90 days. Means in same column with same letter are not significantly different using Student t test at 5% level.

Table 3. Yield data for sweet potatoes grown in sand and NFT systems maintained in greenhouse.

Growing System	Cultivar	Duration (days)	Storage Root		Linear Growth Rate		Edible Biomass Harvest Index (%)
			Fr. Wt. Per Plant (g plant ⁻¹)	Dry Wt. Per Plant (g plant ⁻¹)	Edible Biomass Canopy Area (g m ⁻² d ⁻¹)	Edible Biomass Root Area (g m ⁻² d ⁻¹)	
Sand, pot	Georgia Jet	120	869*	203	14.1	52.0	89.2
			748**	175	12.1	44.8	87.6
Sand, pot	Jewel	120	606*	162	11.3	41.5	82.4
			505**	135	9.4	34.6	79.6
NFT	Georgia Jet	105	1308*	235	16.9	48.4	61.9
			949**	190	13.7	39.1	60.0
NFT	TI 155	130	1790*	397	23.1	66.0	-
			1493**	331	19.3	55.0	-

*Highest plant yield
**Mean of four plants

Table 4. Growth responses^z of four sweet potato cultivars grown in the Tuskegee University NFT system.

Cultivar	No.	Storage Roots		Foliage	
		Fresh Wt. (g)	Dry Wt. (g)	Fresh Wt. (g)	Dry Wt. (g)
T2093	6.0 a ^y	322.6 a	29.9 bc	188.4 ab	29.9 bc
TU 52	3.0 b	384.0 a	58.8 a	366.4 a	58.8 a
TU 80	3.0 b	305.9 a	14.1 c	74.8 b	14.1 c
TU 50	0.5 c	116.7 b	50.7 ab	347.1 a	50.7 ab

^zMean of four plants

^yMean separation in columns by DMRT (5% level). Means followed by the same letter are not significantly different.

Table 5. Proximate analysis and comparison^x of 'Georgia Jet' sweet potato storage roots grown in the Tuskegee University NFT system and field grown roots.

Component	NFT System (g)	Field Grown (g)
Moisture	78.5	77.4
Protein	1.0	- ⁺
Starch	12.9	18.6
Sugar	- ⁺	4.0
Vitamin B ₁ **	0.136	- ⁺
Vitamin B ₂ **	0.064	- ⁺
Vitamin C**	28.0	10.5
Carotenoids (Vitamin A)**	7.5	7.5

^xNFT system measurements made on fresh roots immediately after harvest; field grown measurements made on raw roots following curing and two weeks of storage.

*Wet basis

**mg per 100g

⁺Not measured in this study

CLONING CROPS IN A CELSS VIA TISSUE CULTURE:

PROSPECTS AND PROBLEMS

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ABSTRACT

Micropropagation is currently used to clone fruits, nuts and vegetables and involves controlling the outgrowth *in vitro* of basal, axillary or adventitious buds. Following clonal multiplication shoots are divided and rooted. This process has greatly reduced space and energy requirements in greenhouses and field nurseries and has increased multiplication rates by greater than 20-fold for some vegetatively-propagated crops and breeding lines. Cereal and legume crops also can be cloned by tissue culture through somatic embryogenesis. Somatic embryos can be used to produce "synthetic seed", which can tolerate desiccation and storage and germinate upon rehydration.

Synthetic seed of hybrid wheat, rice, soybean and other crops could be produced in a controlled environment life support system (CELSS). Thus yield advantages of hybrids over inbreds (10% to 20%) would be exploited without having to provide additional facilities and energy for parental-line and hybrid seed nurseries. In our laboratory media costs for producing 1000 viable somatic embryos of wheat are about \$ 0.12. This compares to \$ 0.02 per 1000 for hybrid seed produced commercially and \$ 0.40 per 1000 when seeds are produced in controlled environments with artificial lighting. Mass and energy requirements for seed and propagule production in a lunar or martian CELSS will be substantially reduced by innovations in micropropagation and synthetic seed technology.

INTRODUCTION

The list of agricultural crops cloned *in vitro* for research, breeding or commercial purposes has expanded rapidly in recent years (Table 1). The rationale for using tissue culture for terrestrial applications includes rapid multiplication rates, low energy and space requirements, and maintenance of specific genotypes. This rationale will likely be of even greater importance in an extraterrestrial controlled environment life support system

Table 1. Partial listing of crops that are cloned *in vitro* for either research, breeding, hybrid seed production, commercial production, or multiplication of virus-free nuclear stock.

<u>Crop</u>	<u>Explant of choice</u>	<u><i>In vitro</i> system¹</u>	<u>Purpose²</u>	<u>References</u>
<u>Cereals</u>				
Wheat	Immature embryo & inflorescence	SE	GE, SV, SS	1, 2
Barley	Immature embryo	SE	GE, SV	3
Maize	Immature embryo	SE	GE, SV	4, 5
Rice	Immature embryo & inflorescence	SE	GE, SV	6
Sorghum	Embryos, shoot tip	SE	GE, SV	7
<u>Legumes</u>				
Soybean	Immature embryo	SE	GE	8, 9, 10
<u>Vegetables</u>				
Carrot	Hypocotyl, meristem	SE	BR, SS	11, 12, 13
Cassava	meristem tip, mature embryo	AvB, SE	VE, MNS, SS	14, 15
Cocoyam	shoot tip	AvB	VE, MNS	14
Hausa potato	leaf	AvB	MNS	14
Taro	shoot tip	AvB	VE, MNS	14
Potato	Axillary bud, petiole, tuber disc, meristem	ABB, AvB	VE, MNS, SV	16, 20
Sweet potato	Meristem, tuber disc, petiole	SE, AdB	SS, MNS	14, 17, 18
Sweet yam	corm segment	AdB	MNS	14

Table 1 (cont.).

<u>Crop</u>	<u>Explant of choice</u>	<u>In vitro system¹</u>	<u>Purpose²</u>	<u>References</u>
<u>Vegetable crops cont.</u>				
White yam	Mature embryo, nodal cutting	SE, AdB	VE, CM, SS	14, 19
Papaya	Axillary bud	AdB	CM	20
Artichoke	Apical bud	ABB, AdB	CM	21
Asparagus	Basal bud	ABB, AdB	CM, MNS	20, 22
Celery	Immature petiole	SE	SV, SS	23
Lettuce	Leaf	SE	SV	24
Mustard	Immature embryo	SE	SS	25
Cucumber	Leaf	SE	SS	26
<u>Sugar and oil crops</u>				
Sugarcane	Meristem, leaf	SE	SV, SS	27, 28
Sugarbeet	Lateral bud, petiole	ABB, AdB	MB	29
Oil palm	Embryo, leaf	SE, AdB	MB	30
<u>Fruit and nut crops</u>				
Tomato	Embryo, leaf, hypocotyl	SE, AvB	GE, SV, MB, SS	31, 32
Strawberry	Immature embryo, meristem	SE, ABB, AvB	VE, SS, CM, SV, MPN	16, 22, 33, 34, 35
Raspberry	Apical and axillary bud	ABB, AvB	VE, CM	22, 35
Blackberry	Root, Apical & axillary bud	ABB, AvB	VE, CM	22, 35, 36
Blueberry	Axillary bud	ABB, AvB	CM	22, 35

Table 1 (cont.).

Crop	Explant of choice	<i>In vitro</i> system ¹	Purpose ²	References
Fruit and nut crops cont.				
Peach & nectarine	Shoot tip	AvB	CM	37
Apricot	Shoot tip	AvB	CM	37
Apple and crabapple	Bud	AvB	CM	37, 38
Cherry	Root	AvB	CM	37, 39
Plum, prune	Shoot tip	AvB	CM	37
Pineapple	Shoot tip & axillary bud	AvB	CM, MNS	14, 16, 40
Banana	Axillary bud, corm	AvB	CM	14, 16, 41
Grape	Anther, ovary, node	SE, ABB, AvB	SS, CM	35, 42, 43
Date palm	Lateral bud	SE, AvB	SS, CM	44
Pecan walnut chestnut filbert	Shoot tip	AvB	CM	37
<u>Spice and fiber crops</u>				
Caraway	hypocotyl	SE	SS	45
Cacao	Immature embryo	SE	SS	46
Coffee	Leaf	SE, AvB, ABB	SS, CM	47
Cotton	Cotyledon	SE	BR, GE	48

¹ABB, Axillary or basis buds; AvB, adventitious buds; SE, somatic embryogenesis

²BR, basic research; CM, commercial micropropagation; GE, genetic engineering; MB, micropropagation for breeding purposes; MNS, micropropagation for nuclear stock; SS, synthetic seed research; SV, somaclonal variation; VE, virus eradication

(CELSS). The purpose of this article is to summarize the commercial use of *in vitro* cloning with today's crops and to describe areas of research important to the application of *in vitro* cloning for food production in a CELSS.

TISSUE CULTURE AND CROP PRODUCTION

Plant regeneration *in vitro* occurs through one of three developmentally-distinct processes: branching from normally-formed basal or axillary buds, branching from adventitiously-formed buds, or somatic embryogenesis. Cloning from basal, axillary or adventitious buds involves cuttings from vegetative tissues and is thus a form of vegetative propagation. The term "micropropagation" is reserved for these processes. In contrast multiplication by somatic embryogenesis is not a vegetative process but involves the formation of entirely new plants, usually of single or near single cell origin, without the cutting and rooting procedures associated with micropropagation. Procedures for cloning horticultural, agricultural or forestry crops by somatic embryogenesis are still largely in a developmental stage.

Shoot formation (or branching) from preexistent basal or axillary buds occurs when dormancy or quiescence of buds is released *in vitro* by hormone treatments. Tissues generally do not dedifferentiate and there is no callus intermediate. Hence this process is considered to be genetically stable. Shoots produced are cut into pieces that contain axillary or basal nodes and the process is repeated. The multiplication potential is calculated

by the formula $x=n^c$, where x = the total number of plants produced, n = the average number of propagules produced per explant during each cycle of multiplication, and c = the number of multiplication cycles. Generally values of n range from 5 to 10 and multiplication cycles range from 3 to 6 weeks in duration.

In the second process adventitious buds form directly from dedifferentiated cells of the explant or from cells of a callus intermediate. Genetic and epigenetic stability are more readily compromised in this process, which may cause somaclonal variation (49), especially when a callus intermediate is involved. The multiplication procedure is similar to that described above. While multiplication cycles are typically of a long duration (4 to 10 weeks), the n value from the formula listed above can be much higher (50 to 100), which results in a higher overall multiplication rate. Most micropropagation systems involve shoot formation from a mixture of both preexistent and adventitious buds. Rates of multiplication by tissue culture are often far superior to those obtained by conventional procedures (Table 2).

Micropropagation is important not only for commercial production but for cloning male sterile, gynoecious or polyploid parental lines used to produce hybrid seed. For example male sterility is a homozygous recessive trait in tomatoes. Multiplication of these parental lines by seed requires use of heterozygotes as pollinators. Segregation from the required crosses results in an undesirable 1:1 ratio of male sterile to male fertile plants. The latter plants must be manually removed from hybrid seed production nurseries upon their identification at flowering. Micropropagation

Table 2. Multiplication rates of selected crops by micropropagation and conventional propagation.

Crop	Propagules produced ¹	-----years-----		References
		<i>In vitro</i>	Conventional	
Potato	125	0.2	2	16
Pineapple	40-380	1.0	5-9	40
Stone fruit	9-35	0.1	1-3	37
Strawberry	20	0.1	1	22

¹Number of propagules produced is squared or cubed when time invested (*in vitro* or conventional) is doubled or tripled.

of these male sterile lines offers an attractive alternative. Similar situations exist for asparagus, cucumber, broccoli and triploid hybrids such as watermelon and sugar beets (see 50 for a review). *In vitro* procedures are being developed not only for clonal propagation but for somaclonal variation, genetic engineering or other research or breeding purposes (Table 1).

SEED AND PROPAGULE PRODUCTION IN A CELSS

The operation of a CELSS will be limited by availability of human resources. Because of this limitation crops selected for a CELSS will be restricted to those crops where sowing, crop growth, harvesting, and seed or propagule processing can be automated. The CELSS Initial Reference Configuration (Nov. 1988) identifies a plant growth facility, for production of crops, seeds and propa-

gules, and a storage facility, where the cleaning and storage of seeds and propagules will occur. Most procedures associated with these facilities lend themselves to current automation technology.

Micropropagation. Any system proposed to improve or streamline conventional procedures of crop production in a CELSS will need to be automated. On earth, where human resources are plentiful, micropropagation is beginning to replace conventional procedures for seed and propagule production (Table 1). Labor, energy and/or space considerations are driving private-sector decisions in this direction. Costs of skilled labor for micropropagation are becoming cheaper than the greenhouse, nursery and labor costs of conventional propagation. In contrast the human labor variable in a CELSS will be heavily weighted, and micropropagation will need to meet a higher level of automation than is currently employed on earth.

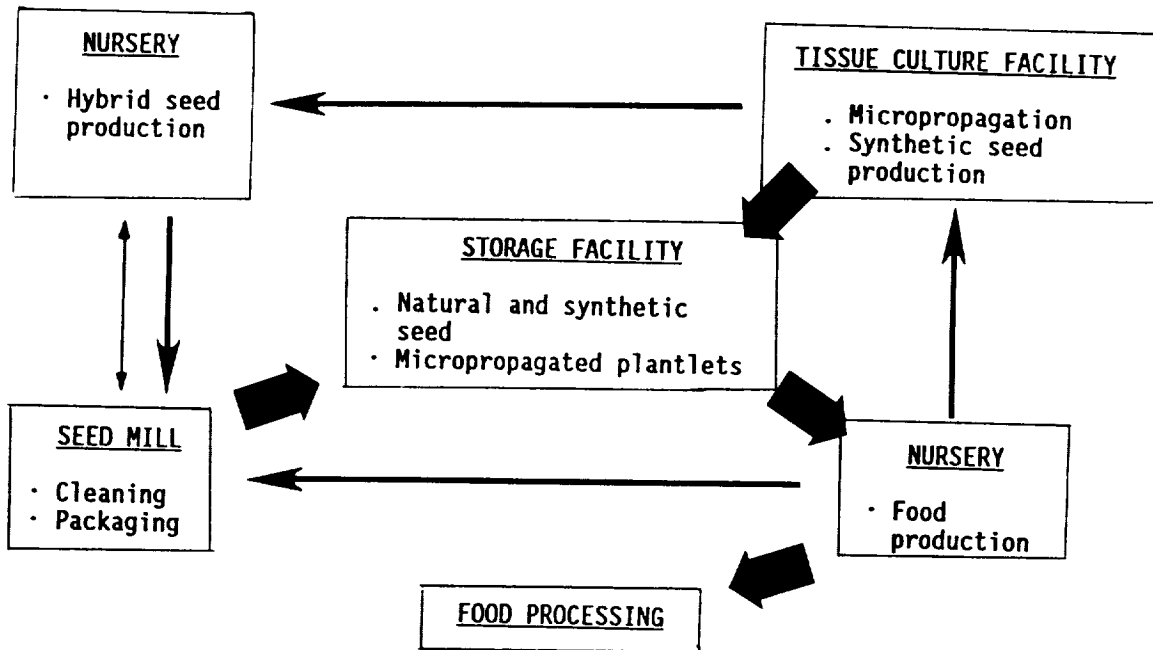
Automating micropropagation will be complicated. Micropropagation usually involves at least one mechanical cutting for every propagule produced. Cuttings often need to be made in precise locations to assure proliferation of additional shoots. Furthermore micropropagated tissues are sensitive to desiccation and mechanical injury. Thus machinery designed to handle these operations must "visualize", to a greater or lesser extent, the tissue to be propagated, make decisions based on "visual" images, and manipulate and slice tissues of varying sizes in a delicate manner. Finally nearly all of these operations must be conducted aseptically. Progress in tissue culture automation is being made (51)

but equipment designs appropriate for a CELSS probably will not be available for some time. An automated micropropagation system could be used in a large-scale CELSS to clone potato, asparagus, cucumber, sugar beet, yam, plantain, papaya, pineapple, banana, raspberry, strawberry, grape, filbert, coffee and others (Table 1).

In addition to being automated, innovations for increasing yields in a CELSS must also be energy efficient. For example nurseries for parental-line and hybrid seed production could be incorporated into the plant growth facility. This would permit the exploitation of hybrid vigor, which often means a 10% to 20% increase in yield. However, even with automation, yield advantages simply might not justify the added mass and energy required to maintain parental and hybrid seed nurseries. In this respect a combined approach where an automated micropropagation system is used to eliminate some of the parental-line nurseries may justify production of hybrid seed (Fig. 1). Currently crops for which this strategy may be of value include broccoli, cauliflower, cucumber, tomato, watermelon and sugar beets (Table 1).

Synthetic seed production. Somatic embryogenesis is the highest expression of cellular totipotency in plant tissue cultures and offers the greatest potential in terms of mass cloning and automation. With carrot as many as 5000 uniform somatic embryos can be obtained within 14 days from 1 ml of packed cells of a cell suspension (52). Unfortunately rates of production of somatic embryos of other crops are generally much lower and, even in the carrot system, most somatic embryos are abnormal and fail to germinate.

Figure 1. Seed and propagule production in a CELSS, current capabilities . Currently tissue culture could reduce space and energy requirements for production of propagules of many crops (Table 1). Most seed and propagules would be produced conventionally by the lower loop, nursery to seed mill to storage facility to nursery.



Nevertheless procedures for selectively multiplying highly totipotent "embryogenic" cells and inducing these to form somatic embryos are being improved for numerous crops (Table 1).

Research efforts are now focusing not only on inducing somatic embryogenesis but on defining conditions that cause normal embryo development. Recently procedures were developed for producing somatic embryos of carrot without use of an exogenous auxin. Embryos so produced are more normal and can be encapsulated in calcium alginate for "synthetic seed" production. Germination

rates are as high as 50 % (13). Synthetic seed technology and its future application have recently been reviewed and discussed by Redenbaugh et al. (53, 54).

A goal in our laboratory is to understand and increase the production of embryogenic cells in wheat tissue cultures. We have observed increases by specifically altering the type of media and auxin used (55, 56, 57), by reducing oxygen availability to tissues (1), and by pretreatments that alter endogenous hormone levels prior to tissue culture (58, 59). We are also exposing embryogenic cells to environments that simulate *in ovulo* conditions. Partial simulation of *in ovulo* oxygen, hormone, and desiccation environments has increased numbers of somatic embryos produced by six-fold (3600 per gm of callus) and have increased germinability of somatic embryos from 10 % to 40 % (1, 60).

Synthetic seed technology may be perfected by the time a lunar CELSS is constructed (approx. 2015). This technology will probably involve 3 to 4 stages. The first stage will occur in suspension culture where embryogenic cells will be mass produced. By definition such cells are capable of immediately beginning to form somatic embryos if exposed to appropriate conditions. However, during the first stage, conditions will remain inappropriate for both embryo formation and for multiplication of nonembryogenic cells.

Proliferation of embryogenic cells will be instantaneously terminated in the second stage. This will be followed by a synchronous initiation of embryogenesis. Conditions appropriate for embryo initiation and early formation may not be satisfactory for

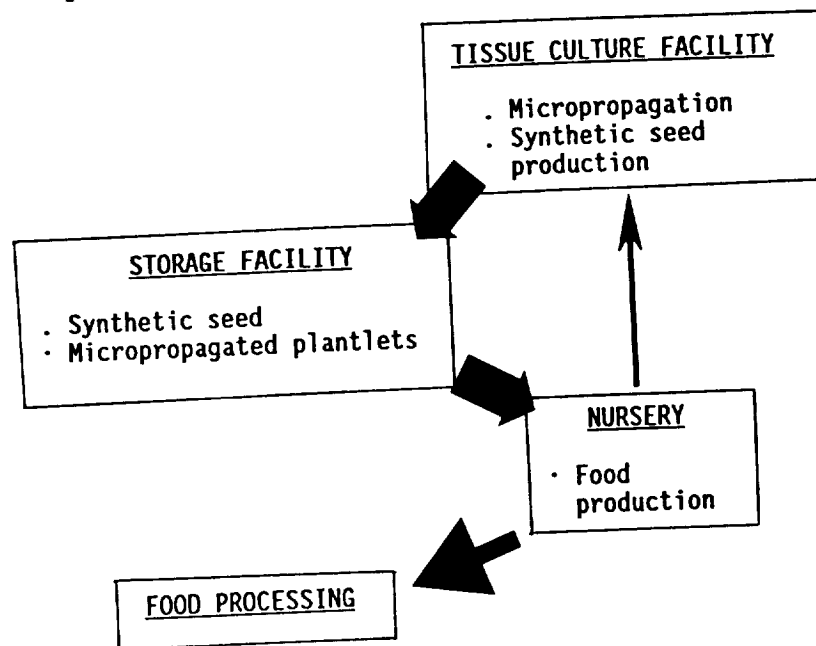
embryo maturation (1, 60, 61, 62). Thus a third stage, for embryo maturation and desiccation, will probably be required. Somatic embryos of albuminous species (food reserves of seeds associated with endosperm) will require encapsulation with an artificial endosperm (53, 54). Encapsulation might not be required for somatic embryos of legume and various other dicotyledonous crops, where food reserves are primarily stored in the cotyledons (63).

Costs of synthetic seed: what to expect. Replacement of true seed with synthetic seed in a lunar CELSS (Fig. 2) could be cost effective. Yields would be higher because harvested material would not be used as seed (3 % to 5 % savings in yield) and hybrids (10 % to 20 % yield advantage) could be used without the mass and energy drains of conventional parental-line and hybrid seed nurseries. Furthermore this technology could be used with nearly all crops.

It is difficult to predict what the costs of synthetic seed will be 25 years from now. However the economics of synthetic seed will certainly be more attractive in a lunar CELSS than on earth. This is because production of true seed in a lunar CELSS will require supplemental lighting for from 50 % to 100 % of the entire production cycle. In contrast somatic embryogenesis requires little or no light.

On earth pure line seed of wheat is purchased for about \$ 0.01 per 1000 while hybrid seed is about twice this much. The energy cost of producing 1000 pure line wheat seed in a controlled environment with 100 % supplemental lighting is 40-fold higher, approx.

Figure 2. Seed and propagule production in a CELSS, future capabilities. By 2015 tissue culture may nearly eliminate space and energy requirements for conventional production of seeds and propagules. Vegetative propagules and synthetic hybrid seed (of numerous crops) would be produced through the upright loop, nursery to tissue culture facility to storage facility to nursery.



\$ 0.40 (calculations assume a \$ 0.05 per KWH energy cost and current production levels at Utah State University, 64). In our laboratory somatic embryos of wheat are produced in the dark at ambient temperatures with a media cost per 1000 viable embryos of \$ 0.12 (calculated from production and germination data in 1). Energy costs are negligible.

If the costs of media and energy rise proportionately when produced in a CELSS, then true seed will remain approx. 4x more expensive. Furthermore production of 1000 viable somatic embryos requires about 75 cm³. The area required to produce 1000 true seed

in a controlled environment is about 200-fold greater, or 15,000 cm³. Clearly if somatic embryogenesis can be perfected and automated, then substantial savings in energy and space should be achievable. Automated systems of micropropagating potato, yam, sweet potato, asparagus and others (Table 1) may also be cost effective in terms of the mass and energy constraints of a lunar or martian CELSS.

Our cost analysis of wheat synthetic seed assumes use of current somatic embryogenesis technology, which is far from optimal. In our system callus is produced on semi-solid medium and nearly 50 % is nonembryogenic. Another problem is a structural interconnection of embryos that reduces germination frequencies and requires that plantlets be separated manually. Our goal is to produce fine suspensions of uniformly-embryogenic cells that will synchronously form singular somatic embryos. Such a system is being approached with carrot (11) where the cost of media per 1000 somatic embryos is approx. \$ 0.01 (based on 5000 somatic embryos per ml packed cells, a 5:1 ratio of embryogenic suspension to packed cells, and a 40:1 ratio of embryo induction medium to packed cells). Development of such a system for wheat could reduce media costs per 1000 somatic embryos to about \$ 0.02.

CONCLUSIONS AND RECOMMENDATIONS

Micropropagation systems are becoming more cost effective than conventional propagation systems, particularly for certain vegetable and fruit crops and for male-sterile, gynoeocious or polyploid parental lines used to produce hybrid seed (Table 1). In a lunar

CELSS conventional propagation will require high light intensities from artificial lights. In contrast micropropagation requires low intensities and can be accomplished in a much smaller area. Such variables could make automated systems of micropropagation attractive for numerous crops.

By the time a lunar CELSS is constructed (approx. 2015), private industry may have replaced many micropropagation and true seed systems with synthetic seed, particularly for high cash-value crops. It is doubtful that private industry will apply these innovations to major field crops, where the cost of natural seed is extremely low. However energy and mass limitations in a lunar CELSS may present a very different scenario. Advantages of producing synthetic seed of wheat over true seed in a CELSS could include a reduction in cost of as high as a 95 %, a reduction in required space of as high as 99.5 %, yield increases of 3 % to 5 % due to harvested seed not being used in the sowing of subsequent crops, and yield increases of 10 % to 20 % due to the use of hybrids. Both micropropagation and synthetic seed technology should receive further investigation in terms of providing mass and energy savings in a future CELSS.

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THE CONVERSION OF LIGNOCELLULOSICS TO FERMENTABLE SUGARS: A
SURVEY OF CURRENT RESEARCH AND APPLICATION TO CELSS

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ABSTRACT

One of the anticipated uses of a bioreactor in CELSS will be to process the waste streams from plant growth chambers that are rich in non-digestible lignocellulosics. This report provides an overview of the options for converting lignocellulosics into fermentable sugars as applied to CELSS. A requirement for pretreatment is shown as well as the many available options. The available physical-chemical and enzymatic hydrolysis processes for producing fermentable sugars (saccharification) are discussed. At present physical/chemical methods are the simplest and best characterized options but enzymatic processes will likely be the method of choice in the future. The use of pentose sugars by microorganisms to produce edibles at levels comparable to conventional plants is shown. The possible use of mycelial food production on pretreated but not hydrolyzed lignocellulosics is also presented. Simple trade off analyses among some of the many possible biological pathways to regeneration of waste lignocellulosics is undertaken. Comparisons with complete oxidation processes are made. It is suggested that the NASA Life Sciences CELSS program maintain relationships with other government agencies involved in lignocellulosic conversions and utilize their expertise when the actual need for such conversion technology arises rather than develop this expertise within NASA.

I. INTRODUCTION

The conversion of cellulose to sugars which are usable directly as food or as chemical reagents in a Controlled Ecological Life Support System (CELSS) is a subject that has been discussed for several years. This concept provides an alternative to the oxidation of all non-edible, fixed carbon from food production processes to carbon dioxide and water. Neither option has clearly been developed relative to the tradeoffs between energy consumption, mass balances and CELSS integration issues. Certainly one of the major reasons that the conversion alternative has not been addressed in any systematic fashion is that the possibilities and challenges associated with this approach have not been examined in a CELSS context. It is the objective of this report to provide a survey of the current research into the conversion of lignocellulosics to usable sugars and to provide an assessment of the application of these technologies in a CELSS. For the purposes of this report, usable sugars are defined as fermentable sugars or sugar process streams containing monosaccharides.

It is not possible to model an actual conversion process since the exact make-up of the waste material is not yet well defined. In this report, we used the conversion of the residue from wheat as a general model for the entire process. If data is not available for this source of biomass, other sources such as municipal solid wastes, cornstover, etc. were used.

The major rationale for examining the alternative of converting lignocellulosics rather than burning them results from the need to "make every photon count" in the confines of a bioregenerative life support system such as CELSS. The overall

process is the conversion of CO₂ to biomass, fixation into edibles by biological sources and the eventual oxidation of fixed carbon back into CO₂ and water. The mass flow can be diverted (essentially held up) by converting polymeric material into usable, high-energy sugars. Thus the questions that need to be examined to evaluate these kinds of alternative pathways in CELSS include the following. What can microbes and/or enzymes do in a CELSS? What can be done with the microbial product? What percentage of the available lignocellulosics can be made available to food processing streams? What are the trade-offs between biological conversions and physico-chemical processes? This survey will provide data and information about these questions and provide some conclusions and recommendations for future CELSS research.

A general, alternative pathway to processing lignocellulosics is depicted in Figure 1. Each area in the flow diagram of Figure 1 will be discussed in this report. Progress and options in these areas will be presented and their applicability to CELSS discussed. The major research effort in the U.S. for the conversion of lignocellulosics to monomeric components suitable for fermentation has been provided by the Department of Energy at research institutions, national laboratories and universities. Information was obtained from the literature, private conversations with researchers in the field and from attendance at appropriate meetings and the abstracts from these meetings.

One of the forums for research in this area has been an annual symposium on the biotechnology for fuels and chemicals sponsored by Oak Ridge National Lab and the DOE Energy Conversion and Utilization Technologies Program. At the tenth annual

meeting held in May of 1988, an open discussion of the progress in the field of conversion of cellulose to fuels and chemicals was held. Figure 2 outlines some of the highlights of that discussion. Relative to CELSS, it is obvious that many of the concerns of this body of research are applicable to CELSS waste treatment processing. It will be useful for the NASA CELSS program to keep abreast of developments in this field of research since advances here will be applicable to CELSS problems.

II. COMPOSITION OF CELSS BIOMASS AND ASSOCIATED CHALLENGES

The fixation of carbon dioxide into edible biomass will most likely be performed by typical agronomical species. A set of candidate crops has been defined and includes wheat, potatoes, lettuce, soybeans, and others (1). In every case, the dry weight mass ratio of edible to non-edible material is about 0.5 (harvest index). Even in the highly controlled and confined growth chambers, harvest indices are on the order of 0.5. The composition of these inedible portions is comprised of cellulose, hemicellulose, lignin, crude protein and ash. An example of these compositions is provided in Table 1. The data shows values from wheat cultivars used in actual intensive growth chambers to analyze possible CELSS candidate food crops and on typical wheat straw purchased at a local feed store. The values are very similar except for the lignin content. This value is often variable because of the various methods used to analyze the data and most methods underestimate the actual lignin content because often relatively severe methods are required to isolate the lignin which also degrades this material. More comprehensive explanations of common and accurate analytical techniques can be found in the literature (2). One of the better methods for use

in comparing lignin content is the so-called Klason lignin value. This is derived by dissolving the biomass in 72% sulfuric acid. Any undissolved residue is considered lignin. Values can vary widely as is shown in Table 1 and are a function of method. Note that in the Van Soest data (3), the values are lower than that given by Klason or spectrophotometric data. Other data give values for lignin in straws that more closely approximates the Klason and spectrophotometric data given in the literature. It is known that annual plants do not usually have high lignin contents because they have no need to provide long-term resistance to biodegradation (4). The candidate species chosen for CELSS are all annuals and thus their lignin content will likely be low.

The availability of fermentable sugars from the inedible biomass is on the order of 50-68% of the inedible dry weight. This represents a significant fraction of usable material for food and/or chemicals production. The lignin is the intractable material that must be fractionated from the biomass in order to process the remainder of the polymeric material. Its use as a chemical feedstock is of interest to bioconversion schemes (5) but is less likely to serve any good purpose in CELSS and should probably be considered as a feedstock for process heat, i.e. conversion to CO₂ and water especially since lignin possesses the highest specific heat content of biomass (12,700 BTU/lb). The remaining mineral content of the plant will eventually enter process streams and be recycled. It will be shown below that the process heat and mineral content of plants could be used to provide energy and chemicals for the conversion of inedibles to fermentable sugars.

Although there are more extensive descriptions of the

structure of a lignocellulosics such as wood and straw, the following is a brief but useful summary of such compositions. "Cellulose, hemicellulose, and lignin are found within the wood cell wall... In general, cellulose fibrils are surrounded by hemicellulose, which is embedded in a lignin matrix. Crystalline cellulose is viewed as the reinforcing framework, sort of the steel rods, of the cell walls. The hemicelluloses and lignins support the fibrils." A more detailed description of these components helps to clarify the complexity of plant cells.

"Cellulose is a single polysaccharide. It's a polymer of...glucose. A single, unbranched cellulose molecule, about 3-5 μm in length, comprised of about 7,000 to 12,000 glucose units. Hydrogen bonding occurs between hydroxy groups within the sugar sequence, and also among adjacent cellulose molecules that band together along their long axes to form bundles called elementary fibrils. Glucose molecules in fibrils are regularly aligned; about 70% of the structures are crystalline. Cellulose is the chief structural polysaccharide of plant cells...

"The next major constituent...are the hemicelluloses... Hemicelluloses, comprised mainly of D-glucose, D-mannose, L-arabinose, and D-xylose, have molecular chains that are often branched. There are about 100 to 200 sugar residues per molecule. Hemicelluloses have higher water solubilities than cellulose, are more readily hydrolyzed, and have less ordered structures.

"Lignins are amorphous, cross-linked phenolic polymers that occur uniquely in vascular plants and comprise 20-30% of most wood.' Molecular weights range from the thousands to the hundreds of thousands. Lignins are made from three cinnamyl

alcohols, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. They differ from each other in the number of methoxyl groups substituted on the benzene ring" (6).

Studies have shown that the lignin in plant material presents a real physical barrier to the effective hydrolysis of cellulose into monomeric substrates (4, 7, 8). Considerable effort has been made to produce methods and means to accomplish the clean separation of lignin from polysaccharides without degradation of the polysaccharide material. The need for two general stages of pretreatment has been suggested by research results. One stage usually involves the mechanical pretreatment of the biomass material by sizing and milling but may also include some chemical or physical processes such as acid/base and heat (usually in the form of steam) or even radiation. The next stage involves some form of fractionation of the lignin from the polysaccharides. It has repeatedly been shown that both steps are necessary for the effective extraction of monomeric sugars from the biomass material. It is possible to circumvent the second step through the direct growth of fungal biomass on biomass ground into small particles and this option will be discussed below. However, the more typical processes involve both steps. The delineation between the stage one and stage two pretreatments is not always distinct and more often these two stages are combined. In this report they are discussed under two separate headings but note that many second stage treatments are really combinations of both stage one and two pretreatments and that first stage pretreatments often employ some type of hydrolysis.

III. FIRST STAGE PRETREATMENT

The process of rendering raw biomass suitable for further

pretreatment and enzymatic or microbial hydrolysis usually involves mechanically sizing or thermally disrupting and fractionating the raw material. Chemical treatments are often coupled with these processes or used as the major source of pretreatment. In many cases, these chemicals are added to assist in simultaneously fractionating and hydrolyzing the lignocellulosics. Figures 3 and 4 show simple flow diagrams for pretreatment and hydrolysis for lignocellulosics.

A. Mechanical

Methods have been proposed and studied for the preparation of wheat straw for enzymatic hydrolysis (9). Mechanical grinding permits positive temperature control, wet and dry processing, no chemical cleanup or recycle and is relatively simple to operate. This comminution technique has only recently been applied to pretreatment of lignocellulosics and may include the use of shredding, knife mills, hammer mills, disk refining and vibratory rod milling. Another similar treatment is agitation bead milling. These methods are commonly used in the food and pharmaceutical industries to obtain fine grinds and dispersions. Results from published work (7, 10) showed that only a two fold increase in enzymatic hydrolysis resulted from such pretreatment methods and that fermentability was a function of particle size. Others observed increased ethanol production using such pretreated material indicating increased enzyme susceptibility (11). These methods all require relatively high power consumption and replacement of mechanical parts both of which are drawbacks in a CELSS context.

B. Thermochemical pulping

The use of steam at moderate temperatures ($>160^{\circ}$ C and 100

psig) solubilizes cellulose and delignifies lignocellulosics. Combined with the use of caustic this method is widely employed to treat lignocellulosics by the pulp and paper industry. These methods work although they can be energy intensive. Efforts to employ variations of thermochemical methods for pretreatment of lignocellulosics for ethanol production have been conducted and are known as autohydrolysis, and steam explosion. Some studies found thermochemical processes the most workable in pretreating municipal solid waste (MSW) (11). In all cases the chemistry is simple and hydrolysis occurs due to mild, acid-catalyzed reactions which cleave glycosidic bonds and acetate groups in hemi-celluloses; cleave, to varying degrees, the glycosidic linkages in cellulose; and hydrolyze the α -ether linkages in lignin. Autohydrolysis and rapid steam hydrolysis are similar processes. Here temperatures are in the 220 to 275 °C range but residence times are short. The process fractionates the lignocellulosics while steam explosion does not. Some reports of 95% glucose recovery (of available glucose) and 70% pentose recovery (of available pentoses) using an 8% slurry, 100 sec residence times and 240°C have been given (12). At 2% substrate levels, the yields were quantitative. Others have reported similar results (13, 14) and generally claim that this type of process improves enzymatic hydrolysis capability of the thermally treated material and depolymerizes cellulose and hemi-cellulose. Lignin is not affected to any great degree. An example of a variation in this method employed steam heating of lignocellulosics at 200 °C for 15 min followed by acid hydrolysis at pH 1.0 for 4 hours at 100 °C. The result was very fermentable xylose fractions. The hydrolysates were only suitable for

fermentation when the xylose concentration was <30 g/L probably due to the several inhibitors of fermentation that were also produced including acetic acid, lignin monomers, furfural and other volatible fatty acids (15). Additionally, in actual operation, acid hydrolysis processes can produce metal ions and often produce increases in concentration of sugars but not in yield (16). Steam Explosion - In this process, lignocellulosics are superheated and extruded which causes tremendous disruption of the fibrous material when the pressure changes. The cellulose is rendered more enzymatically susceptible to hydrolysis by this procedure although about 10% is lost in the process (17). The process tends to disrupt the amorphous regions of the cellulose and increase surface area although the crystallinity of the material is not greatly disrupted. Substrate concentrations on the order of 6% solids are generally used. Considerable work has been conducted to examine the best methods to enzymatically treat steam exploded wood and recover the enzyme. This will be discussed further below.

The energy intensive requirements of thermochemical approaches are not conducive to CELSS, but overall these processes are simple, workable, and some are relatively mature technologies. The possible applications in a CELSS should probably be more closely examined with emphasis on trade-off analyses for energy and weight compared to the weight of cellulosic conversion systems.

C. Chemical pretreatment

Agents such as sodium hydroxide, ammonia, chorite, sulfur dioxide, amines and acid (both dilute and concentrated) have been proposed and used. In the forest products industry, the use of

alkaline pretreatments prevails. Other solvating agents known to solubilize cellulose include iron/tartrate, cadmium or copper and ethylene diamine and others (7, 11). Chemical pretreatments were not considered good candidates for treating MSW largely due to the unknown effect of chemicals and solvating agents on the diverse composition of MSW (11).

An example of a chemical pretreatment process using high acid concentrations illustrates the difficulties in using such processes. Acid (20% HCL or 60% H₂SO₄) treatment of lignocellulosics produces a very good product that is free from many of the side products resulting from thermochemical or mild acid hydrolysis processes. Electrodialysis has been proposed as a method to recover the sulfuric acid (18). An initial calculation showed that it would require 1.1 faraday to move one mole of H₂SO₄ across a membrane and that current efficiencies of 0.26 are required to make the process feasible. Another major expense is the cost of membranes. Commercially, the cost of membranes is roughly equal to the entire capital cost of the rest of the system. These membranes must be able to withstand 20% HCL and 60% H₂SO₄.

In biomass conversion technologies the arguments for acid treatments always must address the main drawback to this approach: the high cost of recycle necessary to make this pretreatment cost effective. There are additional problems which are of minor economic importance in the commercial arena but which may be major factors in a CELSS. These include equipment maintenance, storage of residual acid, and the production of fermentation inhibitors (which presumably would be unsuitable for both fermentation and inclusion into food items). The use of

these agents presents another problem - the toxicity of agents (containing elements such as cadmium) that are used to process biomass that eventually becomes food. Technically for chemical pretreatments, the major problems for CELSS would be the recovery and recycle of acids, bases and other chemicals, the durability and replacement of operating and storage vessels, and the environmental containment of reagents. If chemical pretreatment processes were deemed useful or possible in CELSS, it is at least clear where the developmental work is required. Such definition is not as obvious for the less well understood enzymatic and other pretreatment processes.

D. Electron Irradiation

This method has been used to render cellulose more susceptible to hydrolysis. Emert and co-workers treated MSW with 10-100 Mrad of a 100 kW electron beam accelerator (11). Alcohol production decreased with increased irradiation. The complex effects of radiation on a diverse material leading to unknown products and reactions were considered to be the cause of this poor result. A major drawback for CELSS applications would be the power requirement. With increased understanding of the effects of space radiation on the more homogeneous waste streams in CELSS this method may be useful. However, considerable research would be required since even the effect of radiation on simple tissues is incompletely understood and lignocellulosic waste streams are much more complex.

IV. DIRECT USE OF FIRST STAGE PRETREATED LIGNOCELLULOSICS AS SUBSTRATES FOR MICROBIAL GROWTH

The direct use of only moderately pretreated biomass as substrates for fungal growth has actually been employed in

commercial processes. Many organisms possess hemi-cellulases and efforts have been made to isolate such organisms and use them to directly degrade ligno-cellulosics (19). Fungal species are usually able to attack the cellulose complex while simpler eucaryotes and procaryotes require solubilized materials. Two examples, both from Canadian sources, illustrate the use of the fungal process.

A. Mushroom production

An integrated process using the fungus, Pleurotus sajor-caju growing on corn stover in submerged cultural conditions has been proposed (20). Some moderate pretreatment is required and involves base hydrolysis of the corn stover to solubilize hemi-celluloses and some lignin. The species is also known to fix nitrogen although initial experiments did not exhibit this property. The product produces 40-45% protein of moderate quality (i.e., low in sulfur amino acids). Additional research is being carried out to move this process towards commercialization and including fuel production as an added bonus.

B. Waterloo SCP I & II Processes

Early work by Canadian researchers demonstrated that from ground, milled and thermally/chemically pretreated (hot water and/or alkali) biomass such as cornstover, straw, or bagasse a semi-solid substrate could be provided for cellulolytic fungi (21). The fungi could be grown to high density under relatively simple conditions. Original efforts concentrated on the fungus Chaetomium cellulolyticum but have progressed to a type II process using Neurospora sitophila (22). This was done because work with the former strain showed that unless growth conditions were carefully controlled, mycotoxins were formed (as was also

the case with another fungal food, Fusarium graminearum often suggested as a source of SCP - single cell protein).

These candidate processes are attractive to CELSS food production largely because of their inherent simplicity. Few pretreatment requirements, simple growth requirements and a safe, "mushroomy" type product that is more acceptable as food provide advantages in a CELSS system. The negatives include high energy requirements for mixing and harvesting mycelial mats, low growth rates and mycotoxin production if the wrong strains are used. However, the growth rates are still higher than plants. These processes also claim to produce BOD (biological oxygen demand) free water which would be an advantage in CELSS.

Twelve years ago, a survey of the existing SCP production facilities world wide was made. This was at the height of the interest in SCP. There were several processes either under development or in production phase that employed the following types of biomass substrates: whey, sulfite liquor, coconut, cellulose, and wood pulp (23). Few of these remain today because of the poor economics however their use suggests that direct utilization of CELSS waste streams is possible.

V. SECOND STAGE PRETREATMENT

Pretreatments that are specifically designed to disrupt lignocellulosics, fractionate components and carry out simultaneously the hydrolysis of the cellulose comprise what can be called second-stage pretreatment. Figure 4 depicts the two generally accepted methods for hydrolyzing lignocellulosics. Since most of the effort in this field has been carried out by laboratories interested in fuels and chemicals, it must be remembered that most efforts are evaluated relative to the

fermentability of the resultant "pretreated biomass." Although fuels and chemicals are not our primary objective, the fact that the polymeric sugars are rendered fermentable meets the CELSS criterion for usable sugars. The main thrust of current efforts in this field of research involve acid and/or enzymatic hydrolysis. Research and development into base hydrolysis and solvent assisted base or acid catalysis has also been conducted. As noted above, the caustic treatment of wood is an old technology for the paper and pulp industry and was the basis for the advanced studies being carried out today. There is a significant body of literature and experience that describes these issues and this report will make no attempt to comprehensively examine them. This report will highlight the main features of the processes and the reader may consult the references for further details.

A. Mechanical/Acid Catalyzed

Acid hydrolysis of cellulose is an old technology that is relatively well understood. Integration of mechanically pretreated biomass into reactors and the efficient recovery of the product are major areas of research (5). Although in DOE Biochemical conversion program and its research teams and contractors do not consider all aspects of lignocellulosic conversion research they certainly address most of the major issues in these bioconversion processes. The following are short summaries of some of the major findings in this DOE sponsored program.

- o The size of the pretreated material is crucial in yielding efficient acid hydrolysis conversion.
- o Incorporation of xylose fermentation improves overall

process kinetics.

- o Concentrated acid processes produce high yields (up to 100%) but acid consumption and recovery are expensive.
- o Dilute acid hydrolysis processes exhibit sugar degradation problems and hence lower yields. Research into understanding this phenomenon is underway as well as developing efficient reactor designs.
- o Removal of by-products inhibitory to fermentation may be necessary to overall process economics (The economics may not be of concern to CELSS but the presence of undesirable by-products will be).
- o Methods to improve process economics include use of resins, solvent extraction and adsorption to isolate the product from the process streams.

1. Example of one process

The following is a more detailed description of one kind of acid hydrolysis process and illustrates some of its features. A correlation between mechanical pretreatment and subsequent hydrolysis of cellulose was examined. Coarse shredding of wheat straw was accomplished with hammer-type shredders followed by further comminution with rotary knife mills, hammer mills or laboratory knife mills. The water soaked material (3%) was then fed into a disk refiner and this output was examined for response to acid and enzyme hydrolysis. Breakage of the lignin-hemicellulose-cellulose barrier and subsequent accessibility of the cellulose to enzyme and acid hydrolysis was a primary concern. The data revealed that mechanical treatments could increase digestability only about two fold even under extended treatments but that chemical pretreatments produced dramatic

effects even at rather mild conditions (95°C). They showed that the lignin-hemicellulose-cellulose complex could be completely broken and that the removal of residual lignin was required for successful enzyme hydrolysis. It did appear that removal of hemi-cellulose was required and necessary for good digestability. Acid-detergent pretreatments were not sufficiently better to warrant further use (10, 24).

Additional work has led to a better description of the kinetics of hemicellulose degradation (24, 25). Here it was found that at low temperatures, 95°C, complete xylan degradation could occur if long incubation times, 1-2 days, were used. Further results were obtained which strengthened the relationship between hemi-cellulose removal and cellulose digestability. In addition, the use of other more vigorous pretreatment methods such as explosive decompression appear to increase surface area of the biomass but are not prerequisites for high enzyme digestability. Dilute acid pretreatments could also be carried out successfully in slurries of 20-40 wt % solids (25). (Flow diagrams and equipment requirements are found in all of these published studies). The yield of solubilized xylose was only 70-80%. For CELSS, such yields might border on satisfactory.

Economics will not be the main driver for a CELSS waste processing subsystem and thus concentrated acid hydrolysis might be more favorable for inclusion in CELSS than dilute acid hydrolysis since the production of by-products is negligible. However, the recycling of acid may prove to be an insurmountable problem not from an economic or technical point of view, but from an environmental perspective. The other advantages and drawbacks to this process are discussed above (III.C.).

B. Mechanical/enzymatic hydrolysis

Whereas acid hydrolysis involves two basic steps to obtain fermentables - pretreatment and hydrolysis, the process of enzymatic hydrolysis must also consider enzyme production and recovery (recovery in this instance is analogous to recovery of the acid). This process is well known but not as well understood and significant amounts of research have been conducted to understand the basic chemical/biochemical reactions (5). The following list also summarizes the research efforts for DOE laboratories and is typical of the research into this topic worldwide.

- o The key to increasing digestibility of lignocellulosics appears to be directly related to increasing the cellulose surface area available to the enzymes.
- o Prehydrolysis to a) open surface pore sizes and permit more enzymatic attachments and b) remove xylans is important.
- o Steam explosion usually is not employed as a pretreatment method since it degrades hemi-cellulosics into furan compounds.
- o Staged prehydrolysis steps using acid followed by base permit the removal of hemi-cellulose and lignin which then enhances enzyme digestibility.
- o Understanding the role of feed-back inhibition by glucose and cellobiose are important aspects of improving process flow and economics.
- o Simultaneous saccharification and fermentation (SSF) of pretreated biomass is a possible route to direct utilization of lignocellulosics. For fuels production, the low rates of enzyme production make this process expensive.

However for CELSS, this would not be as much of a consideration (other considerations are discussed below).

- o Research into improving the production of cellulolytic enzymes has yielded good results and currently a few commercial companies are marketing low cost cellulolytic enzyme preparations at reasonable costs (the market here is interestingly enough the food processing industry).
- o Methods to reduce power consumption in mixing highly viscous suspensions of cellulose, lignin and yeast have shown early promise.
- o New organisms which operate at higher temperatures or that are genetically modified to improve some aspect of the process are being examined.

This area of research has recently (10 years) had a heavy investment of time and effort. Only now have some of the basic questions regarding the biochemistry of the cellulolytic hydrolysis process begun to be answered. Again the major problem in this type of hydrolysis is the recovery of the enzyme. Work in Sweden has shown that it may be possible to recycle the enzymes and efficiently obtain glucose in reasonable quantities. By washing and recirculating the undigested cellulose enzyme recovery factors of near 90% have been obtained (17). This is the minimum level of recovery at which the overall process becomes economically feasible. The actual machinery and equipment is relatively simple but would likely require a considerable amount of water and power.

C. Mechanical/base catalyzed hydrolysis

This is the method long used by the pulp and paper industry. It involves the milling of lignocellulosics and

treatmenting them with alkali (5%) at 130 to 180°C. Complete delignification occurs but the hemicellulosic fraction is rendered non-fermentable. Milder conditions, 25°C and long treatment times, result in partial delignification but no degradation of pentosans (26). This process does, however, require high chemical consumption (estimated at 5-20% w/w oven dry biomass) (10, 27). This is a low technology approach using mild conditions that does produce the desired results. This approach does not lend itself to production type processes which are favored by industry. However, it may be applicable to CELSS if sufficient quantities of caustic can be produced from the ash of oxidized lignin and unhydrolyzed biomass (i.e., from fractionated lignin used for process heat). Neutralization is another factor that must be considered for CELSS and the source of acid in a CELSS is not obvious.

D. Mechanical/base catalyzed hydrolysis with solvents

The solvent pretreatment or organosolv processes have been employed to ameliorate the negative effects of direct base catalyzed delignification of biomass material. To avoid large losses of fermentable hemicellulosic degradation products and to reduce the costs involved with large consumption of base, alcohols have been employed along with base catalysis. The hemicellulose is less soluble in alcoholic solutions and thus is not degraded.

In initial experiments, it was demonstrated that 70% of the lignin could be removed using mild alcohol-alkali-water mixtures [50% (v/v), 25°C for 72 hrs] with only 5% loss of pentosans. Base requirements were found to be 0.1g NaOH consumed per gram of cornstover for adequate lignin removal. The enzymatic hydrolysis

of the treated cornstover was increased by four-fold over the untreated cornstover. The extent of utilization of the treated material by bacterial fermentations was 87% (27).

Since these initial studies, the organic solvent method has been expanded to examine the kinetics and wider applications of this process including the use of mild acid catalyzed processes. In the case where no acid is added, the relatively high temperatures and eventual degradation of the lignocellulosics, results in production of organic acids such as acetic acid, which then catalyze the further hydrolysis of hemi-celluloses and promote delignification. In the cases where acid is added, researchers have found that the use of acid assists in rendering the cellulose more digestible by enzymes. In one study, H_3PO_4 acid at 0.02 to 0.008 M and 130 °C was found to make the cellulose nearly 100% digestible by enzymes (28).

VI. QUALITY OF OUTPUT FOR CELSS APPLICATIONS

The definition of usable sugars as fermentable sugars is easily demonstrated by those wishing to produce fuels or chemicals by microbes. Usable sugars in a "food" context requires a further definition. Many of the monosaccharides can be directly used as a food source or processed into edible components. These are largely the hexoses. The pentose sugars are not of themselves a good source of carbohydrate and must be further processed in some fashion. A typical, commercial method is to use these sugars as substrates for microbial food production. An example of such a process is yeast grown on sulfite waste liquors in paper processing plants which liquors are largely comprised of pentose sugars (23). Additionally, reports have been published which describe the actual food value

from two or three yeast strains that might be candidates for CELSS unconventional food production (29). The conversion factor or harvest index for converting monosaccharide to edible components by these candidate yeast strains was on the order of 0.5. This edible portion is comprised of about 6-20% protein and the balance in carbohydrate, usually in the form of trehalose and glycogen with some small fraction of lipid. The overall microbial "harvest index" value can be increased if only protein is desired. These results coupled with the conversion of lignocellulosics to monosaccharides show that microbial food production can serve as a useful food production and waste processing subsystem for CELSS. The mycelial or fungal food produced as described in Section IV above is very similar to that produced in mushroom production. This microbial food is easily processed into food products suitable for man (30). Its quality is similar to other microbial food with a likely deficiency in the sulfur amino acids (29).

VII. APPLICATIONS TO CELSS

The major purpose of this survey was to examine the field of biomass conversion and determine areas that might be applicable to the CELSS system. There were two aspects of this survey: 1) identifying the lignocellulosic conversion systems that are available and 2) making some evaluation as to the relevance of these conversion systems to CELSS food production and waste processing systems. The material presented above largely addressed item 1 and some of item 2. What follows are additional comments on that survey and on the applicability of the conversion alternative to CELSS.

1. The list of candidate food production species for CELSS

food production schemes is largely made up of annuals and/or plants with low lignin content. This is an advantage since this will lessen the pretreatment requirements and ease isolation of polysaccharides for hydrolysis into usable sugars. The use of low lignin plants will be advantageous from a waste processing point of view.

2. It does not appear possible to avoid any pretreatment of the biomass prior to hydrolysis. Some sort of mechanical and other (thermochemical, chemical, etc.) pretreatment processes will be necessary to render the biomass suitable for hydrolysis to the monosaccharides. This will require equipment and varying amounts of water, depending upon the process used and the possible regeneration of some chemicals such as caustic. Some of these processes are quite simple but most are usually energy intensive. Some will require additional research before they can be considered as possible candidates (electron radiation). While some processes may be very effective (e.g. concentrated acid pretreatments), they also increase the complexity of processing since environmental problems of acids, base for neutralization and possible toxic by-products such as metal ions must be considered. Trade-off analysis are clearly necessary but will not be useful until candidate systems are chosen and designs are implemented. From an engineering point of view in systems where pressurized vessels are employed, the cost of building such systems rises geometrically with the increase in pressure. It should be noted that in the Soviet Union a large industry is in place that utilizes acid-hydrolyzed biomass for animal feed, although not for CELSS type applications (Grohmann, personal communication).

3. The hydrolysis of lignocellulosic fractions can occur with any one of the following methods acid, base, enzymatic and organosolvents after the lignin has been fractionated out of the lignocellulose. This fractionation step is a prerequisite to full recovery of usable sugars from the lignocellulosic materials. The hemi-cellulosic fractions are generally quickly solubilized followed by the slower hydrolysis of the cellulosic fractions. To avoid the attendant problems of using acids and acid leachate products and the environmental problems of solvents, the base-catalyzed hydrolysis or enzymatic hydrolysis procedures appear to be best suited for CELSS.

Some other aspects of these hydrolysis processes are important to consider in evaluating the best choice for CELSS. The base catalyzed or enzymatic processes require lots of water and power for mixing. If water becomes limiting, then acid hydrolysis is actually less water intensive. Acid hydrolysis using 20-30% slurries and acid hydrolysis giving 10% sugar solutions have been reported (25). In all of these processes, cellobiose is produced and acts as an inhibitor and its conversion to glucose must be considered. In the area of enzymatic hydrolysis, it is not possible to immobilize these enzymes since they are acting on a solid substrate, i.e. solid-solid reactions don't readily occur. Some suggestions have been made to employ simultaneous saccharification and fermentation (SSF processes). Commercially, this is one area of interest in the alcohol fuels area for it proposes to eliminate some problems in multi-step processing. In many ways the Waterloo Process is analogous to this approach. However, in the production of usable sugars no consideration has been given to finding a strain of

microbial species that is both cellulolytic and edible. Thus, a strain would have to be found that meets the following initial criteria: it produces cellulases and hemi-cellulases and no toxins. In addition, it will probably have to meet some minimum production requirements such as tolerance to high concentrations of sugars and alcohols, etc. Again, trade-off analyses will be required when the CELSS system is better defined.

The use of base catalyzed hydrolysis is low technology but is effective and caustic may be easier to deal with than acid from an environmental perspective. Enzymatic processes are environmentally suited for hydrolyzing cellulosic fractions. Enzymatic processes have not reached their full potential largely due to the lack of understanding of the basic chemistry and biochemistry of these systems. As this research area matures, it is expected that it will become more suitable for CELSS applications.

4. The use of fungal decomposition, fractionation hydrolysis and production of single-cell food is a low technology process that is very attractive. Some pretreatment is necessary, but grinding, hot water and some caustic can usually render the biomass suitable for fungal growth. This presumes that the end product-fungal food-is the desired output rather than glucose which could then be processed into food items. There are some problems with power (for mixing) and some water requirements but they would be small compared to other processes. The main consideration to be made here is the trade-off on the output of the waste processing stream. Is it to be glucose or a microbial food product?

5. With DOE supporting this work, it would appear that the

CELSS program will be the beneficiary of results supported by another government agency. This will reduce the cost to NASA. However, it would be necessary to keep abreast of the developments by DOE researchers and determine how new advancements might aid CELSS researchers. When design scenarios for CELSS begin to be implemented, it would be prudent for NASA to involve DOE R&D people rather than try and develop all the expertise for waste processing of lignocellulosics themselves.

VIII. TRADE-OFF ANALYSES AND CONCLUSIONS

The accompanying analysis is a compendium of "napkin" calculations trying to relate the quantity of energy necessary to make food verses that which is necessary to decompose it. As a necessity there are many assumptions that one needs to rely on in order to carry out such an analysis. Even though the results obtained here are only approximations, they do offer some insight into the complex trade-offs that will be necessary in developing a CELSS model system. Most of the values cited here come from two NASA publications: NASA Conference Publication 2480 (31) and NASA Contractor Report 177422 (32) and a published report (33).

The principles in this analysis are the food source wheat and the inedible waste lignin. Given the best of all worlds 100 g (426 Kcal) of wheat (all values are for dried material) could give, if properly processed, 84 g (314 Kcal) of edible material and 16 g (112 Kcal) of inedible lignin. From this it can be calculated that the lignin comprises about 16% of the weight and contains 26% of the original energy (31). Unfortunately it is difficult to interpret the magnitude of these values within the CELSS system unless these can be related to some other parameter. This leads use to the second stage of analysis.

In order to start, we'll assume that the lignin produced by the plant needs to be recycled. To do so, we use the process of wet oxidation. Analysis of this process (32) shows that the process requires 1.3 Kw of power (303 w electricity + 275 btu/h heat) to process a waste mixture of 10% feces/90% urine at 330 cc/h. If 10% of the slurry is feces then in one hour we would process 33 g giving a final energy cost of 0.039 Kw/g/h. If we assume the composition, by mass, of feces and lignin to be the same then the 16g of lignin waste would require 0.624 Kw/h or 536 Kcal. Remember the original lignin has trapped only 112 Kcal of energy. Thus you need almost 5 times the amount of energy to get rid of lignin by wet oxidation than what it originally contained.

Another way to look at the problem is to see if one can relate the amount of energy involved in both making and disposing of lignin in some other terms (32). What we chose was surface area necessary to grow the food source in the first place. In order to supply the daily energy requirement for an astronaut (2800 Kcal/d), one would need 680 g of seed/d of wheat seed. A typical cultivator gives 15 g of seed/m²/d. In order to produce the 680 g/d, 45 m² of surface area would be required. If light for photosynthesis is generated by electricity, then 27 Kw of electricity would be required per day (0.6 Kw/m² x 45 m²). Now let's transgress a little. If a harvest index of 0.45 is chosen for wheat, then the 680 g/d of seed comes from 1511 g of wheat. Sixteen percent or 242 g of the wheat is lignin which amounts to approximately 1694 Kcal or 26% of the wheat energy content. 26% of the wheat energy is in lignin or 7 Kw or 11.7 m² of surface area (7 Kw x 0.6 Kw/m²). It takes by wet oxidation about 5 times the energy to break lignin down than it takes to make it,

therefore wet oxidation would take 35 Kw of photosynthetic energy or 58 m² of growing surface area. The total area for lignin both to make and break would be about 70 m² or about 1.5 times the original area for cultivation.

In conclusion, the above mentioned analysis suggests that the amount of energy necessary for growth and disposal of lignin is significant. By using this type of analysis, it might be possible to get a better understanding of the trade-offs that will be necessary in order to make CELSS successful. It is also possible that this type of analysis might be helpful in describing areas of future work and thus help direct the overall effort.

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Table 1 Composition of Wheat cultivars (straw, roots, stems, leaves, etc.) and other Selected Plant Materials

Source (reference)	Crude Protein	Hemi-Cellulose	Cellulose	Klason or Spectrop.	Lignin	Van Hoest
All values are shown as a % of the source material only						
Yecora Rojo (3)						
Overall	—	—	—	15.7	—	7.7
Stems	6.6	22.9	32.6	—	—	5.7
Leaves	11.8	27.2	25.8	—	—	3.7
Roots	—	43.7	25.2	—	—	4.3
Veery 10 (3)						
Stems	3.7	22.8	35.0	—	—	7.3
Leaves	9.0	24.0	26.9	—	—	3.6
Wheat Straw (10,24)	—	24.8	42.7	18	—	—
Wheat Straw (2)	—	—	—	13.9	—	—
Oat Straw (2)	—	—	—	14-22	—	—
Rice Hulls (2)	—	—	—	40	—	—

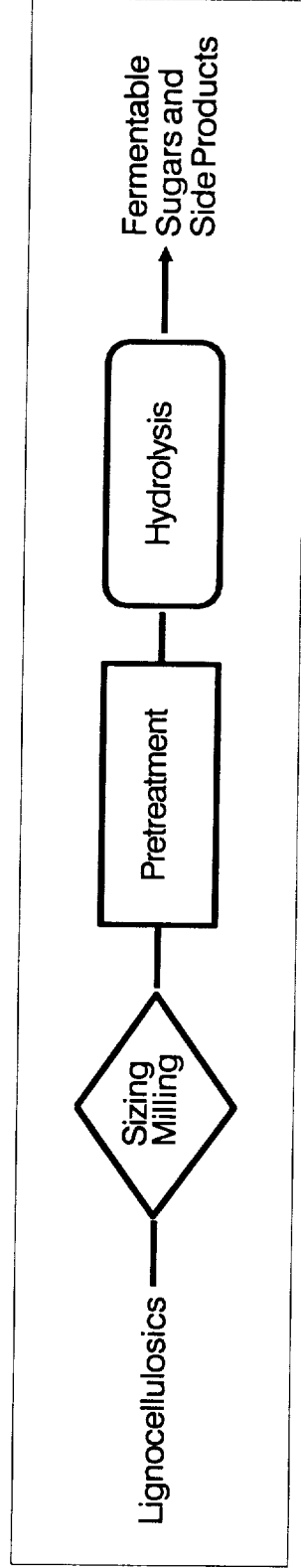


Figure 1 . Methods for the conversion of lignocellulosics to usable sugars
all involve the same basic steps

The most important processes that are under investigation are as follows:

1. Pretreatment of raw materials
2. Making cellulolytic and other enzymes
3. Hydrolyzing cellulose to glucose
4. Fermentation of xylose to alcohols

Separating hydrolysis and fermentation is favored because it gives more control in the mixing and matching of enzymes

Most research in the study of enzymatic processes has been done on the enzyme from the fungus, *Trichoderma*

Most fermentation studies have involved yeasts because of their consistent ability to ferment sugars at high efficiencies.

Areas needing more study:

- Better biological catalysts and better biological reactors (design and operational concerns)
- Developing a standardized measure of digestability

Figure 2. Conversion of Cellulosics to Sugars: Overview of Current Research and Challenges

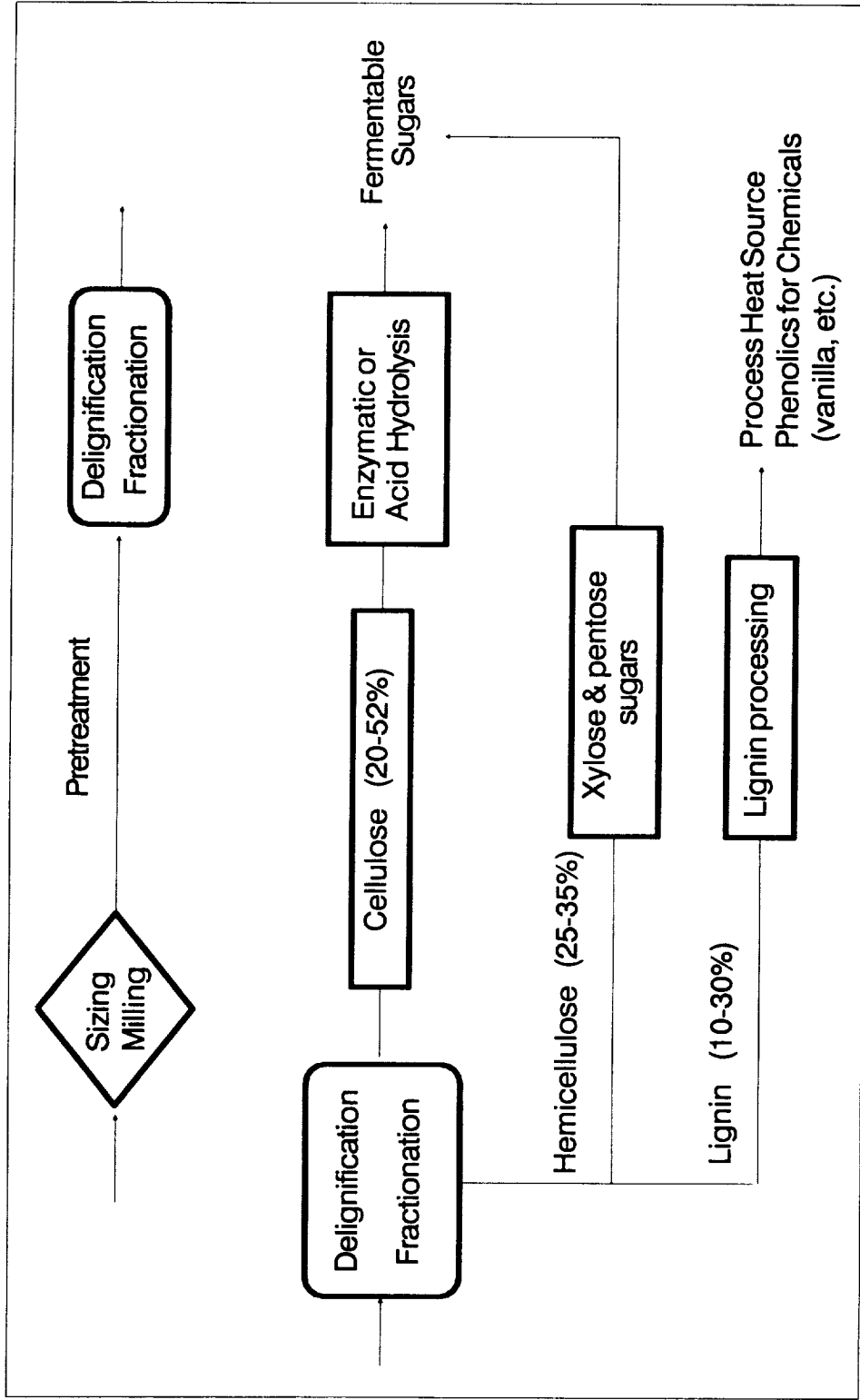
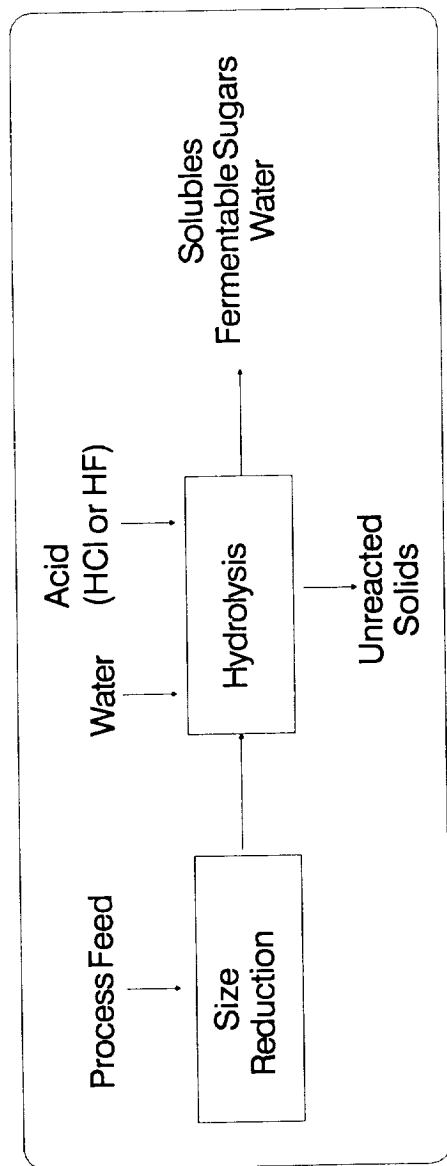


Figure 3: Pretreatment and pretreatment with fractionation

Acid Hydrolysis Processing



Fungal Enzyme Hydrolysis Processes

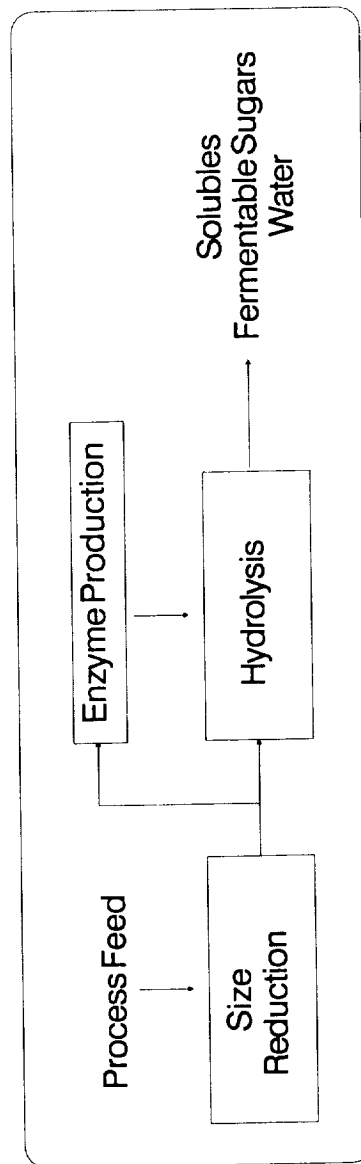


Figure 4. Hydrolysis of Sized, Milled and Pretreated Lignocellulosics



USE OF INEDIBLE WHEAT RESIDUES FROM
THE KSC-CELSS BREADBOARD FACILITY FOR
PRODUCTION OF FUNGAL CELLULASE

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ABSTRACT

Cellulose and xylan (a hemicellulose) comprise 50% of inedible wheat residue (which is 60% of total wheat biomass) produced in the KSC-CELSS Breadboard Biomass Production Chamber (BPC). These polysaccharides can be converted by enzymatic hydrolysis into useful monosaccharides, thus maximizing the use of BPC volume and energy, and minimizing waste material to be treated. The evaluation of CELSS-derived wheat residues for production of cellulase enzyme complex by *Trichoderma reesei* and supplemental β -glucosidase by *Aspergillus phoenicis* is in progress. A cellulase hyperproducing strain of *T. reesei* and *A. phoenicis* were grown in monoculture vs. coculture with different substrates--alpha cellulose and starch{controls}, wheat residue, and residue treated to remove lignin and hemicellulose. Enzyme activities were measured by standard filter paper and β -glucosidase assays and by hydrolysis (1 to 4 days) of CELSS wheat straw. When compared to *T. reesei* monoculture, coculture of *A. phoenicis* with *T. reesei* resulted in increased levels of β -glucosidase (0.27 vs 0.94 IU/mL) and filter paper activity (0.09 vs 0.13) but did not significantly alter the amount of glucose produced (21 g/L) during 48 hr wheat residue hydrolysis. Current monoculture studies suggest that β -glucosidase production by *A. phoenicis* is higher on treated or untreated residue (~0.6 IU/mL) than on starch (0.13 IU/mL), but cellulase production by *T. reesei* is lower on residue (0.03 to 0.08 IU/mL) than on alpha cellulose (0.13 IU/mL). Separate preliminary bench scale studies have examined conditions for enzymatic hydrolysis of wheat residue cellulose and identified optimal enzyme loading rates (3 to 5 IU of *T. reesei* cellulase per g of CELSS wheat residue) and substrate concentration (5% w/v wheat residue) to yield high residue cellulose conversion efficiencies (up to 70%) and high product glucose concentrations (up to 23 g/L).

INTRODUCTION

The composition of wheat grown under the controlled conditions of the KSC-CELSS Breadboard facility Biomass Production Chamber (BPC) is shown in Figure 1. Edible biomass, e.g., harvest index, is typically 40%, with the remaining

60% of little nutritional value to humans. Nearly 35% of this inedible residue (or 21 % of total biomass) can be leached from the biomass by a simple, 4 hr water extraction (25°C), with soluble organics and inorganics both removed by this treatment. The characterization of these extracts and the potential to recycle the inorganics and further utilize the organics in a CELSS is another topic of current research at KSC. Preliminary results of this research were presented at the 1988 Annual Meeting of the American Society of Gravitational and Space Biology and, thus, will not be included in this paper.

The water-insoluble, particulate matter of the wheat residue is predominantly cellulose, a polysaccharide containing glucose subunits, and hemicellulose, which in wheat is mostly xylan containing xylose subunits and side chains of arabinose and glucuronic acid. Although these polysaccharides are not of direct use by humans, they can be readily hydrolyzed into monosaccharides. The sugar produced may be consumed directly by the crew or be incorporated into edible biomass by yeasts or fungi (See introductory paper by Knott concerning the ongoing research at KSC, this publication).

The extremely low lignin content of wheat grown in a controlled environment noted in Figure 1 is of importance, because lignin complicates the conversion of cellulose and hemicellulose to monosaccharides, and because lignin is a true waste in CELSS. The same variety of wheat (cv. Yecora rojo) grown under field conditions will typically contain three to five times more lignin than noted in Figure 1 (Bugbee, personal communication).

The primary goal of biomass processing research at KSC is the minimization of the volume and energy required to (a) grow plants, (b) store the biomass produced and (c) recycle elements (C, N, etc.) through the waste processing systems while maximizing the utilization of volume, space, weight, light energy. To accomplish this goal, an efficient means of converting inedible crop residues into usable, preferably edible, products was researched.

Enzymatic hydrolysis of crop residue cellulose into glucose offered many advantages over other biomass conversion processes and, thus, was selected as the

first to be considered. The cellulase enzyme complex, especially of hyperproducing mutants of *Trichoderma reesei*, has an extensive literature database(1). In addition, the technology needed to convert cellulose to glucose enzymatically seemed to be readily available, without the need for extensive developmental research. Compared to acid hydrolysis of cellulose, enzymatic conversion avoids production of toxic byproducts, especially from the hemicellulose fraction of crop residue (2,3). Furthermore, the enzyme complex would be easier to produce and manage in the confines of a spacecraft than hot dilute acid, which would tend to corrode equipment and need neutralization and disposal after the conversion process was completed.

METHODS

Organisms

Aspergillus phoenicis QM329 and *Trichoderma reesei* QM9414 were obtained from the U.S. Army Natick Research Laboratory. Organisms were maintained on potato dextrose agar slants (PDA, Difco) at 4°C, and transferred every 2 weeks.

Culture Conditions

The basal medium for production of enzyme and preparation of mycelial inocula contained the following compounds (g/L): 2.0 KH₂PO₄, 1.4 (NH₄)₂SO₄, 0.3 urea(Sigma), 1.0 proteose peptone (Sigma), 0.3 MgSO₄·7H₂O, 0.3 CaCl₂, 0.005 FeSO₄·7H₂O, 0.0016 MnSO₄·H₂O, 0.0014 ZnSO₄, 0.002 CoCl₂, 0.005 citric acid, 0.00025 CuSO₄·5H₂O, 0.00005 H₃BO₃, 0.00005 Na₂MoO₄·2H₂O. Tween 80 was added at 1 mL/L. This medium is a modification of that of Ryu et al. (4), with the addition of selected micronutrients (3). Alpha-cellulose (Sigma) was added as carbon source for *T. reesei* QM9414 and potato starch (Sigma) for *A. phoenicis* QM329. Actively growing mycelial cultures were used to inoculate cultures for enzyme production. Erlenmeyer flasks of basal medium plus 1% (w/v)

alpha-cellulose (for *T. reesei*) or starch (for *A. phoenicis*) were inoculated with a 2% spore suspension (v/v) of the respective fungus. The suspension was prepared by washing two week old, sporulated fungal PDA plates with sterile deionized water. This suspension was then adjusted to a density of 0.4 absorbance units (660 nm wavelength). Mycelial cultures were incubated for 72 hrs at 25 degrees C while being shaken at 125 rpm on a rotary shaker. The resulting *T. reesei* mycelial suspensions could be used directly to inoculate enzyme production flasks, but the *A. phoenicis* mycelium grew in clumps and was gently dispersed with a glass tissue homogenizer to create a homogeneous inoculum.

Erlenmeyer flasks containing basal medium were also used for enzyme production. The medium was inoculated with the active, 72 hr mycelial suspensions, at selected densities, of *T. reesei* and *A. phoenicis*. Alpha-cellulose and starch concentrations varied with the experiment. Enzyme production flasks were incubated at 25 ° C and 125 rpm. At the end of 7 days incubation, the fungal mycelia were removed from the medium by centrifugation at 3000 x g for 10 min, and the resulting supernatant was assayed for enzyme activities and for saccharification of wheat straw.

Assays

All enzyme assays were performed on the supernatant of seven-day old cultures (except for the timecourse of enzyme production depicted in Figure 2) after centrifugation at 3000 x g for 10 min to remove the fungal mycelia. Cellulase activity was estimated by the conventional filter paper assay (hydrolysis of purified cellulose contained in Whatman No. 1 filter paper) under standard assay conditions (4). Glucose (glucose oxidase/peroxidase, Sigma), instead of reducing sugar, was measured as the hydrolysis end product. β -glucosidase activity was estimated with cellobiose as substrate according to Sternberg et al. (5) Results of all enzyme assays are reported as μ moles glucose produced per (minute x mL).

Saccharification

Saccharification of wheat straw was assayed by incubation of equivalent volumes of enzyme solution obtained from enzyme production supernatants and citrate buffer (pH=4.8) with 5% (w/v) ground wheat straw (50 mesh size) pretreated with alkaline peroxide to remove lignin and hemicellulose 6. The wheat straw was grown, hydroponically, to maturity (ca. 70 days) in the BPC (23°C, 24 hr light at 300-500 $\mu\text{moles}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 65% relative humidity) at KSC as part of a separate study. Sodium azide (0.02%) was added to the saccharification assay tubes to inhibit microbial growth. Saccharification assay tubes were incubated horizontally in a 50°C water bath and shaken at 100 cycles/min. Tubes were removed at specified sampling times and centrifuged at 1000 x g for 2 min to remove particulates. Samples (0.1 mL) were removed from the supernatant, diluted 100 fold, and assayed for glucose.

RESULTS AND DISCUSSION

Studies of cellulase enzyme production.

When a monoculture of *T. reesei* QM 9414 was grown on alpha cellulose, a typical timecourse of cellulase enzyme production occurred (Figure 2). β -glucosidase activity reached a maximum at about 96 hrs and decreased slightly after 144 hrs. Cellulase activity reached a plateau at 96 hrs and then trended upwards at 192 to 240 hrs (240 hr data not shown). Relative to cellulase activity, β -glucosidase activity was low, with a ratio of the two at about 1:1 near the typical enzyme harvest at 168 hrs. A number of published studies have recommended an optimal ratio of β -glucosidase to cellulase for cellulose hydrolysis at 4:1 or greater (6,8).

This result was not unexpected, as hypercellulolytic mutant strains of *T. reesei*, such as QM9414, are known to produce an extracellular cellulase complex which is relatively deficient in β -glucosidase (7,8). In practical saccharification reactions, this deficiency has caused a buildup of cellobiose, a competitive inhibi-

tor of the exoglucanase component of cellulase (9). A solution to this problem was suggested in the literature. The rate and extent of cellulose hydrolysis by the *T. reesei* cellulase complex can be increased by the addition of supplemental β -glucosidase, produced by *Aspergillus phoenicis* in separate (8) or mixed (10,11,12) culture with *T. reesei*.

However, the benefits of increased glucose production must be weighed against the the added cost of *Aspergillus* production. Although mixed cultivation of *Trichoderma* and *Aspergillus* eliminates the expense of a separate fermentation step, starch is required as an additional carbon source for production of β -glucosidase in *Aspergillus*. Furthermore, mixed culture may also involve problems resulting from interactions between the two fungi during growth. For instance, Duff et al.(11) noted a decrease in cellulase activity with increasing starch concentration in cocultures of these two fungi, and suggested competition for medium components, or production of inhibitory metabolites by *A. phoenicis* might be responsible.

An experiment was designed to evaluate the effects of relative inoculum density of the two fungi and starch concentration (substrate used for *A. phoenicis* growth and β -glucosidase production) on the hydrolytic capacity of the cellulase enzyme complex produced in mixed cultures of *Trichoderma reesei* QM 9414 and *Aspergillus phoenicis* QM 329. A 4 x 4 factorial design was utilized, with starch concentration (0, 0.25, 0.5, and 1 % w/v) and *A. phoenicis* mycelial inoculum (0, 0.2, 1, and 5 % v/v) as factors. Alpha-cellulose concentration (1% w/v) and *T. reesei* mycelial inoculum (5% v/v) were held constant. The sixteen experimental treatments were repeated three times. All response variables were analyzed by three-way ANOVA with starch concentration and *A. phoenicis* inoculum as factors and replicates as a block. Bonferroni Multiple Comparisons were run when any significant differences occurred with either factor. Linear regressions were also performed on the data and correlation coefficients were obtained between all response variables.

The results of this experiment are shown in Figure 3 (for clarity, only the high and low starch concentrations are plotted). Both β -glucosidase and cellulase

activities (Figure 3A and 3B, respectively) were affected significantly by the two factors: enzyme activities increased with increases in both starch and *A. phoenicis* inoculum density. Even at exceptionally low *A. phoenicis* inoculum (0.02%, v/v), significant increases in enzyme activities occurred at all starch concentrations tested.

The effects of starch concentration and *A. phoenicis* inoculum density on the extent of wheat residue (harvested from the KSC-CELSS BPC) hydrolysis after 48 hr (Figure 3C) were distinctive from those observed with the enzyme assays. A small, but significant, increase in cellulose hydrolysis with increasing *A. phoenicis* inoculum still occurred at 0% starch. Higher glucose yields from enzymes produced by cocultures containing *A. phoenicis* probably were a result of increased β -glucosidase activity, as has been shown previously (12). When starch was included as an experimental factor, however, residue cellulose hydrolysis was unaffected. Correlations between glucose production from wheat residue and the enzyme assays were low (data not presented), and revealed the limitations of depending on enzyme assays alone to predict the hydrolytic potential of a cellulase complex acting on a natural cellulosic substrate.

An interspecies interaction, e.g., competition or antagonism, between the two fungi in cocultures containing starch and cellulose may be responsible for the results observed in Figure 3. Soluble protein levels (data not shown) indicate that *A. phoenicis* may have grown at the expense of *T. reesei*, whatever the reason. This interaction is apparently not significant in cocultures that contain only cellulose, with growth of *A. phoenicis* greatly reduced compared with cocultures containing starch. Although more precise definition of the nature of this interaction is beyond the scope of the present study, changes in media or other growth parameters could potentially reduce such competition.

To better understand the apparent need for increased β -glucosidase for cellulose hydrolysis, enzyme production by monocultures of each fungus was examined. In addition to growing the fungi in monoculture for production of enzyme, the experiment also examined the substrate used for preparation of the inocula for enzyme production. Alpha cellulose and starch, purified substrates unavaila-

ble in a functioning CELSS, had been used in previous studies for fungal growth and production of the enzymes. Table 1 lists the factors examined in this experiment.

Table 1. Experimental design for monoculture production of β -glucosidase by *A. phoenicis* and of cellulase by *T. reesei*. with different substrates used for generating inocula for enzyme production from wheat residue.

FACTOR	LEVEL
Inoculum substrate	
<i>A. phoenicis</i>	Starch, wheat residue, or base treated residue
<i>T. reesei</i>	Cellulose, wheat residue, or base treated residue
Enzyme ratios for wheat residue hydrolysis	
β -glucosidase:cellulase	0:1, 1:4, 1:1, 4:1

Wheat residue was used for enzyme production.

To date, only one replicate of three has been completed, so conclusions drawn from the data are tentative. Figure 4 shows the enzyme activities for both fungi with inocula grown on different substrates and with enzyme production from wheat residue. β -glucosidase production (Figure 4A) by *A. phoenicis* grown on wheat residue appeared to be considerably greater with inocula prepared from either wheat residue or base extracted wheat residue when compared with inocula grown on starch. β -glucosidase production by monocultures of *T. reesei* appeared to be slightly greater for inocula prepared with cellulose, but enzyme activity from inocula prepared with treated wheat residue may not be substantially less.

Activities of cellulase produced by the fungal monocultures are presented in Figure 4B. Surprisingly, *A. phoenicis* produced measurable cellulase activity when cultured on wheat residue if the inocula were prepared with wheat residue

or treated wheat residue. To our knowledge, cellulase production by this fungus has not been demonstrated previously, but substrates such as starch and monosaccharides were used for growth of the inocula and enzyme production, instead of the complex lignocellulosic materials of our study (10). Cellulase activity of *T. reesei* monocultures grown on wheat residue (Figure 4B) were greatest when inocula were prepared with cellulose, but inocula prepared with treated wheat residue also caused significant production of cellulase.

To test the enzymes produced by the two fungal monocultures against natural substrates, culture filtrates were combined in different ratios of *A. phoenicis* β -glucosidase to *T. reesei* cellulase and used to hydrolyze wheat residue for up to 168 hrs. Figure 5 shows the results of one set of these assays for *T. reesei* cellulase from wheat residue inoculated with mycelia grown on base-treated wheat residue. All three experimental sources of *A. phoenicis* β -glucosidase are shown, i.e., monocultures of wheat residue inoculated with *A. phoenicis* mycelia grown on either starch (control), untreated wheat residue, or wheat residue treated with base to solublize the lignin and hemicellulose. Only the two extreme β -glucosidase:cellulase ratios are plotted, i.e., 0:1 and 4:1. After 24 hrs of hydrolysis, glucose production from the wheat residue cellulose appears to be stimulated by the addition of *A. phoenicis* β -glucosidase regardless of source, whereas at 168 hr, the addition of β -glucosidase causes only a small increase in cellulose hydrolysis. These preliminary results (more replications to be run) indicate that supplementation of *T. reesei* cellulase complex by β -glucosidase from *A. phoenicis* may not be needed. However, enzyme loading rates (ratio of cellulase to substrate cellulose) were kept low for this study as a result of poor enzyme yields from one of the *T. reesei* monocultures (inoculated with mycelia grown on wheat residue). At these low loading rates, the need for additional β -glucosidase at later times may have been diminished by the lack of cellobiose buildup. Additional studies at higher loading rates are needed before the role of supplemental β -glucosidase can be determined.

In conclusion, *Trichoderma reesei* QM 9414 can be used to produce sufficient cellulase from treated wheat residue for the conversion of CELSS derived crop wastes. Contrary to current belief, the *T. reesei* cellulase complex may not need

supplemental β -glucosidase, especially if long hydrolyses with high conversion efficiencies are desired. If supplemental β -glucosidase is needed, then separate monocultures of *Aspergillus phoenicis* could be used. However, the effects observed may not apply to cocultures grown on other substrates.

Integration of biomass conversion into the KSC-CELSS Breadboard Facility:

Estimates of reactor sizes are needed for scale-up of enzyme production and of enzymatic hydrolysis of crop residues (saccharification) for preliminary design considerations in anticipation of integrating biomass conversion processes with the output/harvest from the BPC. To make these estimates, data were needed concerning substrate concentration, e.g., wheat residue, and conversion efficiency at various enzyme (e.g., cellulase) loading rates (amount of enzyme per g of substrate). For these early studies, hydrolysis substrate was alkaline peroxide treated wheat residue, with the lignin and hemicellulose being chemically removed by the procedure of Gould and co-workers (7). The enzyme was prepared by cultivation of *T. reesei* on alpha cellulose. Results of 48 hr residue hydrolysis assays (Figure 6) indicate that the best conditions for optimal conversion efficiency were 5% residue concentration at an enzyme loading of 3 IU/g residue {IU = international units of enzyme = g glucose produced / (mL x min)}

These values were used to estimate reactor sizes. For convenience, continuous operation of the BPC was assumed, with one tray of mature wheat harvested per day. From the data shown in Figure 6, the anticipated hydrolysis (or saccharification) reactor size was estimated at 16 L (containing 5% residue for 48 hr, or the equivalent of 2 days harvested wheat residue). The amount of cellulase enzyme needed to support this saccharification would be 900 IU, with an assumed enzyme loading of 3 IU / g cellulose and residue with a cellulose content of 40% (Calculated from Figure 1, for cold water extracted residue). With an enzyme yield of 4 IU/ g of residue (calculated from results presented in Figure 5), then the enzyme production reactor would be 9 L and contain 225 g of residue. This quantity is nearly 29% of the residue available for both enzyme production and saccharification.

To better estimate reactor sizes and other parameters needed to scale-up enzyme production and residue saccharification, the data presented in Figure 6 should be repeated with cellulase enzyme produced from wheat residue instead of from alpha cellulose. Nevertheless, improvements can be suggested: (a) cellulase enzyme in the hydrolysis reactor should be recycled, thus reducing the requirement for use of residue to produce enzyme, (b) the enzyme production yield should be increased, and (c) the saccharification conversion efficiency should be increased through pretreatments to remove lignin and hemicellulose.

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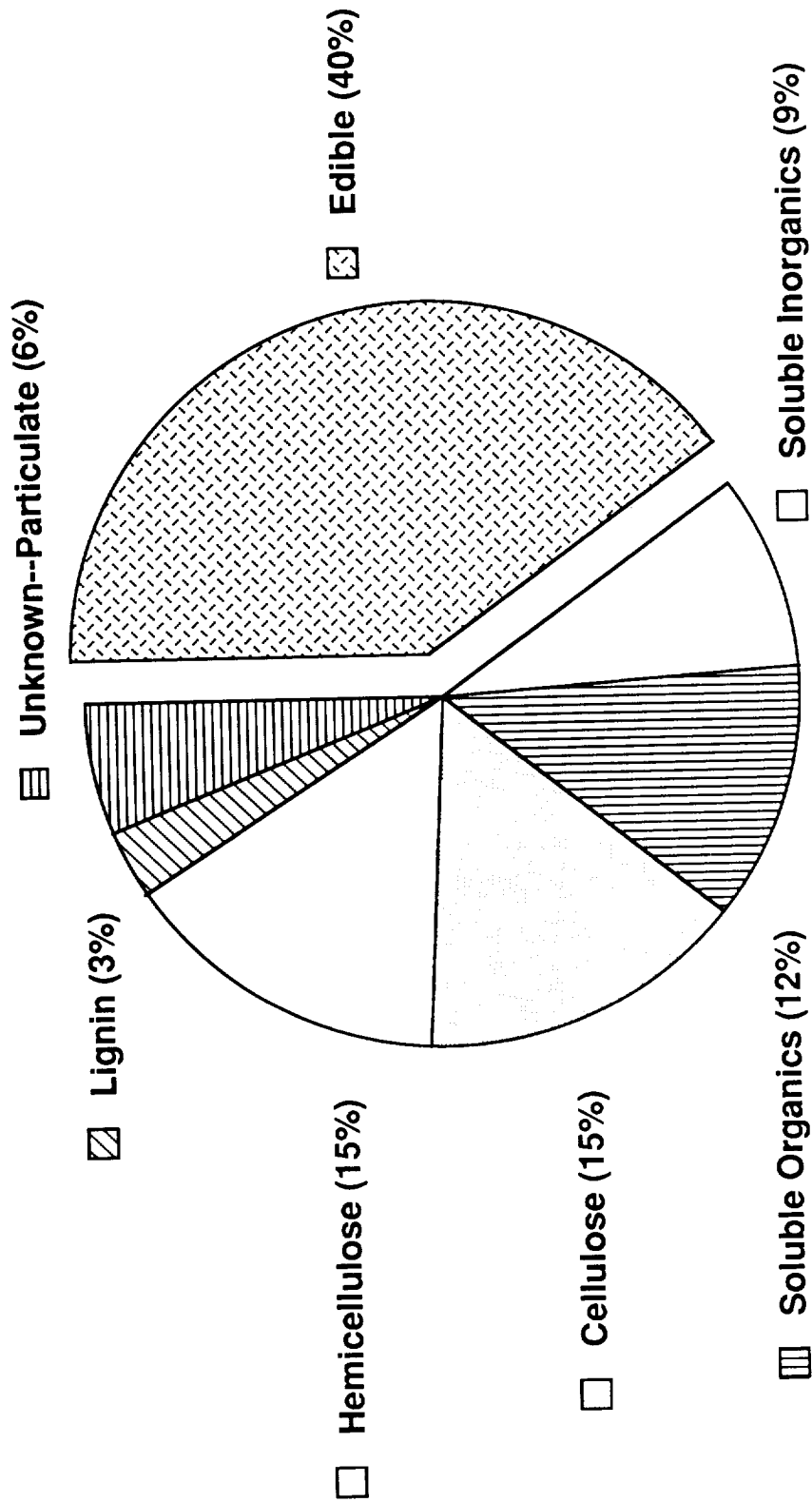


Figure 1. Composition of KSC-CELSS Breadboard wheat at harvest from the Biomass Production Chamber. Fiber analysis by Nutritional International Inc.

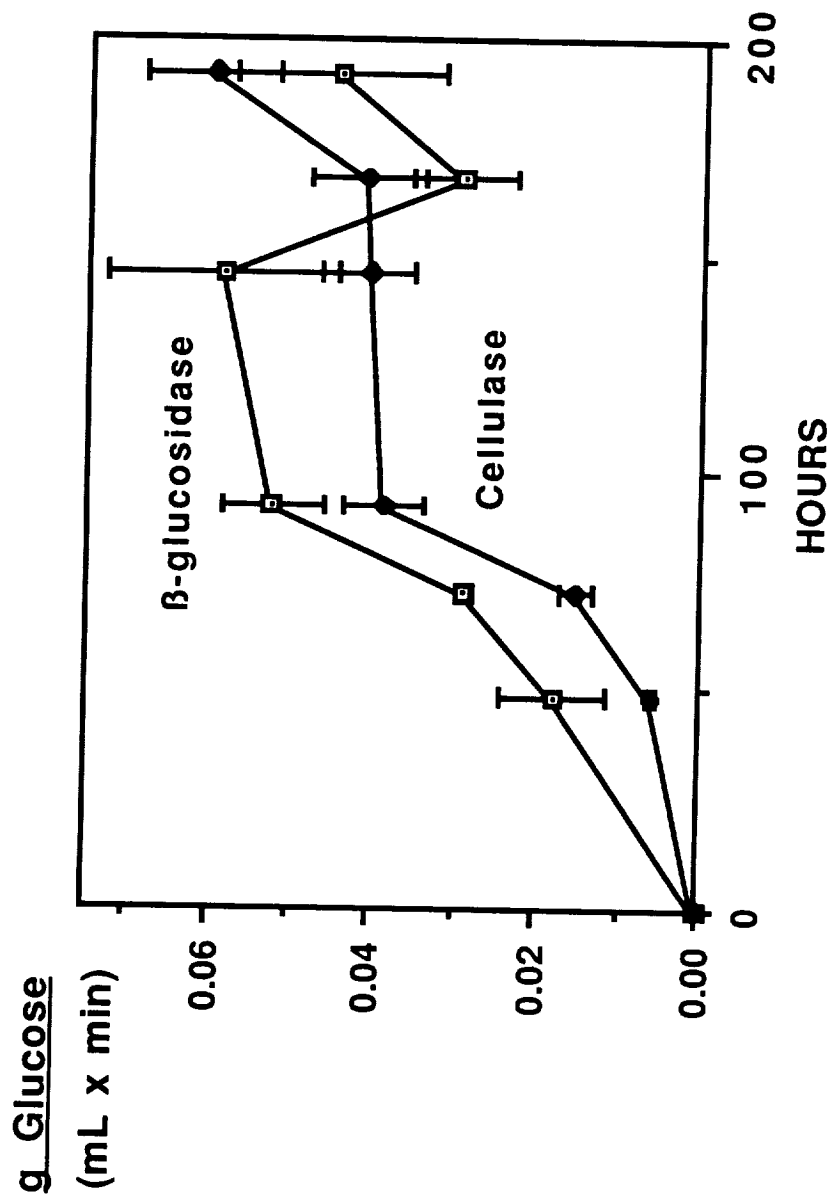


Figure 2. Time-course of soluble enzyme production by a monoculture of *Trichoderma reesei* QM 9414 grown on alpha cellulose. Average of three replicates and standard deviations are plotted.

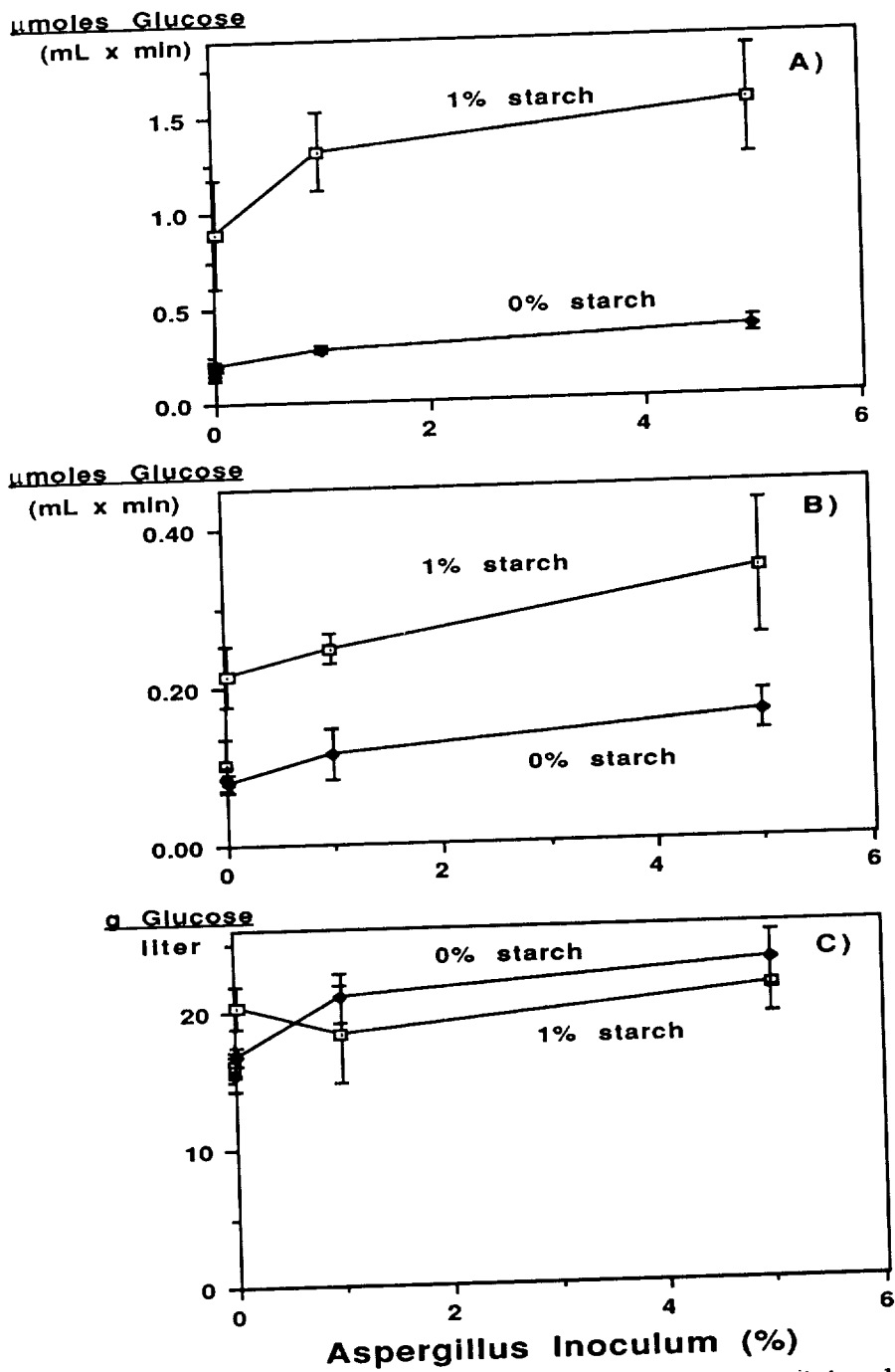


Figure 3. Effects of two starch concentrations and *A. phoenicis* relative inoculum concentration (v/v) on β -glucosidase activity (A), cellulase activity (B) and 48 hr. wheat residue hydrolysis (C) for soluble enzymes produced in 7 days by cocultures of *T. reesei* and *A. phoenicis*. All cocultures were inoculated with the same inoculum concentration of *T. reesei* (1%) and contained 1% alpha cellulose.

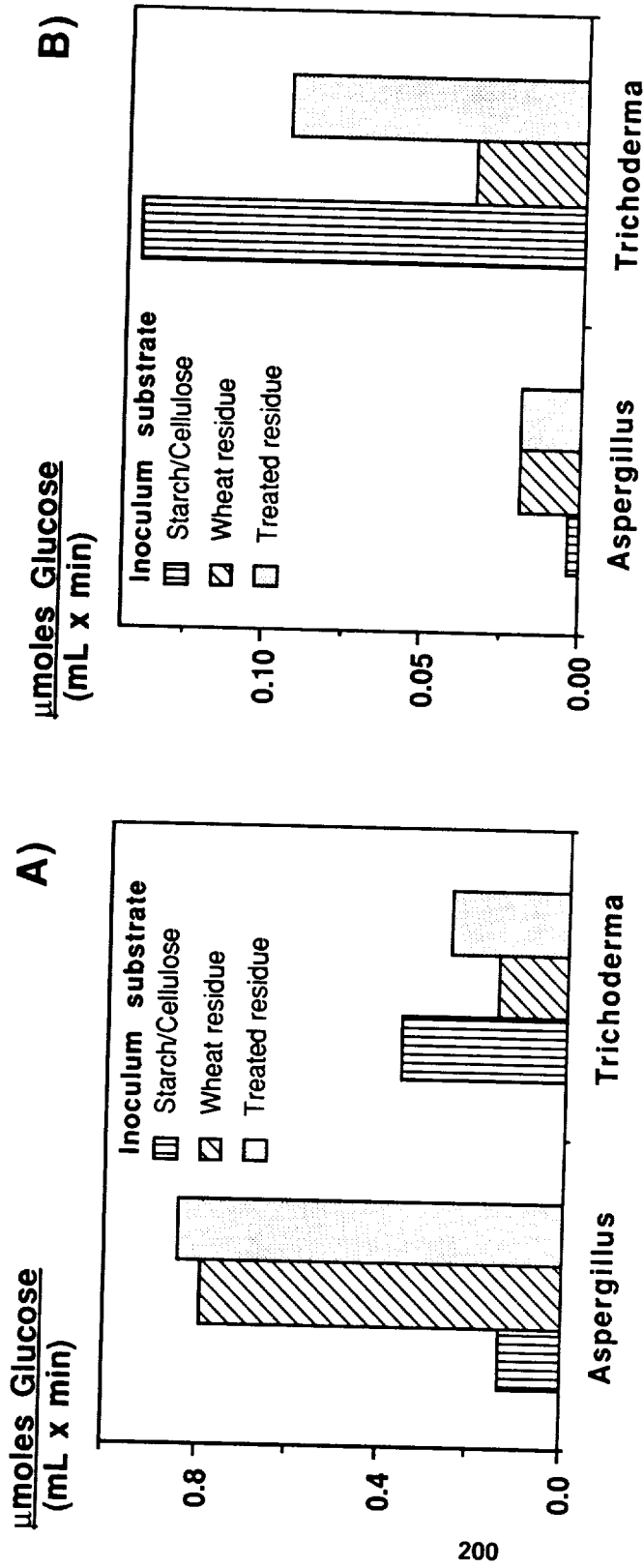


Figure 4. Activities of β -glucosidase (A) and cellulase (B) produced in 7 days by monocultures of *T. reesei* and *A. phoenicis* grown on wheat residue. *A. phoenicis* monocultures were inoculated with mycelia grown for 3 days on either starch, untreated wheat residue or wheat residue treated with base to solublize lignin and hemicellulose. *T. reesei* monocultures were inoculated with 3 day old mycelia grown on either cellulose, untreated wheat residue or base treated wheat residue.

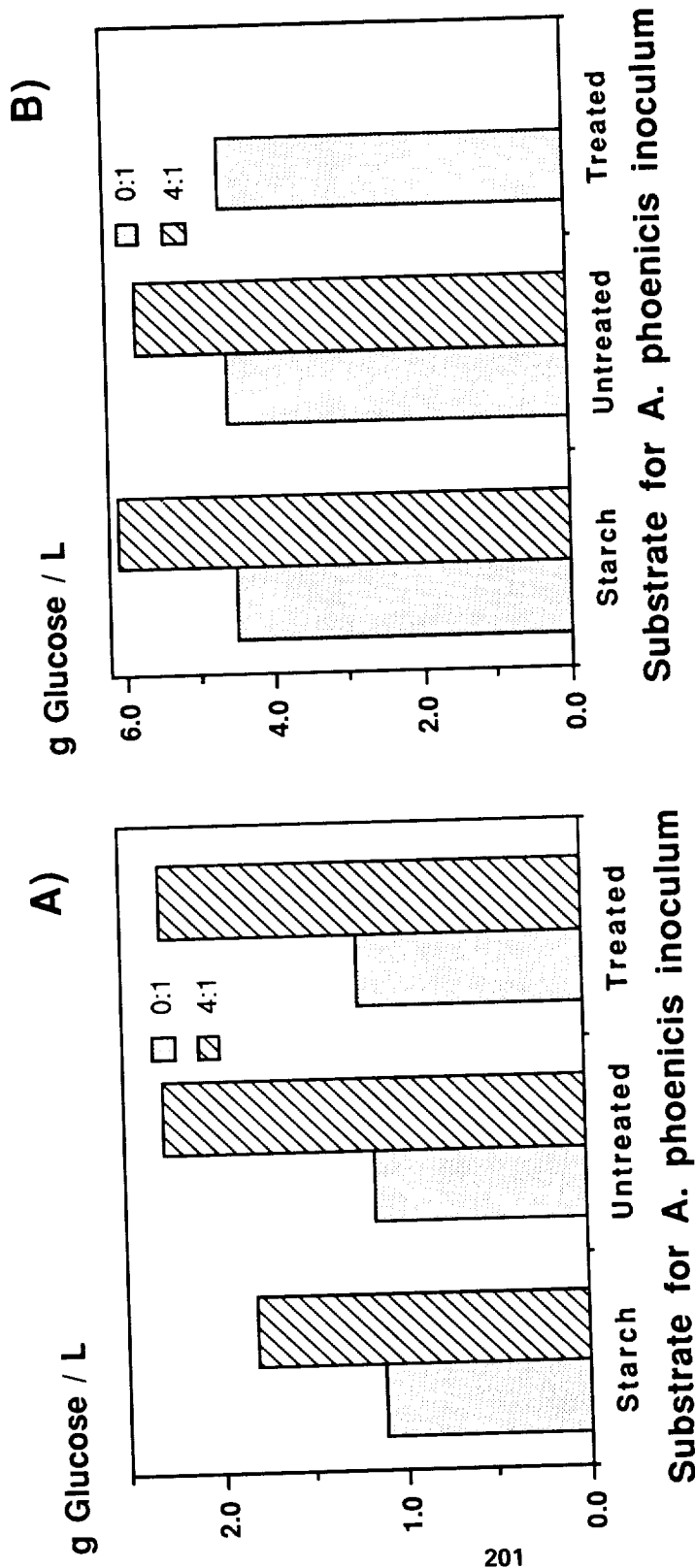


Figure 5. 24 hr (A) and 168 hr (B) wheat residue hydrolysis by soluble enzymes produced by monocultures of *A. phoenicis* (predominantly β -glucosidase, Figure 4A) and *T. reesei* (predominantly cellulase, Figure 4B). Enzymes from the two monocultures were combined in different ratios (0:1 and 4:1 shown, *A. phoenicis* β -glucosidase to *T. reesei* cellulase) according to the enzyme activities as determined for Figure 4. Monoculture conditions are described in the legend for Figure 4.

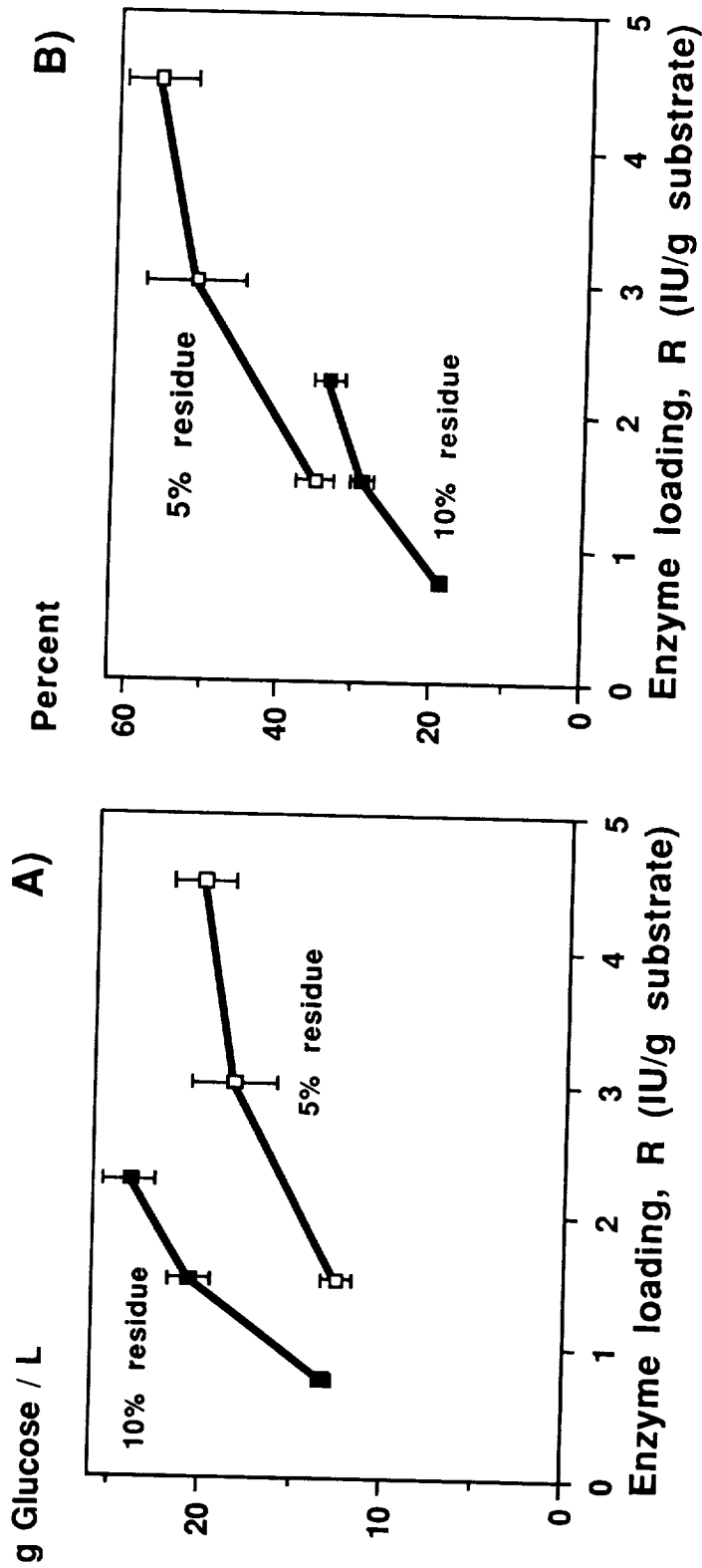


Figure 6. Effects of enzyme loading (IU/g substrate) on glucose production (A) and conversion efficiency (B) at the end of 48 hr saccharification/hydrolysis at two substrate concentrations. *T. reesei* cellulase was produced in monoculture from alpha cellulose. Saccharification/hydrolysis substrate was KSC-CELSS produced wheat residue that had been treated with alkaline peroxide to remove lignin and hemicellulose.

CYANOBACTERIA IN CELSS: GROWTH STRATEGIES FOR NUTRITIONAL VARIATION AND NITROGEN CYCLING

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ABSTRACT.

Cyanobacteria (Blue-Green Algae) are versatile organisms which are capable of adjusting their cellular levels of carbohydrate, protein and lipid in response to changes in the environment. Under "stress" conditions (salinity, cold shock) there is an imbalance between nitrogen metabolism and carbohydrate/lipid synthesis. The lesion in nitrogen assimilation is at the level of transport: the "stress" condition diverts energy (trans-membrane pH gradient) from the active accumulation of nitrate to the extrusion of salt; and probably inhibits a cold-labile ATP'ase in the case of cold-shock. Both situations affect the bioenergetic status of the cell such that the nitrogenous precursors for protein synthesis are depleted. Despite the inhibition of protein synthesis and growth, photosynthetic reductant generation is relatively unaffected. The high O_2 and reductant would normally lead to photo-oxidative damage of cellular components, however the organism copes by channeling the "excess" reductant into carbon storage products. The increase in glycogen (28-35% dry weight increase) and the elongation of lipid fatty acid side chains (2-5% dry weight increase) at the expense of protein synthesis (25-34% dry weight decrease) results in carbohydrate, lipid and protein ratios that are closer to those required in the human diet. In addition, the selection of nitrogen fixing mutants which excrete ammonium ions present an opportunity to "tailor" these micro-organisms to meet the specific need for a sub-system to reverse potential loss of fixed nitrogen material.

INTRODUCTION.

To date, life support systems in manned space flights have consisted of consumables transported in a spacecraft for the duration of the flight and periodically replenished by subsequent space flights during longer missions such as Skylab. This has worked well on short missions. However, a contained self-regenerating system (1) that produces edible biomass (1,2) from crew waste products and sunlight would have clear advantages and has been proposed by the Controlled Ecological Life Support System (CELSS; 2). The proposed CELSS is an integration of several unifunctional, tightly

controlled sub-systems such that the output of one sub-system provides the required input of the next sub-system in the cycle. The major sub-systems are shown in figure 1. However, in this "closed-cycle" not all components are inherently stable but must be controlled or regenerated. One obstacle to maintaining system stability is posed by the loss of fixed nitrogen during waste processing and biological denitrification.

Photosynthetic organisms are of prime consideration (1) in a CELSS since they are capable of producing biomass from simple inorganic compounds at the expense of light energy by the so-called oxygenic photosynthesis. Since these photosynthetic systems generate O_2 (from the photolysis of H_2O), they could augment the physical/chemical air revitalization subsystems.

One group of photosynthetic organisms under consideration are the highly versatile blue green algae (3, 4). These microscopic blue green algae blend the advantages of higher plants (photosynthesis) with the ease of handling of bacteria (axenic cultures grown in fermentors). These organisms produce a high percentage of their biomass as protein (table 1), and we have demonstrated that the metabolic energy produced by photosynthesis can be redirected to carbohydrate and lipid synthesis and away from protein, by simple manipulations of environmental factors. With these methods, we are able to manipulate the productivity of protein, lipid, and carbohydrate in a single organism to levels which are compatible with the human dietary requirements.

One distinct advantage of some species of blue green algae is their ability, under nitrogen limitation, to reduce atmospheric N_2 to a biologically useful form. This would provide the CELSS with a sub-system for maintaining the nitrogen balance, countering potential losses due to

denitrifying bacteria or physical processing, projected in the waste processing subsystems (5, 6).

CARBON-NITROGEN BALANCE AND CONTROL.

A basic concept of photosynthesis, outlined in Figure 2, is the competition between N_2/NO_3^- and CO_2 for reductant and ATP. That is, of course, a rather simplistic picture, since CO_2 reduction is required to provide the carbon skeleton for assimilation of NH_3 into amino acids (proteins), and nitrogenous material is incorporated into some lipids, nucleic acids and cell-wall components. Generally, however, the competition between CO_2 and nitrogen reduction does hold true; and since photosynthetic electron flux (and hence reductant supply) is not shown to be improved by environmental factors, the theoretical productivity of one component of the biomass (e.g. carbohydrates) could only be increased at the expense of another (e.g. protein). Our results show this to be the case. Using "shock" (i.e. salinity or cold treatment), we have been able to demonstrate the partitioning of reductant away from (excess) protein to (required) carbohydrate and lipid. Growth of the unicellular Synechococcus 6311 as measured by increments in chlorophyll, was inhibited ~30% with 0.5M NaCl (figure 3a), and since the protein content per cell also decreased, this demonstrates an approximate 50% reduction in total protein productivity. Although the growth rate is inhibited, the glycogen productivity increases markedly, by a factor of 10 over control cells (Figure 3b). In addition, the lipid content was also found to change. Not only did the total lipid increase by 5%, but the fatty acid composition was also altered. Under salt shock the length of the fatty acids were increased from 16:1 to 18:1. Analysis of the total caloric content of the carbohydrate (glycogen plus soluble sugars), protein and lipid per gram of cells under "stress" is shown in table II. The

total caloric value per gram of cells remains fairly constant, but the source of the calories changes, this redirection of reductant truly represents a shift of metabolism from protein to carbohydrate synthesis.

The mechanism involved in this shift in metabolism seems to involve the energy status of the cell. We have extensively documented the physiological and biochemical response of Synechococcus 6311 to salt (7-14), and recent results point conclusively to a depolarization of the trans cytoplasmic membrane pH gradient as a primary event. We have shown that the membrane pH gradient, generated by a membrane bound ATP'ase under non-stress conditions and directly by respiration under stress conditions, is responsible for driving the accumulation of nitrate into the cell. This pH gradient is also utilized to remove excess intracellular sodium ions via a Na^+/H^+ antiporter (13). The presence of high concentrations of intracellular salt compete with nitrate for the pH gradient (table III), with the net result that the cell's uptake of nitrate is severely inhibited and photosynthetic reductant is channeled into CO_2 fixation. The mechanism involved in the case of cold shock is less clear, but there are indications that the cold-labile ATP'ase may be partially inhibited, which would deplete the magnitude of the pH gradient and hence inhibit the uptake of nitrate.

In summary, the utilization of energy (ATP) for non-growth functions (salt removal) or energy depletion (ATP'ase inhibition) results in the inhibition of the nitrate uptake mechanism, presenting a nitrogen starvation situation which results in "over-production" of fixed carbon compounds. This scenario may be capitalized upon in future research, for example, one can envisage the selection of suitable mutants defective in their ATP'ase function, which would synthesize sufficient carbohydrate for human dietary

requirements without the need for environmental manipulations.

Selection of mutants as a way to "tailor" micro-organisms to meet specific needs of sub-systems within the proposed CELSS presents us with a powerful tool. In the next section we will present an overview of how a specific mutant of nitrogen-fixing cyanobacteria may be used as one component in the CELSS.

NITROGEN CYCLING

Organic (fixed) N_2 could be lost from a CELSS due to denitrifying bacteria in stored waste material or by oxidation to N_2 in the proposed waste management system such as catalytic wet oxidation (CWO; 5) and/or supercritical water oxidation (SCWO; 6). Current physical/chemical systems for the reduction or oxidation of N_2 to NH_4^+ or NO_3^-/NO_2^- have a high energy expenditure (approximately 20,000 KWh per ton of nitrogen fixed) in the case of the Haber process, a low efficiency (2%) in the case of the Birkland-Eyde electric arc process, or system instability in the case of metal complexes as catalysts (for a recent review on man-made N_2 -fixing systems see 15). Photosynthetic nitrogen fixation by cyanobacteria is a plausible means of generating a pool of biologically usable fixed nitrogen. Cyanobacteria are a rich source of nitrogen, the majority of which is in a biologically useful form, protein (table IV). However, the mode of transfer of the fixed nitrogen back into the cycle is a critical consideration. The simplest method would be to use the cyanobacteria as a protein supplement for the crew, who become the "nitrogen processing sub-system" (figure 1). An attractive alternative is to select a mutant with the ability to excrete a nitrogenous product that can be used directly by the plant growth chamber. There are several reports in the literature in which versatile cyanobacterial strains have been used with the

specific aim of excreting NH_4^+ ions (produced by nitrogen fixation) into the medium (16-20), and NH_4^+ ions are an ideal nitrogen source for hydroponics. The filamentous cyanobacteria can convert atmospheric N_2 (as a sole nitrogen source; 16, 21, 22) into NH_4^+ using only minerals, CO_2 and light (see ref. 8 and 13 for relevant reviews of N_2 fixation). In these bacteria photosynthesis occurs simultaneously with the oxygen sensitive nitrogen fixation process. To achieve this state, the nitrogen fixing apparatus (nitrogenase) is housed in a specialized, differentiated cell (the heterocyst; 23) where the partial pressure of O_2 is maintained at a low level (24). This unique arrangement allows the cyanobacteria to photo-produce NH_4^+ ions and carbohydrates and, therefore grow on a minimal medium without the need for added complex carbon compounds. Under conditions where alternative fixed nitrogen sources (NH_4^+ , NO_3^-) are available to the cell, the nitrogenase complex is not expressed and heterocyst differentiation is inhibited (23, 26).

The second key enzyme in the metabolic pathway of nitrogen is glutamine synthetase (GS). Glutamine synthetase is expressed irrespective of the source of nitrogenous material, and is the first step in the incorporation of NH_4^+ ions into amino acids. This enzyme removes NH_4^+ ions from the cytoplasm, combining then with glutamate to produce glutamine (25-28) which can be incorporated into protein or transaminated by glutamine 2-oxoglutarate amino transferase (GOGAT; 16) to regenerate the glutamate and synthesize the required amino acids from carbon precursors. To achieve a build-up of NH_4^+ ion concentration, it is necessary to inhibit GS activity. One of the major techniques which has been used to induce strains of nitrogen fixing bacteria to excrete NH_4^+ ions, is the GS inhibitor methionine sulfoximine (MSX; 17, 18). However, MSX is extremely toxic and its removal from the recycled

nutrients would prove problematic. An alternative technique is the production and selection of mutants that excrete high levels of NH_4^+ ions. The use of NH_4^+ analogs such as ethylenediamine or methylamine has been used successfully to select mutants deficient in GS activity, while retaining high nitrogen fixing capabilities (16, 19, 20). The technique is based on exposure of cells (previously grown on nitrogen deficient media) to a mutagen (nitrosoguanidine; 19). The mutated cells are then exposed to an NH_4^+ analog (ethylenediamine) at pH 9 which allows the ethylenediamine to passively diffuse into the cell and prevents selection of NH_4^+ transport mutants (20). Because ethylenediamine is metabolized by GS to produce aminoethylglutamine, a compound which is not metabolized further and accumulates, mutants with a low GS activity accumulate aminoethylglutamine more slowly. Thus, the survival rate of the cells is higher, presumably because less of their glutamate is "tied-up" as aminoethylglutamate and they can assimilate NH_4^+ ions produced by the nitrogenase complex.

A second effect is that the accumulation of aminoethylglutamate causes inhibition of nitrogenase activity (20). In mutants with the *nif* genes derepressed, accumulation of the aminoethylglutamine will have little effect, allowing N_2 reduction to continue. The net result of this procedure is to select for a double mutant. In such a system (in the absence of NH_4^+ analogs) there is an imbalance between the rate of NH_4^+ supplied by the nitrogenase complex and the rate of assimilation into amino acids. This results in a release of NH_4^+ ions into the medium (see figure 4), and the longevity of these mutants is promising (up to 600 hr. tested so far; 16).

Stewart *et al* have considered the use of such mutants to supply nitrogen directly to crops in the field, but have concluded that competition from the faster growing wild type cyanobacteria would probably reduce their

effectiveness (16). However, such competition would not exist in a controlled bioreactor, making this an ideal system for inclusion as part of a CELSS.

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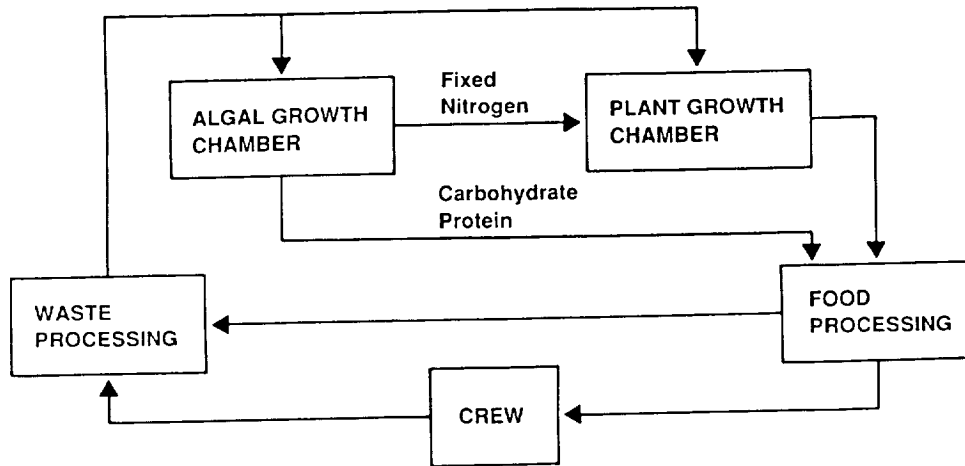


Figure 1. Major components within a CELSS.

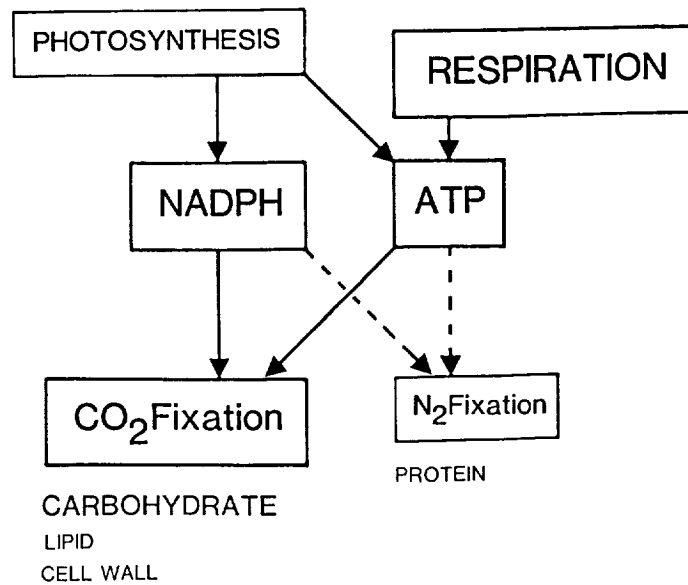


Figure 2. Energy flow diagram, competition between CO₂ and N₂ for reductant and ATP.

Table I. Storage granules in cyanobacteria.

Storage granule (Cell inclusion)	% dry weight	Present in <u>Synechococcus</u>	Present in <u>Spirulina</u>
GLYCOGEN	5-60	yes	yes
POLY-B-HYDROXY- BUTYRATE (lipid)	6(a)	n.a.	yes
MEMBRANES	12-16 (b)	yes	yes
CARBOXYSOMES (protein)	25	yes	yes
PHYCOBILSOMES (protein)	10-20 (a,b)	yes	yes
CYANOPHYCIN (protein)	8-12 (a,c)	no	yes?
GAS VESICLES (protein)	2 (d)	no	yes

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b) Results from our laboratory.

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Table II.

Cellular composition and energy content of Synechococcus 6311.

	Control		Salt shock		Cold shock	
	%Dwt cells	Kcal/g cells	%Dwt cells	Kcal/g cells	%Dwt cells	Kcal/g cells
CARBOHYDRATE	6.0	0.24	29.2	1.17	38.2	1.53
PROTEIN	67.0	2.68	43.6	1.74	33.2	1.33
LIPID	15.0	1.35	15.2	1.37	16.6	1.49
		4.27		4.28		4.35

Energy content (Kcal/g Dwt cells) calculated assuming conversion factors of 4, 4 and 9 Kcal/g for carbohydrate, protein and lipid respectively (Bugbee, B. G. and Salisbury, F. B. in; Controlled Ecological Life Support Systems: CELSS'85 Workshop (MacElroy, Martello and Smernoff eds.) pp447-486, 1986)

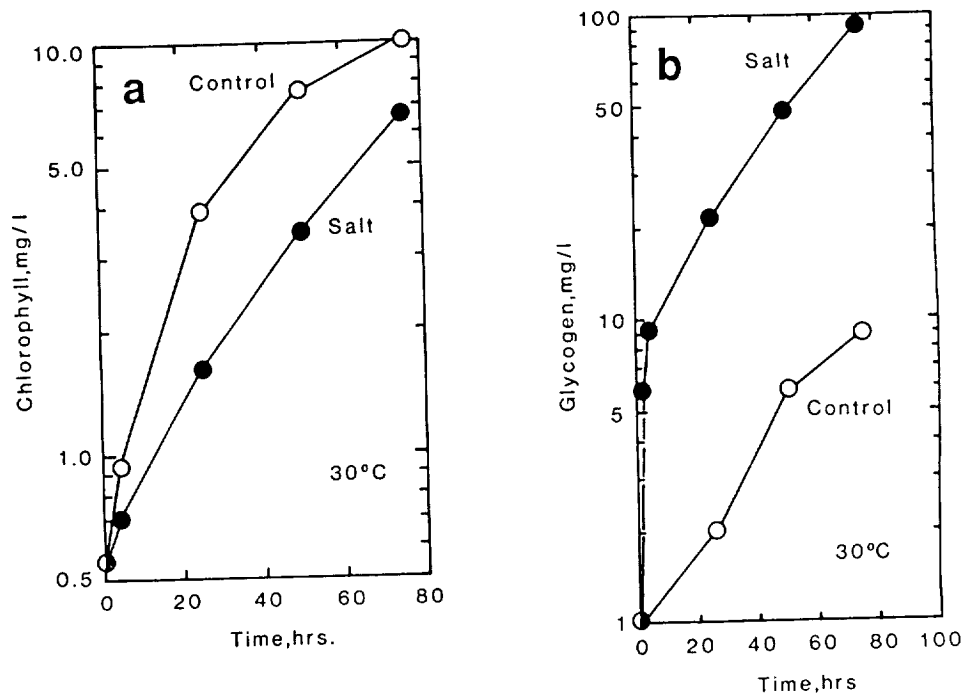


Figure 3. Effect of salt (0.5M NaCl) on the growth (a) and glycogen productivity (b) in *Synechococcus* 6311.

Table III.

Rate of nitrate uptake by *Synechococcus* 6311.

Additions	NO ₃ ⁻ uptake, umoles/mg chlorophyll/hr.
none	0.75
NaCl, 10 mM	0.57
NaCl, 100 mM	0.12

with an imposed pH gradient of 2 units (acid outside) and in the presence of KCN (2 mM) and DCCD (2 umoles/mg chlorophyll).

Table IV.

Nitrogen in Cyanobacteria

Protein	60-70 % Dry Weight
Nitrogen	87 % as Protein 13 % as Nucleic Acid and Peptidoglycan

8-9g N per 100g cells (Dry Weight)

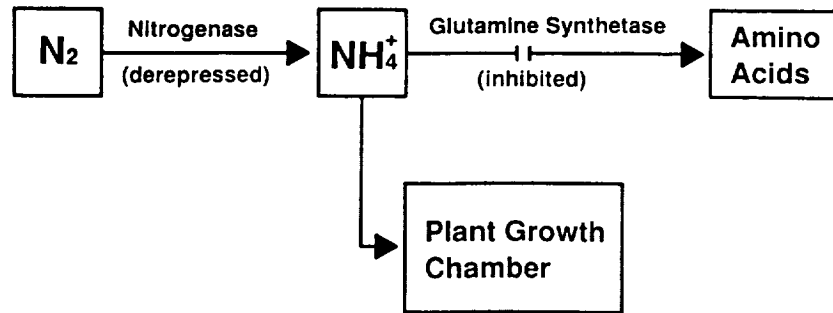


Figure 4. Nitrogen flow diagram in the proposed mutant of a nitrogen-fixing cyanobacterium.

ALGAE FOR CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM DIET
CHARACTERIZATION OF CYANOBACTERIA "SPIRULINA" IN BATCH CULTURES.

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ABSTRACT

Spirulina sp. as a bioregenerative photosynthetic and an edible alga for space craft crew in a CELSS, was characterized for growth rate and biomass yield in batch cultures, under various environmental conditions. The cell characteristics were identified for one strain of Spirulina: S. maxima. Fast growth rate and high yield were obtained under the following conditions: temperature (30°C-35°C), light irradiance 60-100 $\text{uE m}^{-2} \text{s}^{-1}$, nitrate 30mM, phosphate 2mM, aeration 300 ml/min, and pH 9-10. The partitioning of the assimilatory products (proteins, carbohydrates, lipids) were manipulated by varying the environmental growth conditions. Our experiments with Spirulina have demonstrated that under "stress" conditions (i.e. high light 160 $\text{uE m}^{-2} \text{s}^{-1}$, temperature 38°C, nitrogen or phosphate limitation; 0.1 M sodium chloride) carbohydrate increased at the expense of protein. In other experiments, where the growth media were sufficient in nutrients and incubated under optimum growth conditions, the total proteins were increased up to almost 70% of the organic weight. In other words the nutritional quality of the alga could be manipulated by growth conditions. These results support the feasibility of considering Spirulina as a subsystem in CELSS because of the ease with which its nutrient content be manipulated.

INTRODUCTION

Pursuit of our national goals in space exploration will eventually require man's long-duration tenancy of celestial vehicles and planetary base. Requirements for life support could be met through expenditure of stored supplies and by regeneration and reuse of the waste products of human metabolism. The logistics necessary of regeneration for extended space missions are well documented (1). The primary source of all man's food and organic raw materials is solar energy. Conventional food sources consist of higher plants and animals. Unconventional food sources for human consumption are photosynthetic algae and bacteria and non-photosynthetic bacteria, yeast and fungi. Conventional food sources are highly palatable, but require a long time to produce. Algae, on the other hand, grow rapidly; their metabolism can be controlled; they produce a high ratio of edible to nonedible biomass; and their gas-exchange characteristics are compatible with human requirements. The biological components of Controlled Ecological Life Support System (CELSS) will serve as subsystems for the revitalization of air, for the long term space flight. Studies of bioregenerative life support systems for use in space indicated that they are scientifically feasible. Support of a crew in space, whether in an orbiter or on the surface of a planetary body requires that oxygen, potable water and food be supplied and that waste material be removed. Employment of photosynthetic organisms (Algae: Cyanobacteria) allows biomass production from relatively simple components which are readily recycled in a CELSS system, namely carbon dioxide, minerals (NO_3^- , PO_4^{3-} , K^+ , etc.) and micronutrients.

Cyanobacteria single cell protein (SCP) has been used as a food source in various parts of the world (e.g. Mexico, China and Africa) since ancient times; in fact, dried cyanobacteria and cyanobacterial tablets are now sold in health food stores in Japan, North America and Europe because they are recognized for their nutritional value. The nutritional quality of all cyanobacteria which have been tested appears

to be very high. The protein of S. maxima is easily digestible and approximately 65% of the protein is assimilatable.

The semi-microscopic blue-green algae (Cyanophyta; Cyanobacteria) occupy a taxonomic position, since they combine an autotrophic mode of growth that is common to eukaryotic plant cells with a metabolic system that is generally regarded as bacterial, rather than plant-like.

Changes in the supply and consumption of metabolites may have considerable effects on metabolic patterns. The accumulation of photosynthetic products in algae can be induced by manipulating the environmental conditions under which the algae are grown (2). The most difficult problem in using algae as food is the conversion of algal biomass into products that a space crew could actually eat over a long period of time. If algae are to be considered as a primary food source, it will be necessary to determine that they can be converted into a wide enough range of a palatable complete diet. Therefore, Spirulina, an edible alga with less nucleic acids and no cell wall, offers a good prospect for further studies by manipulating growth parameters.

In order to evaluate the potential of Spirulina for a CELSS diet, it is essential to have background information on the environmental tolerance of the species and eventually the responses of physiological characteristics. This background will be obtained from studying the species in batch and continuous cultures.

The purpose of this project was to evaluate the growth and chemical composition of Spirulina under different growth conditions in batch cultures.

MATERIALS AND METHODS

Spirulina maxima (UTEX LB 2342) was cultured in Zarrouk medium (3). The culture medium was modified for nutrient limitations. Studies for nitrogen limited cultures, sodium nitrate was replaced by potassium chloride and nitrate,

ammonia and urea were tested. For P-limited medium the P was replaced by sodium chloride and phosphoric acid was used as P-source. For salinity studies, sodium chloride was used. The pH was maintained in all cases at 9 with 2N NaOH. All experiments were incubated in 30ml medium of continuous light, in small bottles and bubbled with air. These culture were used for evaluating the growth parameters of the alga. Cultures were placed on shelves, illuminated with cool white florescence tubes of light intensity $80 \text{ uE m}^{-2} \text{ s}^{-1}$ in culture room kept at $25^\circ\text{C} \pm 1$. Light irradiation measurements were made with a Li-Cor Model Li-185 (Lambda Instruments) Meter equipped with a spherical quantum sensor.

For mass culturing, algal cells were grown in bottles. Cultures were illuminated continuously by placing them in front of a bank of two cool white fluorescent lamps (40W). Light irradiation, measured at the surface of culture bottles was $80 \text{ uE m}^{-2} \text{ s}^{-1}$. The cultures were grown in a water bath kept at $29\text{--}30^\circ\text{C}$ by the use of a heater-thermostat combination.

The cultures were aerated with air (0.03% CO_2) or air enriched with carbon dioxide. Mixtures of air (0.03%) and carbon dioxide were obtained by blending gases to a desired mixture in a two-gas proportioner. The flow rate of the mixed gas delivered to the culture was maintained at 300 ml/min.

Analysis:

Growth Rate: Growth was measured by monitoring change in absorbance (O.D.) at 560 nm with spectrophotometer (Perkin Elmer Lambda I) and expressed as doublings day^{-1} . The mean daily division rate t , K , was calculated from: $\bar{K} = \frac{3.32}{t} (\log_{10} \text{OD}_t - \log_{10} \text{OD}_0)$, Where, t = days since inoculation, OD_t = optical density after t days, OD_0 = optical density when $t = 0$.

Harvesting of Cells: Cells were collected by filtration using filter paper 10um pore size (Gelman). Cells were washed with buffer solution (pH 8), diluted to known volume and processed for further analysis. Cultures were harvested at O.D. 0.1 units, to avoid light limitation.

Total Chlorophyll: An aliquot from the culture was centrifuged for 2 min at 2000g. The precipitate was suspended in methanol for 5 min in a water bath at 70°C, and therefore centrifuged. The optical density of the supernatant was determined at 655 nm.

Dry Weight Measurements (DW): A volume from the culture was filtered through a filter 10um pore size, dried in previously dried, preweighed filter paper and then weighed after cooling in a desiccator.

Ash-Free Dry Weight (AFDA): After recording the dryweight, the dried cells were ashed. The difference between dry weight and ash weight gave the organic weight of the sample.

Total Carbohydrates: The anthrone sulphuric acid method was followed.

Total lipids: Cellular lipids were solubilized by repeated extraction with methanol and methanol-chloroform (1:1), then phase separated after adjustment of the solvent rations to 10:10:9 (methanol: chloroform: water, v/v).

Total Nitrogen and Protein using Kjeldahl methods. The value of the readings was calculated in ug N, from a standard curve for nitrogen source as ammonium sulfate, which has been treated by the same method. Total protein was calculated from total N x 6.25.

Triplicate samples of the algal suspension were taken for each determination. The mean value of these triplicates was recorded.

Nutrients Requirements:

Cultures were incubated in small bottles under the same conditions as described in Section A. The original growth medium was modified by changing the concentration of one nutrient. Nitrogen, phosphorus, iron, bicarbonate and sodium chloride were studied in sufficient and limiting concentrations. The bicarbonate effect was studied together with the aeration effect.

In all experiments triplicate culture bottles were inoculated from stock cultures in the exponential phase. Growth response was measured as optical density and the growth rate was expressed as doublings per day. The yield of cultures was expressed as the total dry weight after five days of growth. The total dry weight was determined by harvesting the cells and drying it.

B. Physiological Characterization of Spirulina in Batch Cultures:

For this experiment, the alga was grown in batch cultures (Roux bottles) as mentioned in "Methods". The cultures were maintained under optimum growth conditions and monitored in the exponential phase by the absorption measurement.

O.D. of Cell Suspension versus D.W. and Chlorophyll: The species was grown in triplicate Roux bottles under the same conditions described before (see Methods). Twenty ml of culture samples were taken daily for measurements of the D.W., and chlorophyll. The experiment was continued for one week.

Under Optimum Growth Conditions: The species was grown in duplicate Roux bottles under the same conditions described before (see Methods). Cultures were analyzed for growth parameters during the eight days.

Stress Conditions:

Light and Temperature: Batch cultures were incubated in Roux bottles irradiation and others at high temperatures (38°C) in water bath.

EXPERIMENTAL DESIGN

A. Growth Parameters Characterization

Temperature, Light: The algal growth was evaluated for temperature and light tolerance on a gradient plate. Temperature could be adjusted in range from 10°C and 50°C. Illumination was provided by eight cool white fluorescent tubes (40W). The algal species was cultured in small bottles (60 ml capacity) containing 30 ml growth medium. Triplicate cultures were placed on the gradient plate, at temperatures: 20°C, 25°C, 35°C and 40°C. The cultures were exposed to two light intensities and were aerated with air (0.03% CO₂).

pH Effect:

The alga was incubated in small bottles at 35°C on a temperature gradient plate and 80 uE m⁻¹ s⁻² irradiance. The original medium was used for culturing, except the pH used for culturing was varied by using NaOH or HCl. The pH of cultures was adjusted daily to the original pH. The cultures were aerated with air (0.03% CO₂).

Aeration Rate, Carbon Dioxide Enrichment, Bicarbonate Concentration:

The alga was incubated in small bottles at 35°C on a temperature gradient plate and 80 uE m⁻¹ s⁻² irradiance. Three sets of cultures were treated differently:

- a. Cultures were aerated with different flow rates (air 0.03% CO₂).
- b. The flow rate which gave the best growth rate, was selected from "a". The cultures were aerated with air enriched with carbon dioxide in different concentrations 1%, 3%, 5% and 10%.
- c. Cultures were treated with different bicarbonate concentrations in which one set was aerated with air (0.03% CO₂) and other set was aerated with air containing 1% CO₂. The pH of all was adjusted twice daily.

Nutrients:

Batch cultures were grown in Roux bottles in duplicate until the exponential phase was reached. One batch was analyzed and represented the culture sufficient in nutrients. Batch cultures were concentrated and diluted to the original batch volume but with a new medium modified in one element. The cultures were incubated under stressed conditions for two days and then harvested for analysis.

RESULTS AND DISCUSSION

Temperature and Light:

Figure 1 depict the growth and yield of Spirulina at two light irradiations and different temperatures ranging from 20°C to 40°C. The strain did not grow at 20°C but it started to grow at 25°C at very slow rate. Temperatures 30 and 35°C enabled the algal fastest growth rate and highest yield of cells. When the temperature was raised to 40°C, the algal cells turned yellow and gave a lower yield. The alga tolerated light irradiance $120 \text{ uE m}^{-2} \text{ s}^{-1}$.

Aeration Rate:

The effects of air agitation rate on the growth rate and cell yield are depicted in Figure 2. The growth rate of Spirulina increased with increasing the flow rate of air in range of 150 ml/min and 500 ml/min. When the flow rate of aeration was increased to 2000 ml/min, the growth rate started to decline and cells turned yellow. On the other hand the cell yield in terms of dry weight was not affected. The pH of all cultures increased to 11. The cell yield of the strain showed parallel fluctuation to the growth rate of the alga.

Air Enrichment with Carbon Dioxide:

Figure 3 shows the effect of air enriched with different concentrations of carbon dioxide on the growth rate of Spirulina:

. Cultures aerated with 10% CO₂ - in air, did not grow and turned yellow. The pH of the cultures were maintained at 9.4 by the addition of sodium hydroxide. How-

ever, the pH of cultures aerated with 1% CO₂ - enriched air was maintained stable. . Cultures aerated with air (0.03% CO₂) grew at more or less the same growth rate of those aerated with 1% CO₂ - enriched air. The yield of cultures treated with different CO₂ concentrations, in terms of dry weight, was equivalent to the growth rate.

The results of this experiment are in agreement with those of Faucher and Coupal (4). They reported that Sparging 1% CO₂ - air in Spirulina cultures could maintain a constant pH of the culture medium, and at the same time generate HCO₃ ions which were used as carbon source for S. maxima. In a similar study with green algae, Golden and Graham (5), reported that in batch cultures, maximum growth rates were achieved at the CO₂ levels present in atmospheric air and at HCO₃ concentrations of 3 mM.

pH Effect:

The growth rate of Spirulina strain was clearly affected by the pH of the growth medium as is shown in Figure 4. The alga exhibited higher growth rate in media of pH range of 9 to 10. The growth rate decreased with increasing pH above 10 and the cells turned yellow. The cell concentration increased when increasing the pH of the medium from 8 to 10 and then decreased above pH 10.

Nutrient Requirements:

Nitrogen: Nitrogen sources in the form of nitrate and urea were tested in different concentrations in order to determine their effectiveness as N-sources. The results of nitrate-N and urea-N are represented (Figure 5). The growth rate of Spirulina was enhanced with increasing the concentration of urea-N and nitrate-N. The urea-N at 20mM concentration enhanced the growth rate, while further increase in its concentration limited the growth of the strain.

On the other hand, nitrate-N at concentration 30 mM, enabled the strain to reach fast growth rate and high yield in terms of dry weight. The experiment demonstrated that the least amount of nitrate-N necessary to maintain the growth of Spirulina in culture was 10 mM. Microscopically, the trichomes became shorter with average 6 turns/trichome, in media limited in nitrogen concentration. In agreement with our results, Faucher (4) reported that urea-N in low concentration could support the growth of S. maxima, at high concentration of nitrate-N.

Phosphate: Increasing the phosphate-P concentration in the culturing media to 1 mM to 5 mM, enhanced the growth rate of the strain (Figure 6). But as the concentration increased to 10 mM, the growth rate declined. The mass yield showed similar responses coinciding with the growth rate. Microscopically, the trichomes became shorter in media of phosphate-P concentration below 1 mM and with few number of turns in case of S. maxima (5 turns/trichome). Generally, cyanobacteria require small concentrations of phosphate-P for growth. They grow in phosphorus-limited media (6).

Sodium Chloride: Spirulina grew in media lacking sodium chloride (Figure 7). The growth rate increased as the sodium chloride concentration increased to 0.01M. Further increase in sodium chloride concentration (0.1M) affected the growth rate and resulted in lower yield of cells. In addition, microscopic examination of the strain indicated that in media treated with a high concentration of sodium chloride 0.1M, the trichomes were short and with less turns, the average turns per trichome was 6. The results of this experiment, indicate that Spirulina tolerate increases in sodium chloride concentration up to 100 mM. Spirulina tolerance to salt had been previously reported (4).

Iron: Iron concentrations (FeSO_4) influenced the growth and yield of the culture (Figure 8). Concentration of 0.05 mM was sufficient for the growth, although media deficient in iron did not show any growth response. Increasing the concentration of iron beyond 0.1 mM lowered the yield of alga and cells turned yellow.

Bicarbonate Concentration: Figure 9 shows that S. maxima grows in the medium even without bicarbonate salt, providing that the culture was aerated with air (0.03% CO_2). As the bicarbonate concentration increased, the growth rate as well as productivity increased. Further increase in bicarbonate concentration above 16g/L (190 mM) did not affect the growth rate. When the carbon dioxide concentration in the air increased from 0.03% to 1%, as shown in Figure 9, the growth rate increased remarkably by decreasing the bicarbonate concentration in the medium as low as 4g/L i.e. one quarter of the concentration in the Zarrouk medium (see Methods). The results of this experiment indicate that Spirulina can utilize atmospheric carbon dioxide when the media bicarbonate concentration is minimum in the culture medium. The pH of all cultures was adjusted daily to 9.4.

Physiological Characterization of Spirulina in Batch Cultures:

Batch Cultures:

Optical Density (O.D.) of Cell Suspension versus Dry Weight (D.W.) and Chlorophyll: Results are presented in Figure 10. For all samples within the first three days of cultivation, which contain relatively small concentrations of biomass (400 mg DW/L or less), readings fell within the accurate range of the O.D. scale and they could be read directly from the spectrophotometer without dilution. However, for all samples during the later cultivation periods which contained high concentration of biomass (500 mg DW/L), dilution of the samples with distilled water was necessary prior to OD readings. The graph show linearity between OD and dry weight.

Each OD unit is equivalent to a concentration of 700 mg/L in the case of S. maxima. It is obvious from this experiment that other reliable indicators of estimating algal productivity can be computed from OD measurements. Therefore, OD measurements can be translated into biomass yield in terms of dry weight or chlorophyll.

Physiological Characteristics of Culture, under Optimum Growth Conditions:

The cultures were assayed for growth parameters during the eight days. (Fig. 11) Increments of carbohydrates, proteins, dry weight and chlorophyll are expressed as ug/ml culture. The results show that increases in the synthesis of chlorophyll, protein and yield of the culture are correlated. Growth parameters of cultures analyzed after eight days started to level off, due the nutrient exhaustion and light limitation caused by increasing cell concentration.

Physiological Characterization of Cultures, under Stress Conditions:

The results of analysis were expressed on the basis of organic weight (Ash Free Dry Weight: AFDW) and represented in Table 1.

Light Irradiance and Temperature: Increasing the light irradiance to $120 \text{ uE m}^{-2} \text{ s}^{-1}$, led to an increase in the total carbohydrate content and a decrease in protein content: S. maxima 19.58%, 29.06%. Increasing the temperature of culture incubation to 38°C , influenced the composition of the strain, in a similar manner to the light irradiation experiment: S. maxima, 45.28%, 18.75%, for protein and carbohydrates, respectively. The culture produced a low percentage of lipids, when grown in high temperature experiments. Cells turned yellow green in color. Studies with light-limited cyanobacteria showed a high level of polysaccharide formation when they exposed to high light intensities (8).

Nutrient Limitation: Media limited in nitrate-N and phosphate-P, favored the accumulation of carbohydrate rather than protein. Nitrate and phosphate

limited cultures: S. maxima had 37.52%, 35.21% carbohydrate and 21.56%, 41.25% protein. When the cultures were transferred to media limited in nitrogen and phosphate, cultures changed in color from blue to yellow-green. N-limited cultures of Anacystis nidulans (7), and P-limited cultures of Oscillatoria agardhii (8), showed elevated levels of polysaccharide storage.

Sodium Chloride: As Zarrouk (3) media were enriched with 0.1M and 0.5M NaCl, the carbohydrate content of the cells increased, when compared to that of the control (Zarrouk: 0.01M NaCl), to 26.25%, 36.73% in S. maxima. On the other hand, the total protein decreased respectively to: 52.62%, 45.64% in S. maxima. The lipid percentages showed little increase when compared to those of the complete media (control). Many cyanobacteria are capable of adapting to a range of salinity in the environment by synthesizing internal osmotic support in the form of carbohydrates.

Bicarbonate: When bicarbonate concentration of Zarrouk media was reduced to one quarter (4.g/L), the culture showed much difference in the chemical composition as compared with the control media except their yield was somewhat below the control. The carbohydrate increased to 38.53% when 0.03% CO₂ in air was used for aeration and to 40.23% when 1% CO₂ air was used for aeration.

Conclusions of this study are summarized as following:

- . The lipid percentage, in particular, did not show much increase in different culture treatments. But, increasing the temperature of culturing to 38°C or light irradiance to 120 $\mu\text{E m}^{-2} \text{s}^{-1}$, reduced the total lipids drastically. However, increasing sodium chloride to 0.1M in the culturing media, the lipids increased somewhat higher than in the control media.
- . The ability of the alga to utilize macroelements and microelements, and to convert it into biomass.

- . A slight inverse relationship was observed between the protein content and carbohydrates which means that one increased at the expenses of the other. This suggests that quality of biomass may be manipulated for dietary purposes. An adequate supply of nutrients is therefore a pre-requisite for producing a uniform quality of biomass, which in turn could then be used in the formation of diets. (see Sufficient Nutrients). The possibility of manipulating the quality of the biomass could have potential for the NASA/CELSS Program, when specific diet formulation is needed (e.g. low protein content).
- . Overall algal productivity and quality could be manipulated by means of varying nutrient concentrations or temperature and light irradiance.

It can be concluded that through manipulating environmental conditions of the algal growth, one can modify the photosynthetic products. Thus, Spirulina can be, through manipulating growth factors, used as palatable diet comparable to higher plants.

Further work is needed to characterize the efficiency of the algal cells under such environmental conditions in terms of gas exchange and energy loss or gain in steady state.

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Table 1. Molecular Composition of *Spirulina maxima*

Growth Conditions	% Organic Wt. (AFDW)		
	Protein	Carbohydrate	Lipids
*Sufficient Nutrients	69.75	11.5	4.68
High Light (160uEm ⁻² s ⁻¹)	29.06	19.58	3.56
High Temperature (38°C)	45.28	18.75	3.75
N-limited	21.56	37.52	4.68
P-limited	41.25	35.21	5.20
Sodium Chloride 0.1M	52.62	26.25	4.68
0.1M	45.64	36.73	7.52
Bicarbonate (4.4g/L) (0.03% CO ₂)	45.67	38.53	6.22
1% CO ₂)	43.52	40.23	6.53

* Experimental conditions were:
temperature 30°C; light irradiance 80uEm⁻²s⁻¹;
air flow rate 300 ml/min;
The values shown are averages of four independent determinations.

SPIRULINA maxima

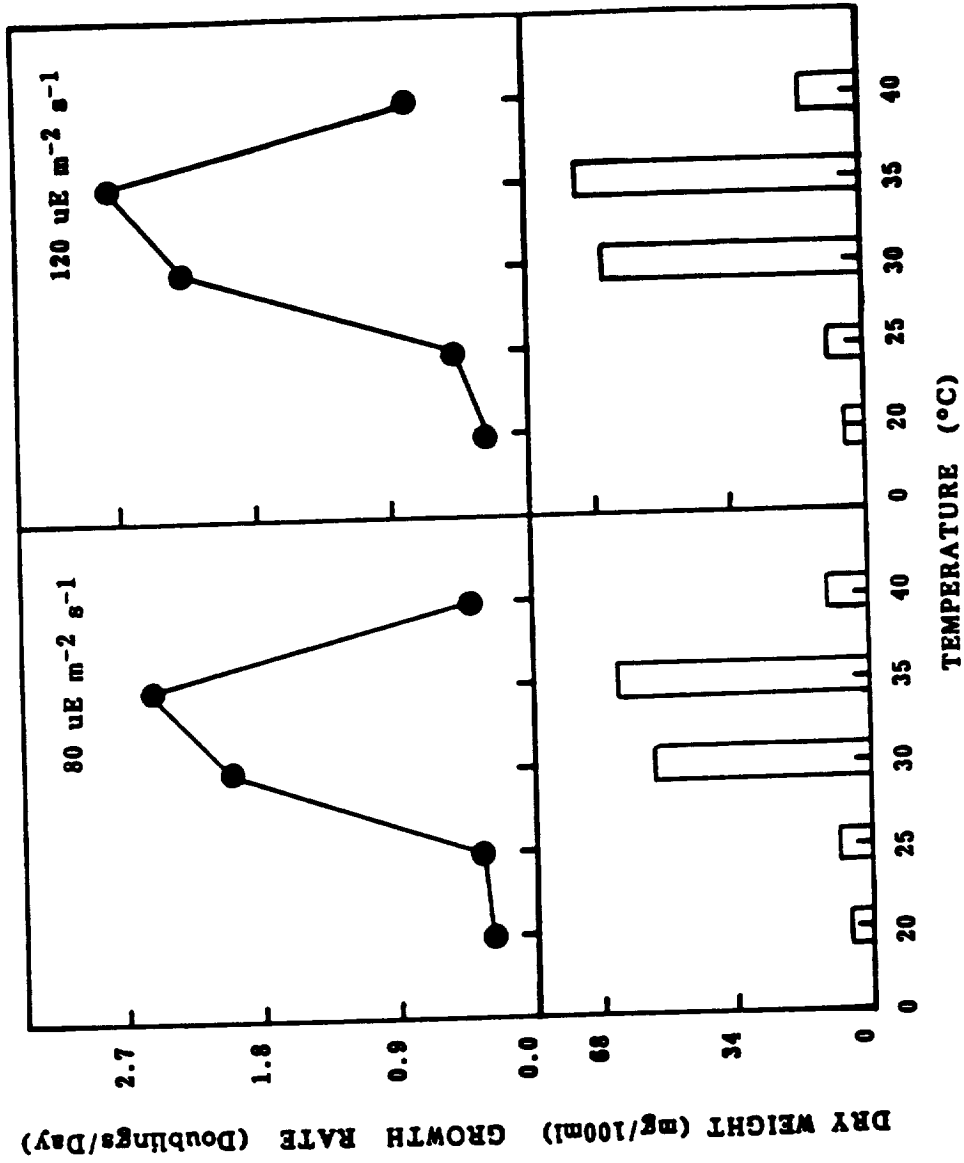


Figure 1. Growth Rate and Yield of *S. maxima* as Function of Temperature and Light Irradiance

SPIRULINA maxima

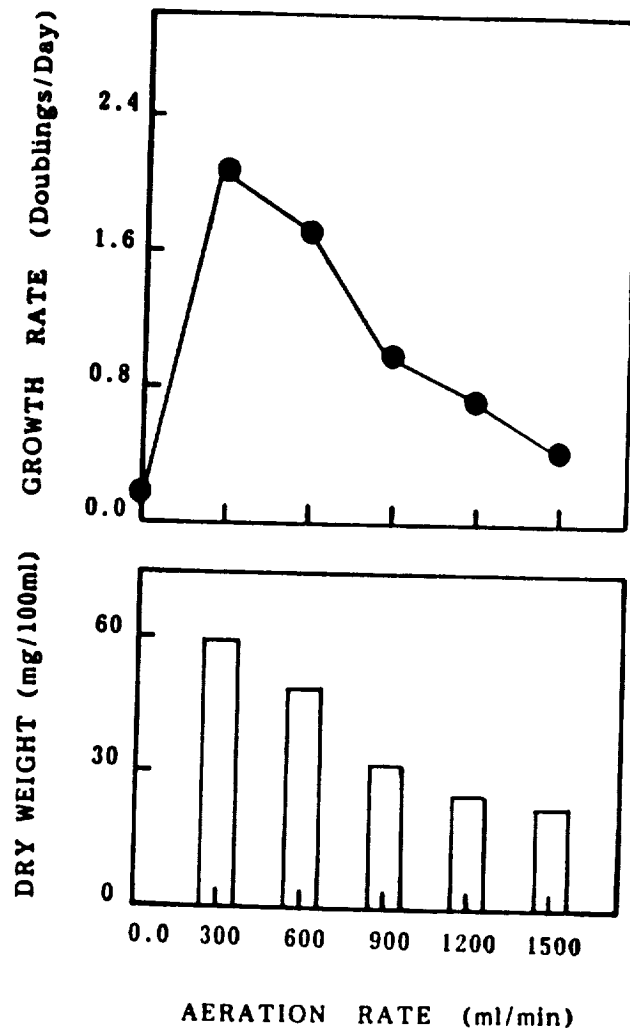


Figure 2. Growth Rate and Yield of *S. maxima* as a Function of Aeration Rate

SPIRULINA maxima

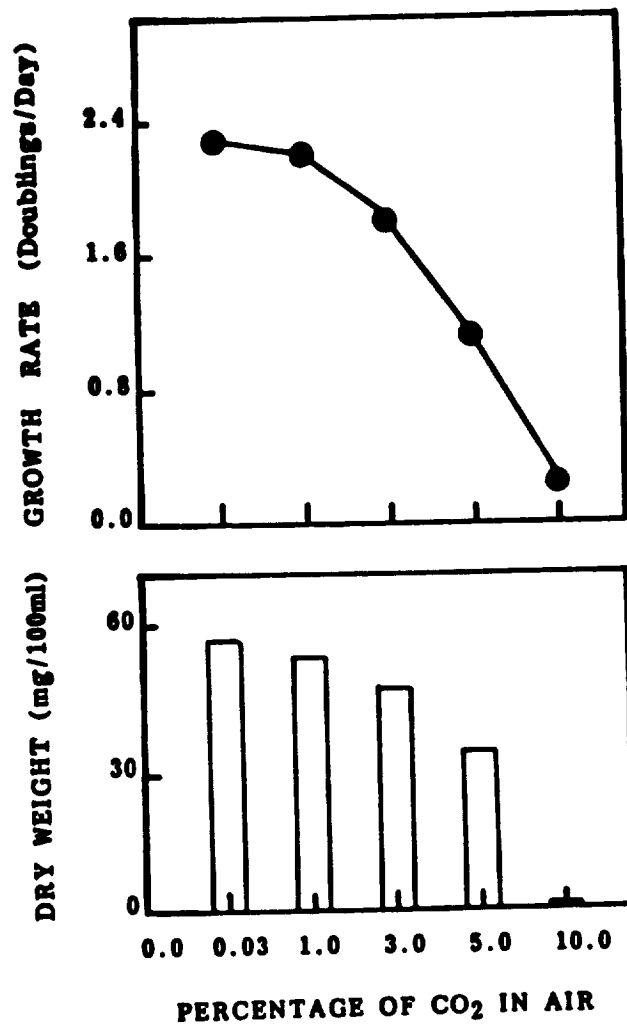


Figure 3. Growth Rate and Yield of *S. maxima* as a Function of Carbon Dioxide Concentration in Air

SPIRULINA maxima

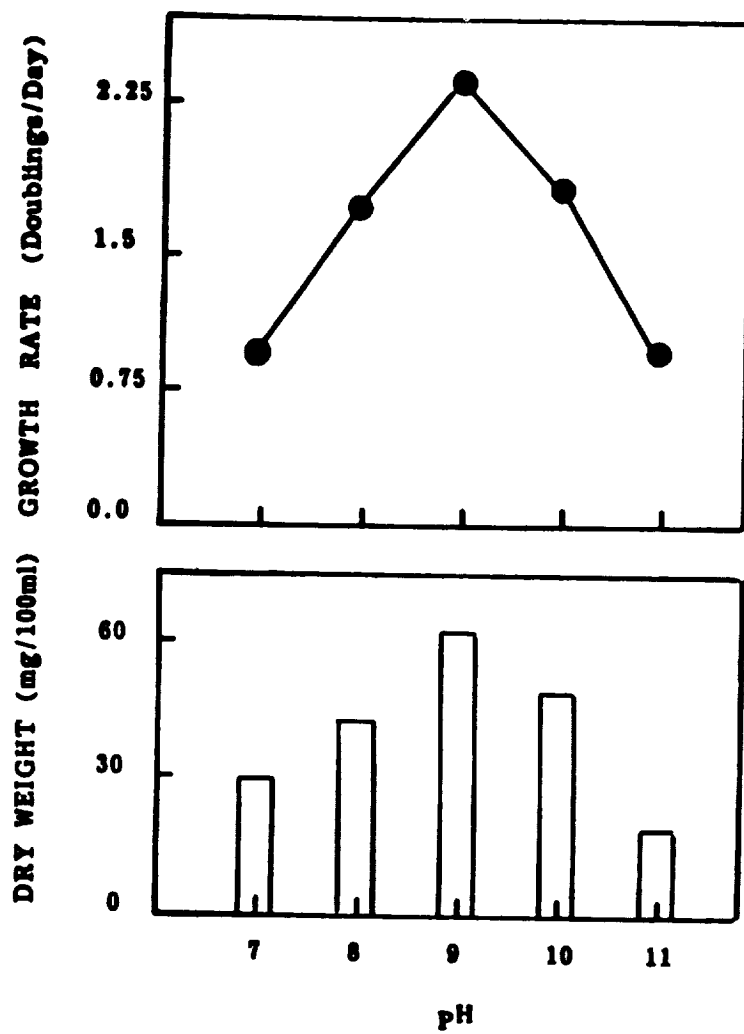


Figure 4. Growth Rate and Yield of S. maxima as a Function of pH.

SPIRULINA maxima

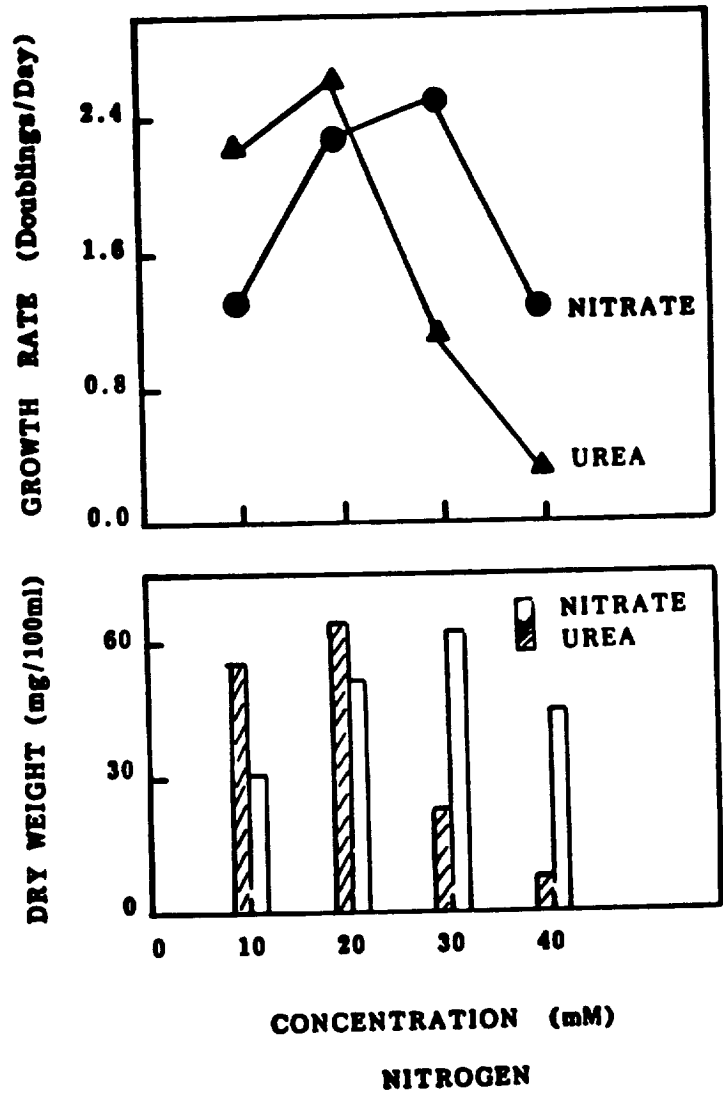


Figure 5. Growth Rate and Yield of *S. maxima* as a Function of Nitrogen Concentration

SPIRULINA maxima

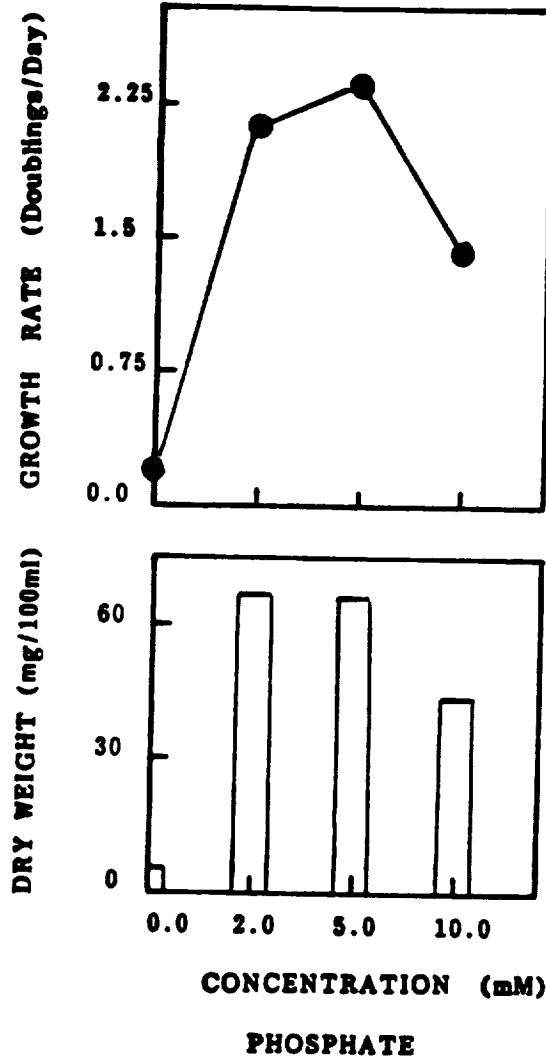


Figure 6. Growth Rate and Yield of *S. maxima* as a Function of Phosphate Concentration

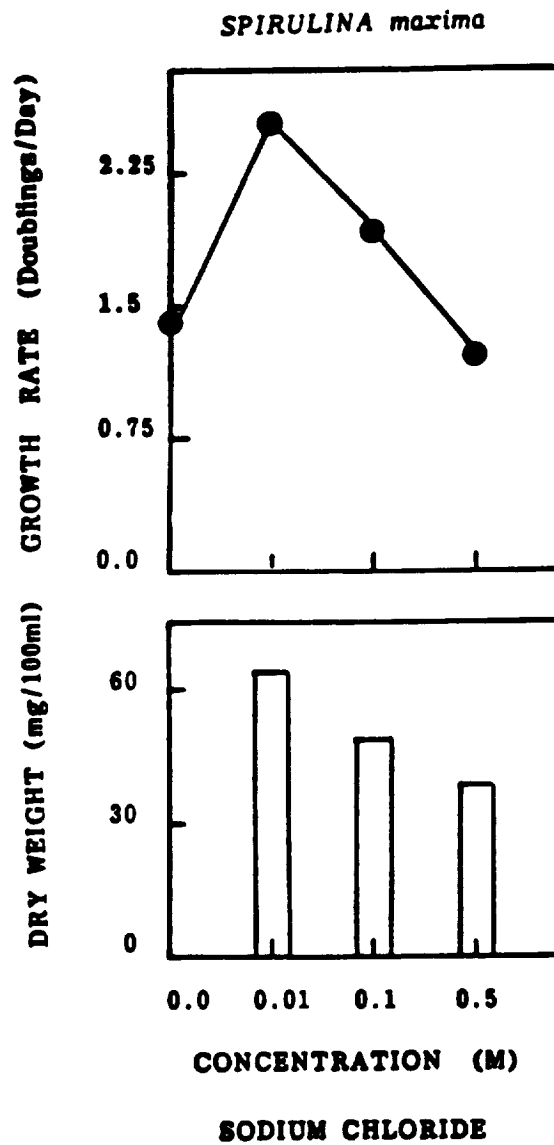


Figure 7. Growth Rate and Yield of *S. maxima* as a Function of Sodium Chloride Concentration

SPIRULINA maxima

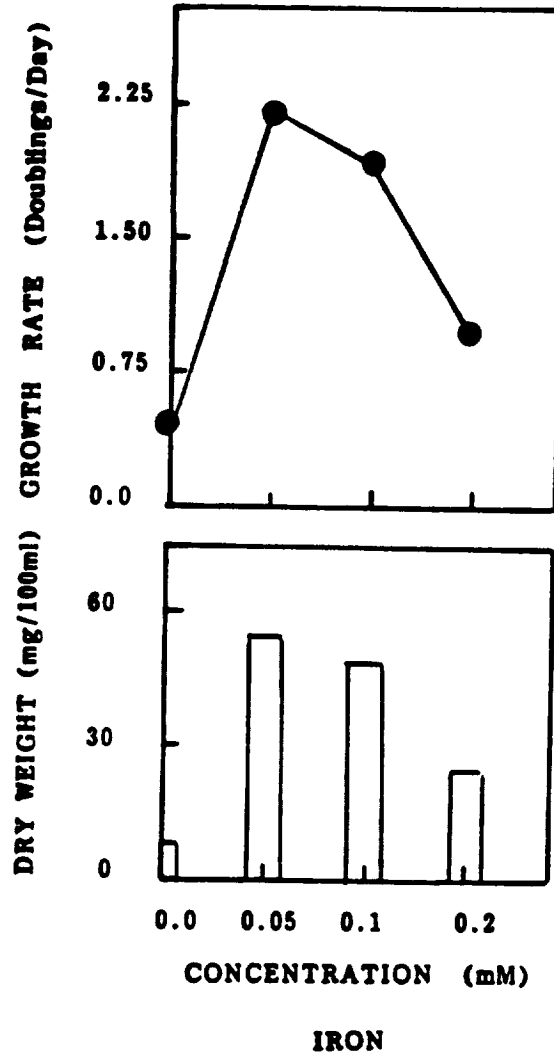


Figure 8. Growth Rate and Yield of *S. maxima* as a Function of Iron Concentrations

SPIRULINA maxima

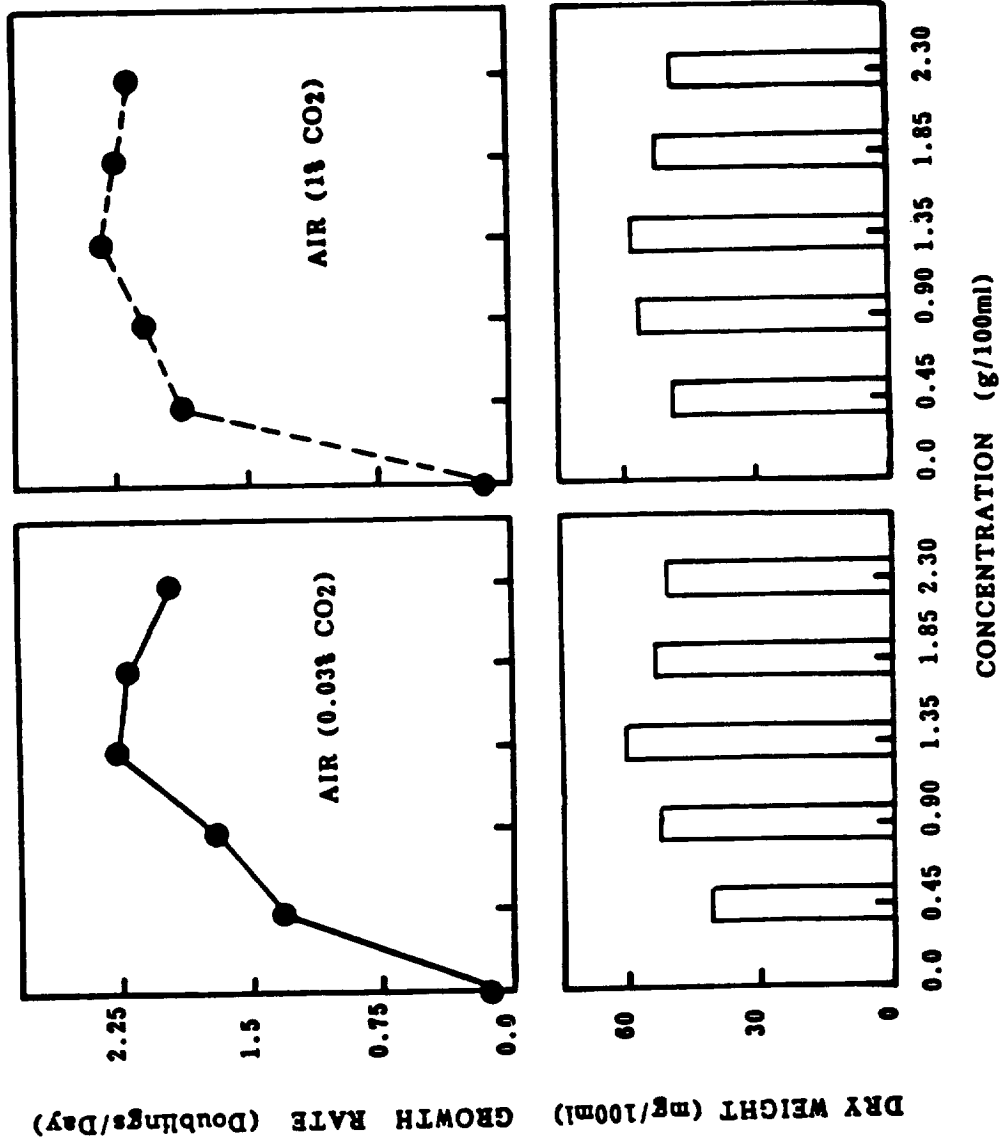


Figure 9. Growth Rate and Yield of *S. maxima* as a Function of Bicarbonate Concentration

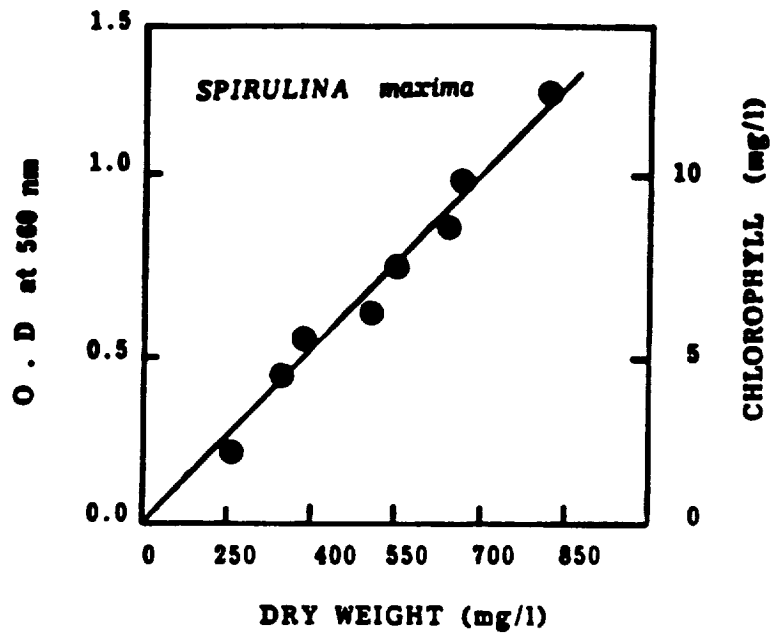


Figure 10. Optical Density versus Dry Weight S. maxima and Total Chlorophyll

SPIRULINA maxima

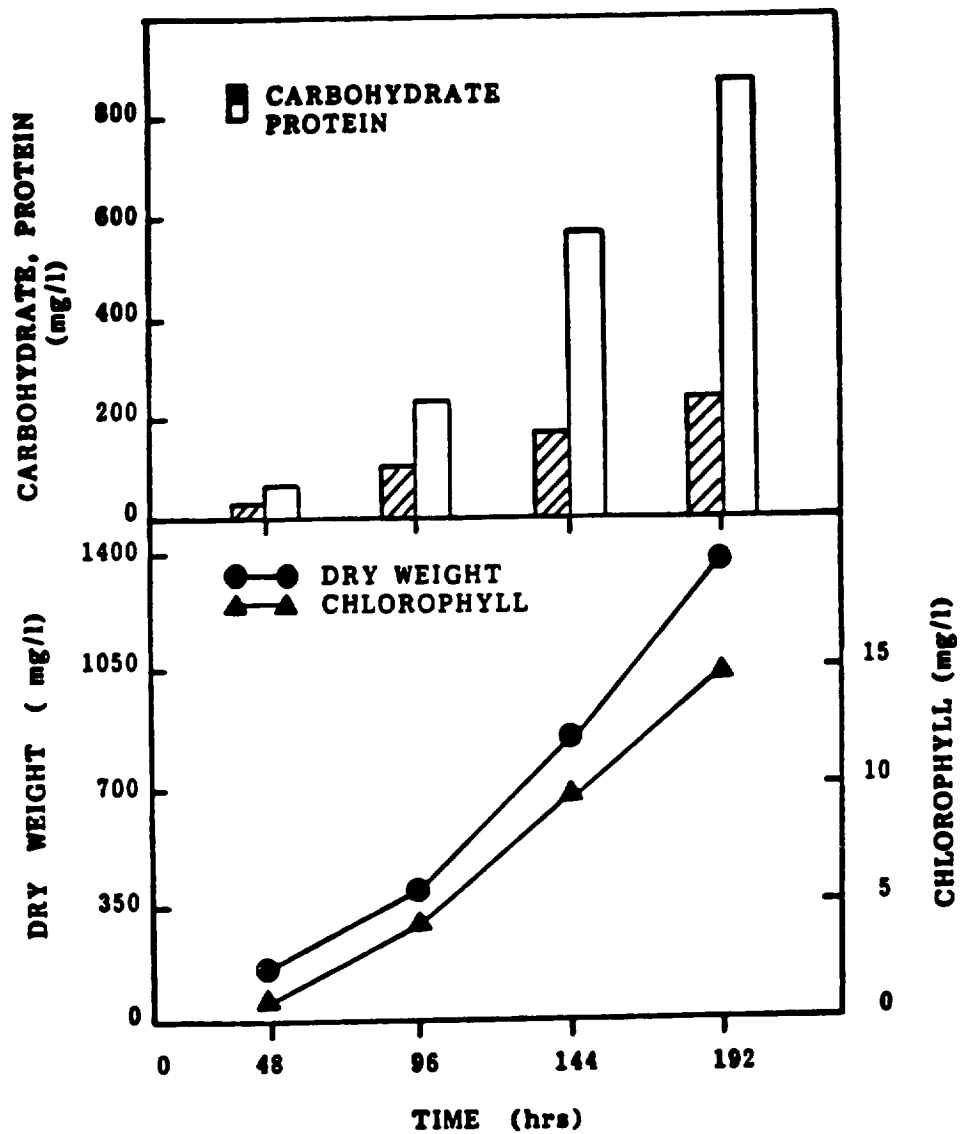


Figure 11. Physiological Characteristics of *S. maxima* under Optimum Growth Conditions

PRELIMINARY EVALUATION OF WASTE PROCESSING IN A CELSS

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ABSTRACT

Physical/chemical, biological, and hybrid methods can be used in a space environment for processing wastes generated by a CELSS. The waste materials in a bioregenerative life support system will be generated by numerous sources. Representative examples of waste components include: the inedible materials from higher plants; volatile organics produced by humans, plants, algae, fungi, and bacteria; CO₂; water vapor; urine; feces; waste water from washing and hygiene; and trash that includes a wide variety of solid materials. To develop systems that are capable of recycling these materials it is necessary to know their composition, the rates at which they are produced, the advantages to be gained in separating them before processing, and the fates of their constituents during various oxidation regimes. Two recycling scenarios, derived from qualitative considerations as opposed to quantitative mass and energy balances, tradeoff studies, etc., will be presented; they reflect differing emphases on and responses to the waste stream formation rates and their composition, as well as indicate the required products from waste treatment that are needed in a life support system. The data presented demonstrate the magnitude of the challenge to developing a life support system for a space habitat requiring a high degree of closure.

INTRODUCTION

Renewed interest in long duration human space missions, particularly the establishment of a Lunar base or a mission to Mars, has prompted a critical evaluation of advanced life support systems (1). This evaluation has revealed that current methods available for nearly complete recycling of water, oxygen, and food in space are technologically and economically impractical. Such limitations will prevent humans from spending long periods of time

in space. Therefore, research emphasis should be placed on developing improved recycling techniques to overcome these limitations.

Nearly complete recycling can be theoretically accomplished by life support subsystems which are dependent either on physical or chemical (P/C) principles or by subsystems which include a living or biological component, such as a Controlled Ecological Life Support System (CELLS). A subsystem which is based on a physical or chemical principle, for example, is a water electrolysis unit which provides oxygen for respiration by using electrical energy to decompose water into hydrogen and oxygen. Higher plants are an example of a biological subsystem that produces oxygen for respiration through photosynthesis, using sunlight for its source of energy. If a high degree of closure of the life support system is required, wherein most of the water, oxygen, and food is recycled, then a hybrid system consisting of a combination of P/C and biological subsystems will undoubtedly be needed.

Recycling in a space habitat implies the conversion of waste streams derived from several different sources into useable products. Some of the waste streams are common to both the P/C and CELSS based life support systems. For example, all of the wastes derived from a human are common to both systems. Certain waste streams are present only in space habitats that use living subsystems as an integral part of the life support system. To illustrate: if higher plants are used to produce food, then inedible biomass (in substantial quantity), water derived from the transpiration of plants, and the spent, plant nutrient solution are wastes not found in a solely P/C life support system.

Not only are the input waste streams different in P/C and biological systems, but the required outputs are also different. A CELSS requires plant nutrients as an output stream, a requirement unique to a photosynthetic-based food production and life support system.

For either the development of a computer model of a waste treatment or recycling subsystem or the functional design itself, it is desirable to have well defined input feed streams, including production rates and composition. Recent data are presented, as specifically as possible, on the nature of the waste streams that could be encountered in a human space habitat. Those streams that are characteristic of a given type of life support system are identified, and two representative scenarios for recycling wastes and nitrogen in a bioregenerative life support system are described.

WASTE SOURCES

In determining the treatment to be applied to any waste stream of a life support system, at least three factors must be considered: 1) stream composition, 2) rate of stream production, and 3) required end product(s).

WASTES FROM GENERAL HUMAN ACTIVITIES

Parker and Gallagher (2) reported results from a comprehensive study of human wastes in which over 25,000 person-days of data was analyzed. They reported mean values for the dry and wet weight of human feces, the volume of human urine (2,066 milliliters/person-day), solids per menstrual period (10 grams), the average number of pads or tampons used per period (15.2), the

average weight of pads (10.65 grams) and tampons (2.60grams) from different manufacturers, and the total amount of toilet paper usage for women for bowel movements and urination (41.1 grams/woman-day). The solids content of human urine was obtained from previous work (3). It should be noted that the values reported by Parker and Gallagher are mean values and they emphasize that a space habitat waste handling and treatment subsystem must be designed to accomodate extremes and should not be designed on the basis of mean values.

The type and amounts of organic and inorganic constituents in human urine can be identified in previous work (3 and 4). The elemental composition of human feces derived from subjects fed a specified diet is also available (3).

The amount of urinal flush water shown in Table 1 is being used for designing or sizing the environmental control and life support system (ECLSS) for the US Space Station (5). The volumes of dish, laundry, shower, and hand wash water were obtained in a private communication (6) and these amounts are also being used for designing the Space Station ECLSS. The amount of cabin humidity condensate and its contaminant concentration were derived from Space Shuttle data and are also part of the design load for the Space Station ECLSS.

The amount of food preparation waste and details concerning its composition are available (3). The work of M. Karel of the Massachusetts Institute of Technology, was employed for designing a model food processing and preparation waste for the US CELSS program (3). In designing this model waste, it was assumed that

Table 1. Waste Feed Stream Production Rates and Solids Content in a Manned Space Habitat containing a Higher Plant Growth Chamber			
Stream ID	Wet Weight Formation Rate, lb/person-day	Dry Weight Formation Rate, lb/person-day	Weight Percent Solids, %
Toilet Waste			
Urine (2, 3)	4.59 (a)	0.14	3.1
Feces (2)	0.21	0.0452	21.4
Wipes (2)	0.091	Unknown	Unknown
Urinal Water (5)	1.09	NA	NA
Hygiene Water			
Dish (6)	12	2.6×10^{-3}	0.022 (b)
Shower & Hand (6)	12	3.4×10^{-3}	0.028 (c)
Laundry (6)	28	1.5×10^{-3}	0.0054 (b)
Humidity Condensate (6)	8.26	1.3×10^{-3}	0.016
Food Preparation Waste (3)	0.13	0.044	34
Trash	2.2	Unknown	Unknown
Respired CO ₂ in Air (5)	NA	2.2	NA
Contaminated Cabin Air (11)		See Table 3	
Inedible Bio-mass (Wheat Chaff)	14	1.4	10
Transpiration Water (7)	136 - 678	See Note (d)	

Footnotes

- (a) The density of urine was taken as 1.008 g/ml (4) to convert urine volume to weight.
- (b) Detergent only; sodium dodecyl benzene sulfonate (an anionic detergent).
- (c) Cleansing agent only; Economics Laboratory Cleansing Agent Formulation 6503.54.4 (an anionic detergent).
- (d) The contaminant load in transpired water from plants is unknown.

the CELSS population would be small, that plants would be grown hydroponically, and that animals would not be part of a CELSS.

In 1985, the NASA-Ames laboratory analyzed the trash brought back to Earth aboard Space Shuttle Flight 51D. The objective of this analysis was to gain insight into the composition, amount, and volume of trash produced during a representative human space mission. This type of information will be needed for the design of a long term human space mission waste handling and treatment subsystems. The results from this analysis are shown in Table 2.

Table 2. Composition and amount of trash derived from Space Shuttle Flight 51D (49 person-day flight)		
Trash Constituent	Weight, lbs	Volume, ft³
Food Containers (a)	50.8	3.3 (b)
Paper	14	1.3
Biomedical	14	1.0
Leftover Food & Garbage	10.5	0.3
Plastic Bags	7	2.2
Grey or Duct Tape	3.5	0.3
Cans, Aluminum & Bimetallic	2.8	0.5
Miscellaneous	5.8	0.8
Total	108	9.9

Footnotes

- (a) Includes 27 lbs of uneaten food and beverages.
- (b) After cleaning and stacking.

AIR CONTAMINANTS

Contaminated air from crew quarters is another waste stream that must be treated in the closed environment of a space habitat. Wastes in cabin air include water and carbon dioxide from perspiration and respiration, volatile contaminants from people and equipment, and airborne particles. The average amount of carbon dioxide produced by an adult each day is shown in Table 1 (5). A contaminant load model (including contaminant type and concentration) is a prerequisite for the design and sizing of the contaminant control subsystem for a space habitat. The load model being used for designing the Space Station contaminant removal subsystem has been developed (11). An extensive list of representative volatile contaminants illustrating the broad spectrum of compounds one can expect to find in a closed habitat is described. Specific information also includes the space maximum allowable concentrations (SMAC) for continuous exposure to a given contaminant.

The estimated concentration and size of airborne particles which are expected to be found aboard the Space Station are given in Table 3 (11). These estimates are being used for the design and sizing of the contaminant control subsystem. In estimating the rate of generation of airborne particles expected aboard the Space Station, it was assumed that about 90 percent of the particles would be derived from humans and their activities. To obtain the total generation rate of particles or dust expected

aboard the Space Station, the numbers in Table 3 must be multiplied by the crew size and the factor 1.1 to account for particle generation by sources other than people (assumed to be 10 percent of the total).

Table 3. Estimation of Space Station Particle or Dust Generation Rate by Humans (11)	
Particle Size, (microns)	Particle Generation, (particles/hr/person)
0.3 - 0.5	81,341,426
0.5 - 1	34,570,164
1 - 2	4,270,366
2 - 5	1,565,870
5 - 10	211,548
Above 10	40,626

WASTES FROM PLANT PRODUCTION ACTIVITIES

In estimating the amount of inedible biomass and transpiration water (Table 1) that must be handled by the waste treatment subsystem in a CELSS, the following assumptions were made: a) the average amount of dry food required by each adult per day is 0.617 kilograms (5), b) wheat alone can meet a person's daily caloric but not necessarily nutritional requirement, c) only 50 percent of the dry mass of a mature wheat plant is inedible (i.e., an optimistic harvest index of 50 percent), d) 90 percent of the wet weight of the inedible portion of a wheat plant is comprised of

water, and e) depending upon carbon dioxide concentration, the amount of transpired water ranges from 50-250 grams per gram of plant (edible plus inedible) dry weight (7).

Transpiration water may contain volatile organic compounds that must be removed before recycling the water. These compounds may come from plants or materials in the plant growth chamber. Currently, the type and concentration of contaminants in transpiration water are poorly defined and therefore this stream must be considered as a waste stream that will require some processing.

WASTES FROM EXPERIMENTAL SYSTEMS

Experiments being conducted in a space habitat will also contribute waste of varying types and amounts that will require handling and treatment. The broad spectrum of wastes that might be derived from experiments precludes this source of waste from being considered here. However, a study has been conducted in which waste derived from potential flight experiments is defined (8).

WASTE PROCESSING

Given the quality and quantity of the waste streams presented in Table 1, there are a number of different waste processing methods that one might consider for handling and treating these wastes. These methods include both biological and physical/chemical processes. The optimum combination of processing technologies remains to be determined. However, a scenario based on qualitative considerations (as opposed to detailed mass and energy balance calculations, tradeoff studies, etc.) is shown in Figure 1.

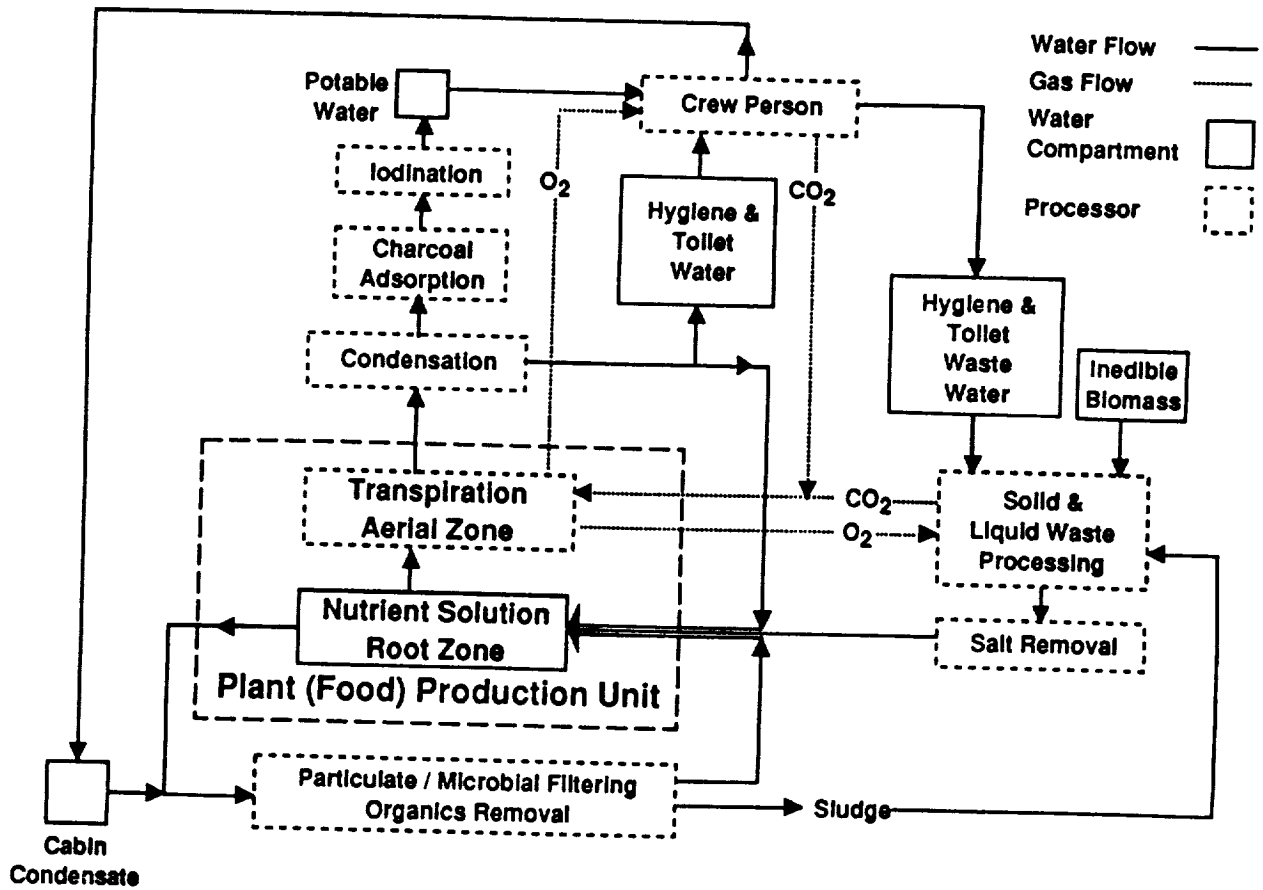


Fig. 1. Representative Water Pathways in a CELSS.

GENERAL WATER AND GAS PROCESSING SCENARIO

Figure 1 graphically depicts a water pathway scenario in a CELSS. The scenario shows how water may be processed or treated in order to attain the required quality standards of each of the depicted water compartments. The different sizes of the solid boxes in Figure 1 reflect proportional volumes (derived principally from Table 1) of the major water compartments on a per day per person basis (actual sizes are dependent upon the rate of throughput and storage considerations).

It should be noted that the range for transpiration water production set forth in Table 1 is determined by the environmental conditions, predominantly the concentration of carbon dioxide in the plant growth chamber. Transpiration water production is inversely proportional to the carbon dioxide partial pressure. When the CO₂ pressure is low, the stomates, openings in the leaves through which gas and water exchange occur, open, and the transpiration rate is high (up to 250 grams water transpired per gram of dry biomass produced); the reverse is true for high CO₂ concentrations.

The ability to change the transpiration rate by varying the CO₂ concentration can be an important control factor in a CELSS. For example, under optional plant growth conditions, more than enough water is provided to meet crew requirements even though the transpiration rate is low. Should an emergency occur whereby more water is needed by the crew, the transpiration rate and the amount of water provided to the crew could be quickly increased, by merely decreasing the CO₂ concentration in the plant growth

chamber.

It is expected that transpiration water, having been derived from a phase change process, will be relatively clean. Therefore, this waste stream may need only minimal filtering and bacterial control to yield high quality water for drinking and other applications. However, most of the condensed transpiration water will be used to replenish water lost from the plant nutrient solution. This cycle as illustrated in Figure 1 shows nutrient solution make-up water also being introduced from other processors.

The condensate collected from the cabin environmental control system, having passed through a phase change, is also expected to be quite clean (see Table 1). However, humidity condensate may contain a high population of microbes derived from microbial growth on the condenser or heat exchanger. Although the amount of the condensate collected is not enough to meet the hygiene and toilet water requirement, the water recovered can be combined and treated along with transpiration water and spent nutrient solution. The nutrient solution will contain an unknown number of microbes as well as organic compounds produced by root metabolism and detritus breakdown. These contaminants can be filtered out and useable salts can be returned to the nutrient solution. The filtrate or sludge would be treated in the solid waste processor.

Toilet water containing feces and the inedible biomass waste streams have relatively high solids concentrations and also may contain potentially harmful microbes. Therefore, these streams will require a more rigorous treatment. High temperature and

pressure processes, such as wet oxidation or supercritical water oxidation may be used to treat these more concentrated streams and to assist in closing the water cycle between the crew person and the plant production unit. The water produced by the solid and liquid waste processor (see Figure 1) includes the yield from inedible biomass, hygiene and toilet water treatment, and also from a certain amount inherent in some of the items listed in Table 2, as well as from the root zone filtrate. This water is not necessarily potable, but after salts and potentially toxic metals (derived from corrosion) are removed, it is benign to the plants growing from the nutrient solution to which it is returned.

Gas exchange between the plant growth chamber and other parts of a CELSS is also an important part of recycling. For example, oxygen produced by photosynthesis in the plant growth chamber may be used for oxidation in the waste processor and for respiration by the crew. Likewise, the CO₂ produced by both crew and waste oxidation is needed by the plants for their growth.

The crew consumes water-containing edible biomass, drinks potable water, and produces waste. Consequently, the crew closes the life support loop in this generally described scenario.

NITROGEN RECYCLE THROUGH A HYBRID WASTE PROCESSING SYSTEM

Figure 2 illustrates a more specific waste recycling scenario which includes methods for recycling nitrogen and converting it into forms desirable for plant metabolism. In this scenario, organic nitrogen is converted into ammonia (NH₃) and nitrate ions (NO₃⁻) which are species desired by plants for their nutrition.

As mentioned previously, waste processing, applied to closing

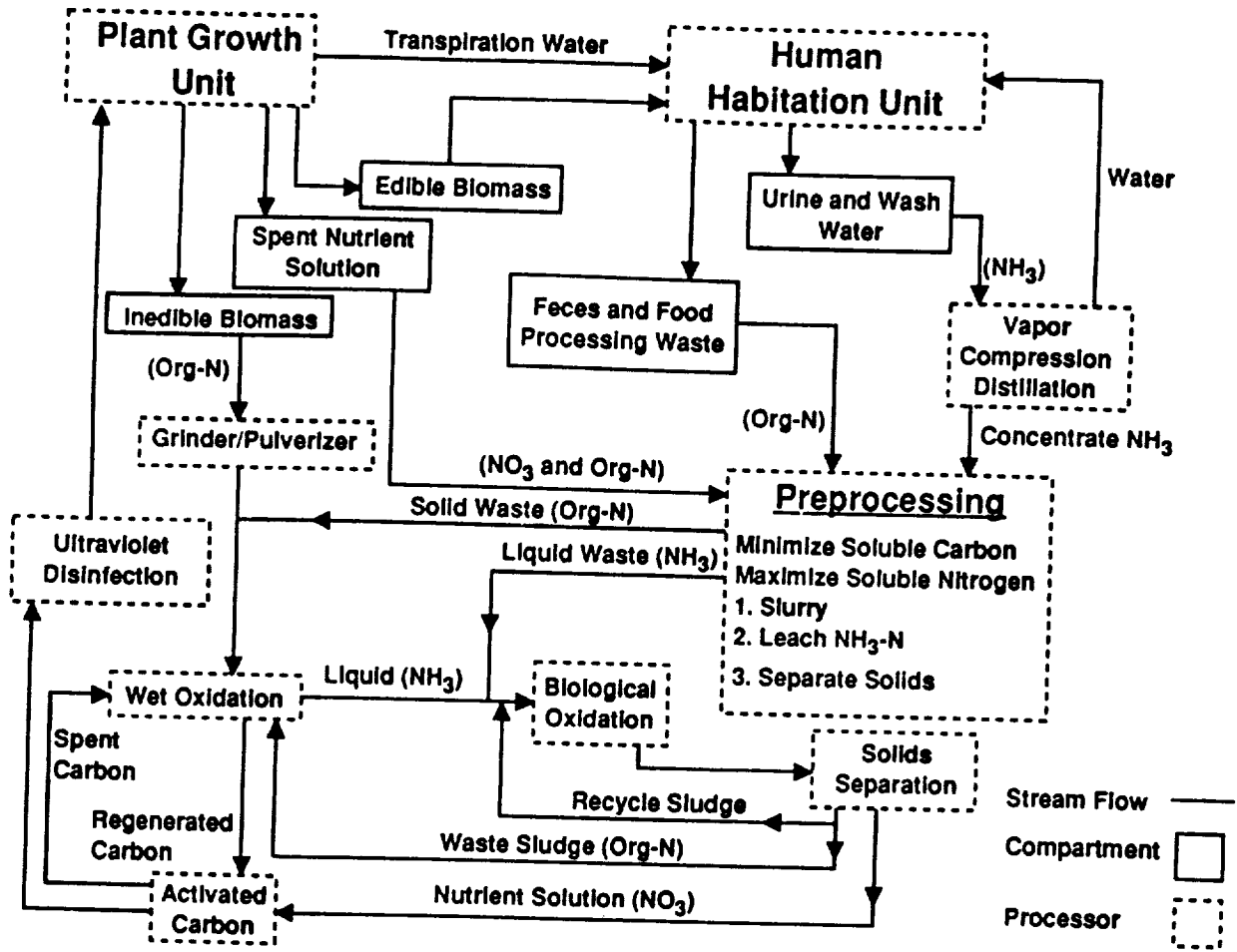


Fig. 2. Waste Processing in a CELSS: Nitrogen Recovery.

a life support system, can be achieved by three basic methods. Physical/chemical methods are directly applicable to handling liquids as well as large quantities of solid materials, but suffer a significant limitation; the inability to produce a form of nitrogen which is reuseable directly by higher plants. The physical/chemical waste processing methods characteristically produce nitrogen gases (N_2 and N_2O) while growing plants for a CELSS require either nitrate or ammonium ions (NH_4^+) as a source of nutrition. Aerobic biological waste processing systems can produce either or both ions as a final end product but do not handle solid wastes efficiently. With the above requirements and characteristics in mind, a scenario of an integrated waste processing system is discussed below. The hybrid system includes vapor compression distillation, wet oxidation, biological oxidation, activated carbon adsorption, and ultraviolet disinfection. A schematic diagram of the proposed system is presented in Figure 2. The treatment scheme is simplified to show the flow of nitrogen only.

The treatment scenario assumes processing of wastes generated by the plant growth and human habitation units. Wastes from the plant growth unit include the inedible biomass and the spent nutrient solution. The inedible biomass is principally solid waste (wheat chaff, etc.) low in organic nitrogen (Org-N) content (3) but overall represents a potentially high mass of nitrogen due to the large amount of material generated. The inedible biomass would first be ground and pulverized to reduce the total volume and particle size. Final processing could be handled by wet

oxidation operated at a temperature less than 300 C and a pressure of 1500 psig to minimize loss of nitrogen as N_2 gas and maximize recovery of ammonia nitrogen (NH_3-N) (9). The spent nutrient solution is primarily water, inorganic salts, and organic residues exuded by the plants. The spent nutrient solution would be sent to the preprocessing stage to act as a wetting agent to slurry and maximize leaching of Org-N and NH_3-N from the solid wastes generated at the human habitation unit. Nitrogen leaching can be accomplished through a combination of physical solids disintegration and an anaerobic fermentation-like process.

The solid wastes from the human habitation unit include feces and food preparation or processing waste. After preprocessing of the solid wastes, the slurry would be physically separated into liquid and solid streams. The solid portion containing Org-N could be further processed by wet oxidation to convert the Org-N to NH_3-N . Liquid wastes from the human habitation unit include urine and hygiene or wash water. These two waste streams would be combined for processing by vapor compression distillation (VCD). The final product of vapor compression distillation could be returned to the human habitation unit as drinking and hygiene water. Current state-of-the-art vapor compression distillation technology requires that pre- and post-treatment be implemented to optimize treatment efficiency and insure potable water quality (10). Pretreatment by pH adjustment will maximize separation of NH_3-N and other salts while post-treatment by ultraviolet (UV) disinfection will significantly improve the bacteriological quality of the final product. Additional potable water could be

supplied by water condensed from the evaporation/transpiration process occurring in the plant growth unit. The concentrate from vapor compression distillation would be sent to the preprocessing stage to be combined with the concentrated $\text{NH}_3\text{-N}$ liquid leachate. The liquid leachate from the preprocessing stage as well as the liquid effluent from the wet oxidation step, both of which will contain highly concentrated $\text{NH}_3\text{-N}$, could be processed by biological (microbial) oxidation such as suspended growth (activated sludge) or fixed film (rotating biological contactor, trickling filter, etc.) systems. Carbon and nitrogen oxidation would occur, transforming the majority of the organic carbon to CO_2 and water and the $\text{NH}_3\text{-N}$ and Org-N to nitrate nitrogen ($\text{NO}_3\text{-N}$). Following separation of the microbial solids, the liquid effluent from the biological oxidation unit would be returned to the plant growth unit to supply water, as well as nitrogen and other necessary nutrients. The microbial solids could be processed by wet oxidation to convert Org-N to $\text{NH}_3\text{-N}$. Due to the incomplete oxidation of carbon and separation of microbial cells during biological processing, the liquid effluent will require additional polishing by activated carbon adsorption and UV disinfection before being transferred to the plant growth unit. The activated carbon system would remove residual carbon which can stimulate the growth of bacteria while UV disinfection would destroy bacteria and viruses including potential plant pathogens. The wet oxidation system would also be used to regenerate the spent activated carbon.

CONCLUSION

The production rate and solid content of waste streams found in a life support system for a space habitat (in which plants are grown for food) have been discussed. Two recycling scenarios (Figures 1 and 2), derived from qualitative considerations as opposed to quantitative mass and energy balances, tradeoff studies, etc., have been presented; they reflect differing emphases on and responses to the waste stream formation rates and their composition, as well as indicate the required products from waste treatment that are needed in a life support system. The data presented here also demonstrate the magnitude of the challenge to developing a life support system with a high degree of closure.

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BIOMASS PRODUCTION AND NITROGEN DYNAMICS IN AN
INTEGRATED AQUACULTURE/AGRICULTURE SYSTEM

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A combined aquaculture/agriculture system that brings together the three major components of a Controlled Ecological Life Support System (CELSS) - biomass production, biomass processing, and waste recycling, was developed to evaluate ecological processes and hardware requirements necessary to assess feasibility of and define design criteria for integration into the KSC Breadboard Project. The system consists of a 1 m² plant growth area, a 500 liter fish culture tank, and computerized monitoring and control hardware. Nutrients in the hydroponic solution were derived from fish metabolites and fish food leachate. In five months of continuous operation, 27.0 kg of lettuce (Lactuca sativa cv. Waldmann's Green) tops, 39.9 kg of roots and biofilm, and 6.6 kg of fish (tilapia, Oreochromis aureus), wet weights, were produced with 12.7 kg of fish food input. Based on dry weights, a biomass conversion index of 0.52 was achieved. A nitrogen budget was derived to determine partitioning of nitrogen within various compartments of the system. Accumulating nitrogen in the hydroponic solution indicated a need to enlarge the plant growth area, potentially increasing biomass production and improving the biomass conversion index. A computer simulation model is being developed to project production potentials and define data needs for more effective management of the system.

INTRODUCTION

One objective of CELSS research is the development of systems that recycle inedible waste into high quality edible biomass. The concept of recycling mass for the purpose of optimizing secondary production of edible food stuffs and minimizing waste has been the subject of several investigations utilizing combined aquaculture/hydroponic systems. Zweig(1), Serfling and Mendola(2), Pierce(3) and Bender(4) reported on "soft technology" systems suitable for home use, that produced

high quality vegetables and fish protein while effectively utilizing space, nutrients and water. These systems generally consisted of a fish culture tank, a vegetable growing area and in some cases a biological filtration system for the microbial conversion of ammonia (NH_4) to nitrites (NO_2) and nitrates (NO_3). The systems were designed primarily for use in greenhouse environments in temperate climates. The use of hydroponic systems to prevent the accumulation of nitrate nitrogen and orthophosphorus in recirculated water in fish culture systems was evaluated by Lewis et al(5) who found acceptable water quality could be maintained by allowing plant growth to serve as a sink for undesirable levels of nutrients. McMurtry et al(6) found that the integration of fish culture, biological filtration, and hydroponic vegetable culture was an economical way to commercially produce bush bean, cucumber, tomato, and tilapia.

Development of secondary production combined aquaculture/hydroponic systems in CELSS would optimize energy and materials utilization, provide resiliency and stability, and maximize the use of available space. Fish were selected as candidates for secondary producers in CELSS for the following reasons. They occupy a low position in the food chain, are a good source of high quality animal protein, and can be cultured in nutrient solution tanks already in use. Tilapia (*Oreochromis aureus*) were chosen because they are tolerant to extremes in water quality, resistant to disease, and fast growing, reaching a harvestable

size in 6 months (7). This species of tilapia is also omnivorous, easy to handle, and populations are readily manageable in controlled environments (7).

As part of ongoing CELSS research, system scale-up and integration, we designed a combined aquaculture/agriculture system that incorporated the three major components of CELSS - biomass production, biomass processing, and waste processing. In this system biomass production included fish, higher plants, and algae. Biomass processing based on the incorporation of inedible plant biomass, fish processing waste, biomass conversion by-products, and table scraps into a fish diet is being evaluated. This system will potentially allow for waste management by the use of condensate and gray water, control of oxygen and carbon dioxide balance, and the recycling of nutrient solution.

One goal of this research was to quantify the biomass production potential of fish, lettuce, and biofilm (organic matter and attached microbial community). Other objectives were to describe the partitioning of nitrogen between system compartments, evaluate the response of biological components, and develop a computer simulation model of nutrient dynamics and biomass production. A computer monitoring and control system was designed and system hardware and software were also tested and evaluated during this study.

MATERIALS AND METHODS

The integrated system consisted of a 500 liter conical bottom fish culture tank and a 1 m² plant growing area. Thirty various sized (27-144g) tilapia were stocked at the beginning of the study. Twenty additional fish were stocked after 28 days to give a total fish biomass of 4.9 kg at that time. Plans are to operate the system at the CELSS Breadboard Project scale of one person. This stocking density should provide three filets per week. A commercial fish chow was fed at the rate of 2% body weight per day divided into three feedings. Every 2 weeks fish were weighed to monitor growth and adjust feeding rate.

Lettuce (Lactuca sativa, cv. Waldmann's Green) was planted on six polyvinyl chloride (PVC) pipes which were 2 inches in diameter and 1.5 m long. Plants were spaced 15 cm apart, 9-10 plants per pipe. The lettuce was planted and harvested approximately every 7 days.

Water from the fish culture tank was pumped through the PVC pipe where it served as the nutrient solution for the plants. Lettuce roots were in direct contact with the solution where they functioned as a biofilter substrate for bacterial colonization, allowing for nitrification of ammonia. Free ammonia (NH₃) is toxic to fish (8), therefore the biofilter component is important in this system. Solution flow through each PVC pipe ranged between 0.5 and 1.5 L/min. Water samples were collected weekly from the tank for chemical analyses. Parameters monitored included pH, conductivity, macronutrients, micronutrients,

ammonia, total kjeldahl nitrogen, total suspended solids and total organic carbon. Light was provided by three high pressure sodium (HPS) lamps which produced an average photosynthetic photon flux (PPF) of $245 \text{ umol s}^{-1} \text{ m}^{-2}$, with an 18 hours on and 6 hours off photoperiod. Air temperature over the plant canopy was ambient, averaging $29.2 \pm 2.1 \text{ }^{\circ}\text{C}$. Relative humidity was controlled at 65% and tank water temperature was $24.1 \pm 1.4 \text{ }^{\circ}\text{C}$. Solution pH was controlled at a maximum of 6.2 units and averaged 5.8 ± 0.5 units. Dissolved oxygen was measured daily and remained at 6.6 ± 0.6 ppm.

At the start of the study the nutrient solution was one quarter strength Hoagland's and was replenished twice during the next two weeks until the fish food leachate and fish metabolites provided enough plant nutrients. Total minerals added during the first two weeks of the study, exclusive of fish feed were as follows:

<u>Element</u>	<u>mg</u>
N	7.35
P	1.09
K	8.19
Ca	7.00
Mg	1.68
S	2.24
Fe	4.34
Mn	0.031
Zn	0.0031
Cu	0.0012
B	0.033
Mo	0.00062

After the second replenishment, the addition of nutrients to the solution was totally through fish feed wastes and fish metabolites.

A simulation model was developed to examine biomass production and nutrient dynamics. The model diagram was drawn using energy circuit language symbols (9). Programming was conducted with Lotus 1-2-3 (10).

RESULTS AND DISCUSSION

After 5 months of continuous operation the system produced 27.0 kg of lettuce tops, 39.9 kg of roots and biofilm, and 6.6 kg of fish on a wet weight basis. During this 5 month period 12.7 kg of feed was added. This resulted in a biomass conversion index, based on dry weights, of 0.52. The partitioning of nitrogen added to the system over the 5 month period is described in Figure 1. Fish tissue incorporated 35.2% of the nitrogen while lettuce tops contained 7.9%, and the roots and biofilm accounted for 12.5%. The nutrient solution retained 35.5% of the nitrogen. Loss of nitrogen gas through denitrification and from ammonia volatilization, in addition to unquantified biofilm inside system plumbing was believed to account for the remaining 8.9%.

Figure 2 shows the behavior of NO_3 , NO_2 , and NH_4 in the solution over time. A dramatic increase in NH_4 and NO_3 occurred after the addition of 20 fish on day 28. The relationship between ammonium ion (NH_4^+) and free ammonia is pH dependent (11). In order to keep the NH_3 below toxic levels we maintained

the solution pH below 7.0. Ammonium and NO_3 increased at equal rates until about day 84, after which time NH_4 levels fell and NO_3 continued to rise, suggesting the development of an adequate community of nitrifying bacteria. Nitrite, the end product of the first stage of nitrification, stabilized after a small peak around day 28 which coincided with the addition of fish.

Preliminary work on a biomass production model of the system is shown in Figure 3 which depicts the producer module with its lettuce storage, the consumer module with fish storage, and the biofilm with attached microbes. Results of the simulation run depicted in Figure 4a show production in the system over a 100 day period. Lettuce tops and roots were harvested every 7 days along with the attached biofilm. Fish biomass was shown to increase steadily at a rate that was similar to the observed growth. Since the plant area and production rate were held constant and fish were growing and being fed more, the biofilm compartment increases rapidly around day 85. Based on observation, fish wastes and waste feed which compose the substrate for the biological film began to accumulate not only on the roots but also as suspended solids in the tank. Figure 4b shows a simulation where the lettuce biomass on the system was doubled which allowed it to run 110 days before the biofilm began its rapid increase. The increase in the biofilm component was slowed by the additional harvest of lettuce roots and biofilm. The response of fish biomass to biweekly harvest can be seen in this simulation. Fish production with harvest remains fairly constant.

CONCLUSIONS AND RECOMMENDATIONS

After 5 months of system operation it was concluded that nitrogen accumulated in the solution, and therefore the plant growth area needs to be increased. This concept was also supported in the simulation model runs. A larger plant growth area should allow for more production and better biofiltration. Fish growth rates and productivity should also increase with improved water quality resulting from better filtration, and by operating the system at a higher temperature. In addition, system improvements such as oxygen injection and foam fractionation to remove organics, biological oxygen demand (BOD), and solids could also improve production. The simulation model will continue to be developed and tested and used for system management. The revised model will incorporate not only biomass but also energy, and nutrient dynamics.

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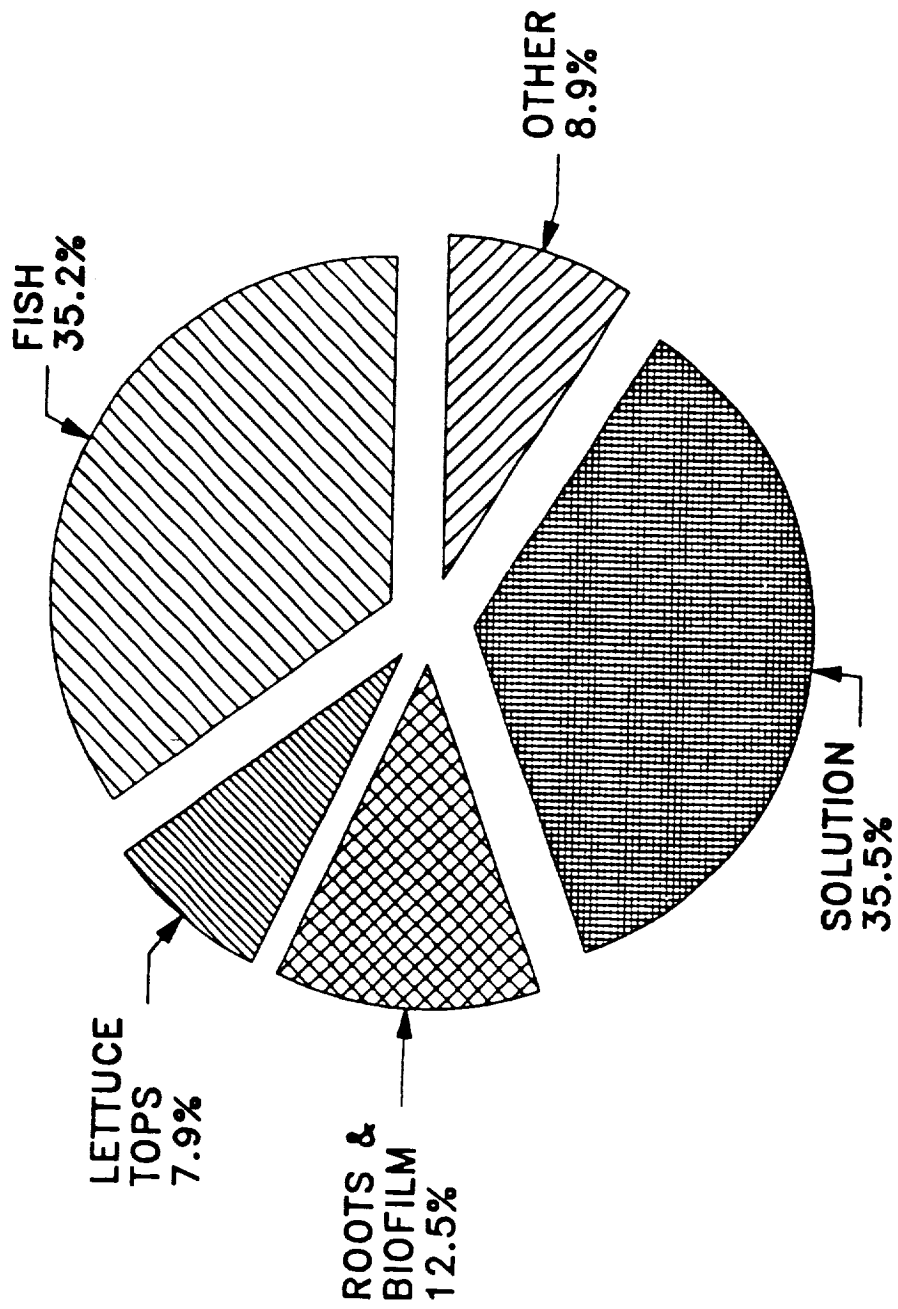


Figure 1. Partitioning of nitrogen added to the system during 5 months of operation.

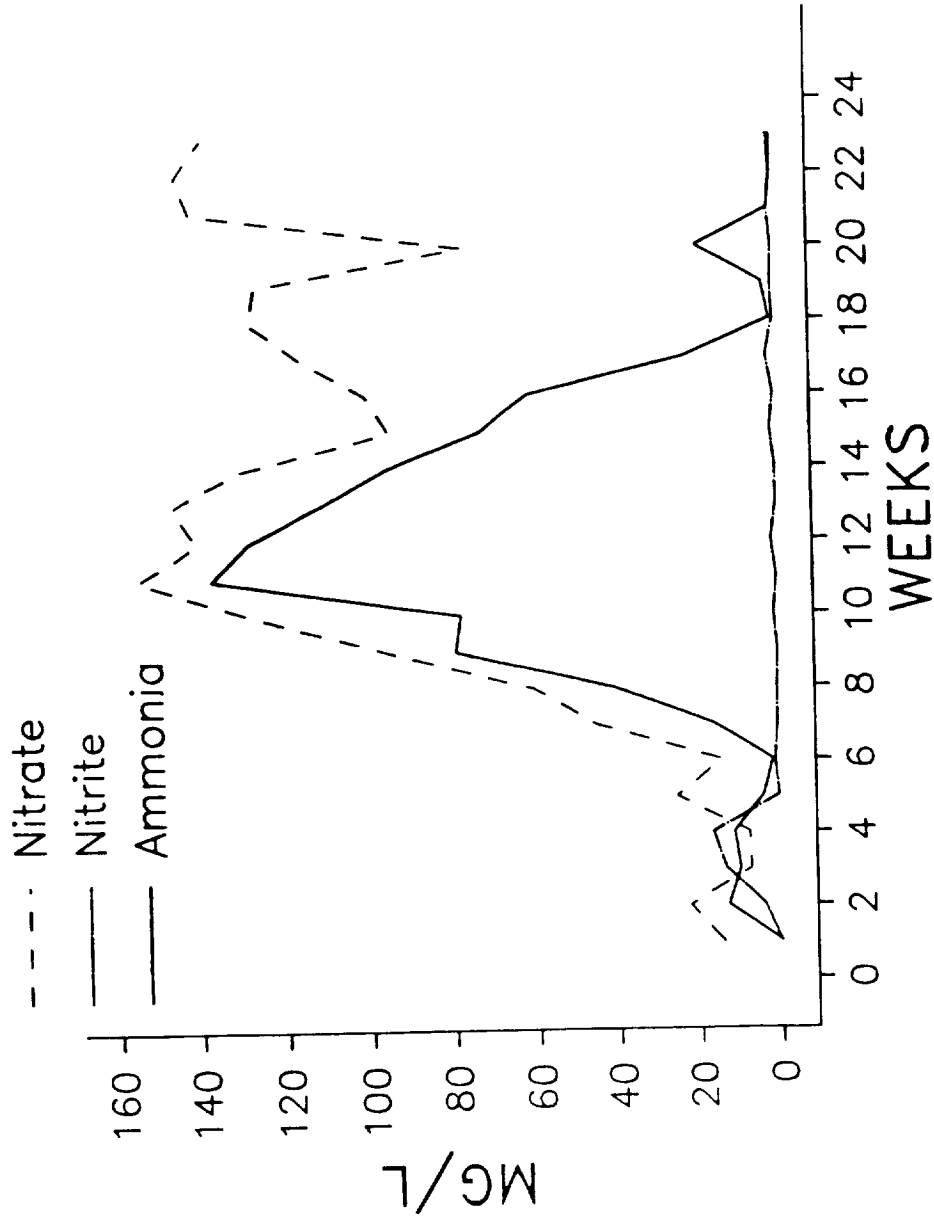


Figure 2. Nitrate (NO_3), nitrite (NO_2), and ammonia (NH_4) dynamics in solution over time.

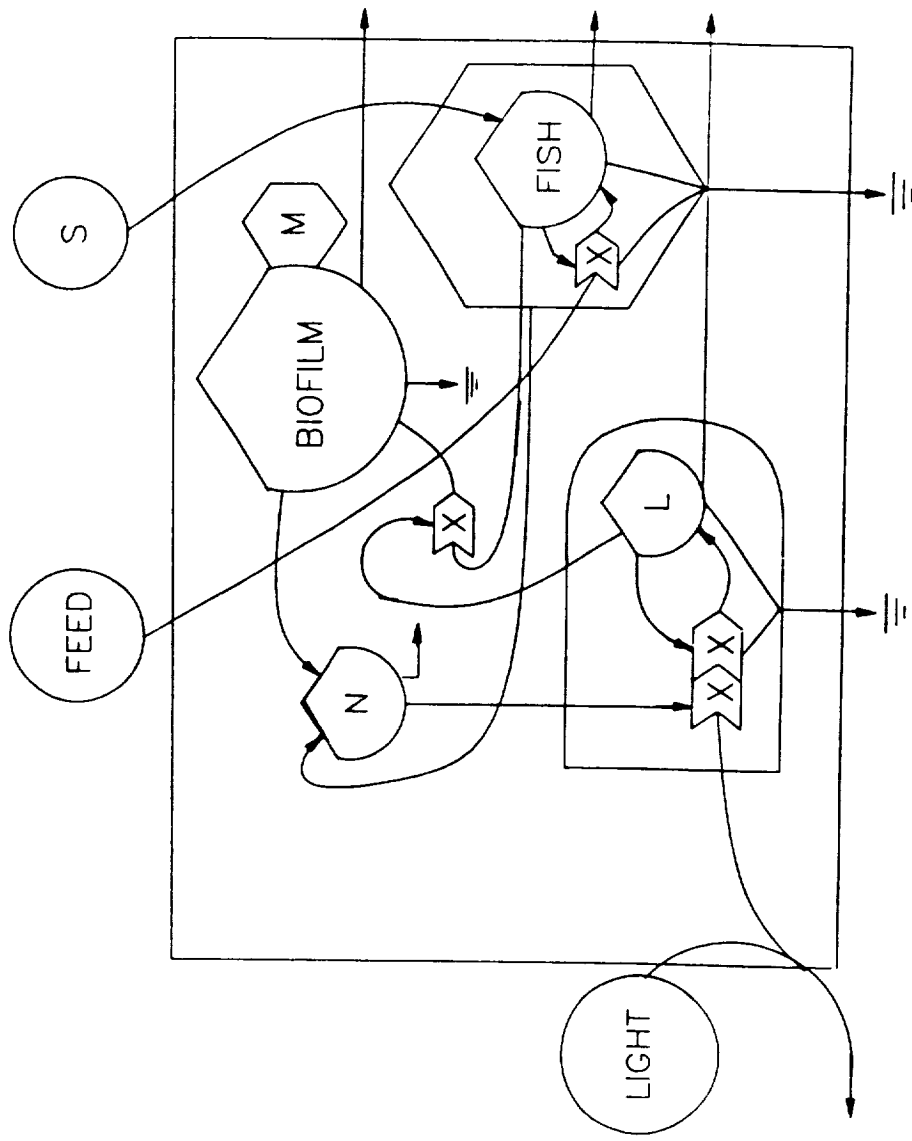


Figure 3. Diagram of the integrated aquaculture/agriculture system biomass production simulation model. (N=nitrogen, l=lettuce, M=microbes, and S=stock, for fish replacement)

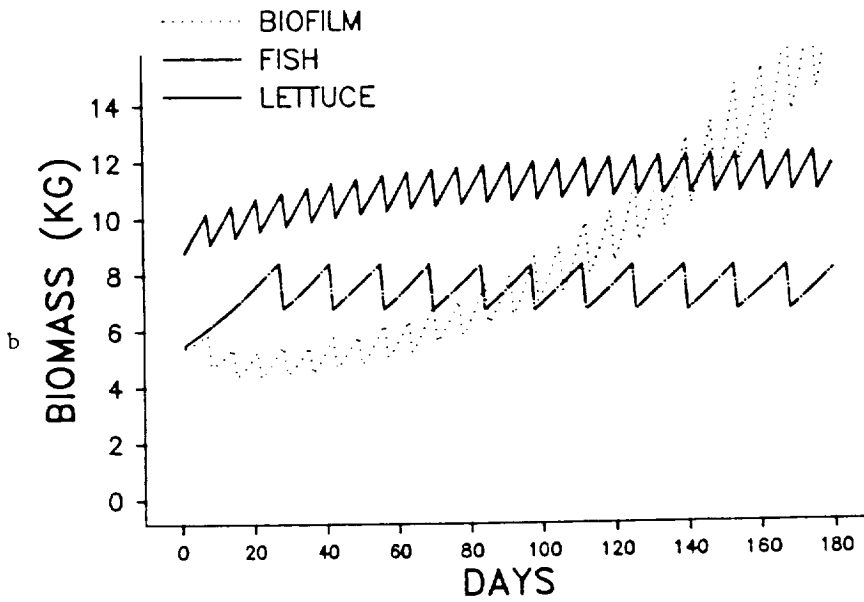
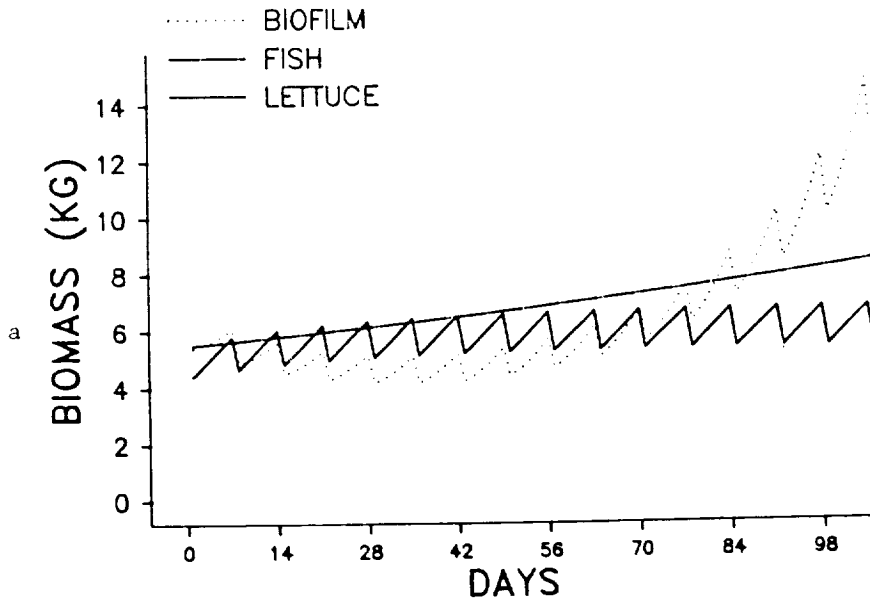


Figure 4. a. Initial model simulation run showing biofilm, fish, and lettuce production over 100 days. b. Model simulation run with increased lettuce biomass, and biweekly fish harvest over 180 days.

SEED SPROUT PRODUCTION: CONSUMABLES AND A FOUNDATION FOR HIGHER
PLANT GROWTH IN SPACE

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ABSTRACT

Seed sprouts can be produced as a source of fresh vegetable material and higher plant seedlings in space. Sprout production was undertaken to evaluate the mass accumulations possible, the technologies needed and the reliability of the overall process. Baseline experiments corroborated the utility of sprout production protocols for a variety of seed types. The automated delivery of saturated humidity effectively supplants labor-intensive manual soaking techniques. Automated humidification also lends itself to modest centrifugal sprout growth environments. Under unprotected growth conditions, bacterial and/or fungal contamination occurred in about 10% of the growth cups. A small amount of ultraviolet irradiation effectively suppressed the contamination and the sprouts were suitable for consumption. These experiments have provided the foundations for designing an automated sprout production facility for space use. Scaled from the laboratory experiments, the sprout production needs of a crew could be handled in a volume equivalent to that of a mid-deck locker.

INTRODUCTION

Seed sprouts have been seen as an opportunity to produce fresh consumable biomass in zero gravity for use as both a dietary supplement and a source for higher plant seedlings (1, 2). Many technical, methodological and reliability questions are raised when biological systems are considered as major components for space life support systems. Space experience and research with most biological systems has been too limited to provide suitable answers to such questions. Opportunities to remedy this situation, however, do exist. The production of seed sprouts in space is a means to satisfy two NASA needs. Sprouts, from a

variety of seeds, can be used to produce fresh consumables in space. At the same time, sprout production can be used to answer a variety of questions raised regarding the use of biological systems in space to provide life support (3).

Sprout production in space can meet the austere volume and power budgets typical of current space missions. The technology is relatively simple and the production is quite high. Ground-based studies have been undertaken to assess ground production. These studies quantify (1) the amount of biomass accumulated (wet weight) for each of a variety of seeds, (2) the methods through which human interaction and effort can be minimized, and (3) the methods through which contamination (bacterial, fungal) can be minimized. Sprout production and consumption in space would document the utility of biological systems in an overall life support scenario. Suitable measures taken during production would extend our understanding of space environment effects on biological systems and plant germination, particularly. The tests undertaken here allow the specification of design parameters needed for space sprout production.

METHODS

Baseline:

Standard sprout production techniques were used to establish a data base against which a variety of technique modifications could be compared. Seed soaking times and water delivery schedules were evaluated for impact on sprout mass accumulation. Using alfalfa (0.715 g, approximately 300 seeds), mung bean

(2.464 g, approximately 40 seeds), and spicy clover/radish mix (0.924 g, approximately 200 seeds), individual plastic cups (32 ml volume, 3 cm bottom diameter, 4 cm top diameter, 3.3 cm height) were prepared with the indicated seed amounts for these experiments. Small holes (2 mm diameter) penetrated the bottom of the cups to permit draining of excess water. With eight holes in each cup, the bottom of the cup remaining unpenetrated was approximately 6.82 cm². When not being watered, weighed or otherwise treated the cups were maintained in a low light environment at 22-24 degrees C with 60-80% relative humidity levels. Ambient air was not forced to move across the openings to the cups. For soaking, approximately 20 volumes of tap water (22-24 degrees C) were added to the seed volumes: soaking times were evaluated hourly for one to twenty-four hour soak periods. The seeds were weighed prior to and following these soaking periods. Prior to weighing, the excess water was "wicked" from the seed cups using absorbant paper towels. Wicking was permitted for five minutes. After the initial soak period, all seeds were sprayed with 30 ml per cup of distilled water at eight hour intervals. Daily, after three intervening sprayings, all seed cups were "wicked" and weighed. These procedures were followed until the fifth day following initial soaking. At this time, the formation of secondary leaves and a bitter taste factor had begun.

Humidity:

Automatic, controlled humidification was considered as an option for watering that required minimal human interaction. For this

system, all that was required was to fill the humidifier with water daily. The seeds were weighed before soaking as in the above baseline studies using the following amounts of seeds: mung (2.464 g, approximately 40 seeds), alfalfa (0.715 g, approximately 300 seeds), spicy (0.693 g, approximately 150 seeds). A smaller amount of spicy seeds were used in the humidity experiments because, in the baseline studies, it was determined that 200 seeds caused severe crowding in the growing sprouts. Following the initial weighing of the seeds and the cups containing them, all of the seeds were soaked for eight hours. The wicking system used in baseline was employed to drain the seeds after soaking, at which time they were weighed. Then they were placed into a humidity chamber (53 cm x 24 cm x 12 cm) and humidified for the following amounts of time: three hours (0.25 gallons of water delivered daily), six hours (0.5 gallons of water delivered daily), twelve hours (1 gallon of water delivered daily) and twenty-four hours (2 gallons of water delivered daily). The humidity chamber was a cast acrylic box in which the humidity was delivered at the top center portion of the lid. The humidity cycles were determined by an automated timer assembly so that the sprouts would get humidity at regular, pre-determined intervals. During the times in the cycle when humidity was not being administered, a fan circulated air (50-70% relative humidity) across the sprouts. For five days, the sprouts were weighed daily after draining. The sprout growth was compared with that achieved in baseline experiments.

Bacterial and Fungal Suppression:

Broad spectrum ultraviolet light was used to suppress the contamination of sprouts with bacteria and fungus. Treatments ranged from 2 minutes to 24 hours daily. Two sources of radiation were used: (1) 12" Sylvania Germicidal Lamps (8W each) at 10" above sprouts and (2) Black Ray UV Lamp using 100W mercury spot bulb at 14" above the sprouts.

RESULTS

Baseline:

After the variations in soaking periods, the seeds weighed at 24 hours after the beginning of soaking showed mass accumulations directly related to soaking times (Fig 1). The accumulated mass appeared to slowly approach asymptotic values as soaking times extended beyond about eight hours. Clearly, however, most mass was accumulated during the first hour of soaking.

Not all of the seed types responded in the same ways to soaking time variations. The alfalfa seeds clearly showed asymptotic accumulations whereas the mung seeds showed less evidence for asymptotic accumulations.

Interestingly, the large differences in seed size (and volume to surface ratios) were not reflected in differential mass accumulation. The three varieties of common sprout seed examined here all showed mass increments of about 200% regardless of soaking times.

When total mass accumulation is plotted for five days, the sprout production continues to reflect slightly higher accumulated masses for those seeds exposed to longer initial periods of soaking (Fig. 2). The effect is most pronounced in spicy seeds and is almost nonexistent in alfalfa seeds. In any event, the spicy seeds yielded an eight-fold accumulation in mass compared to initial starting mass of seeds. Alfalfa accumulations were just below a six-fold level, and mung at approximately a four-fold level.

Humidity:

Provision of water for sprout production can be achieved by direct humidification systems. Such systems eliminate labor intensive steps and assume uniform water delivery. Also, humidification systems are readily automated to handle a variety of water and gas transport requirements of the sprouts.

The results of different humidification delivery schedules are shown in Figs. 3, 4, and 5 for mung, alfalfa, and spicy sprout production, respectively. Baseline values are shown, as well, for comparative purposes.

The mung sprouts (Fig. 3) clearly required almost continuous humidity delivery to assure maximum mass accumulation. It is interesting that 12 and 6 hour humidification times yielded mass accumulations that approached the 24 hour and baseline values on days 4 and 5. Clearly, 3 hours of humidity delivery was insufficient for significant mass accumulation in mung seeds.

Alfalfa sprouts (Fig. 4) were even more dependent on high levels of humidification delivery. Despite the apparent variance in the data, it seems clear that neither 3 or 6 hours of humidification adequately supported alfalfa sprout production.

The spicy seeds (Fig. 5) were similarly dependent on high levels of humidification. Interestingly, both the 12 and 24 hour humidification treatments yielded spicy mass accumulations in excess of those recorded in baseline studies.

Table 1 compares the total mass accumulation of the mung, alfalfa, and spicy sprouts over the whole five day growth period in percent form of the humidity and baseline experiments (as compared with dry seed weight).

The Table 1 baseline and humidification data suggest that, whereas the seed volumes do not have a systematic influence on water uptake during soaking, the volume to surface ratios may be critical to seed drying and thus to overall mass accumulation. The small seeds may benefit, accordingly, more from water delivered by saturated humidification than the large seeds.

Bacterial and Fungal Suppression:

Two kinds of suppression information were sought in using ultraviolet radiation during sprout production. First, what amounts of ultraviolet light were needed to suppress sprout contamination during the five days of production and, second,

what amounts of ultraviolet irradiation would be tolerated by the sprouts during growth such that neither mass accumulations nor taste would be adversely affected.

Under baseline production conditions fewer than 10% of the sprout production cups exhibited either bacterial or fungal contamination. With a single, two minute period of ultraviolet irradiation daily, the contamination either did not develop or was sufficiently suppressed to permit full term sprout production in the absence of contamination levels that would make the sprouts unacceptable for consumption. Shorter periods of irradiation (tested at 30 second intervals) were only partially effective. Longer periods yielded no significant improvements in the observed suppression. It should be noted that no attempt was made to disturb the sprouts during irradiation. Thus, shadowed areas and residual water undoubtedly accounted for decreased ultraviolet effectiveness.

To examine sprout tolerance to ultraviolet irradiation, the sprouts were exposed to continuous ultraviolet light. Such irradiation, at the levels used in the present studies, did not inhibit sprout mass accumulation. Since these sprouts were not grown to mature plants, effects on growth remain to be determined.

These studies demonstrated that modest levels of ultraviolet irradiation readily controlled sprout contamination. The required ultraviolet light levels were two orders of magnitude

less than those demonstrably tolerated by the sprouts. These observations demonstrate an effective means for controlling contamination and, accordingly, the reliability of sprout production in space.

CONCLUSIONS

The baseline studies of sprout production undertaken here show that any of several sprout producing seeds can be used to reliably yield consumable vegetable materials using little space and specialized equipment. A couple of 32 ml cups yield sufficient fresh biomass for a sprout salad. Given the production times of 4-5 days, the daily salad needs of an astronaut might be met with 10 of these small cups and the needs of a crew could be met within the volume of a mid-deck locker. Although the systematic results shown here involve only three seed types, more than 15 types of sprouts have been produced under similar conditions. Thus, a good deal of sprout variety is assured.

To deal with the labor intensive watering demands of sprout production, tests were done using a saturated humidification delivery system that was fully automated. With sufficiently long periods of humidification, the measured accumulation of fresh biomass actually exceeded that produced by conventional sprout production methods.

Finally, the occurrence of contamination raised issues of sprout production reliability. With small amounts of irradiation by

ultraviolet light, the contamination problems were readily suppressed. These treatments were much less intense than those having any observable consequence on sprout production.

The goal of producing fresh biomass can be reached through experimentation using either the humidity chamber and/or baseline methodology. These studies and follow up studies will lay the foundation for sprout production and consumption in space. As indicated, many other seed types are being investigated. Humidity delivery has proven to be effective, and centrifugal systems are being evaluated for use in both soaking cycle needs and in gravitational needs of the sprouts. All of these technologies are being developed to serve the two NASA needs cited above: the production of fresh edible biomass and the production of plants in space as a subsystem for CELSS.

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TABLE AND FIGURES

Table 1			
<u>Hours of humidity</u>	<u>Mung</u>	<u>Alfalpa</u>	<u>Spicy</u>
3	81%	206%	229%
6	382%	790%	675%
12	243%	992%	846%
24	357%	1147%	1184%
Baseline results (average)	340%	560%	762%

Fig. 1 Graph of 24 Hour Mass Accumulation

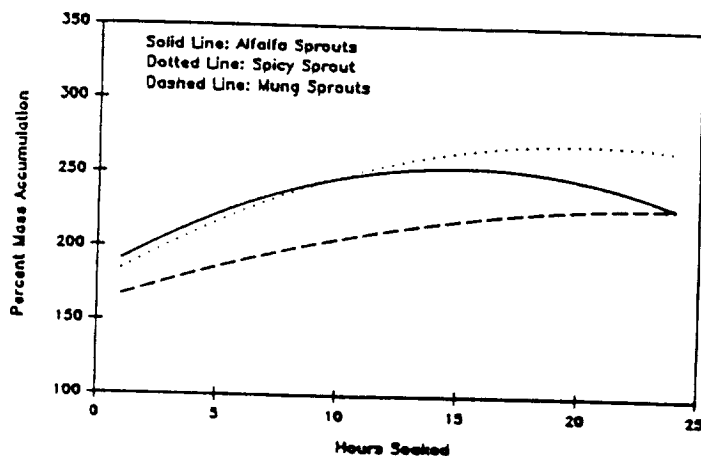


Fig.1 Linear regression summary of the accumulation of seed mass based on soaking times ranging from 1 to 24 hours in duration. Triplicate sample cups were soaked at each of the one hour intervals. The 72 samples yielded significant correlation coefficients for each of the three regression lines representing alfalfa, mung and spicy seeds, respectively. All data show results at 24 hours.

Fig. 2 Graph of Five Day Mass Accumulation

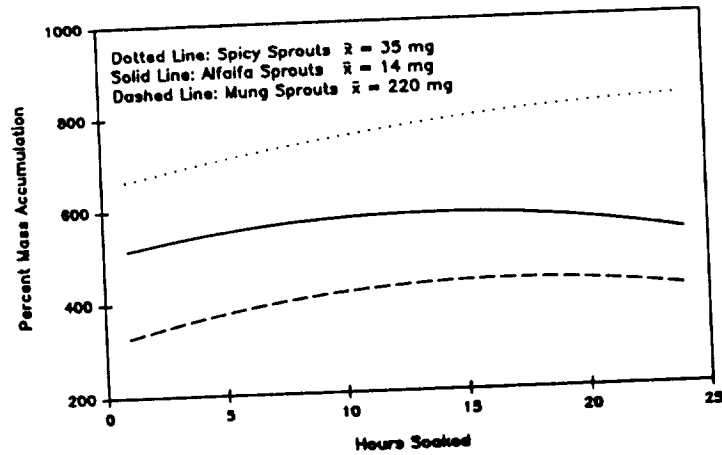


Fig. 2 Linear regression summary of the accumulation of mass based on soaking times. Conditions as in Fig. 1. Total sprout masses for measurements obtained after five days of baseline treatment.

Fig. 3 Graph of Mass Accumulation vs. Time
Mung Sprouts

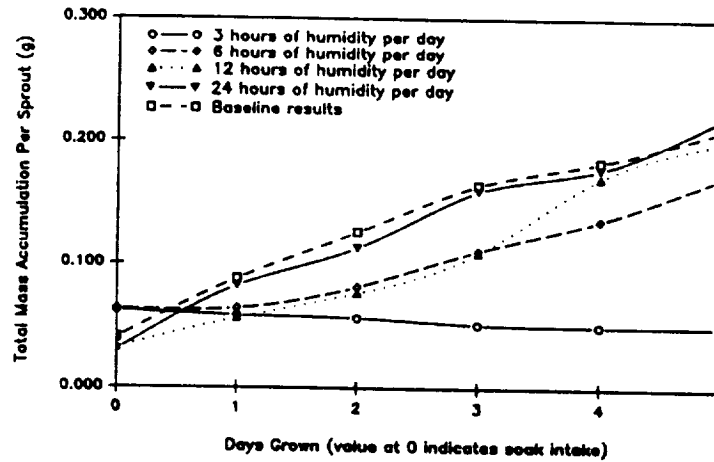


Fig. 3 Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.

Fig. 4 Graph of Mass Accumulation vs. Time
Alfalfa Sprouts

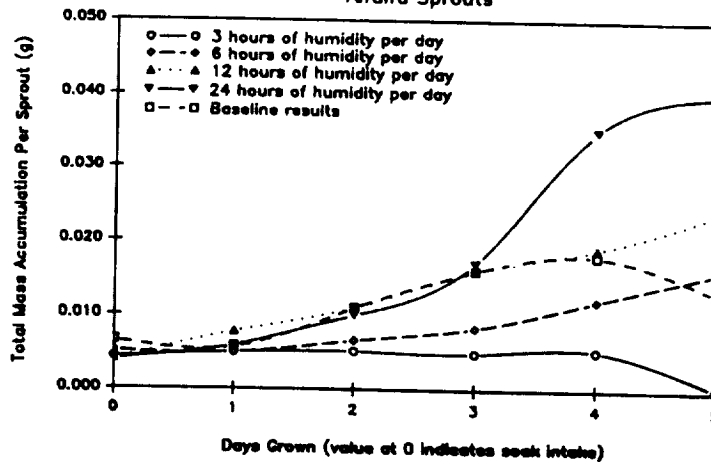


Fig. 4 Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.

Fig. 5 Graph of Mass Accumulation vs. Time
Spicy Sprouts

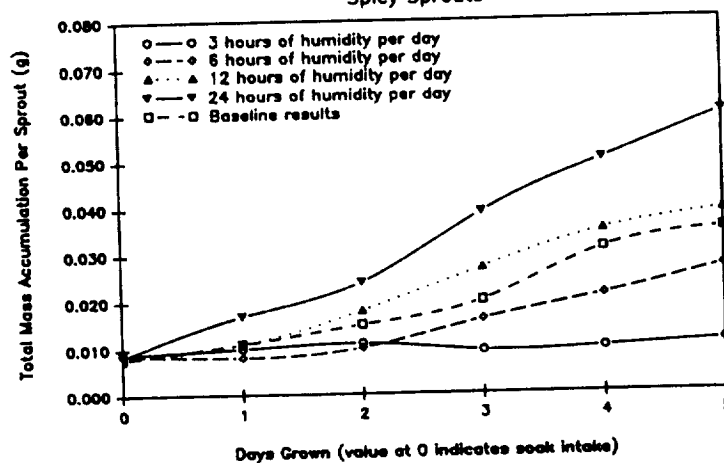


Fig. 5 Summary of the accumulation of seed mass based on a soaking time of eight hours and humidity cycles of three, six, twelve, and twenty-four hours with baseline data shown for comparative use. Four sample cups were used for each experiment iteration.

N91-31791

CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM

BREADBOARD PROJECT - 1988

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ABSTRACT

The goal of the first phase of the Controlled Ecological Life Support System (CELSS) Breadboard Project is to develop, build, and test a ground based CELSS at a one-person scale. The successful integration and operation of such a system should establish the feasibility of developing a CELSS that will meet life support requirements for crews during long duration space flights. Over the past two years, personnel of the Breadboard Project have concentrated on the construction and initial testing of a large biomass production chamber, the primary module for the plant growth and atmospheric revitalization for this CELSS. This large plant growth chamber is now functional and the first full biological test of the facility was completed in January 1989 using wheat as the test specimen. Associated laboratory research during the past year has concentrated on production studies with other crop species, microbiological characterization of hydroponic solutions, enzyme conversion of cellulose to sugar, aquaculture techniques, recovery of inorganic elements from plant waste through water leachate, and the development of a nutrient delivery system for microgravity. This paper will summarize this work and present future plans; especially those that are important to establishing modules or a complete CELSS, including food processing and waste management.

INTRODUCTION

A plan for the Controlled Ecological Life Support System (CELSS) Breadboard Project was written and approved in 1986. This project was initiated primarily because research results on some crops suggested that productivity was sufficiently high to allow a CELSS to be economically feasible. But these productivity

estimates based on scaling upward small laboratory level studies, as history indicates, can not always be relied upon to yield operational level data. The Breadboard Project was initiated to empirically demonstrate the feasibility of a functioning CELSS.

Project Description

During the first phase of the project (five years), we plan to construct and operate NASA's first ground based CELSS. Currently, the working concept of this activity is as depicted in Figure 1. The development of this CELSS will depend on available proven technology and will therefore establish what we are capable of doing today with little attention to innovation or required new technology. Crop production trials on a functional level will be the primary focus during the initial period. Activities centered in the biomass production chamber (BPC), involve the growth of crops under environmentally controlled conditions including thin film hydroponics, a sealed atmosphere, and artificial lighting systems. Biomass produced in this chamber will be removed and transported to laboratories adjacent to the BPC for processing. Biomass processing will include the production of food and/or meals from edible plant material and the conversion of inedible biomass into edible products or usable raw materials. In these adjacent laboratories, wastes will be converted to plant nutrients, inorganic elements to plant fertilizer and organic material to CO₂ and water. Total mass will be carefully measured through all these laboratory activities. Three major modules of a CELSS: biomass production, biomass processing, and waste

conversion will be integrated and validated during this first phase of the Breadboard Project.

Table 1 summarizes the major activities that are planned in the CELSS Breadboard Project over the next five years. At least three crops will be tested in the BPC; wheat, soybean, and potato; including multicroping studies and continuous production trials. Condensate water will be recycled through the nutrient delivery system and a trace atmospheric gas contaminant control system will be added to the BPC as required to control trace organics.

Food processing and waste management activities will concentrate on measuring and analyzing the biomass, condensate water, and atmospheric contamination produced within the BPC. Processing of edible biomass into food at a kitchen level will occur later in the project at which time meals will be prepared and tested. Reactors and subsystems to produce alternate food sources by utilizing the inedible biomass will be integrated with the BPC. These reactors will include enzymatic conversion of cellulose to sugar and the production of single cell organisms. Another one of the subsystems will probably be based on aquaculture.

The waste conversion module will first consist of a soluble mineral reactor that will remove elements from stem and leaves for use as a fertilizer. Combustion and biological reactors will be added to the Breadboard Project as it matures. Integrated tests of the completed Phase I Breadboard are scheduled to occur in 1993 and 1994.

The primary output from the Breadboard Project will be data on energy use and rates of mass flux through the CELSS. The copious amounts of data generated by the Breadboard tests will be reduced and analyzed through multivariate statistics. Appropriate models which will establish the utility of a CELSS in space will be selected and validated. These models will be useful in developing design requirements for more complex Breadboard facilities, including ones that involve humans as active participants.

Project Status

Over the last year, the BPC became operational and tests of wheat as a single crop are nearing completion. The environmental control subsystems have been programmed, thoroughly tested, and mated with a computerized monitoring subsystem and a data display software package. The atmospheric leak rate for the BPC was established to be $< 5\%$ of its volume per day. Extensive microbial sampling and analysis during the wheat studies baselined the microbiological characteristics of the chamber, the atmosphere, and the hydroponic nutrient solutions.

Crop research in the laboratories during the last year has concentrated on the preparation of soybean and potato for inclusion in the BPC. Most of the research on soybean involved tests of two cultivars at various CO_2 levels and studies of irradiance intensities on internode elongation. Our knowledge of soybean growth is almost at a point where this crop is ready for testing in the BPC. Methods to monitor the microbial populations present with these crops were also established during these tests.

In anticipation of the time when a CELSS will be functional in microgravity, a membrane nutrient delivery system was tested with several crops. This system will deliver water and nutrients to the roots of plants in a manner that will prevent loss of the nutrient solution to the surrounding atmosphere in microgravity.

Biomass processing research has thus far concentrated on enzymatic conversion of cellulose to sugar. Some success in this conversion using fungal species as a source of the enzyme was obtained. An integrated aquaculture system with lettuce as the plant component and Tilapia as the fish was operated for over six months. The flow of nitrogen through this system was well documented. Feeding trials with Tilapia were also conducted so that the feeding requirements of this species could be established in hopes that inedible plant biomass could be used.

Food preparation activities included the identification of at least 25 meals that could be generated from six crops. Food processing equipment required for the six primary CELSS crops was also identified.

Waste management research involved a few studies that used water leachate of wheat straw as a fertilizer for growing plants hydroponically. Results suggest that such leachate may supply much of the inorganic elements required for plant growth.

SUMMARY

If humans are to become settlers in space, then a recycling life support system is a prerequisite to the success of such a venture. Through its Life Sciences Division, the NASA continues to support research and development of a CELSS. The BPC is in

operation and promises to allow us to evaluate atmospheric and liquid subsystems during plant growth in a sealed environment. When waste and food processing modules are integrated with the biomass production component, we will begin to better understand the mass and energy requirements of a functioning CELSS. A primary product from this project will be conceptual designs for future facilities that will be constructed in the next phase of the CELSS Breadboard Project. This will permit a logical progression in testing a bioregenerative life support system in a complete operating state on the ground, and ultimately in space.

Table 1. Major activities FY 89-93 of the CELSS Breadboard Project, Phase 1.

PROJECT MATRIX

TASK	ACCOMPLISHMENTS				
	FY89	FY90	FY91	FY92	FY93
CROP PRODUCTION	Wheat	Wheat, Soybean	Wheat, Soybean, Potato	Multiple Crops	Continuous Production
WATER RECYCLING	Design	Fabricate And Install	Operate	Modify	Operate
ATMOSPHERIC GAS CONTROL	Measure And Analyze	Design and Fabricate	Install	Operate	Operate
BIOMASS PRODUCTION CHAMBER					
SUPPORT LABORATORIES					
FOOD PROCESSING	Harvest, Store, Measure, Analyze	Establish Laboratory	Process Edible	Process Inedible	Evaluate
WASTE CONVERSION	Store, Measure Analyze	Establish Laboratory	Recycle minerals, Design Combustion System	Install	Evaluate
DATA MANAGEMENT	Environmental Nutrient Delivery, Atmospheric	Energy, Trace gas	Multivariant Analysis	Models	Validate

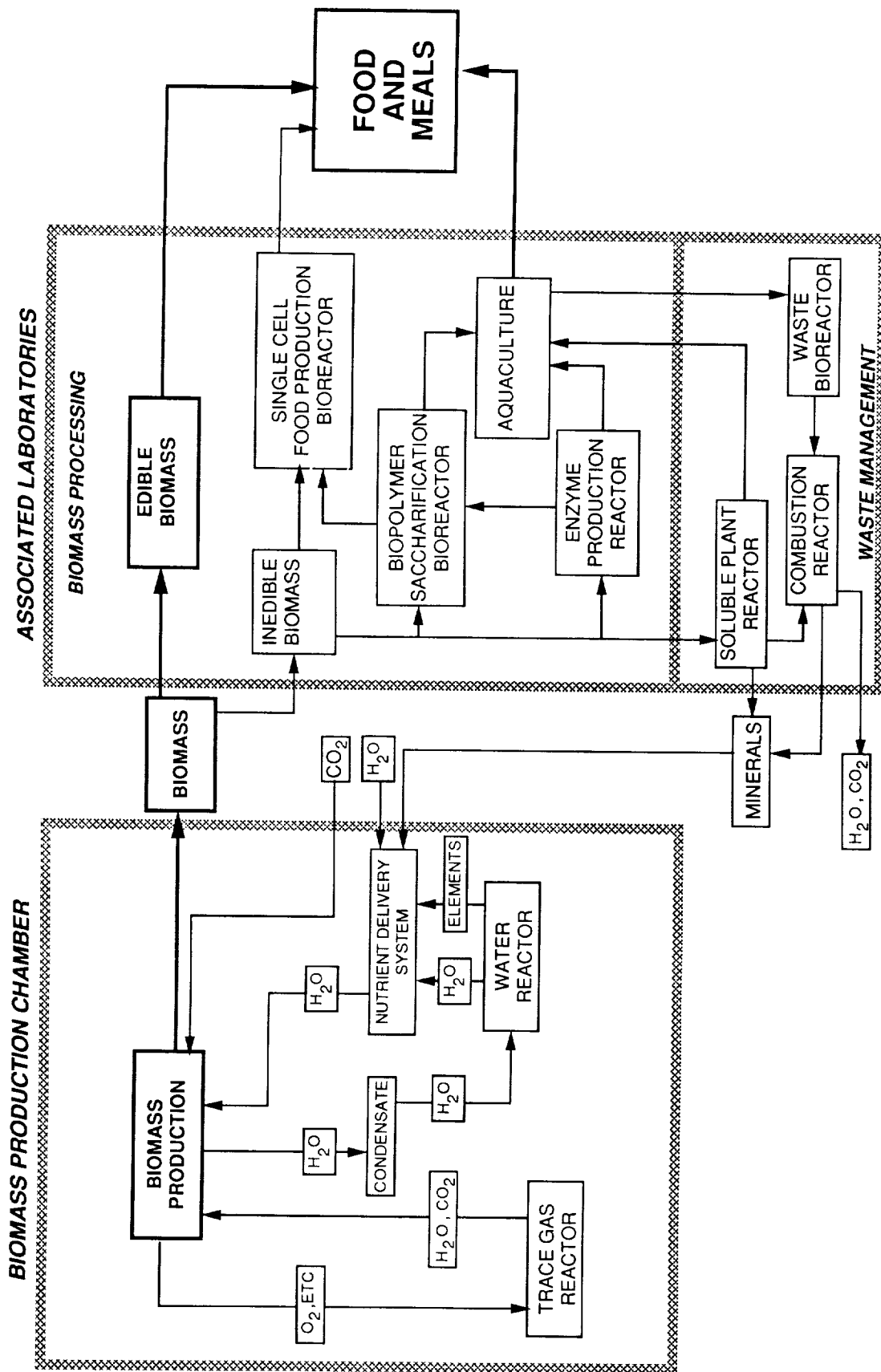


Figure 1. Schematic of the relationship between proposed components in CELSS Breadboard Project, Phase I.

THE CROP GROWTH RESEARCH CHAMBER:
A GROUND-BASED FACILITY FOR CELSS RESEARCH

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ABSTRACT. A ground-based facility for the study of plant growth and development under stringently controlled environments is being developed by the CELSS program at the Ames Research Center. Several Crop Growth Research Chambers (CGRC) and laboratory support equipment provide the core of this facility. The CGRC is a closed (sealed) system with separate recirculating atmosphere and nutrient delivery systems. Environmental influences on gas exchange, growth and development, and biomass and food production of crop plants growing within the closed environment will be investigated. Laboratory size of the CGRC will be small enough to allow treatment replication but large enough to provide information representative of larger plant communities. Water recovery and management systems and gas management systems will be included. Eventual integration of candidate waste processing technologies into the cycling system is intended.

Development of a Controlled Ecological Life Support System (CELSS) requires identification of the critical requirements that will allow the system to operate with stability and efficiency. Identifying and meeting those requirements will be accomplished through scientific experimentation and technology development on the ground. A Crop Growth Research Facility has been defined by CELSS principal investigators and science advisory panels as necessary for the development of a bioregenerative life support system (1,2).

The crop growth research facility is for the study of plant growth and development under stringently controlled environments isolated from the external environment. This facility is important for three CELSS activities: 1) research, 2) system control and integration,

and 3) flight hardware design and experimentation. Several Crop Growth Research Chambers and laboratory support equipment provide the core of this facility. The Crop Growth Research Chamber (CGRC) is a closed (sealed) controlled environment system designed for the growth of a community of crop plants with separate recirculating atmosphere and nutrient delivery systems.

CGRC HISTORY

The CGRC concept was first discussed during a 1984 meeting at the Ames Research Center (ARC) attended by CELSS principal investigators and NASA scientists and engineers. The purpose of the meeting was to define requirements for the development of a CELSS. Equipment needed for research to be conducted on earth and in extraterrestrial environments was discussed. A need was identified for a plant growth chamber where crops could be subjected to ground-based verification of flight environments. Science requirements for chamber performance were developed (1). A design concept for a Plant Growth Module (PGM) was produced in 1985 based on those science requirements.

During 1984 and 1985 a decision was made to construct the Biomass Production Chamber (BPC) at the Kennedy Space Center with the purpose of scale-up and verification of crop production results from research laboratories. The verification goal for the BPC serves several important purposes in the CELSS program but the need for research in closed environments is not adequately addressed by that chamber.

CELSS principal investigators and NASA scientists met at ARC in 1986 to confirm the need for a research facility to investigate plant growth in systems isolated from the external environment. At this meeting a need was identified for a unique research facility to address topics in a manner not possible in the BPC. Emphasis was placed on the need for such a facility to have the capabilities for tightly sealing the plant growth chamber, having minimal exchange of mass with the external environment, and conduct experiments

with appropriate replication and control treatments. The general feeling of the principal investigators was that a better facility would be built if all had input into the development of one facility rather than each building their own version with unique problems and with great cost. As a result, a CGRC Research Consortium was formed to act as an advisory panel during development of the research facility. The individual chambers were suggested to have the title Crop Growth Research Chamber to indicate the primary goal.

Requirements for a Space Station based unit capable of conducting CELSS experiments were defined at a workshop in 1987 (2). The group at the workshop, many of whom were members of the CGRC Research Consortium, agreed that the CGRC could and should serve as the developmental unit for flight prototype hardware and technology development.

By early 1988 the need for a controlled environment research facility for CELSS had been clearly stated. The CGRC had been identified as the individual unit where various combinations of environmental factors could be selected and the influence on biomass and food production of a community of crop plants investigated.

With the need of a crop growth research facility identified, and the research priority in design specifications clearly stated, in March of 1988 Ames scientists and engineers formed a working group to define the personnel and facility needs for CGRC development. The scientists of the group represented the needs for ground-based and in-flight CELSS research, the engineers represented the design, mechanical, and electrical engineering disciplines.

In June of 1988 the ARC science and engineering working group met with the CGRC Research Consortium to further develop and achieve consensus on the science requirements for the CGRC. There was a general discussion of the research efforts required for CELSS development and each principal investigator discussed their unique desires for potential utilization and application of the CGRC. The meeting was very informative and was particularly effective in exposing the engineers to the wide range of applications of the CGRC

and the necessity for strict environmental control. After reviewing the science requirements the ARC working group defined the personnel needs for CGRC design and development, and so in October of 1988 the ARC Engineering and Science Team for CGRC development was assembled.

The tasks assigned to the CGRC engineering and science team were to, 1) develop clear understanding of science requirements, 2) develop and produce an engineering requirements and specifications document, 3) begin system and subsystem conceptual design, and 4) meet with the CGRC Research Consortium and controlled environment engineers to review the engineering requirements document and first level system/subsystem conceptual design.

The engineering and science team has utilized the CGRC science requirements (task 1) to develop and complete the "Crop Growth Research Chamber Requirements Specification" (task 2) (3). This engineering specification document is now being used to direct system and subsystem design (task 3). A series of engineering review panels within ARC will evaluate conceptual designs with CGRC Research Consortium and controlled environment engineers review of preliminary designs to follow (task 4). Completion of the final design and review process will lead to fabrication of a CGRC prototype. This prototype will then be subjected to a series of tests to evaluate performance and verify that design goals have been accomplished. Following verification of CGRC prototype performance several units will be constructed and integrated with laboratory and analytical equipment at ARC to make up the Crop Growth Research Facility.

CGRC OBJECTIVES

Objectives of CGRC use can be separated according to three areas of application: 1) research, 2) system control and integration, and 3) flight hardware design and experimentation. In reality, however, all work conducted in the Crop Growth Research Facility will have application to CELSS development. Work will be conducted

to define the production efficiency of human usable products per unit input to the system and to define the hardware performance requirements for a CELSS in extraterrestrial environments. As mission scenarios are developed with clearly defined input limitations constrained optimization can be performed using the information acquired in the Crop Growth Research Facility to choose the optimum system design.

Research - The laboratory size of the CGRC will be small enough to allow duplication of the unit, treatment replication, and conduct of controlled experiments, but large enough to provide information representative of larger plant communities. An important feature for research is that the CGRC be of manageable size so that tasks can be performed in a timely fashion. Single plant chambers are potentially the easiest system to manage but we know that single plant growth is not an adequate nor acceptable indicator of community performance (4,5).

The CGRC will provide a range of environments not available to individual CELSS investigators in the form of unique or commercially available equipment. The CGRC represents the next generation of controlled environments. Capabilities include the potential for uninterrupted photosynthetic photon flux levels of $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$ or greater (full noon sun is approximately $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and "closure" so that mass within the chamber is conserved. The CGRC will provide the capability for manipulation and control of atmospheric constituents allowing the study of individual and interactive effects on gas exchange and biomass and food production of plants. Closure of the system will allow identification of plant produced volatiles and soluble organics. Quantification of the effects of volatile and soluble organic compounds and trace gas contamination on crop performance will be possible. Microbial population dynamics and pathogen challenge to the system are intended applications of the CGRC.

System Control and Integration - Operation and control of a stable system is essential for development of a reliable life support

system. The crop growth unit is only one portion of a CELSS but the crop plants function as several unique component processors. Carbon dioxide is removed from the atmosphere while oxygen is introduced through photosynthesis. Plant transpired water has been filtered through uptake by the root and incorporation of solutes into tissue before being evaporated from the interior of stomata of the leaves to the atmosphere. Transpiration rate can be manipulated over a wide range by environmental conditions. Carbon dioxide utilization and oxygen and water production are dynamic systems with short response times and the rate at which these processes operate can be varied as needs for a particular product vary. Of course food is being produced by the plants at the same time; the response time for expression of perturbations in the food production process is much greater than that observed for the other plants processes.

Edible plant yield is the integration of development during several unique phases between germination and harvest. Understanding the dynamics of yield development, i.e. having knowledge of crop responses to environmental manipulation during yield critical phases, is essential to predicting system performance.

Carbon dioxide uptake, and oxygen, water, and food can all be considered as products of the plant component of a CELSS. Information required for trade-off analysis to determine the short- and long-term gains and losses resulting from environmental manipulation during the life cycle of a crop as required for the desired plant product will be provided by the Crop Growth Research Facility.

Future interface with candidate unit processors on a laboratory scale will be possible. As candidate processes are developed for such operations as waste processing, oxygen removal and storage, nutrient recycle, and harvest and food processing, laboratory scale prototype units could be interfaced with the CGRC. Performance of these processors and requirements for interface with a crop growing unit could be evaluated. Unacceptable processors or designs could be discarded before the time and expense is expended on scale-up and

interface with the Biomass Production Chamber (BPC) at the Kennedy Space Center.

Ames Research Center was recently assigned as lead center for the physical chemical/closed loop life support system (PC/CLSS) program activities. The CELSS and PC/CLSS groups have been organized into one life support program in the Life Science Division. Since CELSS includes the appropriate physico-chemical processors we are now in a unique position to develop technology necessary for a truly regenerative life support system.

Mathematical models are important to CELSS for prediction of biomass, food, water, and oxygen production and CO₂ consumption by the crop plants, integration of biological and physico-chemical subsystems, and implementation of control theory. The Crop Growth Research Facility will supply information required for model development and will provide the vehicle for model validation.

Control in a CELSS will not be a trivial matter. The fully functional extraterrestrial CELSS, as well as the ground-based research and breadboard CELSS projects, represent very complex and dynamic systems with processes that can be varied. The CGRC is being utilized as a test bed for control theory application and verification (6).

Flight Hardware Design and Experimentation - The CGRC serves as the mechanism for setting science and engineering specifications, technology assessment, and potentially serves as the ground-based control unit or reference for the CELSS Test Facility of the Space Station Freedom. The CGRC science requirements for performance and the CGRC Engineering Specifications Requirements document (3) are being utilized for development of the CELSS Test Facility (CTF) and for estimation of system complexity, mass, volume, and cost.

THE CGRC SYSTEM

The CGRC system has two main components, the atmospheric environment and the hydroponic environment. Both environments must be maintained independent of the other; the goal is to have no

movement or migration of materials between the environments except for what is conducted through the plants which are continuous between the environments.

Atmospheric Environment - The atmospheric environment has several component subsystems and physical zones as shown in Figure 1. The growing volume makes up the majority of the atmospheric environment. The shoot, root, and subroot zones are included in the growing volume. The root zone, while located within the growing volume is a component of the hydroponic environment. The shoot zone is the area above the root zone. The size of the shoot zone will vary as the the plants are raised or lowered as required for experimental treatment or as the plants increase in size. The subroot zone is a very small volume which accommodates the flexible tubing and connections for the hydroponic system. The subroot zone is a very small volume when the root zone is in the lowest position and the shoot zone is near maximum volume.

Located external to the growing volume are the gas makeup system, heating/cooling/ventilation system, pressure control system, gas removal and separation system, lamp system, hydroponic reservoir, water condensation and collection system, and the associated ducting. The entire atmospheric system will be sealed to minimize leaks and the system will be positively pressured throughout.

Photosynthetically active radiation will be supplied to the growing volume by a lamp system located external to the closed atmospheric environment. A transparent barrier placed between lamps and plants will maintain the seal while minimizing the amount of non-photosynthetically active radiation entering the growing volume (7).

The recirculating hydroponic solution in the root zone will remain isolated from the gaseous atmosphere of the shoot zone. Water will be conducted from the root zone by the plant and enter the shoot zone by transpiration. Transpired water will be collected

for analysis and eventually recycled to the hydroponic or the humidification systems.

Hydroponic Environment - The hydroponic system will supply a source of temperature controlled and oxygenated nutrient solution to the plant roots. The composition of the solution will be monitored and controlled. CGRC operation in the facility will allow a common solution to be circulated in all CGRCs or several experimental solutions to be circulated among representative solution containers in each CGRC.

System Control and Data Acquisition - All systems must function in concert to maintain control, within tight tolerance, the settings for all environmental parameters. While the CGRC is made up of several subsystems and components, it is itself an analytical research instrument which must function as designed with stability and repeatability. Measurements made within the chamber will serve as feedback to the control system and as scientific data that will be further utilized in calculations and manipulations. The control system and data acquisition systems may physically be the same system with redundant sensor placement or each system may be unique.

CGRC SCIENCE REQUIREMENTS

For the Crop Growth Research Facility to be able to accomplish the stated goals each individual unit in the facility must be capable of providing a wide range of environmental conditions to accommodate the studies to be conducted. A set of science requirements, better referred to as hardware performance requirements, for the CGRC were determined by the CGRC Research Consortium (Table 1).

The functional design in figure 2 represents the processes necessary to accomplish the performance requirements of the CGRC. System component characteristics will be determined during the design process. A potential physical appearance of the CGRC is portrayed in figure 3.

SUMMARY

The Crop Growth Research Chamber represents a unique controlled environment research and developmental tool available to the CELSS program. The integration of several CGRCs and laboratory analytical equipment provides a facility useful for ground-based and flight research, technology development, CELSS component subsystem integration, and system control. The Crop Growth Research Facility will allow evaluation of the stability of a CELSS and determination of the quality of the human useable products.

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Table 1. Crop Growth Research Chamber Science Requirements for Environmental Control.

<u>Parameter</u>	<u>Control Range</u>	<u>Accuracy</u>
Air Temperature	5-40°C	±1°C
Air Pressure	+ 15 mm H ₂ O(gage)	± 5 mm H ₂ O(gage)
Relative Humidity	35-90%	±2%
Air Composition		
Nitrogen	75-95%	±5%
Oxygen	5-25%	±5%
Carbon Dioxide	25-5000 μmol mol ⁻¹	±0.2%
Air Flow Rate	0.1-1.0 m s ⁻¹	± 0.1 m s ⁻¹
Photosynthetic Photon Flux	0-3000 μmol m ⁻² s ⁻¹	±2%

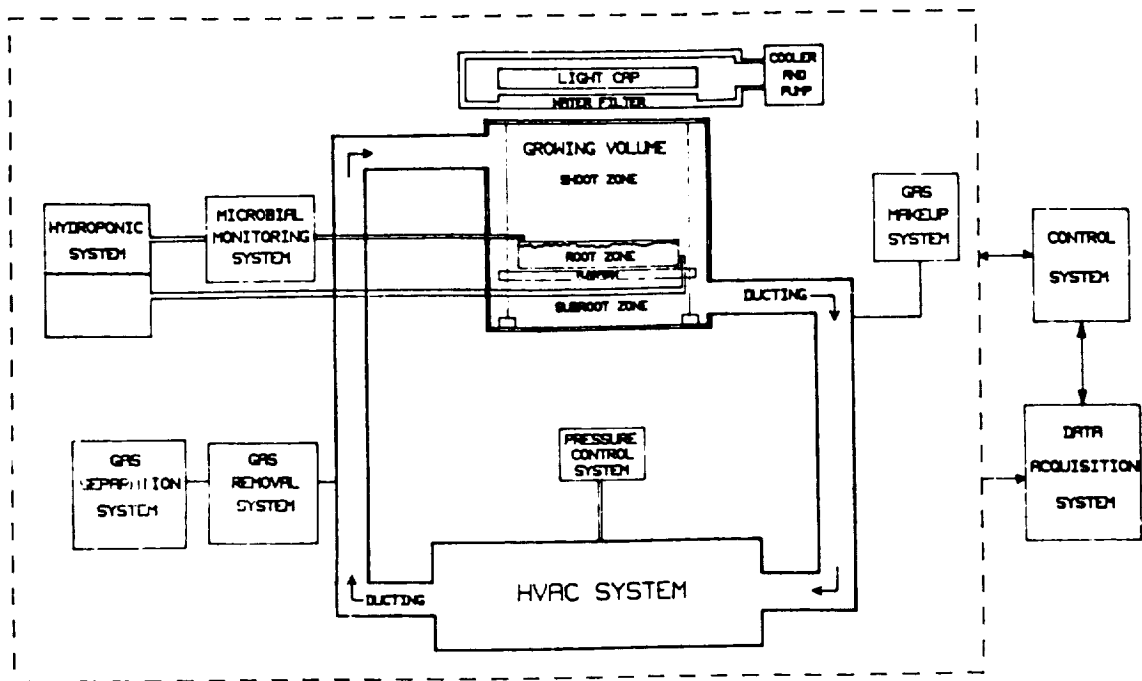


Figure 1. Block diagram of component subsystems and physical zones of the Crop Growth Research Chamber (CGRC).

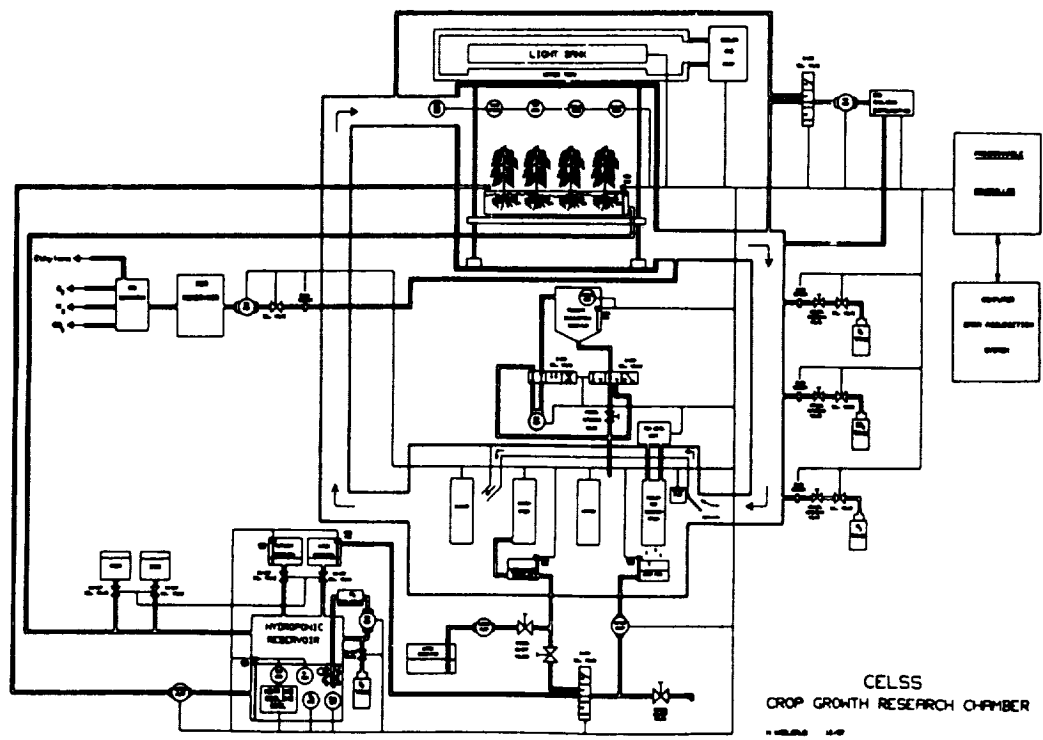


Figure 2. Functional design of the Crop Growth Research Chamber.

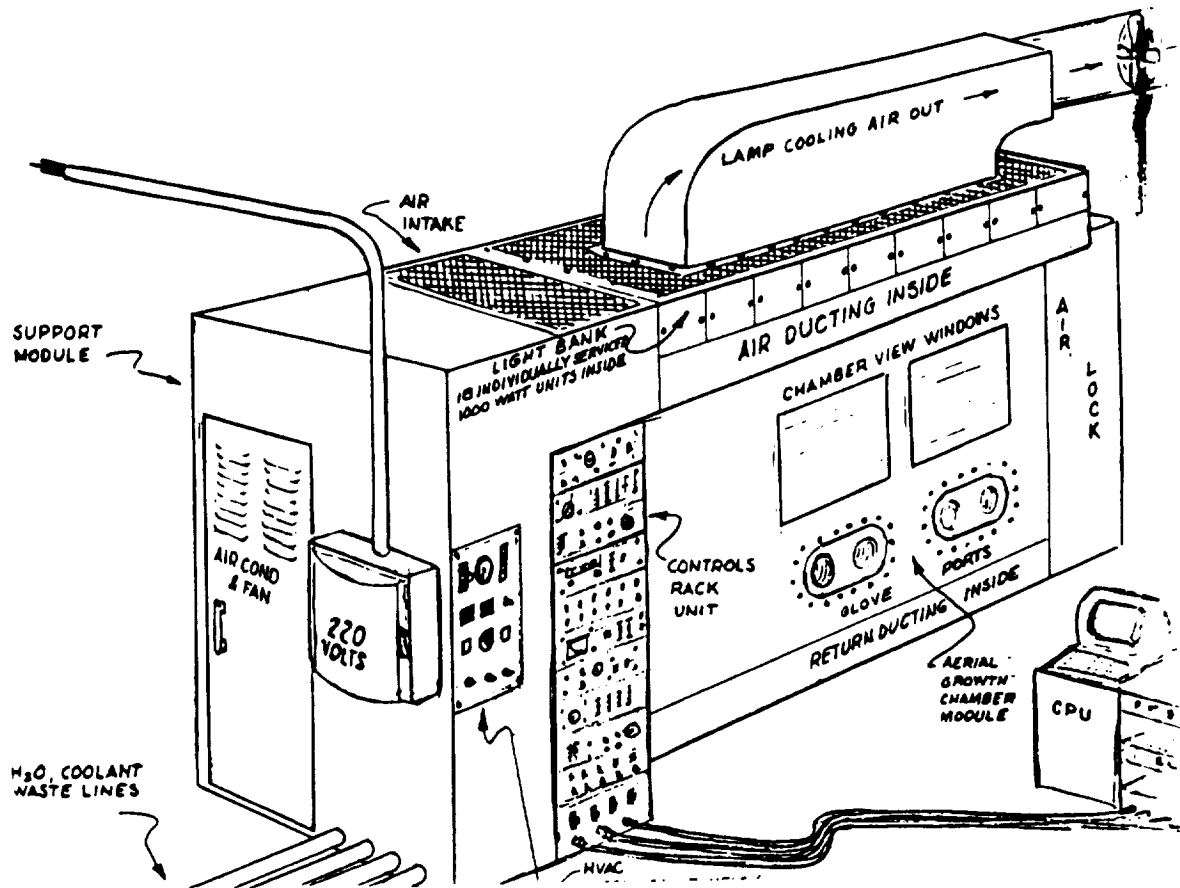


Figure 3. Potential physical appearance of the Crop Growth Research Chamber.

Development of the CELSS Emulator at NASA JSC

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ABSTRACT

The Controlled Ecological Life Support System (CELSS) Emulator is under development at the NASA Johnson Space Center (JSC) with the purpose to investigate computer simulations of integrated CELSS operations involving humans, plants, and process machinery. This paper describes Version 1.0 of the CELSS Emulator that was initiated in 1988 on the JSC Multi Purpose Applications Console Test Bed as the simulation framework. The run module of the simulation system now contains a CELSS model called BLSS. The CELSS Emulator empowers us to generate model data sets, store libraries of results for further analysis, and also display plots of model variables as a function of time. The progress of the project is presented with sample test runs and simulation display pages.

THE LONG-DURATION FUTURE SPACE MISSIONS including settlements on lunar and planetary surfaces will require a spectrum of human life support systems that regenerate air, water, and food from process wastes. Such life support systems i.e. Controlled Ecological Life Support Systems (CELSS) will be expected to provide highly reliable service with integrated biological and nonbiological components of limited reliability.

The conceptual design of a CELSS to sustain a crew of 6 was reported by the author last year for a piloted Mars sprint (1,2). An integrated operation was achieved in this study with air, water, and waste processing and supplemental food production. A computer model of the specific life support system design was developed by utilizing the SINDA '85 code to support the analysis. The system performance was analyzed in terms of the physical-chemical subsystems or components and a greenhouse. Two crops, lettuce and winged beans, were chosen for bioregeneration. The crew cabin and the greenhouse were physically separated but dynamically interfaced with mass and energy flows. The plants provided 9, 29, 22, and 50 percent of air revitalization, water reclamation, wet food supply, and waste processing functions, respectively.

On the other hand, a computer model called BLSS was developed earlier by Drs. John Rummel and Tyler Volk to analyze a generic CELSS with a wheat-based crop cycle (3,4). BLSS uses a reservoir approach in modeling, i.e.

subsystems are tracked not phenomenologically but instead in their response to the reservoir inputs and outputs. High-productivity wheat is the sole driver for bioregeneration functions including the crew diet.

Currently, the CELSS Emulator of the Solar System Exploration Division at JSC is being developed on a computer test bed system designed at JSC for the Space Station Freedom, called the Multi Purpose Applications Console, or MPAC Test Bed, with the incorporation of the BLSS model for first CELSS simulation experience. This paper is intended to describe the Emulator and its progress with results.

SIMULATION SYSTEM

The Multi Purpose Applications Console (MPAC) Test Bed was developed by the Systems Development and Simulations Division at JSC to provide an environment for the prototyping of the Space Station Freedom's display and control techniques. The development system consists of a MicroVAX II Computer employing the VAX/VMS Version 4.7 operating system, a Raster Technology Model One/85 graphics terminal, and a Dataviews graphics management system. The MPAC Test Bed provides the display and control of data values generated internally by simulation models or from data collected via one of the networks servicing the MicroVAX Computer. The system currently supports software developed in Fortran, C, Pascal, Ada, and Assembly languages.

The User Interface Language (UIL) Demonstration System (UDS) is the simulation system developed at JSC for the purpose of generating data for a UIL workstation. The software system requirements definition for all model measurements is controlled by a data base file. The realtime software provides a user interface for displays and controls in the Space Station Freedom command and control workstation. All realtime measurement values are found in a common memory designated as Current Value Memory (CVM). All task communication is through common memory and event flag clusters, a service provided by the MicroVMS Operating System. Simulation models drive the values in CVM and provide the output to the Freedom simulation

workstation . An offline system provides the user with the ability to add new measurements to the active data base, or to modify existing measurements. The offline system also enables the user to build the CVM structure based on the current data base. The philosophy in the software development is to generate as much information as possible for the realtime system in an offline mode. This offline mode is used in the production of the global common memory map, the generation of display pages, the identification of measurements for the simulation models, and the generation of parameter tables used to identify measurements being transported between models.

The CVM contains the measurement values for all items in the active data base. The entry for each measurement contains a control word for realtime processing as well as the current value of this measurement. The models can access the CVM which is primarily driven by the outputs of other models .

CELSS EMULATOR DESCRIPTION

The goal of the CELSS Emulator development is to have a smart tool to help define the parameters needed for a real CELSS for future space pioneers. The Emulator allows the user to easily set up data sets and manages the output files produced by model runs. Table 1 lists various steps leading to a CELSS design while Table 2 shows the role of the Emulator in this design process. Our approach has been such that the initial version of the Emulator functions in accordance with the existing BLSS model. Hence, the results discussed in this paper are identical to those of the original version of BLSS.

Figure 1 shows a diagram of the Emulator system. The top portion in the figure deals with the computer system configuration, operation, and offline processing. The middle portion contains the simulation computer system consisting of the BLSS model, a future crop model, the database, the display and control software package, and a future logic processor. The bottom portion of the diagram refers to the

display and control station equipment i.e. the graphics terminal. The dotted lines indicate future implementation.

The Emulator operational interfaces are presented in Figure 2. Note that a Preprocessing Utility module provides the capability to develop, manipulate, and store data sets that can be inputs to the BLSS model within the CELSS Emulator. A Postprocessing Analysis module allows us to generate plot files with the data obtained in a simulation run. Central to all the operations is the BLSS Utility Directory (BUD) to organize the storage and retrieval of the setup files and the report files.

SAMPLE RUN

Figure 3 presents the CELSS Emulator operational flow. When the Emulator is running, the data change is observable at appropriate display pages. Any time duration can be chosen for the BLSS calculations. Figure 4 shows a display page for BLSS summary at the end of a 200-day run. There are six distinct panels on this page. The top panel indicates time in days and crew size. The second panel lists the current values for dry food storage and process mass. The BLSS model assumes no leakage, so the process mass remains constant. The next panel on the summary display page shows edible and inedible biomass being produced. The fourth panel indicates the amount of waste being processed.

Displayed right below it are the flow rates of air and water for the plant growth module and the crew compartment. Finally, the Emulator displays information on how much water, oxygen, carbon dioxide, and plant nutrient are available in storage.

Figure 5 shows another display page, the Crew Compartment Atmosphere Status. To change the crew size (see Figures 2 and 3), for example to 6, the number across "CREWSIZE" is replaced with "6" by means of either the slider or the keyboard.

By using the Postprocessing Analysis module, plots such as

Mission Definition	(Objectives, external interfaces, environment)
System Requirements	(Performance objectives, measures of system value)
System Concepts	(System elements and interfaces, element-level performance objectives, parametric evaluations, trade studies)
Feasibility Evaluation	(Alternative approaches to subsystems, comparison of performance with existing technology, formulation of prototypes)
System Definition	(System- and subsystem-level specifications, subsystem and system operation description)
System Design	(Technical drawings and configurations, performance parameters, operational procedures)

Table 1: Steps Leading to CELSS Design

Phase	Input	Output
Mission definition	Scenarios Environment	
System requirements	Performance measures	
System concepts	Element definitions Interface definitions	Performance objectives Parametric studies System performance
Feasibility evaluation		Alternative approaches Comparison of technologies Evaluation of prototypes
System definition		Compatibility evaluations Validation of interfaces
System design		Fidelity evaluation Crew training - later

Table 2: Role of CELSS Emulator in Design Process

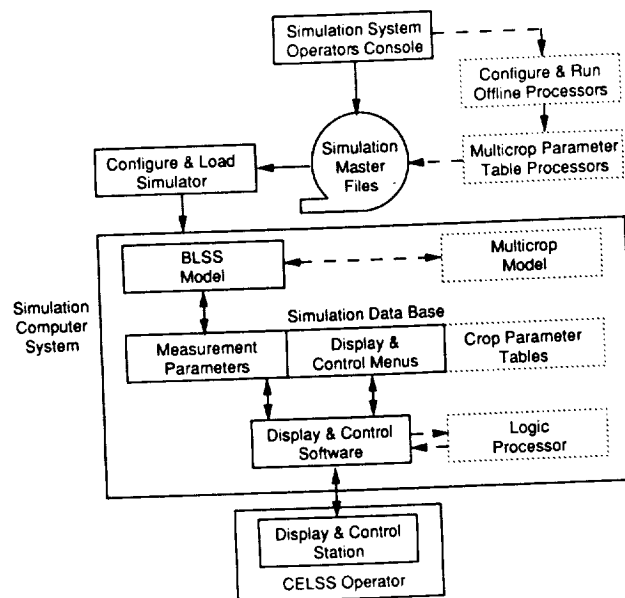


Figure 1 — CELSS Emulator System Diagram

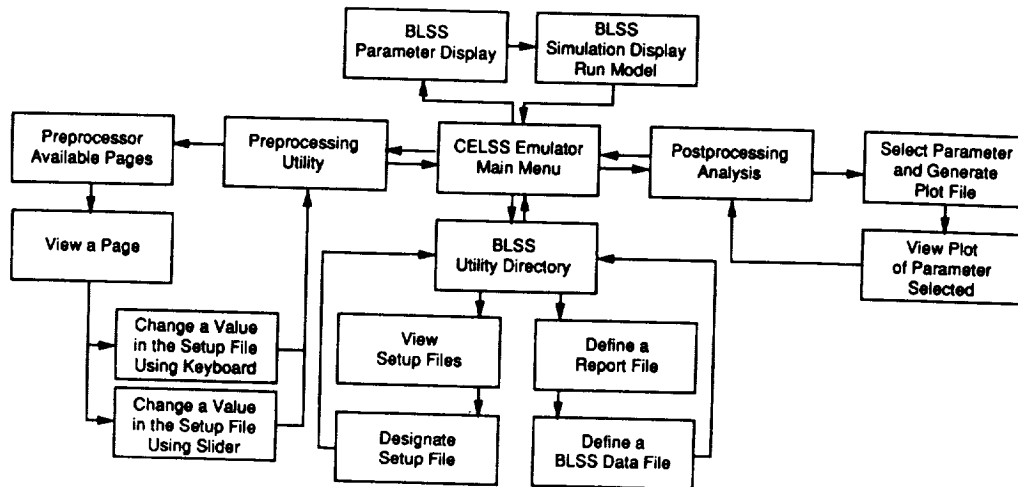


Figure 2 — CELSS Emulator Operational Interfaces

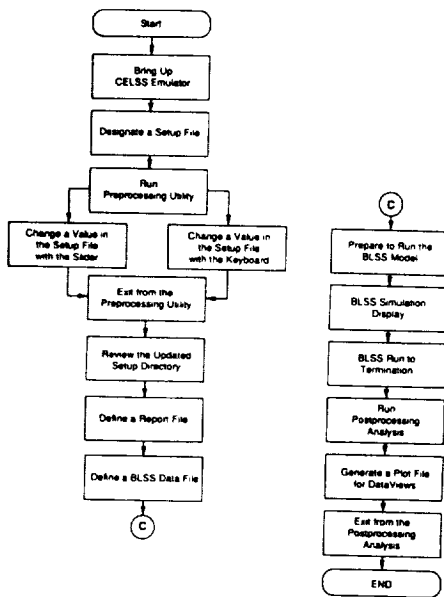


Figure 3 — CELSS Emulator Operational Flow Diagram

those given in Figures 6, 7, and 8 can be generated. Figure 6 shows a time plot for three parameters: dry food, total edible biomass, and total inedible biomass. Note the fifty-five day growth cycle for the wheat crop. The time history for the dry food is represented by the sawtoothed graph. The edible biomass is harvested and stored as dry food. While the next crop is being grown, the dry food in storage decreases as it is eaten by the crew.

Various variables can be compared for different crew sizes. Figure 7 show a plot for dry food storage with a range of crew size from 0 to 6. As calculated, food consumption increases with crew size. Also, at each harvest time, the dry food storage is filled to capacity. The excess harvest is either used as seed or processed as waste. In the first cycle, a small fraction of the dry food is used for seeding the second cycle. This amount is recovered in subsequent harvests.

Similarly, Figure 8 is a plot of the water storage with time for crew size ranging from 0 to 6.

BLSS SIMULATION DISPLAY MODEL EXIT |

DAYS CREW SIZE

DRY FOOD PROCESS MASS

EDIBLE BIOMASS INEDIBLE

TOTAL DRYWASTE TOTAL WASTE

PGM CO2 IN CREW O2 IN

PGM H2O OUT CREW H2O OUT

STORAGE

WATER OXYGEN

CARBON DIOXIDE NUTRIENT

Figure 4 — BLSS Simulation Display Page

CELSS BLSS PARAMETER DISPLAY
 CREW COMPARTMENT ATMOSPHERE STATUS
 READ FROM SETUP FILE
 STANDARD SETUP FILE CREWSIZE 6
 HEADING FOR BLSS FILE REPORT: CELSS RPT

SELECT BOX FOR SLIDER INPUT	INPUT VALUE	BLSS VARIABLE	NOMENCLATURE	UNITS
<input type="checkbox"/>	1	TIMESTART	'START RUN TIME'	'HRS'
<input type="checkbox"/>	280000	VOLOFCCOM	'ATMOSPHERE VOL'	'L'
<input type="checkbox"/>	20	CCOMAMBTMP	'AMBIENT TEMP'	'C'
<input type="checkbox"/>	50	CREWRELHUMIDSET	'RELATIVE HUMIDITY'	'%
<input type="checkbox"/>	20	CREWO2LEVELSET	'O2 LEVEL'	'%
<input type="checkbox"/>	350	CREWCO2LEVELSET	'CO2 LEVEL'	'uL/L'
<input type="checkbox"/>	6	CREWSIZE	'CREW SIZE'	'MAX 6'

DEFAULT INPUT SYSTEM SET CHECK XCODE

Figure 5 — Display Page for Crew Compartment Atmosphere Status

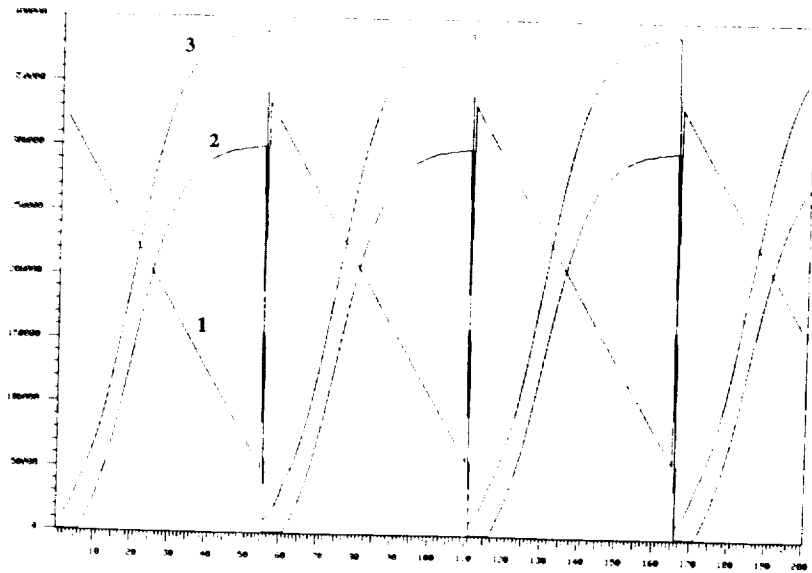


Figure 6 — Plots of Dry Food (1), Total Edible Biomass (2), and Total Inedible Biomass (3) for a BLSS Sample Run of 200 Days.

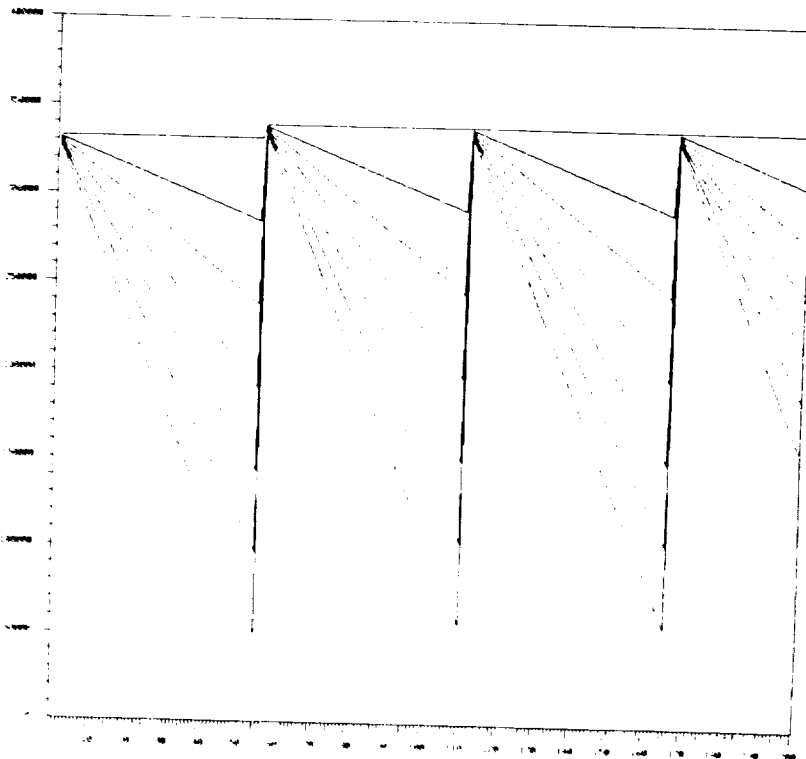


Figure 7 — Plots of Dry Food Storage for Crew Size Ranging from 0 (top) to 6 (bottom).

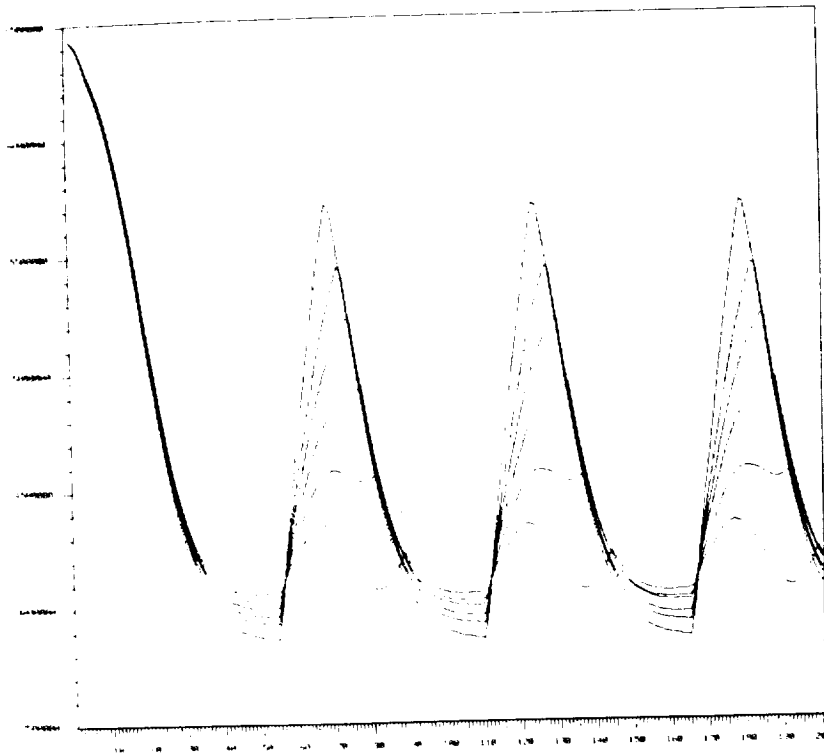


Figure 8 — Plots of Water Storage for Crew Size Ranging from 0 (top) to 6 (bottom).

CONCLUSIONS

The CELSS Emulator Version 1.0 represents the first significant step in the development of a computer-based simulation tool to study stability, monitoring, control, and integration aspects of operating CELSS. Through computer simulation, the CELSS Emulator at JSC will allow researchers to determine the characteristics of future CELSS equipment and its interaction with the crew. CELSS is a very complex system. We need the Emulator to unravel this complexity so we can understand where we are and where we are going.

ACKNOWLEDGEMENT

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A PERSPECTIVE ON CELSS CONTROL ISSUES

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ABSTRACT

Some issues of Controlled Ecological Life Support Systems (CELSS) analysis and design which are effectively addressed from a systems control theoretic perspective are discussed. CELSS system properties which may be elucidated using control theory in conjunction with mathematical and simulation modeling are enumerated. The approach which is being taken to the design of a control strategy for the Crop Growth Research Chamber and the relationship of that approach to CELSS plant growth unit subsystems control is described.

INTRODUCTION

In any life support system, whether it is open, regenerative through strictly physical-chemical processes, or bioregenerative, the primary goal is to provide a support structure for the maintenance of desirable conditions for the humans within the crew compartment. These conditions include the provision of adequate nourishment, potable water, utility water, a suitable thermal environment and properly balanced and pressurized gaseous atmosphere, and the removal of wastes. In the terminology of control theory, the dynamic system whose behavior we desire to influence is called the "plant", which consists, in the context of a life support system, of the crew and their immediate environment. Fig. 1 illustrates the crew compartment as the "plant" with respect to a reference configuration for a Controlled Ecological Life Support System (CELSS). In that bioregenerative system, the remaining portions of the system are the life support system control components. The control concept is realized by considering the dynamics of the plant, the dynamics of the control system components, the behavioral goals of the controlled system and

is described in [2] as "the process of influencing the behavior of a dynamical system so as to achieve a desired goal." Through the mathematics of control theory, control laws are derived which the controllable variables of the system must follow in order to achieve desired system behavior.

The Hierarchy of Control Concerns.

Fig. 1 illustrates that a CELSS consists of a complex interconnection of dynamic subsystems, the behavior of each of which may require management by means of control. The hierarchy of control in a CELSS is illustrated in [3]. Several hierarchical levels are present in a CELSS: components, subsystems, and complete CELSS system. The element defined as the "plant" will differ among hierarchical levels as well as among systems on the same hierarchical level. The goals associated with each system may differ, but the aspects of control issues which will be discussed are applicable on each hierarchical level.

Passive and Active Control.

It has been suggested that the CELSS design approach include the property of modularity, i.e. the processors (dynamic components such as plants, waste processor, crew) interact indirectly through mass storage elements.[4] As noted in [4], the mass storages in a CELSS are equivalent to the reservoirs of inorganic material found on Earth. Storages have been demonstrated to be effective buffers in a CELSS, particularly under component failure conditions. Because of the volume and mass requirements, the use of storage in a CELSS is limited. The dynamic behavior of stable systems can be influenced by using storages as passive control devices. If, however, a dynamic system is not stable, feedback (active control) is required to regulate the behavior of the system.

If the system is stable but is subject to disturbances which are not predictable, feedback control may be required to meet desired performance objectives. Implementation of

feedback control can reduce the sensitivity a system to a variety of disturbances. Newly emerging perspectives of robust control can produce closed loop systems which are significantly independent of the effects of uncertainties and disturbances. In the following sections, attention is focused on the development of active (feedback), and especially robust, control systems for CELSS.

Development of Control Laws.

Rules for appropriate control actions are mathematical. For feedback control, these rules represent the sequence of signals, functions of the states of the system, which are sent to the control actuator in order to achieve the desired system behavior.

The rules for control actions are based upon both a model of the system and the goals which the behavior of the controlled system must achieve. When the system is similar to one with which the control designer already has some familiarity, initial control law design may be based upon his previous experience (i.e. a mental model) and then tuned to accommodate the unique aspects of the system under consideration. When the system or the performance requirements of the system represents a significant departure from familiar systems, a model which will provide the designer with an organized approach to control law development is required.

A means for analyzing the characteristics and behavior of a dynamic system is provided by a mathematical model.¹ The analysis of these characteristics in conjunction with a mathematical statement of the performance objectives of the controlled system leads directly to a mathematical description of the control laws required for that system-performance combination.

¹A distinction is made between mathematical models and other system models such as symbolic or conceptual models, computer or simulation models, physical models or mock ups, etc.

Mathematical Modeling.

The role of mathematics and mathematical modeling in control theory is elegantly developed in [2]. Some particularly relevant passages from that document are excerpted in the following section.

The mathematical modeling issue in control design differs from that in scientific research. The fundamental challenge in control modeling is to find parsimonious representations of complex physical and biophysical phenomena which are adequate for the analytical and computational needs of control design. For scientific understanding, great emphasis is placed on developing microscopically accurate models derived from physical laws. In theory, once such a model is firmly established, the control design based upon it is at least computationally feasible but may be so complex as to be impossible to implement. It may not be possible, however, to write down exact dynamic laws since processes, such as some biophysical responses to the special environments produced in a CELSS, may be poorly understood.

It is well established that feedback reduces the effect of uncertainties including modeling errors. This would imply that, in the extreme, model imperfections are not relevant in the context of control. From such a perspective, what would be needed is a powerful feedback design methodology yielding a robust, fault-tolerant control system. The process of control modeling therefore involves identifying the appropriate mathematical structure - rich enough for adequate problem description yet simple enough for mathematical tractability - and then bringing the power of mathematical machinery to bear on the solution of the control problem. [2]

Mathematical Model Characteristics.

The suitability of the mathematical model for control design is determined by the physical properties of the system and the control objective. The modeler must decide whether the

system is best represented in the continuous or discrete time domain, whether distributed phenomena can be suitably represented by lumped models (i.e. the need for partial differential equations versus the adequacy of ordinary differential equations), and whether the nonlinear phenomena in the system must be fully accounted for. Robustness considerations are involved in the selection of the time scale of the model. Fast stable dynamics, which are usually ignored in conventional control analysis, cannot necessarily be neglected in the design of robust controllers. Robustness considerations also arise in selection of the level of aggregation in modeling, particularly with respect to biological phenomena.

Representing Uncertainty.

Uncertainties in the representation of a dynamic system for control design purposes include those related to parameter values, those related to functionality and those related to external disturbances. Uncertainty in parameters may be due to inherent variability in components of a system, variation in characteristics as components age, variations in response rates as environmental conditions change, etc. Uncertainty in functionality may arise from poorly understood processes, from functional variation with life stage or environmental conditions, from unknown but finite higher order dynamics, from failures, etc. Errors in rate functions due to aggregation must be accounted for in the mathematical representation of uncertainty.

Some controller design techniques [5] require at least partial knowledge of the statistics of the uncertainties. Other techniques require knowledge only of the bounds or the uncertainties [6, 7], but conservatism in design is reduced when the effect of uncertainty can be expressed in terms of a frequency content. [7, 8] The control design method selected is influenced by, among other factors, the information available concerning the uncertainties.

Analysis of the System Model.

Many important questions related to the acceptability of performance of a dynamic system can be evaluated by analyzing its mathematical model. In addition, the analysis process provides information about the system which is fundamental to the synthesis of effective control.

Stability.

A fundamental question to be addressed is whether the system is stable. The definition of stability is not unique. [9] In the sense that it is used here, stability is defined with reference to a region of the system space about the desired operating points. A dynamic system is said to be stable to a region if the states of that system, when perturbed from an equilibrium point within that region, remain within that region for all time thereafter.

The fundamental niche of a living system is described by the range of tolerable environmental (i.e. biotic and abiotic) conditions. The organism will survive within the region described by the full range of that niche. If the environmental system is stable to the region of the niche, the organism will survive. Within the region of the fundamental niche lies a subregion in the organism exhibits some desired characteristics and it is within this subregion, defined by performance objectives in addition to basic survival, that we wish to confine the environmental variables. If an environmental system is stable to this subregion, desirable operation of the living system will occur.

The "plant" for a complete CELSS, i.e. the crew compartment with no inputs, is not stable by this definition. In an open life support system, the states of the crew subsystem can be forced to remain within an acceptable region for the duration of the mission by introducing an environmental control system and providing source and sink reservoirs which are sufficiently large. If sources (stores) can be exhausted

or sinks (storages) can become saturated, the system fails the criterion for stability as defined. If regular resupply is included as a state dependent input, the stability criterion may be met by the controlled system. In the completely closed CELSS, i.e. with no resupply and no "unusable wastes" (as indicated in Fig. 1), the criterion of stability to an acceptable region must be met. The region of acceptability would be defined as the subregion for desirable operation for normal conditions and as the region of the fundamental niche for failure conditions or other emergency conditions.

Robustness.

Analysis of a mathematical model of a system may indicate that it is stable for nominal values of the parameters. Deviations of the parameters from their nominal values may result in an unstable system. Additionally, disturbances to the system may drive the system states or outputs of interest out of the desired region. Robustness measures can be applied to the mathematical model to determine the stability robustness of the system to the expected variations in parameters. [10]

Stabilizability and controllability.

If a system is stable to a desired region or subregion under all uncertainties and if no further optimization of performance is needed, no additional control is required. If this is not true, then the available control variables must be employed to attempt to maintain the system states or outputs within the desired region. The mathematical model may be analyzed to determine whether the control variables which are available can be manipulated in any way to maintain regional stability. If and only if all system states can be brought to specified values in finite time by means of the available inputs, the system is said to be controllable. If all unstable modes can be modified by the controls, the system is said to be stabilizable. If it is necessary to maintain a stabilizable system within a region, the available control variables may be

sufficient to do so. If it is necessary to bring the stabilizable system to another operating region, a restructuring of the control variables will be required. If the system is not stabilizable with the available control variables, a restructuring of the control variables will be required. This restructuring may involve the addition, relocation or resizing of control variables or a redesign of control actuators. (See [11] and references therein.)

Detectibility and observability.

The outputs of the system which are available for measurement can affect the ability to implement effective control algorithms. The likelihood of implementing an effective control decreases progressively from (a) the situation in which all the system states can be measured, to (b) the case in which not all states can be measured but all the information about the dynamics of the system can be reconstructed from the available measurements, to (c) the situation in which only the stable modes of the system are unobservable through the measurements. The mathematical model of the system may be analyzed to determine which of these cases exists as the system is currently designed. As a result of this analysis, the measurement system may need to be restructured in order to provide the information required for implementation of a control algorithm. This restructuring may involve the addition, relocation or redesign of measurement instruments. (See [11] and references therein.)

Coupling.

The mathematical model can be examined to determine the degree of coupling which the system exhibits among the system variables and the control variables. A lightly coupled system may allow the system to be analyzed as a set of single input single output systems, significantly simplifying the control strategy. Strong coupling will require analysis as a unified multiple input multiple output system, possibly resulting in

interrelated control strategies.

Development of Control Algorithms.

As previously noted the development of control algorithms depends upon both the characteristics of the system to be controlled and the performance objective. The objective of a bioregenerative life support system is to provide a self sustaining system for a long duration, in theory indefinitely. The constraints on the deviations of the values of some system variables from some nominal constant or prescribed time dependent reference will be fixed by biological and physical requirements. A CELSS must be at least stable to a region (described by these reference values and deviations) for the design life of the CELSS. This stability must be maintained in the face of parameter variations, external disturbances, and internal functional changes, i.e. any control strategy must be robust to these factors. An analysis of the overall system will assist in determining the performance requirements which this system constraint places upon the subsystems and their components. Such requirements include dynamic response, accuracy, noise sensitivity, control range, etc.

It should be noted that a robust control strategy is not necessarily unique. It is possible that such a strategy could be arrived at by developing an algorithm by some other means. However, without a formal procedure which incorporates establishing robustness as a required property of the design, it is difficult to demonstrate with confidence that robustness has actually been achieved. Whatever control strategies are developed, the hierarchy of control algorithms for subsystems and components should, collectively, not impose excessive requirements for computational intensity and should be numerically well conditioned.

Intelligent Control.

Intelligent control represents the integration of symbolic computation, numeric computation and artificial intelligence

(AI). AI may play an important role in decisions concerning which control strategy to use in view of changes in the control environment. The selection of the most appropriate control strategy for physical systems has been based upon analysis of a system model provided to the AI system using searches for best matches to dynamic response patterns [12]. It is possible that the relationship between biological age and chronological age of a crop in an operational CELSS may differ from the relationship established in previous studies. Because of this potential discrepancy and the fact that control strategies (e.g. harvesting) are related to biological age, it may be necessary to monitor the biological age of a crop using techniques such as image processing in combination with measurements of dynamic biophysical responses. An AI system such as that reported in [12] might be used to search for model matches. Depending upon the best match selected, the most appropriate environmental control strategy for a crop of that particular biological age could be employed to maintain, accelerate or decelerate growth and development as required. The use of this level of response for intelligent autonomous systems is discussed in [13].

Simulation Modeling.

Simulation models are valuable tools for demonstrating and testing the performance of systems. Scenario studies can be conducted to verify the effectiveness of the control design. Recall that it is characteristic of the system control problem to employ simplified models for the purpose of controller synthesis. These models typically (a) employ functionally more simple representations of process behavior and (b) do not describe all stable dynamics. Computer simulations, based on more comprehensive and nearly complete models which include at least the most significant nonlinearities, can be utilized to demonstrate controlled system performance.

Important properties of simulation models are portability and modularity. Portability of a model to various computers

with little modification enhances communication among researchers and makes models more readily adapted to state-of-the-art developments in computer hardware. Modular design of modeling software allows system design option variations to be examined easily without significantly affecting the programming code of the remainder of the model.

Many simulation techniques are currently being developed which allow data entry through graphical techniques for general purpose simulations [14], for generalized environmental control and life support system design and analysis, [15] and for control system design [16, 17, 18]. Graphical interfaces greatly ease the data input process and reduce the problems associated with programming errors. The utilization of a graphical input simulation technique which accommodates the biophysical and physical processes involved in the CELSS system would be valuable in controlled system validation.

Iteration.

Finally, it should be noted that control synthesis is an iterative process of modeling, analysis, control algorithm development, simulation and testing, in which simplifying assumptions are gradually removed in the design process and, as hardware is developed, other assumptions made during theoretical development are altered.

APPLICATIONS TO THE CELSS SUBSYSTEM PLANT GROWTH UNIT

One of the subsystems in Fig. 1 is the Plant Growth Units and its associated components. Four configurations of the Plant Growth Unit subsystem are currently planned: the Crop Growth Research Chamber (CGRC), CROP, the Salad Machine, and EDEN. The CGRC is a ground-based unit in which precision control of environmental conditions will allow scientific research into plant growth in closed environments. CROP is a space-based unit with precision control comparable to the CGRC. The Salad Machine is a space-based unit designed to produce

small amounts of salad vegetables for the crew. EDEN is a fully cycling space-based unit which will produce 10 to 15% of the food supply for the crew. The CGRC prototype instrument is currently under development.

It can be seen that each of these four configurations are specific physical realizations of the generic Plant Growth Unit subsystem. They differ from one another in performance specifications, degree of linkage to the remainder of the CELSS, and specific control component requirements to perform analogous functions in their particular design operating environments. Nevertheless, the functional analogy among them suggests a commonality of approach to modeling and control issues.

The CGRC Concept.

Fig. 2 is adapted from the CELSS reference configuration of Fig. 1. The interfaces of the storages which act as sinks and sources for the Plant Growth Units and associated control units with the remainder of the CELSS system have been removed. It is evident that this subsystem is functionally analogous to the CGRC. In the CGRC, the storages which had provided linkage to the remainder of the CELSS system have been made sufficiently large so that they can supply all the inputs (e.g. water, nutrients, carbon dioxide) and receive all the outputs (e.g. transpired water, oxygen) required for the plant growth subsystem. By providing sufficiently large storages, ideal closure conditions of a complete CELSS can be emulated by the CGRC.

The control issues for the CGRC are related to regulation of air temperature and humidity and atmospheric composition and pressure within the shoot zone and regulation of nutrient solution temperature, composition and pressure within the root zone of the plant growth chamber. The design range and tolerances of the shoot zone and root zone environmental conditions are given in Table 1. By controlling these conditions, the response of plants to conditions within the

ideal subregion of the fundamental niche or to conditions in the remainder of the region of the fundamental niche can be examined. The latter may represent emulation of suboptimal closure conditions. Studies of crop responses to the extent of the range of environmental conditions will assist in establishing uncertainty bounds on the functional response of the plant growth unit. These bounds can be used in the representation of uncertainties for the Plant Growth Unit subsystem as a component in the study of control needs for the complete CELSS. A detailed description of the CGRC is given in [19].

The dynamic processes in both the shoot zone and the root zone of the plant growth chamber are profoundly coupled to those of the plant. Internal processes within the plant couple the shoot zone and root zone dynamics. A complete CGRC system analysis and control system synthesis must take into account the dynamics of the shoot and root zones and those within the plant. An initial simplifying assumption that the within plant coupling processes are weak allows separate preliminary analysis of the shoot zone and root zone dynamics. In later iterations of the design process, this assumption may be modified.

Description of a Proposed System.

Fig. 3 is a schematic model of the plant growth chamber and the components of a proposed system to regulate the shoot zone environment. The plants receive radiant energy from a light source above the chamber (not shown). Air flow into the chamber is assumed to be sufficient to assure that uniform conditions exist in the atmospheric control volume within the chamber and surrounding the plant canopy control volume. Gaseous exchange of carbon dioxide, water vapor and oxygen occurs between the canopy and the atmosphere. Air enters near the top of the upper chamber and is thoroughly mixed with the air in the upper portion of the chamber. The resulting mixture flows between the walls and the baffle formed by the plant

support surface into the lower portion of the chamber and then into the duct work located near the bottom. A filter is provided to remove particulates from the air as it leaves the chamber. Air flow out of the filter is affected by the controllable orifice flow area of a valve. A portion of the air flow is diverted, by means of a controllable flapper valve and fan, into a gas separator which removes excess oxygen or carbon dioxide. A centrifugal pump (blower) serves to compensate for pressure losses within the system and provide the required air movement within the system. Makeup gases are injected into the flow stream to maintain the required atmospheric composition. A portion of the flow is diverted through a dehumidifying heat exchanger, where the condensate is removed from the system. Two variable flow area orifices are present one each in the flow path through the dehumidifier and in the parallel bypass. The orifice flow areas are variable in order to regulate the mass flow ratio of the paths. The air in the two flow paths is mixed and flows through a section of duct work. A portion of the flow is diverted through either a heater or a cooling heat exchanger. Three variable flow area orifices are present one each in the flow path through the cooling heat exchanger, the heater and the parallel bypass. The orifice flow areas are variable in order to regulate the mass flow ratio of the paths. Since meeting performance specification is considered paramount to economic constraints in this design concept, two heat independently controlled heat exchangers are utilized in humidity control and temperature regulation. The flows are mixed and flow through the duct work into the chamber inlet.

Control Focus for the CGRC.

The initial focus of control for the CGRC is in developing the strategy for variation of the available inputs (blower torque, valve apertures, etc.) in order to meet the chamber environmental tolerance requirements. The performance goals contain stringent tolerances on the acceptable region of the state space. The nature of the performance goals for the CGRC

and the degree of closure of the system suggest that it is sufficiently different from other closed environmental chambers [20] that a control design based upon a mathematical model of the system is warranted. Some of the system components, particularly the biological, are inherently variable [21]. Their functional responses to environmental conditions are nonlinear and exhibit a considerable degree of uncertainty. These goals and characteristics suggest robustness as a control objective and a continuous time, state space mathematical modeling approach to the development of the control strategy.

Modeling Procedure.

The primary step in the modeling procedure involves the development of a symbolic model of the system. The symbolic model for the shoot zone of the CGRC represents the thermodynamics and fluid mechanics processes which are assumed to be significant in governing the dynamics of the system. Initially, attention has been focused on dynamics which occur, it is assumed, rapidly in comparison to plant growth. The following processes are accounted for in the symbolic model: mass and energy storage, fluid inertance, pipe friction, flow splitting and merging, duct expansions and contractions, flows through orifices and porous media, gas injection and removal, mechanical energy storage due to rotational inertia in the blower, isentropic compression in the blower, molecular diffusion between the chamber air and storages internal to the plant canopy, water transport within the plant, convective and radiative heat transfer, absorption of photosynthetically active radiation, and binding (release) of energy into (from) biochemical form.

In the following step, the symbols representing the constitutive relationships describing these processes are linked in a structure which illustrates the manner in which the processes interact. The mathematical expressions which describe the physical laws governing these constitutive relationships are combined with the equations which describe

the structure of process interactions.

The details of a symbolic model which has been developed for the shoot zone of the CGRC are contained in [20]. The assumptions used in deriving the primary equations are developed in detail. Sample process equations and structural equations of the molar component and energy component of the symbolic model are also illustrated. The equations which are derived from the symbolic model relate the partial pressures of each atmospheric component, total pressures, mass or molar flow rates (hence velocities, transpiration rates, etc.), air temperatures, plant canopy temperature, etc. for the plant growth chamber and control system components. An illustration of some of the equations describing the thermodynamics and fluid dynamics of the shoot zone of the plant growth chamber of the CGRC which result from this approach appears in Appendix A.

Future Work.

The equations describing the dynamics of the total system, in state variable form, remain to be fully developed. These equations may be used to analyze system properties previously discussed, e.g. (1) location of equilibrium points, (2) stability at equilibrium points, (3) stability robustness at stable equilibrium points, (4) controllability, (5) observability, (6) system variable coupling, etc. The state variable form of the equations, including system uncertainties and disturbances, may then be used to seek robust control algorithms as required. The control system design can then be tested utilizing scenario studies on a simulation model. Should the system configuration as currently proposed fail to meet the required performance, a redesign can be made by reformulating the mathematical model. Additional processes, such as those affecting crop shoot-root interactions, may be added in order to model the behavior of the system in response to root zone environment disturbances and control inputs. Processes which affect longer term phenomena, such as biomass production, may be added in order to model system behavior over

a growth cycle. As the development of the physical system progresses, the model can be modified as necessary to reflect the properties of the actual hardware as determined by system testing.

CONCLUDING REMARKS

Several issues in modeling and control have been discussed as they relate to problems in the analysis and synthesis of CELSS systems. These issues have been couched within a systems control framework in order to demonstrate how they might be addressed effectively utilizing the techniques of that discipline. A perspective has been presented of the Crop Growth Research Chamber (CGRC) as one version of the Plant Growth Units subsystem, one of the hierarchical levels of control to be addressed in the overall CELSS design. The initial steps to CGRC design which have been taken from a systems control theoretic perspective have been presented and an example of the equations which describe the thermal and fluid dynamics of the shoot zone of the plant growth chamber of the CGRC has been illustrated. Suggestions for future efforts to be pursued using that approach have been outlined.

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APPENDIX A

Example Dynamic Equations - Shoot Zone

- Energy

Chamber air

$$\begin{aligned} \dot{T}_c = & (c_{vco_2} M_{co_2} N_{co_2} c + c_{vo_2} M_{o_2} N_{o_2} c + c_{vn_2} M_{n_2} N_{n_2} c \\ & + c_{vh_2o} M_{h_2o} N_{h_2o} c)^{-1} \\ & \{ + q_{conv_{ij}} + q_{conv_s} + \sum_j q_{conv_{sj}} + q_{conv_{cp}} + q_{arad_c} [H_2O, CO_2] \\ & + T_i (c_{pco_2} M_{co_2} n_{co_2} i + c_{po_2} M_{o_2} n_{o_2} i + c_{pn_2} M_{n_2} n_{n_2} i \\ & + c_{ph_2o} M_{h_2o} n_{h_2o} i) \\ & - T_c (c_{pco_2} M_{co_2} n_{co_2} o + c_{po_2} M_{o_2} n_{o_2} o + c_{pn_2} M_{n_2} n_{n_2} o \\ & + c_{ph_2o} M_{h_2o} n_{h_2o} o) \\ & - T_c (c_{pco_2} M_{co_2} n_{co_2} p, p) \\ & + T_{cp} (c_{po_2} M_{o_2} n_{o_2} p, p + c_{ph_2o} M_{h_2o} n_{h_2o} p, tr + c_{pco_2} M_{co_2} n_{co_2} p, r) \\ & + \frac{1}{2} u_i^2 (M_{co_2} n_{co_2} i + M_{o_2} n_{o_2} i + M_{n_2} n_{n_2} i + M_{h_2o} n_{h_2o} i) \\ & - \frac{1}{2} u_o^2 (M_{co_2} n_{co_2} o + M_{o_2} n_{o_2} o + M_{n_2} n_{n_2} o + M_{h_2o} n_{h_2o} o) - P_c \dot{V}_c \} \end{aligned}$$

Canopy

$$\begin{aligned} \dot{T}_{cp} = & \{ q_{arad_{cp}} [PAR] + q_{arad_{cp}} - q_{erad_{cp}} - q_{conv_{cp}} - q_{p,p} \\ & + q_{p,r} - q_{p,tr} \} / C_{T_{cp}} \end{aligned}$$

Growing surface

$$\dot{T}_s = \{ q_{arad_s} - q_{erad_s} - q_{conv_s} + q_{rz} \} / C_{T_s}$$

• Continuity

$$\dot{N}_{n_2 c} = n_{n_2 i} - n_{n_2 o}$$

$$\dot{N}_{co_2 c} = n_{co_2 i} - n_{co_2 o} + n_{co_2 p,r} - n_{co_2 p,p}$$

$$\dot{N}_{o_2 c} = n_{o_2 i} - n_{o_2 o} - n_{o_2 p,r} + n_{o_2 p,p}$$

$$\dot{N}_{h_2o c} = n_{h_2o i} - n_{h_2o o} + n_{h_2o p,r} + n_{h_2o p,p} + n_{h_2o p,tr}$$

Example Functions - Shoot Zone

$$q_{arad_{cp}} = \alpha A_{cp} \sum_j F_{cp,j} q_{erad_j}$$

$$q_{erad_{cp}} = \epsilon \sigma A_{cp} (T_{cp} + 273)^4$$

$$q_{conv_{cp}} = h_{conv_{cp}} A_{cp} (T_{cp} - T_c)$$

$$q_{p,r} = \lambda [T_{cp}] A_{cp} E$$

$$A_{cp} E = n_{h_2o p, tr} M_{h_2o} = A_{cp} \frac{\rho_a (\omega_{cp} - \omega_c)}{r_l^{h_2o} + r_b^{h_2o}}$$

$$r_b^{h_2o} = r_b^{h_2o} [W_l, D_l, u_c]$$

$$r_l^{h_2o} = r_l^{h_2o} [T_{cp}, C_{co_2 cp}, RH_c, \Psi_{cp}]$$

Definitions

T	temperature - °C
N	moles
n	molar flow rate - mol/sec
M	molecular weight
u	velocity of the air mass - m/sec
q	heat/energy transfer rate - watts
c_p	specific heat (constant pressure) - watt sec/gm K
c_v	specific heat (constant volume) - watt sec/gm K
C_T	heat capacitance - watt sec/K
P	static pressure - Pa
V	volume - m ³
PAR	photosynthetically active radiation
R	ideal gas constant
ϵ	emissivity
α	absorptivity
σ	Stephan-Boltzmann constant
A	area - m ²
$F_{i,j}$	radiation shape factor between surfaces i and j
h	heat transfer coefficient - watts/m ² K
λ	latent heat of vaporization - watt sec/gm
E	transpiration rate per unit area - gm/m ² sec
RH	relative humidity
r	diffusion resistance - sec/m
C	concentration - grams/m ³
Ψ	water potential - Pa
W	effective dimension across direction of air flow - m
D	effective dimension in direction of air flow - m
ω	humidity ratio
ρ	density - gm m ⁻³
.	derivative with respect to time - sec ⁻¹

Subscripts

c	chamber atmosphere
cp	plant canopy
s	growing surface
sj	surface j
lf	light filter surface
co ₂	carbon dioxide
o ₂	oxygen
n ₂	nitrogen
h ₂ o	water vapor
i	incoming air mass
o	outgoing air mass
p,r	dark respiration of the plant canopy
p,p	photosynthesis of the plant canopy
p,tr	transpiration of the plant canopy
erad	emitted radiative
arad	absorbed radiative
conv	convective
rz	root zone
l	effective leaf
b	boundary layer
a	dry air

TABLE 1. Design range and tolerances set for the Crop Growth Research Chamber shoot zone and root zone environmental variables.

Shoot Zone

Air temperature	5-40° C ±1° C
Relative humidity	35-90% ±2% of set point
Carbon dioxide concentration	25-5000 ppm ±0.2% of set point
Oxygen concentration	5-25% ±5% of set point
Nitrogen concentration	75-95% ±5% of set point
Gage pressure	0.5" H ₂ O ±0.25"
Air velocity	0.5 m sec ⁻¹ ± *
Photosynthetic photon flux	0-3000 μmoles m ⁻² s ⁻¹ ± 10 μmoles m ⁻² s ⁻¹
Surface temperatures	Air temperature + 2° C

Root Zone

Solution temperature	5-40° C ±1° C air temperature + 2° C
pH	4.0 - 8.0 ±0.2
DO	> 80% saturation
Nutrient concentration	0 - 500 mmol ± *

* tolerance not determined

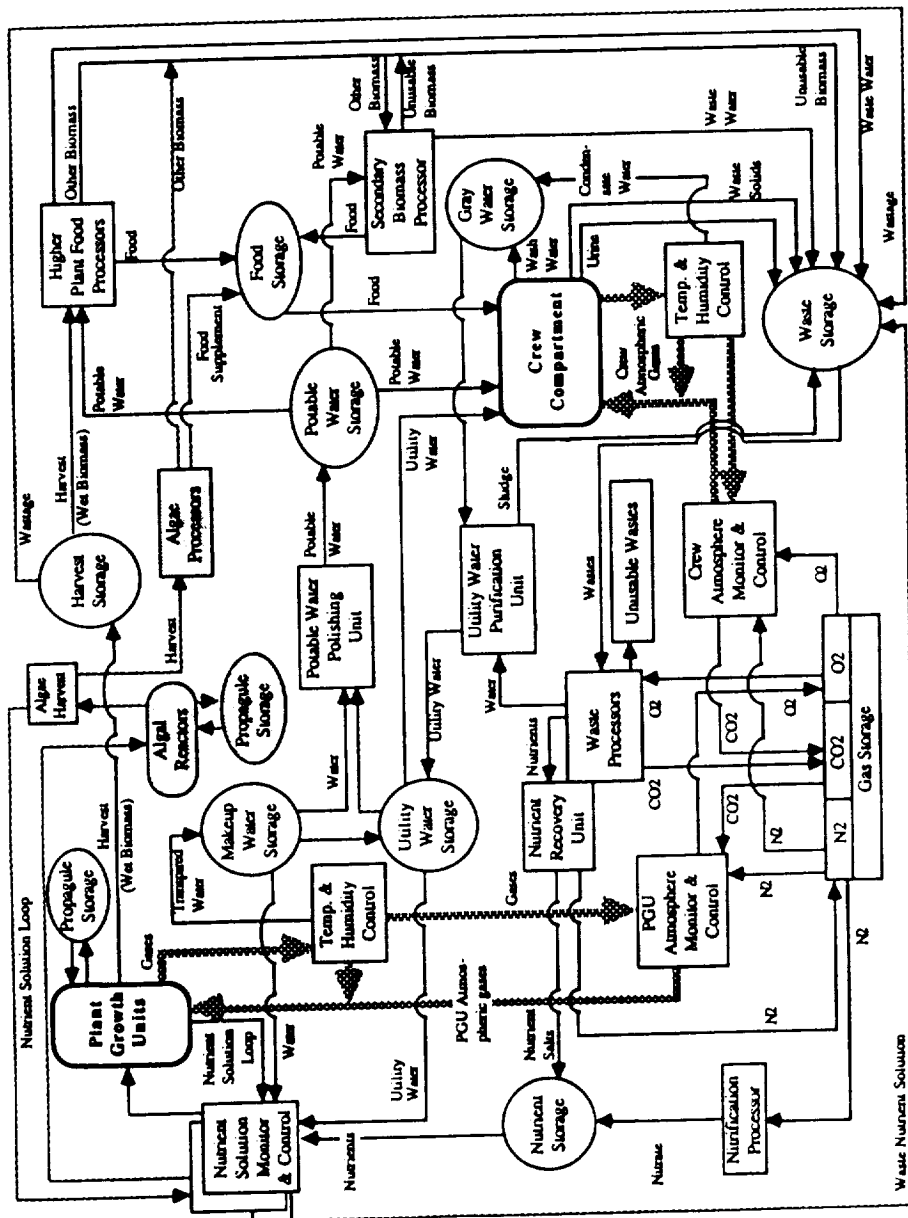


Fig.1. CELSS initial reference configuration. (From [1].)

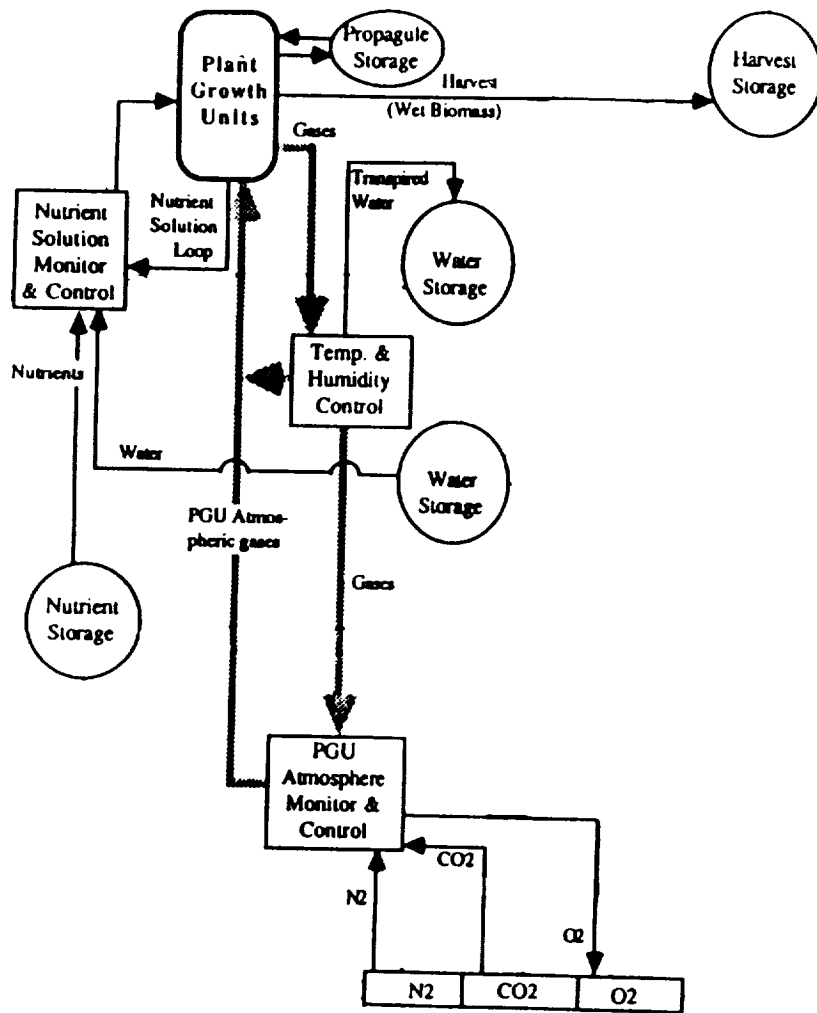


Fig.2. Crop Growth Research Chamber functional analogy to the CELSS Plant Growth Units subsystem.

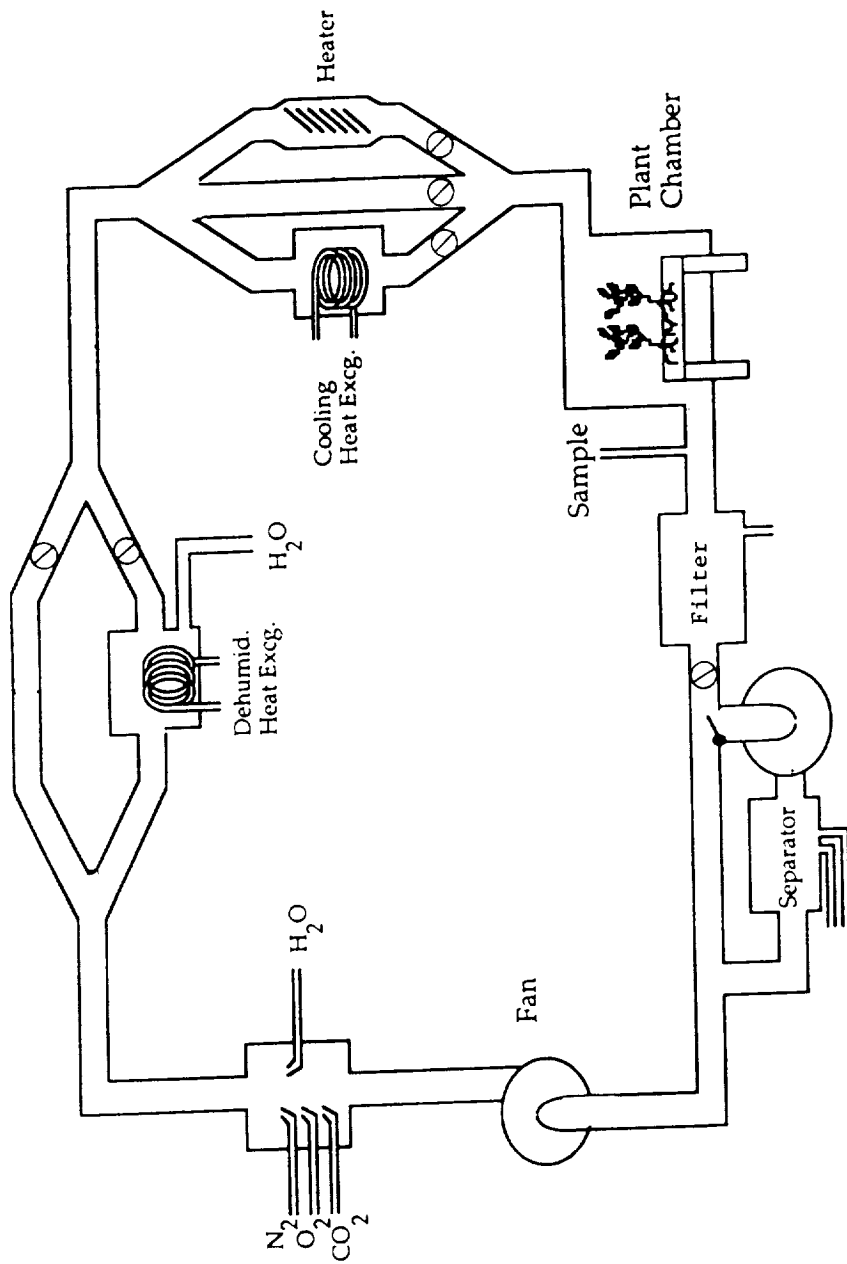


Fig.3. Schematic model of the Crop Growth Research Chamber.

TRANSPIRATION DURING LIFE CYCLE
IN CONTROLLED WHEAT GROWTH

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ABSTRACT

We use a previously-developed model of wheat growth, which was designed for convenient incorporation into system-level models of advanced space life support systems. We apply the model to data from an experiment that grew wheat under controlled conditions and measured fresh biomass and cumulated transpiration as a function of time. We examine the adequacy of modeling the transpiration as proportional to the inedible biomass and an age factor, which varies during the life cycle. Results indicate that during the main phase of vegetative growth in the first half of the life cycle, the rate of transpiration per unit mass of inedible biomass is more than double the rate during the phase of grain development and maturation during latter half of the life cycle.

THE GROWTH MODEL

We developed a model for the growth of wheat /1/, which proved useful for coupling the wheat with other components in a system-level model of a Controlled Ecological Life Support System (CELSS). The rationale for the model's form is that growth curves of most crops prominently show the S-shaped or sigmoidal curve typical of biological systems. The solution to the logistic differential equation imitates this S-shape of exponential growth followed by a leveling-off. In the logistic equation, $dM/dt = rM(1-M/K)$, where M is the biomass and t is time, there are two parameters: r and K . The r is the growth rate for the purely exponential part of the system. K , the carrying-capacity in an ecological system, in this case is the maximum biomass reached by the crop. The logistic equation thus contains some biologically meaningful parameters.

While the logistic equation can be applied directly to the growth of the inedible biomass of a crop, the equation for the edible crop parts is here somewhat differently structured (see also /2,3/). Like the inedible cells, the edible cells reproduce and the total edible growth must be proportional to the edible mass (M_{ed}). However, the edible parts do not produce their growing mass through photosynthesis, but rather receive photosynthetic products from the inedible parts (in particular, the leaves); therefore, the inedible biomass (M_{ined}) should also appear in the edible equation. Furthermore, since the edible growth occurs substantially after the beginning of the inedible growth (about halfway through the life cycle for wheat), a time that initiates the growth of the edible mass (t^*) is incorporated into the edible equation. Before t^* the edible biomass is assumed equal to zero, and its growth is initiated at t^* with a minimum edible mass (E_{min}). The full set of equations is (see also /2,3/):

$$\frac{dM_{ined}}{dt} = r_{ined} M_{ined} \left(1 - \frac{M_{ined}}{K_{ined}} \right) \tag{1}$$

$$t < t^* : \frac{dM_{ed}}{dt} = 0 \tag{2a}$$

$$t > t^* : \frac{dM_{ed}}{dt} = r_{ed} M_{ined} \left(\frac{E_{min} + M_{ed}}{K_{ed}} \right) \left(1 - \frac{M_{ed}}{K_{ed}} \right) \tag{2b}$$

The parameters t and t^* are in units of time, r_{ined} and r_{ed} are in units of time⁻¹, and all other parameters (M_{ed} , M_{ined} , K_{ed} , K_{ined} , E_{min}) are in identical units of either dry mass or dry mass per unit area. The system of equations (1a-c) above was used for wheat, soybean, and potato /2/. The total fresh biomass (B) is the sum of the fresh edible and inedible masses, expressed using their respective ratios (w_i 's) of their wet (fresh) mass to dry mass:

$$B = w_{ed} M_{ed} + w_{ined} M_{ined} \tag{3}$$

We compare the model to data provided by S. Schwartzkopf /4/. He grew wheat at the NASA Ames Research Center under controlled conditions of temperature, humidity, and atmospheric CO₂ (1200 ppm). Since we are not concerned here with how growth is affected by changes in these variables (except for humidity, see below), or other parameters (such as planting density), this data has been normalized to the total fresh biomass at day 60 (B_{60}), which in his experiment was the maximum total fresh biomass reached during the seed maturation. Figure 1a plots this normalized value of B/B_{60} as a function of time for the wheat data.

Equations (1, 2a-b, and 3) are run with $w_{ed} = 1.13$ (gm fresh per gm dry) /5/ and $w_{ined} = 5.7$ (gm fresh per gm dry) /6/. Other parameters used here are $K_{ed} = 2500$, $K_{ined} = 3700$, $E_{min} = 80$, initial $M_{ined} = 10$. In previous models these units have been gm dry mass m^{-2} , but here the units may be considered arbitrary since to facilitate comparison to Schwartzkopf's data, the model's output is normalized as a ratio between total fresh biomass and the total fresh biomass at day 60. This ratio, B/B_{60} , is plotted in figure 1a. Note also that the harvest index, defined as the fraction of the edible dry biomass—here approximately $2500/(2500+3700)$ —is consistent with the value of 0.4 from data /5/. The only major unknowns that can influence the shape of the growth curve significantly are the growth rates; these are adjusted to produce a reasonably accurate fit to data. The model curve shown in figure 1a uses $r_{ed} = r_{ined} = 0.2 \text{ day}^{-1}$.

TRANSPIRATION FORMULATION

Transpiration will probably account for about half the energy balance in the plant growth system of a CELSS. By definition, the total transpiration rate (Γ) is proportional to the transpiration rate per unit of inedible biomass (γ) and to the total inedible biomass. Therefore

$$\Gamma = \gamma M_{ined} \quad (4)$$

Following general reasoning such as that given in Gates /7/, γ is a function of the difference between the partial pressures of water vapor in the leaf ($P_{H_2O,leaf}$) and atmosphere ($P_{H_2O,air}$) and a function of the stomatal resistance (f_s), which itself a complex function of various environmental factors including light, temperature, and CO₂.

$$\gamma = \gamma^* f_h f_s f_a \quad (5)$$

Here we have written γ as a product of a humidity factor (f_h is a function of $P_{H_2O,leaf} - P_{H_2O,air}$), a stomatal resistance factor (f_s), a unit normalizing constant γ^* , and an age factor (f_a), which accounts for changes in the plant's transpiration rate per unit inedible biomass during its life cycle even when all environmental factors (f_h, f_s) are constant. A $\gamma = \text{constant} = 2.4 \text{ gm H}_2\text{O per gm dry inedible biomass per day}$ (this gives a rough average of typical wheat under controlled environments /8/) was used by Rummel and Volk /1/, but could not be tested against data during the plant's life cycle at that time. S. Schwartzkopf has been able to take detailed transpiration data from wheat /4/. To facilitate comparison between model and data the cumulated transpired water at time t ($\int_0^t \Gamma dt$) is normalized to the cumulated transpired water at day 60 ($\int_0^{60} \Gamma dt$). This ratio— $\int_0^t \Gamma dt / (\int_0^{60} \Gamma dt)^{-1}$ —is plotted as a function of time in figure 1b. It is also useful to consider the instantaneous transpiration rate Γ (here calculated from the data on cumulated water for any point using the previous and subsequent points), also normalized to the cumulated transpired water at day 60. This ratio— $\Gamma / (\int_0^{60} \Gamma dt)^{-1}$ —has the units of day^{-1} , and should be read as the fraction of the total transpired water during the life cycle transpired during a given day; this ratio is plotted in figure 1c.

$$\text{Normalized cumulated transpiration} = \frac{\int_0^t \Gamma dt}{\int_0^{60} \Gamma dt} \quad (6a)$$

$$\text{Normalized transpiration rate} = \frac{\Gamma}{\int_0^{60} \Gamma dt} \quad (6b)$$

Schwartzkopf's humidity controls kept the relative humidity (rh) at 0.35 at the beginning of the experiment, but only maintained rh near 0.45 at the end /4/; the change was gradual and approximately linear. Assuming leaf rh = 1.0 and air rh varied from 0.35 to 0.45, the humidity factor f_h , expressed in terms of rh and non-dimensionalized to the final condition, is taken here to have varied linearly from 1.18 at the beginning of the experiment ($(1 - 0.35)/(1 - 0.45)$) to 1.00 at the end. Furthermore, we set $\gamma^* = 2.4 \text{ gm H}_2\text{O per gm dry inedible biomass per day}$, and since environmental conditions were approximately constant, $f_s = 1.0$ for the duration of the experiment. We test several cases of the age factor f_a .

The first case has $f_a = 1.0 = \text{constant}$ (or, $\alpha = 0$, see below). Output from the model for cumulated transpiration and transpiration rate is normalized to the cumulated transpiration at day 60 using equations 6a-b, like the experimental data. Note this normalization effectively eliminates dependence of the results on γ^* . Results with f_a

= constant are plotted against data in figures 1b and 1c. Although the general shape of the cumulated transpiration data is matched by the model (see figure 1b), the empirical value is significantly underestimated during the middle one-third of the life cycle. The underestimation is even clearer in the rate results, shown in figure 1c. For the first one-half of the life cycle, the transpiration rate in the model is much too low.

A second case explores the possibility that the transpiration rate per unit inedible biomass is substantially higher when the plant is younger than when mature. A convenient way of parameterizing this process that takes into account the apparent steadiness of the transpiration rate during the second one-half of the life cycle, when the inedible biomass itself is relatively maximum and constant, is to write f_a as a function of M_{ined} :

$$f_a = 1 + \alpha \left(1 - \frac{M_{ined}}{K_{ined}} \right) \quad (7)$$

Here the term α is an enhancement of transpiration rate per unit biomass when the plant is young. Note $f_a = 1.0$ when $M_{ined} = K_{ined}$. The model output for cumulated transpiration and transpiration rate for this second model—which uses $\alpha = 1$, rather than $\alpha = 0$ —is shown in figures 1b and 1c. Overall better fit to the data is apparent, in particular, improvement in the transpiration rate during the first one-half of the life cycle. However, also clear is that even higher rates (in other words, higher α 's) are needed in the first one-third of the life cycle. Without fitting the data even further, we have nevertheless demonstrated the possibility of representing the transpiration to varying degrees of accuracy with formulations that have physical meaning.

CONCLUSION

Models such as these are the best way of examining the "interactions of assumptions" /9/. Considering the overall results, the logistic growth equations combined with the assumption that the rate of transpiration per unit inedible mass decreases during the life cycle of the crop will generally reproduce the data and will probably be adequate in highly aggregated models of a CELSS, for example, the BLSS model /1/. A physiological interpretation of this transpiration formulation and comparison of these findings to the transpiration formulations in more detailed models of non-hydroponic wheat /10,11/ (which, however, are presently not applicable to a CELSS model) will help the crop model shown here develop more complex dynamics and allow better preliminary designs of space agricultural systems.

ACKNOWLEDGEMENTS

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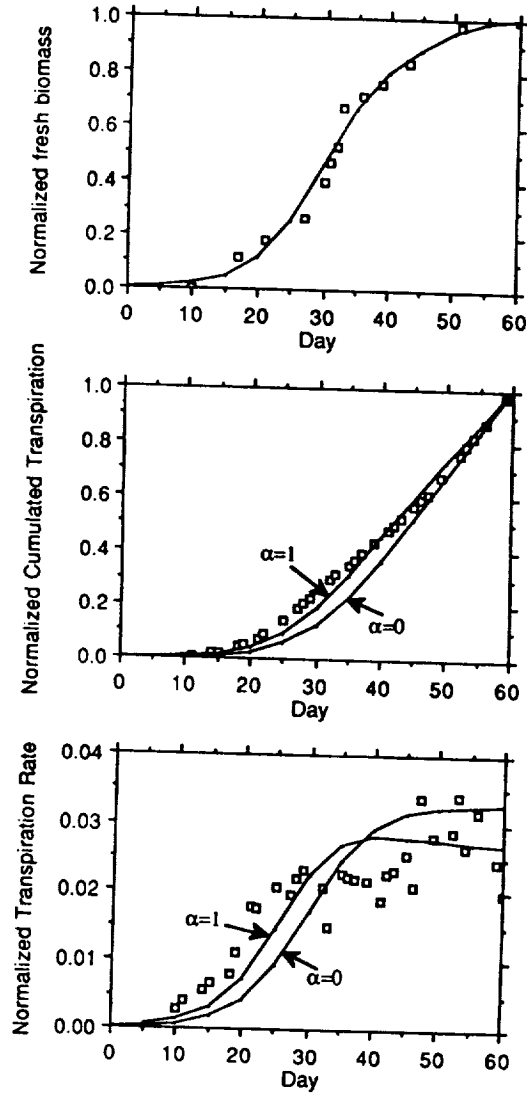


Fig. 1. Model results (lines) and empirical data (points) of (a, top) fresh biomass, (b, middle) cumulated transpiration, and (c, bottom) transpiration rate, all normalized as described in the text. Results in (b) and (c) are shown for two values of α ; α controls the time-dependence of the age factor f_a in the transpiration rate. When $\alpha = 0$, f_a is constant; when $\alpha = 1$, f_a decreases non-linearly from about 2 in early growth to about 1 in late growth (see text). Results for biomass in (a) do not vary as a function of α . See equations 6a-b for definition of the normalized cumulated transpiration and normalized transpiration rate.

PREPARATORY SPACE EXPERIMENTS FOR DEVELOPMENT OF A CELSS

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ABSTRACT

The purpose of a CELSS plant-production facility is to achieve maximum yield and quality in a minimum area (or volume) and with minimum inputs of mass and energy. Research with wheat and other crops has shown that maximum theoretical yields (determined by available light) can be approached if the best cultivars are grown at optimal day and night temperatures, humidity, wind velocity, photoperiod, CO₂, mineral nutrients, and plant density. Yield is nearly a straight-line function of irradiance at least up to sunlight-equivalent levels, but photosynthetic efficiency decreases with increasing irradiance. Will these generalizations hold in the worst-case situation of a microgravity CELSS? Or in the reduced lunar or Martian gravity?

Further space experimentation will be required to find out. So far, the few experiments with plants in space have not had environments truly suitable for CELSS studies. Nevertheless, results of this work suggest that plant growth could be adversely affected by microgravity. The goal of CELSS studies will be to examine effects of microgravity on yield and quality of plant products and on the interactions between irradiance and crop area. Measuring yield and quality of crops as a function of irradiance in microgravity is virtually unique to the CELSS program, as is an emphasis on canopies rather than individual plants. The first step for space experiments is to develop a relatively stress-free environment for plant growth, something that has so far never been achieved. High light levels are essential, and there must be time enough to complete a significant portion of a life cycle. Optimal atmosphere and nutrients must be provided. Such responses as germination, orientation of roots and shoots, photosynthesis and respiration, floral initiation and development, and seed maturation and viability will be studied.

THE PURPOSE AND CHALLENGE OF A CELSS

In developing a CELSS plant-production facility, and thus in CELSS research with plants, the challenge is to obtain maximum crop yield per unit area (or volume) with minimum inputs of mass and energy. It is imperative to calculate the efficiency of the system in terms of food energy produced per unit input of light

energy. The maximum yields achievable per unit area (or volume) and the light energy required to produce them must be determined. With this knowledge, engineers can design future CELSS systems with appropriate light sources.

The mass of the system is a problem for the engineers. Energy required to operate the system beyond the light energy used to irradiate the plants is also largely an engineering matter. Plant researchers allow themselves to use equipment of any size and energy requirement, knowing that clever engineers should ultimately be able to optimize the mass and functional-energy needs.

WHAT CELSS RESEARCH HAS TAUGHT US SO FAR

The plant scientists reporting at this conference have been studying these things with NASA support for nearly a decade. Much has been learned. For one thing, we can calculate the potential crop yields on the basis of photosynthetic efficiencies, and then we can compare the yields that have been achieved with the theoretical ones (1). The theoretical efficiencies depend upon a number of factors. We have used the model discussed by Dr. Bugbee at this meeting. It involves the amount of light absorbed by the plants, the quantum efficiency of the photosynthetic process, the respiration efficiency (percentage of the photosynthetic products that are used up in maintenance respiration necessary for growth and to keep the plant alive -- a somewhat variable factor that makes the final calculation of efficiency also somewhat variable), and the harvest index (edible biomass as a percentage of total biomass -- also a variable figure that depends upon species, cultural practices, and what an

astronaut is willing to eat). Ignoring the harvest index for the moment, and assuming that maintenance respiration might be somewhat less in controlled and optimized environments than it is in the field, we arrive at a figure for photosynthetic efficiency of about 15 percent. If this is a valid figure, the food energy in the biomass will never exceed 15 percent of the light energy delivered to the plants.

In our research with wheat (1), we have found that the theoretical efficiency can be approached if the environment is optimized. The highest efficiency we have been able to measure is about 10 percent, but that is measured over the complete life cycle of the wheat plants. Much light energy is wasted during approximately the first 20 days while the plants are forming a canopy; more light energy is wasted during the final days after most of the leaves have senesced but before the grains are mature. This high efficiency is achieved when day and night temperatures are optimized (in Fig. 1, 20°C day and 15°C night), carbon dioxide is enriched to an optimal level (about 1,000 to 1,200 $\mu\text{mol mol}^{-1}$), irradiance is at an optimum level and spectral balance for maximum efficiency (about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, mostly from high pressure sodium lamps), mineral nutrients are optimally supplied in a well-balanced nutrient solution, the daily period of irradiation is optimized (continuous light in our recent experiments), and plant densities are ideal (dense enough to rapidly form a canopy but not dense enough to reduce yields by competition; 2000 or more plants m^{-2}). Humidity and wind velocity also need to be optimized but seem to be somewhat less important than the factors just enumerated.

Our model has assumed that yield is a function of irradiance when everything else has been optimized, and results have borne that out. In addition, we discover that efficiency as well as yield is a strong function of irradiance. This is illustrated in Figure 1 in which total biomass, seed biomass, and efficiency are plotted as functions of total daily irradiance. The highest yields were produced by an instantaneous irradiance equivalent to noon-day summer sunlight ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) given continuously during a 20-h light period (4-h dark). This is about 2.5 times as much radiant energy as plants could receive anywhere on Earth. Within statistical error, and beginning above the light compensation level and at our lowest irradiance, biomass production increased in a nearly linear fashion with increasing irradiance. There was no sign of saturation. Efficiency, on the other hand, decreased linearly with increasing irradiance from about 10 percent at the lowest light levels to about 7 percent at the highest light level.

IMPORTANT CELSS TRADE OFFS

If these results, obtained with wheat, are valid for other species as well, they suggest some important trade offs in the design of a future CELSS. First, it is obvious that more light means a smaller farm. At our highest light level, we harvested about $60 \text{ g m}^{-2} \text{ d}^{-1}$ of edible wheat. Assuming that a human can function with the energy provided in 780 grams of wheat per day (or its equivalent in other foods), this much food energy ($11,700 \text{ kJ} = 2800 \text{ kcal}$) could be provided in a CELSS farm of only 13 m^2 person⁻¹. This assumes that the crop can always be produced at

maximum efficiency. An actual CELSS will incorporate a safety factor and will be designed for crops besides wheat. With a safety factor of about 4, with which I might be almost comfortable, the CELSS farm would be about 50 m² person⁻¹.

Second, less irradiance means higher photosynthetic efficiency and therefore a smaller power input. This is illustrated in Figure 2. (The power input is based on estimates of light-output efficiencies for sodium vapor lamps housed in highly efficient reflectors.) Although farm size per person decreases with increasing light, the power per person increases by about 50 percent. Thus, if the CELSS farm is located where size is less important than power (on the lunar surface, perhaps?), the farm can be larger and the power supply somewhat smaller. If area or volume are critical (as in an orbiting space station or a space craft on the way to Mars), it will be important to have a large power supply so the farm can be proportionately smaller. Much electrical power is required to produce the light needed to grow plants. The entire contemplated power supply of space station Freedom would be required, for example, if its astronaut occupants were to be fed exclusively from a CELSS farm. Thus, it is clear that large sources of power will have to be developed if CELSS farms are to be used in the future, or ways will have to be devised to utilize the relatively inexpensive light energy from the sun. (The cost of solar energy will be reckoned as the cost of the equipment required to utilize it.) Of course solar energy won't be available on the lunar surface during about 15 earth days of the approximately 29-day lunar day (except close to the lunar poles?).

SPECIAL CELSS PROBLEMS FOR SPACE EXPERIMENTS

Although our CELSS research has so far been conducted on the Earth's surface, a CELSS operated beyond the Earth's atmosphere in the foreseeable future will have to contend with microgravity (space station or spacecraft), lunar gravity, or Martian gravity. Can we achieve maximum crop yields in these gravity conditions? To find out we need to do space experiments. To study lunar or Martian levels of gravitational acceleration, we will either have to go to the moon or Mars or use a centrifuge in the space station. Thus CELSS research in space will probably be initiated with experiments carried out in microgravity. The discussion so far should make it obvious that experiments designed to study primarily CELSS problems will place much emphasis on yield, quality, and the interaction of irradiance and crop area. All steps in the life cycle of a crop plant could affect yield and quality:

1. Germination.
2. Orientation of roots and shoots.
3. Growth and differentiation of roots and shoots.
4. Photosynthesis and respiration.
5. Floral initiation and development.
6. Pollination and fertilization.
7. Seed maturation and viability.

All plant scientists interested in space biology would like to study these steps. Indeed, this could be done in the often discussed seed-to-seed experiment. If we knew that a plant could grow from seed to seed in microgravity, there would seem to be no obvious show stoppers in development of a CELSS for space exploration. But what if plants will grow from seed to seed in micro-

gravity but yield only 10 percent as much as they do on Earth? This would surely be an unexpected show stopper. Thus the process of photosynthesis and other developmental steps that lead to the harvested product must be the crucial topics of study in CELSS space experimentation.

WHAT CAN WE EXPECT?

Considering that it has been possible to do experiments with plants in space for over a quarter of a century, it is discouraging to realize how little has been done. This is especially true of the United States' space program in which well-conceived plant experiments can be counted on the fingers of one's hands. Furthermore, none of these experiments has utilized sufficient light to be of interest from the special standpoint of CELSS. Soviet scientists have carried out many more space experiments with plants, but their experiments have also left much to be desired from the standpoint of CELSS (although most of their plant experiments were justified from that very standpoint!). In an article published in the 1987 Annual Review of Plant Physiology (2), Thora W. Halstead and F. Ronald Dutcher summarize what is known about the response of plants to the space environment, particularly to microgravity. The following paragraphs are a brief summary of their summary.

1. **Germination.** Several species of seeds have been germinated in space. There were no problems with the seeds that were tested, and we do not expect problems with other species.

2. **Orientation of Roots and Shoots.** In microgravity plus darkness, roots and shoots both grow in the direction they assume when they emerge from the seed. This has been observed with

several species. Shoots of many species orient toward the light (phototropism). This is especially true for monocots, but some dicots (e.g., soybeans) have not oriented strongly toward the light in microgravity. Roots are not phototropic and have grown out of the soil in several experiments.

When weightlessness is simulated by rotating plants about a horizontal axis on a clinostat, the most obvious symptom is a downward bending of leaves, called epinasty. Thus it is surprising that the Soviet literature never mentions epinasty of dicots in space, and the point has seldom been discussed by American researchers. Nevertheless, the classic experiment in Biosatellite II with pepper plants showed epinasty in microgravity comparable to that observed on a clinostat, and some photographs of space-grown seedlings also show epinastic leaves.

3. Growth and Differentiation. Growth of some species was inhibited: pine, oat, mung bean. Yet hypocotyls of lettuce, garden cress, and Arabidopsis thaliana were longer in microgravity than those grown on a flight centrifuge. This is one example of several kinds of conflicting data from space experiments.

Maize root caps removed just before a flight did not regenerate in microgravity as they do on Earth (within 48 h). A few other effects on differentiation have also been reported.

Many cytological effects have been observed. In several cases, cell division was reduced or inhibited. Yet there were other cases where cell division did not appear to be affected. Damaged chromosomes were observed in many species but again, not always. There has been much discussion about whether these effects were caused by space radiation, microgravity, or an

interaction of the two. Equivalent radiation doses on Earth do not cause such effects, so it is likely that radiation, if it is responsible, is interacting with microgravity.

Abnormal nuclei, endoplasmic reticulum, ribosomes, mitochondria, plastids, dictyosomes, and cell walls have also been observed in space-grown plants. Again, however, these abnormalities have failed to appear in other plants grown in space.

4. **Photosynthesis and Respiration.** To the best of my knowledge, these processes have not been measured in space. (We hope to do so!) Nevertheless, a disintegration and destruction of grana along with a disorientation of the intergrana and a shrinkage of membranes comprising grana stacks has been observed in chloroplasts from pea and other species, as have a lack of starch and reduced chlorophyll. These observations lead to an expectation of decreased photosynthesis -- except that such effects have not appeared in all species and in all experiments.

5. **Floral Initiation and Development.** The Soviets, who are the only ones who have grown plants for relatively long periods in microgravity, reported that death often occurred at the flowering stage. This was true for wheat, peas, and several other species. Yet the Soviets were able to grow Arabidopsis thaliana from seed to seed. They observed some aborted ovules and a markedly reduced germination percentage of the seeds that had been produced in space. Seedlings that grew from seeds that did germinate were often abnormal although the next generation consisted of normal seedlings. Thus the Soviets have achieved the seed-to-seed experiment but not without encountering several problems and some failures in early attempts along the way.

6. Pollination and Fertilization. I know of no experiments designed to study these important phenomena, but the seed-to-seed experiment with Arabidopsis prove that pollination and fertilization can be achieved in microgravity. (In our pending flight experiments, we will look first at photosynthesis and respiration; then we hope to emphasize floral initiation and development as well as pollination, fertilization, seed maturation, and seed viability.)

7. Seed Maturation and Viability. Again, we have the Arabidopsis experiment to suggest that viable seeds can mature in microgravity.

Although much remains to be learned, it is clear that plants may respond to microgravity in many ways besides having their gravitropic responses upset. While germination seems to be insensitive to microgravity, growth may or may not be affected, and mitosis and cytokinesis appear to be quite sensitive to microgravity or to a combination of microgravity and slightly increased radiation. Chromosomal damage is especially prevalent. Differentiation is influenced in several ways, and polysaccharide metabolism including photosynthesis could be affected; this would be especially true if organelle membranes are sensitive as might be the case.

WHAT CAUSES THESE RESPONSES?

Having surveyed the many responses that have been observed in the relatively few experiments, and noting the often conflicting results, it becomes apparent that we must look for the causes of the discrepancies. Although the situation is complex, five immediate possibilities come to mind:

1. Microgravity.
2. Radiation.
3. The growth chamber environment.
4. Interactions of these.
5. The stresses of launch and landing.

Except for complications of launch and landing stresses, microgravity and radiation have been fairly constant in most of the experiments carried out with plants so far. The growth-chamber environment, on the other hand, has varied greatly, particularly in the Soviet experiments and to a somewhat lesser extent in the American studies. Thus we are entitled to be especially suspicious of chamber environments as possible causes for many of the effects that have been observed. And if chamber environments prove to be responsible, it will be possible to avoid some deleterious effects by providing suitable growth environments. Two aspects of the plant-growth environment in microgravity experiments might have influenced results.

First, environmental factors may not have been optimized for the most ideal plant growth. The United States' experiments have never had enough light to provide an adequate rate of photosynthesis, and these low light levels could lead to other effects besides reduced photosynthesis. Growth and differentiation are known to be highly sensitive to the light environment, both irradiance levels and spectral distribution, not to mention photoperiod. Although light might have been the most limiting factor in the experiments already carried out and thus the most important factor to be considered for future experiments, atmospheric conditions have often been far from ideal. For example,

it is likely that ethylene and perhaps other gasses built up in the plant growth unit used in American experiments. Furthermore, we have no assurance that nutrient or water conditions were as good as they should be for ideal plant growth.

Second, microgravity interacts with other factors of the environment. Because gravitational drainage does not occur through the plant substrate, it is difficult to provide ample water with sufficient root aeration. Furthermore, convection caused by temperature (density) differences does not occur in fluids in microgravity, so movement of both air and water must be by forced convection. All these problems must be solved before CELSS flight experimentation can be meaningful.

THE MOST CRITICAL PROBLEMS FOR CELSS FLIGHT EXPERIMENTS

The most critical problem so far has been opportunities for flight. How can we solve the problems if we never get to go? When we do get to go, we must have adequate growth facilities. We must find ways to provide adequate light! Furthermore, we must have adequate space to grow plants in at least limited canopies as they will surely be grown in a CELSS farm. Initial experiments might utilize individual plants, but somewhere fairly early in CELSS flight experimentation, canopies of plants must be used. In addition, we must have sufficient time for a significant portion of a growth cycle, and we must solve the problems of nutrient flow systems and of atmospheric control.

It should now be apparent that CELSS flight experimentation is more demanding than research in other fields of gravitational biology. Because it is absolutely essential to have adequate light, power sources must be found. These may have to depend on

nuclear reactors in spite of public aversion to them. I'm told that NASA is developing safe nuclear reactors for space experimentation. Perhaps space studies in CELSS plant production wont be completely feasible until such sources are available.

Probably the most serious challenge facing us at the moment is public relations. Because NASA administrators as well as the public were well aware of what could be achieved by telescopes beyond the Earth's atmosphere, the Hubble Telescope came into being. It seems critical for us to make NASA administrators and the public aware that long-term goals such as a lunar colony or a station on Mars cannot be reached without incorporating the CELSS concept. We need to get this message across with high visibility programs such as the Kennedy Space Center Breadboard Project. It is incumbent upon each of us to take every opportunity that is presented to tell our story to the public. Interviews by the Associated Press can certainly interrupt one's day, but without them a truly viable CELSS program, including space experimentation, may not develop within our lifetimes. Eventually, as its importance is realized, it will come into being, but if this is to happen soon we must become personally involved in the effort to make it happen.

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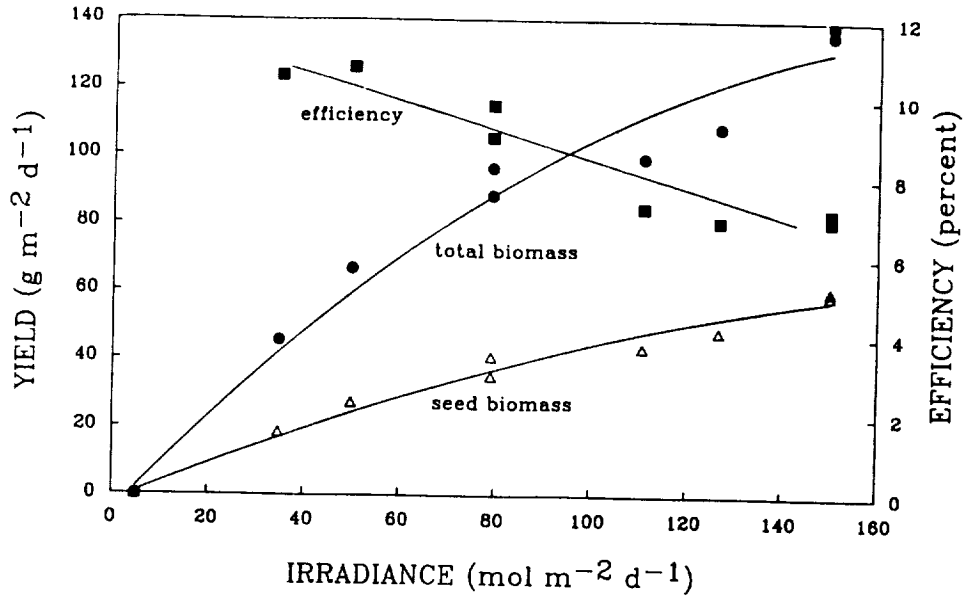


Figure 1. Yield and efficiency of wheat plants grown under different daily photosynthetic photon fluxes. Yields (biomass) were calculated by dividing final yields by the 79-day growth period of the crop. The highest irradiance is equivalent to noon, summer sunlight at the earth's surface, but provided for 20 hours each day (details in reference 1).

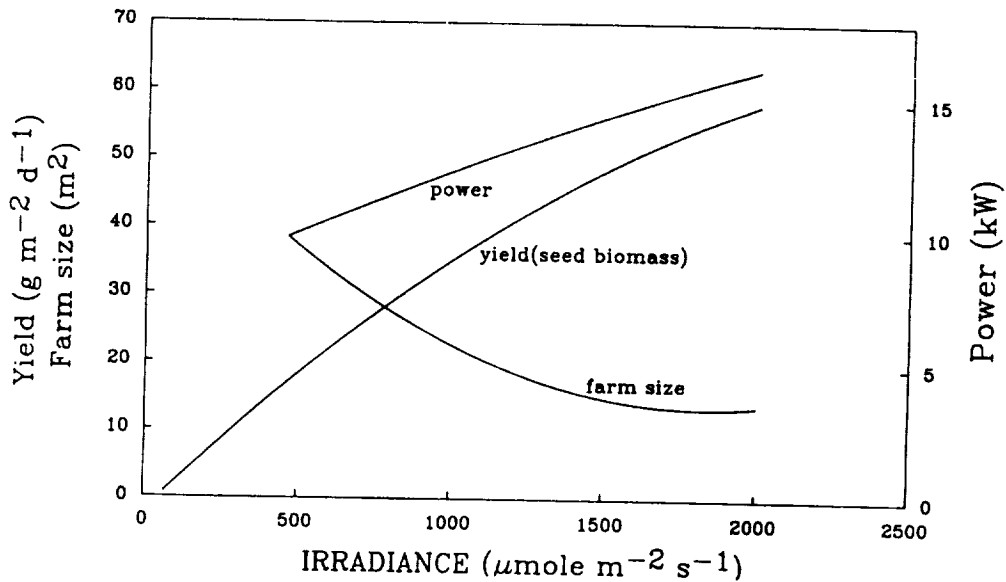


Figure 2. CELSS trade offs based on the experimental data presented in Figure 1 (but showing irradiance integrated for a second instead of a day). As irradiance increases, so does yield, allowing a smaller farm to support a given number of human beings. But as irradiance increases, photosynthetic efficiency decreases, so more light is needed to produce a given yield, and this requires more power.

FACTORS AFFECTING PLANT GROWTH IN MEMBRANE NUTRIENT DELIVERY

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ABSTRACT

The development of the tubular membrane plant growth unit for the delivery of water and nutrients to roots in microgravity has recently focused on measuring the effects of changes in physical variables controlling solution availability to the plants. Significant effects of the membrane pore size and the negative pressure used to contain the solution have been demonstrated. Generally, wheat (Triticum aestivum cv. Yecora Rojo) grew better in units with a larger pore size but equal negative pressure and in units with the same pore size but less negative pressure. Lettuce (Lactuca sativa cv. Waldmann's Green) also exhibited better plant growth at less negative pressure.

INTRODUCTION

A plant nutrient delivery system for microgravity is under development for the Controlled Ecological Life Support System (CELSS) Breadboard Project at the John F. Kennedy Space Center (KSC). This system utilizes a hydrophilic, porous tube to transfer solution (under a slight negative pressure) to plant roots which use capillary action to obtain water and nutrients through the pores (1, 2). Several different materials and configurations of the porous tube have been used to support plant growth and these trials indicated that the amount of negative pressure and the pore size of the material may have a significant effect on plant growth (3, 4). Two trials were conducted to test the effects of pressure and pore size on the growth of wheat

(Triticum aestivum cv. Yecora Rojo) and the effects of pressure on the growth of lettuce (Lactuca sativa cv. Waldmann's Green).

MATERIALS AND METHODS

The tubular membrane plant growth units used in the wheat trial and the porous tube plant growth units used in the lettuce trial were constructed as shown in Figures 1 and 2, respectively. The membrane used was a hydrophilic, acrylic (membrane) filter material and the porous tube a hydrophilic, polyethylene tube. A standpipe manifold system was located in a 1.8 by 2.4 m plant growth chamber. The manifold system utilized a centrifugal pump to maintain a constant solution level. A series of peristaltic pumps, one for each tube, was used to deliver nutrient solution from the plant growth units. The peristaltic pumps also exerted a slight negative pressure, preventing the solution from freely leaking from the porous tube. Negative pressure differential was monitored with a vacuum gauge and adjusted daily, if needed, using a valve located upstream to the plant growth units. Solution level in the reservoir was maintained by daily additions of fresh nutrient solution (modified one-quarter strength Hoagland's), and pH was controlled by the automatic addition of 1 M HNO_3 . The chamber was programmed to provide a 18-h light, 6-h dark photoperiod with corresponding 20 C and 18 C for the wheat and 23 C and 20 C for the lettuce. Relative humidity was set at 65 % with ambient CO_2 concentration and PPF at 300 $\mu\text{mole s}^{-1} \text{m}^{-2}$.

Triplicates of each treatment were operated and maintained among a set of 20 units which were randomly arranged. The pressure treatments for the wheat trial included -0.4 kPa, -1.5 kPa, and -3.0 kPa with a pore size of 0.2 μM . The pore size treatments for the wheat trial were 0.2 μM and 5.0 μM with a pressure of -0.4 kPa. The pressure treatments for the lettuce trial were -0.2 kPa, -0.3 kPa, and -1.2 kPa.

RESULTS AND DISCUSSION

The results of the negative pressure and pore size treatments in the wheat trial are presented as Tables 1 and 2, respectively. Significant differences were found among the negative pressures and pore size treatments in all but three of the harvest variables using Analysis of Variance (ANOVA). The results of the negative pressure treatments in the lettuce trial are presented as Table 3. Significant differences were found in all the variables measured among the treatments. In general, better plant growth occurred at less negative pressures and in the units with the larger pore size material. Recent measurements suggest that leaf photosynthetic gas exchange, transpiration and water potential in wheat are reduced at greater negative pressures, indicating a real-time effect on plant growth. These same measurements on lettuce have been unsuccessful due to the fragile nature and high latex content of lettuce leaves.

CONCLUSIONS

Significant effects of negative pressure on the growth of wheat and lettuce have been observed when these plants were grown in the tubular membrane/porous tube plant growth units. The measurements of leaf transpiration, water potential, and photosynthetic gas exchange rates should provide more insight into causal factors. Pore size was also found to affect wheat growth in the tubular membrane units. We suspect that both factors should exert direct effects on hydraulic conductivity, and consequently water and nutrient availability to the plants, and further tests of physical properties of these materials are underway. If water and nutrient availability are affected, negative pressure and pore size should affect plant growth independent of what configuration is used and may have greater or lesser effects dependent upon the species of plant grown. It is also believed that negative pressure and pore size interact and that adjustments in the suction may be used to overcome the effects of pore size and vice versa. The understanding of these and similar interactions between controlling variables is critical to the development of membrane nutrient delivery systems for crop production in a CELSS.

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Table 1. Data from the pressure treatments of the wheat trial. Significant differences ($\alpha \leq 0.05$) denoted by "S".

Harvest variable	Treatment 1 -0.4 kPa Mean/Std. Er.	Treatment 2 -1.6 kPa Mean/Std. Er.	Treatment 3 -2.4 kPa Mean/Std. Er.
Spikelet No.- Primary heads	16.67/0.13	15.78/0.23	14.08/0.61 S
Spikelet No.- Other heads	15.44/0.05	12.83/0.88	12.22/1.40
Seed No.- Primary heads	18.83/0.62	16.50/1.29	12.93/0.47 S
Seed No.- Other heads	7.67/0.58	3.87/0.64	4.40/1.80
Primary seed- gdw/plant	0.78/0.02	0.67/0.06	0.48/0.03 S
Other seed- gdw/plant	0.29/0.02	0.15/0.03	0.17/0.07
Seed-gdw/plant	1.07/0.04	0.82/0.04	0.66/0.09 S
Root-gdw/plant	0.34/0.03	0.21/0.02	0.19/0.02 S
Chaff-gdw/plant	0.34/0.01	0.29/0.03	0.25/0.02 S
Straw-gdw/plant	0.87/0.02	0.56/0.03	0.45/0.04 S
Total-gdw/plant	2.48/0.12	1.88/0.10	1.55/0.15 S
Primary heads- gfw/plant	1.93/0.07	1.50/0.12	1.01/0.09 S
Other heads- gfw/plant	0.79/0.05	0.38/0.05	0.09/0.03 S
Root-gfw/plant	5.63/0.18	3.53/0.28	2.50/0.06 S
Straw-gfw/plant	2.47/0.07	1.47/0.07	0.99/0.16 S
Total-gfw/plant	10.83/0.22	6.89/0.04	4.59/0.27 S

Table 2. Data from the pore size treatments of the wheat trial. Significant differences ($\alpha \leq 0.05$) are denoted by "S".

Harvest variable	Treatment 1 -0.4 kPa 0.2 micron Mean/Std. Er.	Treatment 4 -0.4 kPa 5.0 micron Mean/Std. Er.
Spikelet No.- Primary heads	16.67/0.13	17.40/0.07 S
Spikelet No.- Other heads	15.44/0.05	16.29/0.39
Seed No.- Primary heads	18.83/0.62	33.03/2.51 S
Seed No.- Other heads	7.67/0.58	41.37/2.81 S
Primary seed- gdw/plant	0.78/0.02	1.28/0.03 S
Other seed- gdw/plant	0.29/0.02	1.57/0.13 S
Seed-gdw/plant	1.07/0.04	2.85/0.10 S
Root-gdw/plant	0.34/0.03	0.33/0.02
Chaff-gdw/plant	0.34/0.01	0.74/0.05 S
Straw-gdw/plant	0.87/0.02	1.48/0.04 S
Total-gdw/plant	2.48/0.12	5.40/0.14 S
Primary heads- gfw/plant	1.93/0.07	2.97/0.15 S
Other heads- gfw/plant	0.79/0.05	3.89/0.38 S
Root-gfw/plant	5.63/0.18	5.53/0.29
Straw-gfw/plant	2.47/0.07	4.80/0.15 S
Total-gfw/plant	10.83/0.22	17.18/0.69 S

Table 3. Data from the pressure treatments of the lettuce trial. Significant differences ($\alpha \leq 0.05$) denoted by "S".

Harvest variable N=3 (mean/unit)	Treatment 1 -0.2 kPa Mean/Std. Er.	Treatment 2 -0.3 kPa Mean/Std. Er.	Treatment 3 -1.2 kPa Mean/Std. Er.
Head fresh weight (g)	5.09/0.79	1.74/0.52	0.72/0.25 S
Head dry weight (g)	0.62/0.05	0.28/0.04	0.09/0.03 S
Root dry weight (g)	0.17/0.03	0.10/0.01	0.04/0.01 S
Total dry weight (g)	0.79/0.07	0.37/0.05	0.13/0.04 S

Tubular membrane plant growth unit

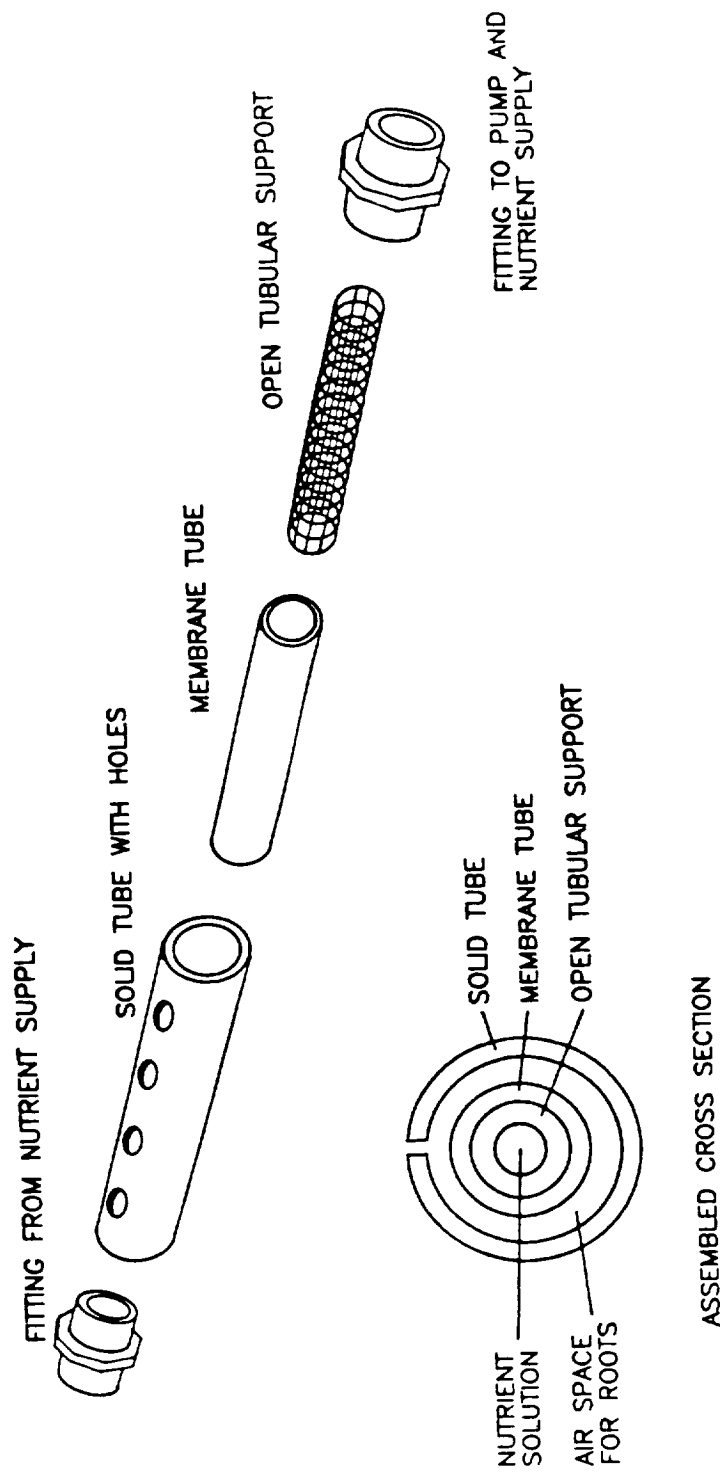


Figure 1. Schematic diagram of the Tubular Membrane Plant Growth Unit used in the wheat trial.

Porous tube plant growth unit

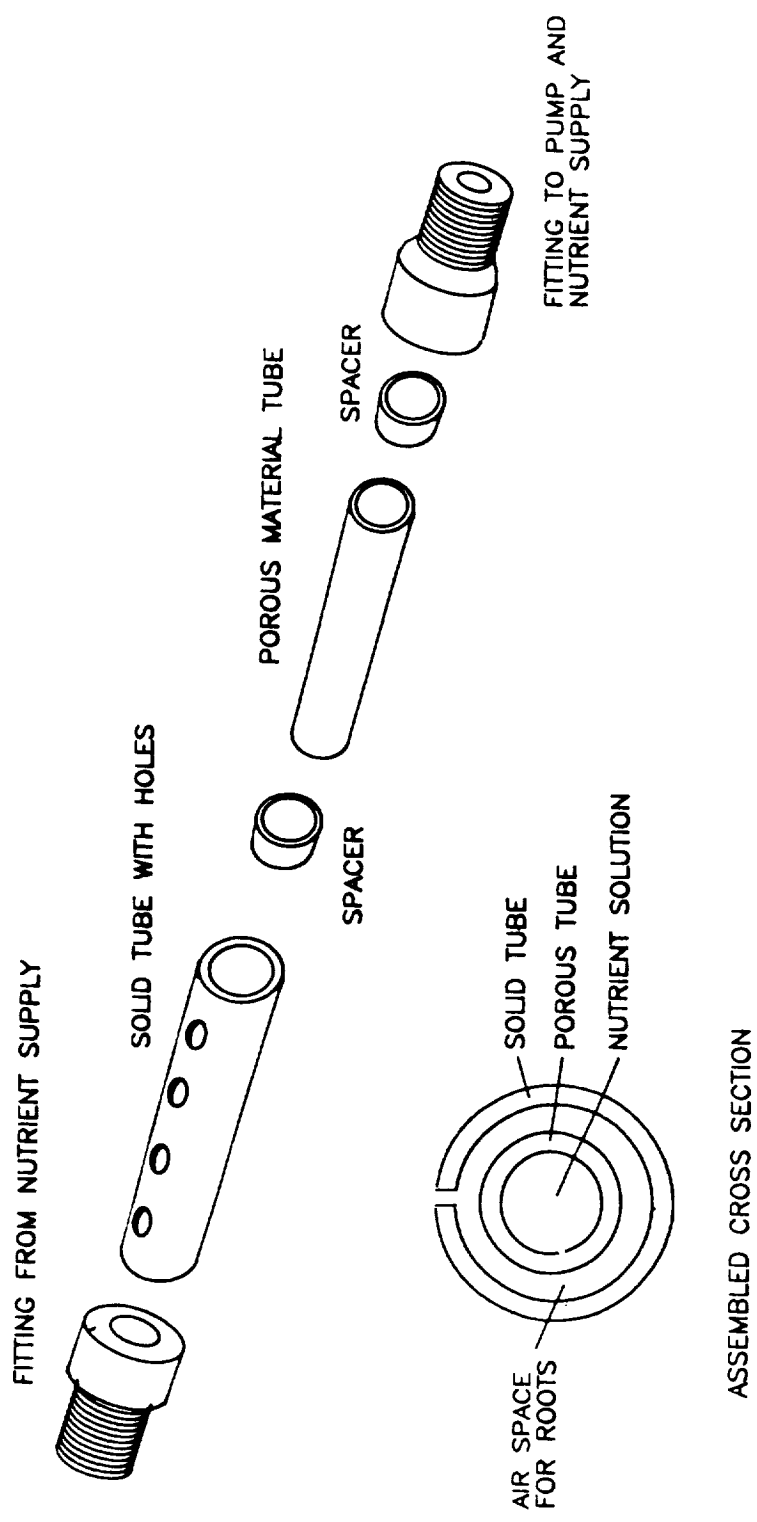


Figure 2. Schematic diagram of the Porous Tube Plant Growth Unit used in the lettuce trial.

CONCEPTUAL DESIGN OF A CLOSED LOOP NUTRIENT SOLUTION DELIVERY SYSTEM FOR CELSS IMPLEMENTATION IN A MICRO-GRAVITY ENVIRONMENT

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ABSTRACT This paper describes the results of a study to develop a conceptual design for an experimental, closed-loop fluid handling system capable of monitoring, controlling, and supplying nutrient solution to higher plants. The Plant Feeder Experiment (PFX) is designed to be flight tested in a micro-gravity (micro-g) environment and was developed under NASA's In-Space Technology Experiments Program (INSTEP). When flown, PFX will provide information on both the generic problems of micro-g fluid handling and the specific problems associated with the delivery of nutrient solution in a micro-g environment. The experimental hardware is designed to fit into two middeck lockers on the Space Shuttle, and incorporates several components that have previously been flight tested.

AMONG THE PROBLEMS encountered in designing devices to function under the micro-gravity (micro-g) conditions of spaceflight, some of the most common involve the handling of fluids. The general fluid handling problem encompasses monitoring and control of fluid composition, mixing of fluids, and fluid transfer. The problem becomes critical in the design and operation of life support systems because of the importance of water and aqueous solutions in such systems. This is especially true in the development of Controlled Ecological Life Support Systems (CELSS) with hydroponic systems for higher plants.

The objective of the study was to develop a conceptual design for an experimental fluid handling system which would be tested in flight under micro-gravity conditions. The Plant Feeder Experiment (PFX) will provide an evaluation of fluid handling capabilities, and supply data on both generic and CELSS-specific fluid handling problems. The specific goals the experiment was designed to address included: 1) measurement of solution monitoring capabilities under micro-g conditions; 2) measurement of solution control capabilities under micro-g conditions; 3) measurement of the capability to condense, collect and recycle water vapor; and 4) measurement of the capability of different nutrient solution delivery/recovery system designs to provide water and nutrient elements to higher plants under micro-g conditions.

PREVIOUS AND ON-GOING WORK

Much of the prior work in the area of micro-gravity nutrient delivery systems has been done by Soviet investigators. In general, they have focused their efforts on solid substrate systems, using either direct soil analogs or fibrous materials such as rock wool [1,2]. Functional operation of these systems has generally been limited to simply adding water or nutrient solution to the rooting substrate, thus avoiding most of the problems associated with the handling of fluids in the micro-g environment. To date, their results with living plants have been very inconsistent; e.g., some species have grown well while others died or grew poorly during flight.

They have theorized that this inconsistency is due to a lack of control over the plant environment including the nutrient supply to the roots.

The European Space Agency (ESA) has also designed and conducted ground testing of several plant growth systems for space flight. These systems have been developed for use both on the Space Shuttle and on the European Recoverable Carrier (EURECA). The ESA designs have stressed the use of soil analogs [3], also avoiding many of the problems associated with fluid handling in the micro-g environment.

Under NASA sponsorship, several U.S. scientists have developed nutrient delivery system designs for micro-g use. One of the first papers on this topic described the use of a sheet of semipermeable membrane as a barrier between nutrient solution and plant roots [4]. This membrane barrier served as a means for maintaining separation of the liquid and gas phases in the root environment, thereby providing a partial solution to one of the micro-g fluid handling problems. This membrane design was modified by a team of scientists working at Kennedy Space Center (KSC), and converted into a tube-within-a-tube arrangement. This modified design has been ground tested [5] at KSC, along with Purdue University and Ames Research Center (ARC). The results of this test program have generally indicated that the design supports adequate plant growth, but that growth is slower than that measured in hydroponic systems of conventional 1-g design.

Two of the NASA-sponsored Centers for the Commercial Development of Space (CCDS), Bioserve Space Technologies at the University of Colorado (Bioserve) and Wisconsin Center for Space Automation and Robotics at the University of Wisconsin (WCSAR), are currently conducting design studies in the area. The work at Bioserve is of a generic nature and has included several studies addressing micro-g plant growth systems in conjunction with ARC. These studies have included evaluation of porous plastic tubes for nutrient solution supply. To date, Bioserve has not published any detailed designs for these systems, but they have discussed their designs at several NASA-sponsored meetings. WCSAR has focused on a hybrid system design which supplies nutrient solution to a solid clay-like substrate by gradually leaking it from a porous, sintered stainless steel tube embedded in the substrate [6]. The WCSAR system is currently undergoing ground tests at the University of Wisconsin.

DESIGN OF EXPERIMENTAL HARDWARE

The initial overall design configuration of the PFX hardware is pictured in Figure 1. The design consists of five subsystems; the reservoir, sensing manifold, control manifold, nutrient solution delivery/recovery subsystem, and the water vapor condensation and recovery system [7]. The development of each subsystem design is discussed in the following sections.

RESERVOIR SUBSYSTEM

Table 1 summarizes the principle methods we identified for organizing the reservoir subsystem. Two categories of subsystem design were developed initially: single reservoir and dual reservoir.

Table 1. Reservoir Configuration Design Options .

1. Single Reservoir
 - a. Stationary
 - b. Rotating
 - c. Divided, Stationary
2. Dual Reservoir
 - a. Large/Small Volumes
 - b. Identical Volumes

Three options were identified for single reservoir systems, two using an open tank (Figure 2 a,b) and one using a divided tank (Figure 2 c) . Two of the single tank options (Figure 2 a,c) require mechanical pumps to transfer the solution. Because of the problems associated with pump priming under micro-g conditions, these design options were identified as the least desirable. One of the open tank options was a centrifugally-powered reservoir in which the tank was rotated about its main axis (Figure 2 c), and a pitot tube pickup was immersed in the liquid film coating the wall of the rotating drum. This design option has several desirable features with regard to mixing, monitoring and control of the solution composition, but requires more volume, has a higher power use, and produces more vibration than the other options.

The two design options we identified as most desirable were the dual reservoir options. One option (Figure 2 d) utilizes a bladdered tank and a small bladdered accumulator (to receive the solution flowing out of the nutrient delivery/recovery system), while the other uses two bladdered tanks of the same volume (Figure 2 e). Another option considered for the accumulator was a piston driven,

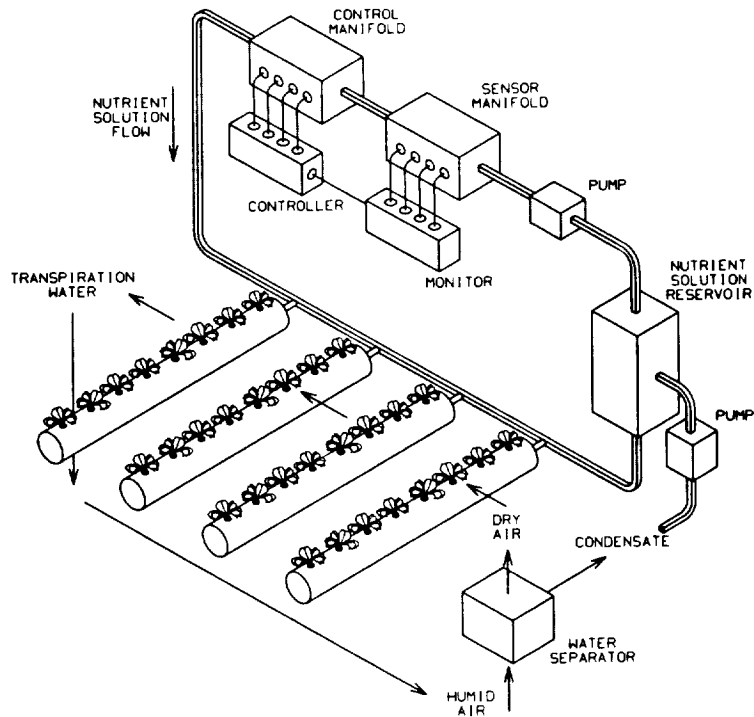


FIGURE 1. Initial PFX Hardware Configuration

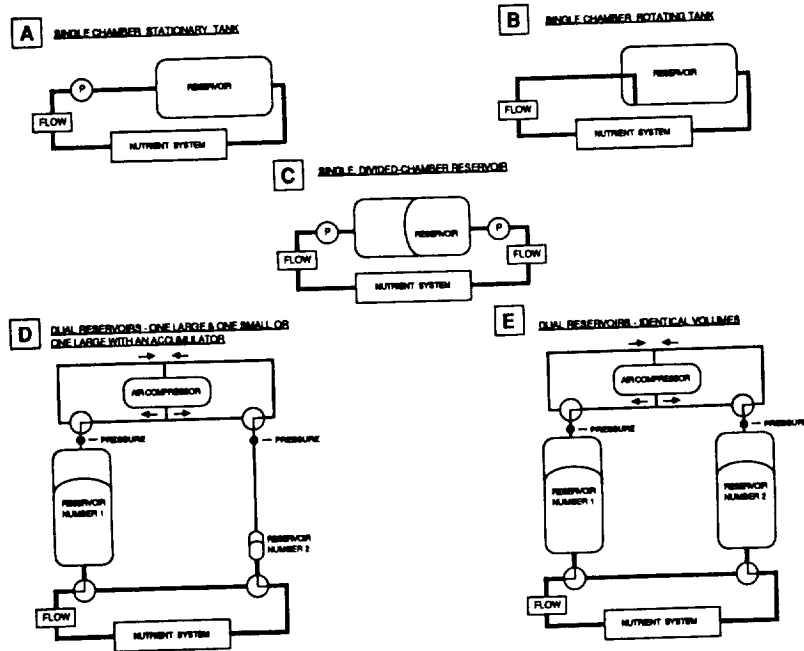


FIGURE 2. Reservoir design options.

syringe-like system. The piston option was eliminated because of the potential problems of sealing around the piston.

In order to support the testing of solution mixing capabilities, we selected the option illustrated in Figure 2 e as the baseline for the experiment design. This option requires an compressor which pumps air from one bladdered tank to the other, thus creating fluid flow through the system. The tanks used in both options are flight-tested hardware derived from Lockheed's Research Animal Holding Facility (RAHF). This design option also provides the capability for precise control of the liquid pressure differential across the nutrient delivery/recovery system under test by control of the reservoir air pressures.

SENSOR MANIFOLD

Monitoring solution composition is complicated by several factors, most notably the lack of liquid/gas phase separation. Phase separation problems as simple as an air bubble adhering to the active portion of a sensor can produce erroneous composition readings. The sensors themselves must be robust enough to withstand launch, with an operating stability that does not require recalibration during flight.

Table 2 lists the kinds of solution measurements required and the types of sensors considered for each. Ultimately, we determined that ion-specific electrodes provided the most rugged, stable devices for monitoring solution ion concentrations. Gas-specific electrodes were chosen to monitor the concentrations of oxygen and carbon dioxide dissolved in the solution. A platinum RTD was chosen for solution temperature monitoring and standard pH and electrical conductivity electrodes chosen for measuring these bulk solution parameters.

The initial design for this manifold is illustrated in Figure 3. A static mixer has been included in the design to assure complete mixing of the solution flowing through the bore of the manifold. By properly positioning the mixer, a homogenous liquid stream is supplied to the sensors, while simultaneously preventing the clogging of sensing elements by air bubbles.

CONTROL MANIFOLD

The control functions incorporated into this design included specific elemental concentrations, temperature, pH, electrical

Table 2. Sensor Manifold Design Options

1. pH Measurement
 - a. Electrochemical
 - b. Fiber Optic
2. Electrical Conductivity
 - a. Conductivity Electrode
3. Ion Concentration Measurement
 - a. Ion Specific Electrodes
 - b. Ion Chromatograph
 - c. Optical Absorbance
4. Temperature
 - a. Thermocouple
 - b. Thermistor
 - c. RTD
 - d. Integrated Circuit
5. Dissolved Gas Concentration
 - a. Semipermeable Membrane Sensor
 - b. Headspace Gas Sampling

conductivity and dissolved oxygen and carbon dioxide concentrations. The control methods we evaluated for use in this experiment are listed in Table 3.

Table 3. Control Manifold Design Options

1. pH Control
 - a. Electrochemical
 - b. Chemical
2. Electrical Conductivity Control
 - a. Direct Solution Addition
 - b. Trans-membrane Solution Addition
3. Ion Concentration Control
 - a. Passive Control by Ion Exchangers
 - b. Direct Chemical Addition
 - c. Electrochemical Addition
4. Temperature Control
 - a. Thermoelectric Unit
 - b. Chilled Water/Electric Heater
5. Dissolved Gas Concentration Control
 - a. Direct Gas Injection/Chemical Absorption
 - b. Trans-Membrane Addition/Removal

The design of the control manifold body is very similar to that of the sensor manifold. Miniature metering pumps are incorporated into the design to add distilled water or concentrated nutrient solution for electrical conductivity and elemental concentration

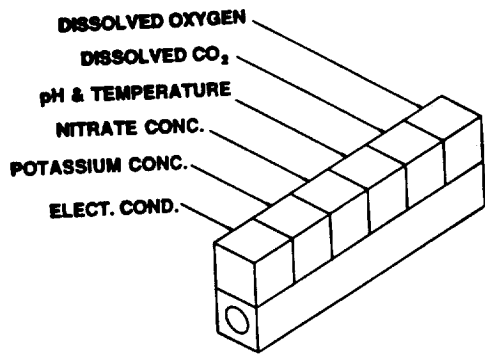


FIGURE 3. Sensor manifold design.

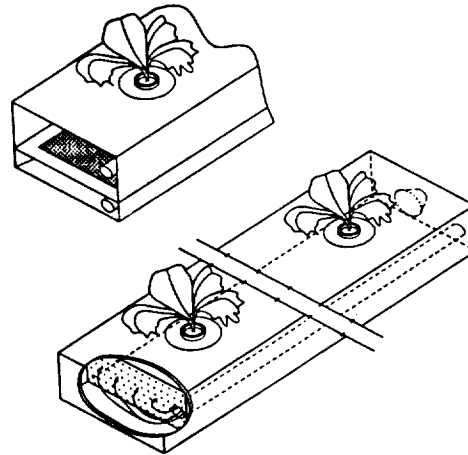


FIGURE 6. Solution culture design.

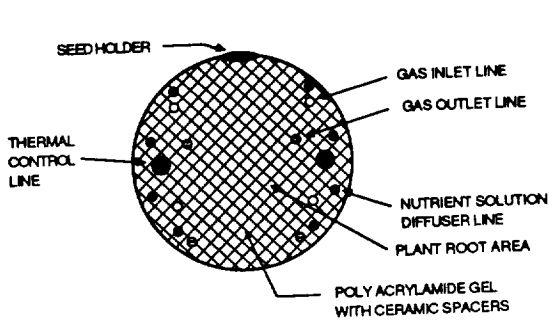


FIGURE 4. Polyacrylamide gel system design.

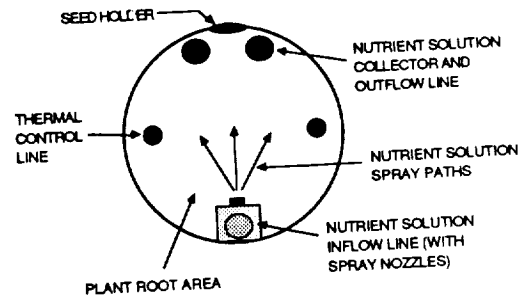


FIGURE 7. Aeroponic design.

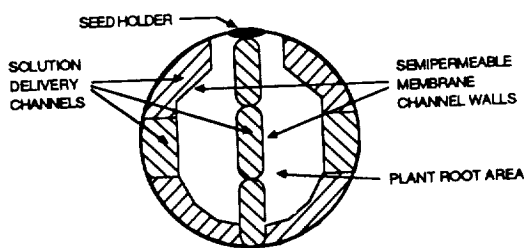


FIGURE 5. Semipermeable membrane design.

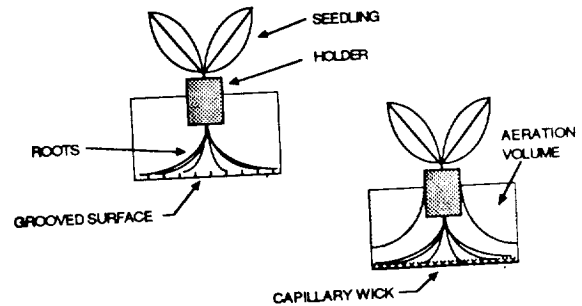


FIGURE 8. Heat pipe designs.

control, and acid or base to control solution pH. Temperature is controlled with an electrical heater bucked against a coolant jacket supplied with cool water from the Space Shuttle. Dissolved gas concentrations (O₂ and CO₂) are controlled by a semipermeable membrane equipped gas/fluid exchangers.

NUTRIENT DELIVERY/RECOVERY SUBSYSTEM

Table 4 lists the different families of nutrient solution delivery/recovery system identified for potential testing in this experiment. Because one of the main objectives of the experiment is to qualitatively determine the functional characteristics of these systems, we plan to support at least four different systems in the design.

Table 4. Nutrient Delivery/Recovery Design Options

1. Solid Substrate
 - a. Soil
 - b. Soil Substrate
 - c. Ion Exchange Resin
 - d. Gel
2. Nutrient Film Technique
3. Solution Culture
4. Aeroponics
5. Hybrid Design
6. New Design
 - a. Tubular Membrane (e.g., KSC)
 - b. Plastic, Ceramic or Metal Wick (e.g., WCSAR)
 - c. Heat Pipe Technology

Figures 4 through 8 illustrate several of the design concepts we feel to be the most promising. Determination of which of these systems will be flight tested will be based on ground evaluations and supporting flight tests (e.g., KC-135).

WATER VAPOR CONDENSATION AND RECOVERY SUBSYSTEM

The efficient condensation and effective collection of water vapor is a particularly difficult problem in the micro-g environment. In any CELSS system with higher plants, however, there will be a strong need to maximize this capability. Table 5 lists the technology options identified for this subsystem. Because it has already been extensively flight tested, we decided to use the Lockheed RAHF condensate collection system in this design.

Table 5. Water Vapor Condensate/Recovery Design Options

1. Hydrophillic/Hydrophobic Separator (e.g., RAHF)
2. Vortex Water Separator
3. Condensing Heat Exchanger with Wick
4. New Design

SYSTEM CONCEPTUAL DESIGN

The detailed conceptual design for the PFX hardware is illustrated in Figures 9 and 10. The current design is baselined to fit into two Shuttle middeck lockers. The main locker houses the reservoirs, monitoring and control manifolds, most of the fluid control components, and the experiment control computer and associated interface electronics (Figure 9). The second locker contains the nutrient delivery/recovery systems to be tested, the water vapor condensate/recovery system, and the remaining fluid control components (Figure 10).

We have also developed a design which integrates and slightly reconfigures the hardware for packaging in a Shuttle double middeck locker. The use of a double locker has several advantages, most notably the reduction in the number of connecting lines (both fluid and electrical) exposed to the middeck environment. The primary drawback is simply the scarcity of double middeck lockers in which the experiment could be packaged.

SUMMARY AND CONCLUSIONS

The flight experiment hardware described here has been developed to support an engineering assessment of several critical functions of micro-gravity fluid handling. The functions we intend to assess include several that are generic to micro-gravity fluid handling as well as some that are specific to the handling of fluids for a CELSS design. To help keep hardware development costs down, the design incorporates existing flight-tested components wherever possible. The PFX hardware design we have developed can be accommodated on the Shuttle middeck, packaged either in two single lockers or in one double locker.

ACKNOWLEDGEMENT

This work was supported by NASA Lyndon B. Johnson Space Center Contract NAS9-17981.

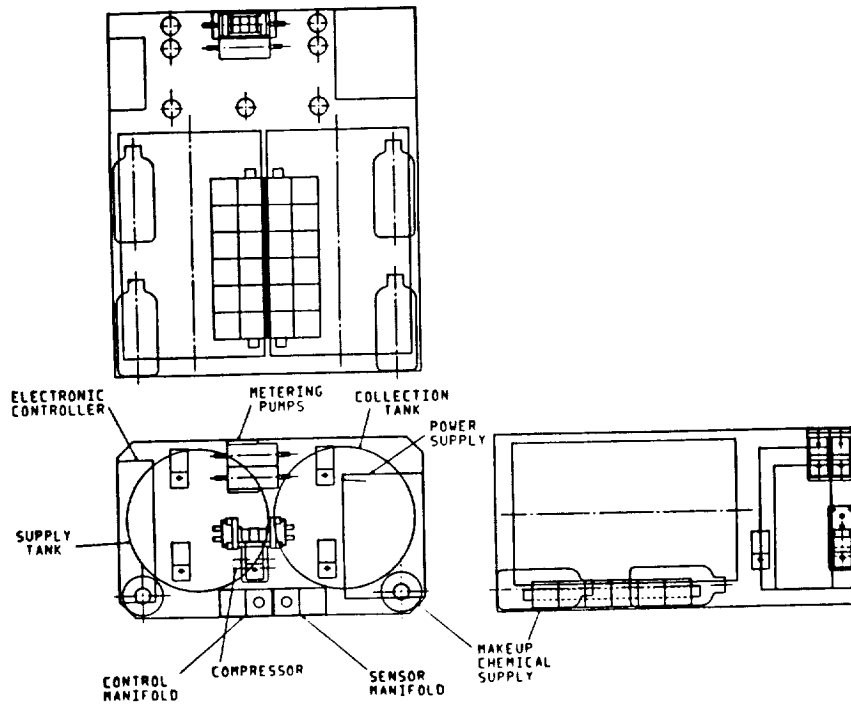


FIGURE 9. Three view layout drawing of main locker.

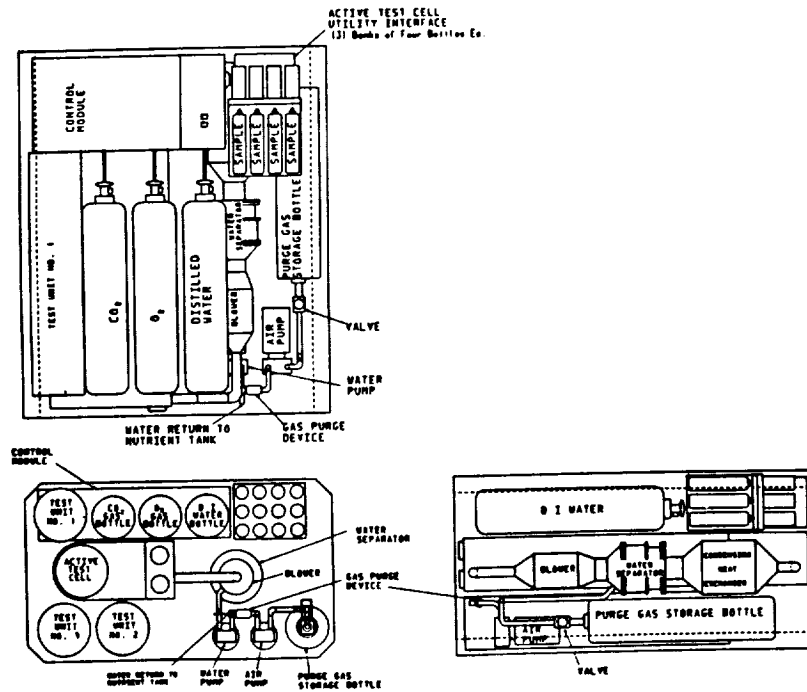


FIGURE 10. Three view layout drawing of second locker.

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TECHNOLOGY FOR SUBSYSTEMS OF SPACE-BASED
PLANT GROWTH FACILITIES

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ABSTRACT

Technologies for different subsystems of space-based plant growth facilities are being developed at the Wisconsin Center for Space Automation and Robotics, a NASA Center for the Commercial Development of Space (CCDS). These include concepts for water and nutrient delivery, for nutrient composition control, and for irradiation. Effort is being concentrated on these subsystems because available technologies can not be effectively utilized for space applications.

WATER AND NUTRIENT DELIVERY SUBSYSTEM

Water and nutrient delivery to plant roots under microgravity conditions is a major concern in the development of a space-based plant growth facility. A concept for utilizing porous stainless steel tubes is being developed to accomplish this task. This concept was evolved from a membrane concept proposed by others (1) which was felt to have several limitations because it did not involve the use of a matrix and it had a limited period of usefulness.

The concept under development includes the use of a non-organic rooting medium in contact with the porous stainless steel tubes. The nutrient solution is circulated through the cavity of the porous tubes under negative pressure. The nutrient solution moves through the porous wall of the tube and into small pores of the rooting medium by capillary action

(adhesion and cohesion of the water molecules). The larger pores in the rooting medium remain filled with air, thereby providing a non-saturated environment that is highly desirable for effective plant root functioning. Since the nutrient solution is contained by negative pressure and capillary forces, the liquid cannot escape into the atmosphere of a space vehicle in a microgravity environment. Thus, the proposed concept provides water and nutrients to plants while meeting three significant requirements for a space-based water and nutrient delivery subsystem for a microgravity environment: [1] provide a non-saturated matrix for plant roots, [2] prevent the escape of the liquid from the root zone to the atmosphere, and [3] allow the tubes and rooting medium to be easily cleaned and reused so that no consumables are involved in repeated growth cycles.

Three models of this concept have been constructed or are nearing completion. Each of these models consist of an array of porous stainless steel tubes (outside diameter of 1.04 cm and an inside diameter of 0.68 cm). The tubes have a pore size of 20 μm and a porosity of 50 percent. The tube size, porosity and pore size do not appear to be critical and were selected for convenience. The pore size has an upper limit that is related to the negative pressure under which the solution can be circulated through the tube cavity without drawing air into the tubes. The tube arrays are connected at each end by manifolds so as to provide a uniform solution flow through each of the tubes in the array.

A proof-of-concept model was constructed to provide preliminary information and experience for the construction of a more complete engineering model. The proof-of-concept model consisted of 10 porous tubes, spaced on 2.5 cm centers and connected to manifolds at both ends.

This provided a plant growing area 47 cm long, 28 cm wide, and 3 cm deep. The nutrient solution was circulated through the porous tubes under a negative pressure of -490 Pa (5 cm H₂O). A flush cycle of -195 Pa (2 cm H₂O) for 1/2 hr each day was imposed to prevent salt accumulation on the surface of the rooting medium. A flow rate of approximately 125 ml per minute was maintained through the porous tube array. Lettuce plants were grown for 28 days and development of these plants was compared with those grown using a standard controlled environment procedure developed for base-line plant growth research (2).

Table 1 shows a comparison of the characteristics of lettuce plants grown in the porous tube nutrient solution delivery model with those grown with the base-line growing procedure. Plant characteristics were essentially equivalent. It does not appear to have been demonstrated that other concepts proposed for providing water and nutrients to plants under microgravity conditions have supported plant growth at equivalent rates. Figure 1 shows a comparison of lettuce plants grown under the two water and nutrient supply methods. Figure 2 shows the root development of lettuce plants grown with the porous tube delivery method. The general root development and distribution indicate that the rooting medium provided a favorable rooting environment that supported the high growth rates of the lettuce plants.

A more complex engineering model has been designed and it's construction recently completed. This model has 20 porous tubes 52 cm long and provides a growing area of 2700 cm². The depth of the rooting zone can be adjusted to a maximum depth of 15 cm to accommodate different types of

plants. Operation of the unit is controlled by a microprocessor. Pressure sensors and flow meters at various sites in the flow path will permit continuous monitoring of the units operation. Sensors for conductivity, pH, temperature, and humidity are also included for monitoring these parameters during the course of an experiment.

A miniature model of the porous tube nutrient delivery system has also been constructed. This model is designed to replace the existing plant growth cells in the NASA flight-approved plant growth unit for the STS middeck locker. This model consists of 4 rooting zone trays each with a single porous tube and having a dimension of 20 cm long, 4.5 cm wide, and 3.5 cm deep. This unit would accommodate small plants proposed for microgravity research in the space biology program.

Future activity will be directed toward evaluation of the engineering model to define the optimum operational parameters of the porous tube water and nutrient delivery concept and to determine growth and productivity characteristics of various CELSS candidate species in this system.

Another activity initiated this year involves the development of a flight experiment to validate the porous tube delivery concept in microgravity. A flight experiment has been proposed and currently is manifested for inclusion in the US Microgravity Laboratory I (USML-1) mission scheduled for March 1992. The experimental goal is to evaluate the rate of capillary movement of water into and out of a matrix using a porous tube based water delivery system and to determine the capability of maintaining this matrix in a non-saturated condition under microgravity conditions.

Data collected from this experiment would not only be useful in defining the water and nutrient delivery requirements for plants growing in microgravity, but also provide information pertinent to the behavior of liquid/gas systems in microgravity. Such systems involve a number of space applications. A diagram of the flight experiment hardware is shown in Figure 3.

USE OF ION EXCHANGE MATERIALS FOR CONTROLLING CHEMICAL COMPOSITION OF NUTRIENT SOLUTION

Plants require that the solution surrounding roots contain specific ions within a certain range of concentrations depending on the nutrient ion and plant species. As the plant uptake of the nutrient ion proceeds, the solution around the roots becomes increasingly depleted. If no mechanism is provided for replenishing the nutrient ions, the solution soon becomes exhausted and plant growth stops.

At present, nutrient solutions balance is maintained by analyzing the solution and providing supplementation when the specific nutrients are depleted. However, accurate analysis of nutrient concentrations in space is fraught with problems. Certain nutrient analysis devices have been proposed for possible use in a CELSS (3). Of these, ion chromatography and specific ion detectors appear to have the best potential for continuous, or near continuous monitoring of ion levels in the nutrient solution.

Unfortunately, ion chromatography requires relatively sophisticated equipment and has not been perfected for all nutrient ions. Specific ion probes require frequent calibration and replacement to provide continuous operation and again are available for only a limited number of nutrient

ions. Thus, there remains a need to develop procedures for controlling nutrient concentration that can be automated and require little or no maintenance.

A concept based on utilizing ion exchange materials for controlling the chemical composition of the nutrient solution has been proposed. Five ion exchange materials, provide control of the 13 essential elements absorbed by plant roots and also control pH. These ion exchange materials maintain both the appropriate ion ratios and the concentrations required for plant growth.

Figure 4 shows a diagram of the components involved in the unit for controlling the chemical composition of a nutrient solution. Table 2 provides a listing of the ion activities controlled by each of the ion exchange materials. The conductivity sensor detects changes in ionic strength of the nutrient solution resulting from nutrient ion uptake by the plants. The pressure sensor detects changes in solution volume resulting from water transpired from plant surfaces. As these changes are detected, a control interface activates pumps to add stock nutrient solutions and water to maintain the ionic strength and volume of the nutrient solution.

Initial evaluations of the ion exchange materials have shown that the ion activities were effectively maintained with the exception of the phosphate ion, which was maintained at a level lower than desired. Additional work is underway to more clearly define the procedure for loading phosphorous onto the ion exchange materials.

Future activity will be directed toward obtaining the documentation required to define the procedures for loading the ion exchange materials so that the desired ion activities are maintained in the nutrient solution. This will be followed by confirmation that the ion exchange materials can effectively supply the proper ratio of nutrient ions required for plant growth and development. This portion of the program will utilize the engineering model of the Water and Nutrient Delivery Subsystem as described in the previous section.

IRRADIATION SUBSYSTEM

We have begun to study the potential of light emitting diodes (LED's) as photosynthetic light sources for plants (4). These devices have potential for use in space because they are solid state and consequently do not contain a gas-filled or vacuum bulb or tube. They also have the potential of greater photon efficiency than presently available lamps.

LEDs consist of a diode encased in transparent material as shown in Figure 5. The diode chip can be made of different materials so that varied wavelengths can be generated. A chip commonly utilized in high output diode construction is of gallium aluminum arsenide and produces a red light output between 620 and 680 nm as shown in Figure 6. This output closely matches the wavelengths of photons that provide maximum photosynthetic efficiency for plants, and is useful for plant growth systems. We are devoting efforts to the identification of LEDs with a spectral output in other regions required for plant growth. Currently, LEDs that emit in the visible region between 500 and 600 nm and in the

far-red region between 700 and 760 nm are readily available. LEDs that emit in the blue spectral region (between 400 and 500 nm) are not readily available and have insufficient output. However, several companies are working to improve the output of these LEDs.

The potential efficiency of the LED chip for generating useful photons per watt of electricity is greater than the efficiency of presently available lamps. However, large losses can occur in passage of the photons from the chip through the transparent casing, resulting in an electrical efficiency that is rather low. Recent developments in LED technology have resulted in devices with an electrical efficiency similar to fluorescent lamps but less than for high intensity discharge lamps.

The capability of high frequency pulsing of LEDs (high nanosecond or low microsecond range) provides the possibility of obtaining significant gains in efficiency of irradiation for plants. These gains will be very large if the on-time and off-time duration of the electric power pulse can be synchronized to the time constants involved in the primary photochemical photon capture by the chlorophyll molecule, and the time constants for the enzymatic reactions of the photosynthetic process. If these photobiological processes can be synchronized, then the on-time of the LED array could be only 25% or less. Under such conditions the electrical efficiency of an LED array would be significantly better than any electric lighting system currently available for plant lighting. In addition, pulsing of the LEDs permits the LEDs to be driven at electric power levels significantly in excess of those permitted if the LEDs are driven in a continuous duty mode.

We have constructed several small arrays, mounting the LEDs on 0.5 to 1.0 cm centers, and have obtained irradiance levels of $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ within a cm of the LEDs. We have initiated growth studies using an array consisting of red emitting LED's and supplemented the irradiation with blue fluorescent bulbs to provide 90% of the photons from the LED's and 10% of the photons from the fluorescent lamp. Growth of 'Grand Rapids' lettuce seedlings utilizing this irradiation has been similar to growth with cool white fluorescent lamps mounted to provide a similar photon level at seedling height.

Future activity will be concentrated on developing arrays with the most efficient LEDs available, utilizing the required balance of different wavelengths to obtain normal plant development. A number of LED manufacturers have introduced new products that exhibit improved performance characteristics and these new devices will need to be evaluated and incorporated into effective units.

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Table 1. Growth characteristics of lettuce plants grown in the porous tube nutrient delivery system compared to plants grown in peat-vermiculite and watered with nutrient solution several times daily.

Treatment	Fresh weight (g±sd)	Dry weight (g±sd)	# Leaves (>1cm)	Length 5th leaf (cm±sd)	Width 5th leaf (cm±sd)
Porous Tube Nutrient Delivery System	45.3±4.0	2.59±0.26	12.2±0.7	14.6±0.8	15.4±1.0
Peat-Vermiculite Watered Daily With Nutrient Solution†	50.8±7.6	2.57±0.38	14.0±1.2	15.2±0.8	15.4±1.2

†Using procedure described in P.A. Hammer, T.W. Tibbitts, R.W. Langhans, and J.C. McFarlane 1978. Base-line growth studies of 'Grand Rapids' lettuce in controlled environments J. Amer. Soc. Hon. Sc. 103 649-655.

Table 2. Specific ion activities controlled by each of the ion exchange materials proposed for the nutrient control subsystem of a space-based growth unit.

	ANION EXCHANGE RESIN	BORON RESIN	CHELATING RESIN	WEAK ACID RESIN	ZEOLITE
pH				■	■
NO ₃ ⁻	■	■			
SO ₄ ²⁻	■	■			
H ₂ PO ₄ ⁻	■	■			■
K ⁺			■	■	■
Mg ²⁺			■	■	■
Ca ²⁺			■	■	■
Mn ²⁺			■	■	■
Zn ²⁺			■	■	■
Fe ²⁺					■
Cu ²⁺			■	■	■
B		■			
Na			■	■	■
Cl	■	■			



Figure 1. Comparison of lettuce plants grown in the porous tube nutrient delivery system and in peat-vermiculite watered automatically with nutrient solution 6 times daily.



Figure 2. Root development of lettuce plants grown with the porous tube nutrient delivery method.

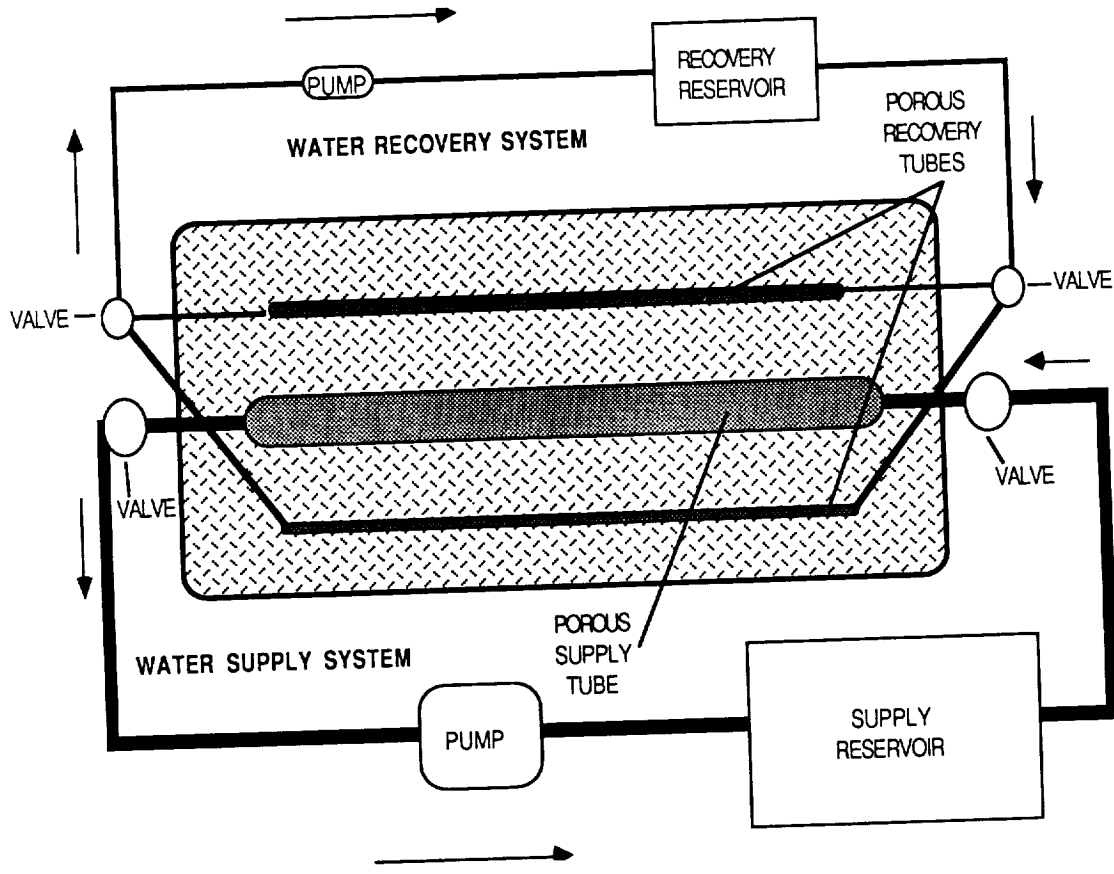


Figure 3. Diagram of hardware for flight experiment proposed to validate the porous tube nutrient delivery concept in microgravity.

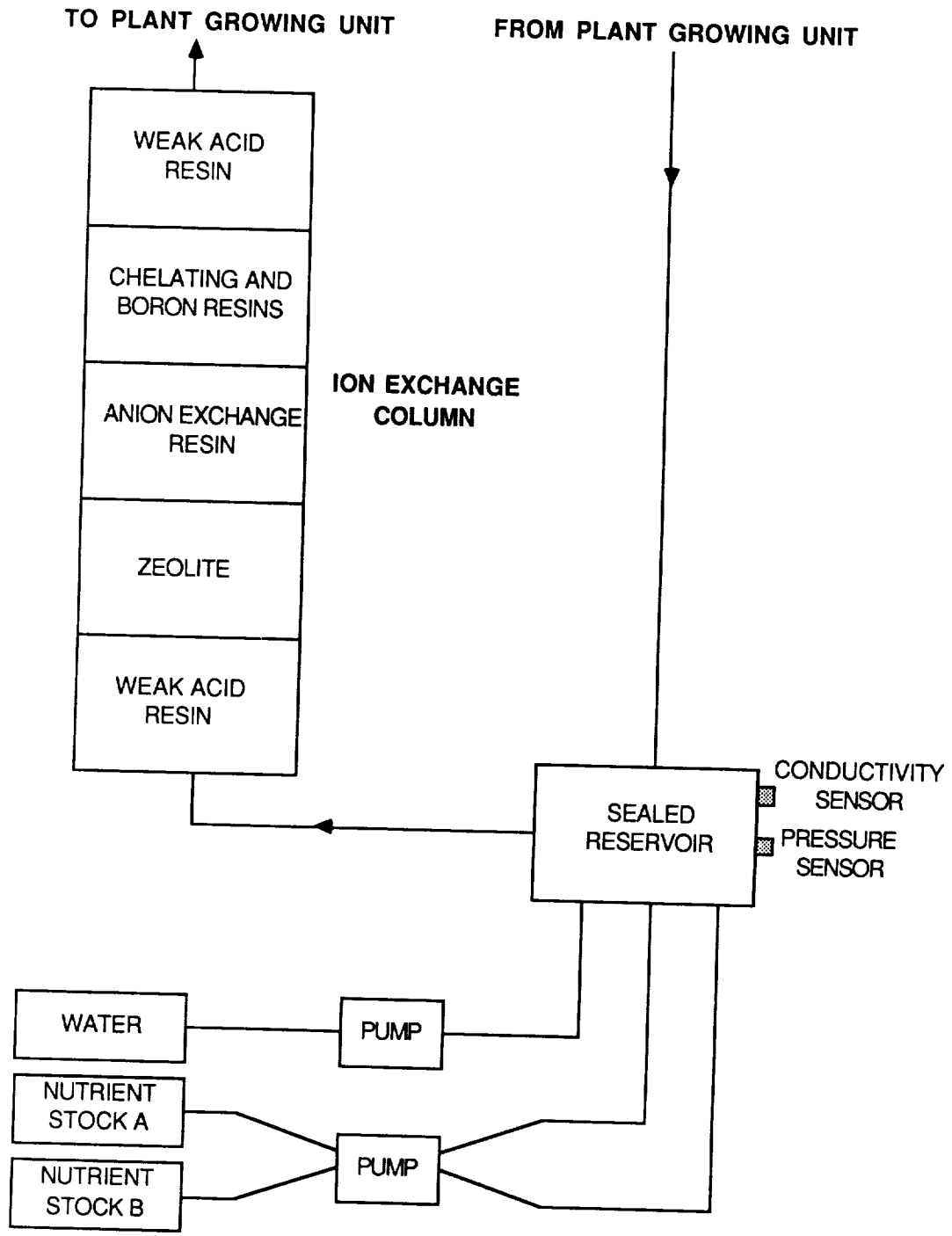


Figure 4. Diagram of the components involved in the unit for controlling the chemical composition of a nutrient solution.

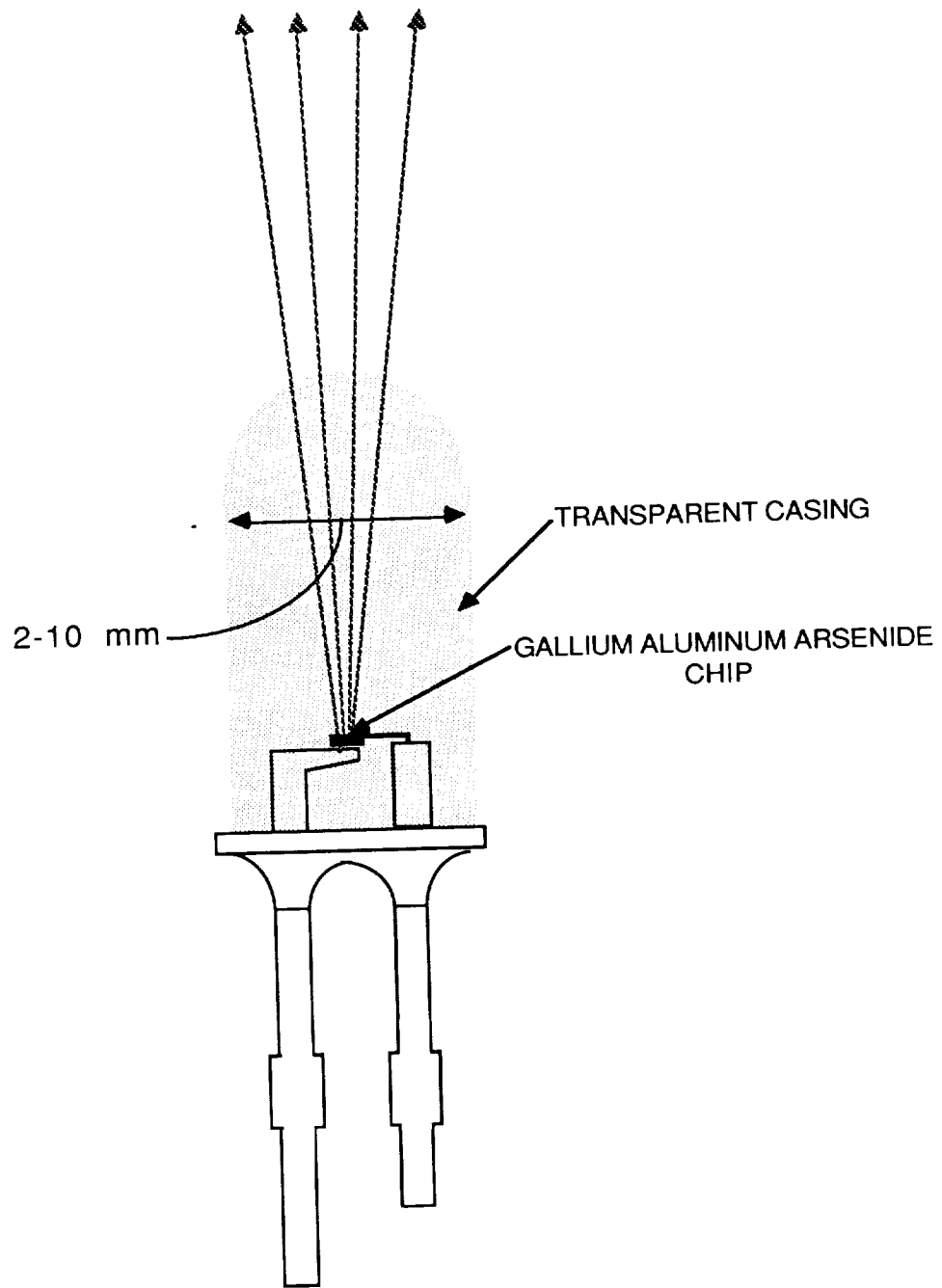


Figure 5. Diagram of a light emitting diode (LED).

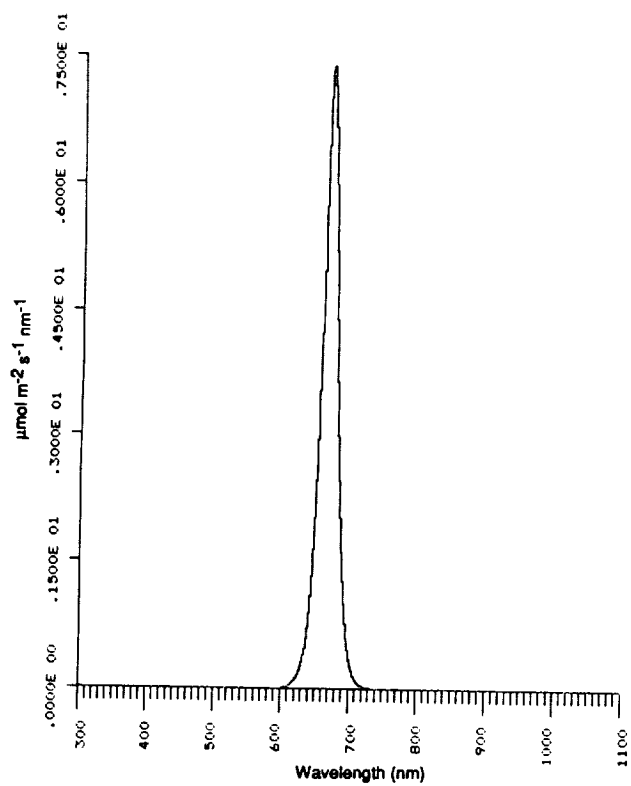


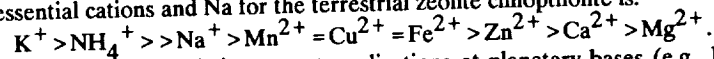
Figure 6. Relative emission of red light emitting LED.

SOLID-SUPPORT SUBSTRATES FOR PLANT GROWTH AT A LUNAR BASE

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ABSTRACT

A variety of *in situ* materials could be used as solid-support substrates for plant growth at planetary bases (primarily lunar bases); including 1) native lunar "soils", 2) sized lunar regolith amended with synthetic materials (e.g., zeolites, smectites) that provide nutrient and water retention, 3) synthetic, inorganic, highly-reactive substrates (e.g., zeoponics), and 4) sized lunar materials or industrial by-products used as inert, solid-support substrates in nutriculture systems. Research at NASA/JSC is being conducted in several areas of plant growth in solid-support substrates; including 1) behavior of lunar materials as "soils"; 2) zeoponic systems for plant growth; and 3) production of lunar simulants to use in plant growth experiments. Presently, most of our attention is being directed toward zeoponic systems and lunar simulants. Zeoponics is only in its developmental stages at JSC and is defined as the cultivation of plants in zeolite substrates that 1) contain essential, plant-growth cations on their exchange sites, and 2) have minor amounts of mineral phases (e.g., apatite) and/or anion-exchange resins (e.g., activated aluminum resins) that supply essential, plant-growth anions. Zeolites are hydrated aluminosilicates of alkali and alkaline earth cations with the ability to exchange most of their constituent exchange cations as well as hydrate/dehydrate without change to their structural framework. Because zeolites have extremely high cation exchange capacities, they are very attractive media for plant growth. It is possible to partially or fully saturate plant-essential cations on zeolites. The apparent selectivity of plant-essential cations and Na for the terrestrial zeolite clinoptilolite is:



Zeoponic systems will probably have their greatest applications at planetary bases (e.g., lunar bases). Lunar raw materials will have to be located that are best suited for the syntheses of zeolites and other exchange resins; however, we have previously shown that zeolites can be synthesized from lunar analog glass subjected to mild hydrothermal conditions. Lunar "soil" simulants have been or are being prepared for zeolite/smectite syntheses and "soil" dissolution studies.

INTRODUCTION

The newly created Office of Exploration at NASA Headquarters is considering several missions that the Agency may undertake as part of the human exploration of our inner solar system. These scenarios include: 1) expeditions to establish the first human presence on another planet (e.g., Mars); 2) lunar outposts to conduct extraterrestrial science; and 3) evolutionary expansion to establish self-sufficient human presence beyond low Earth orbit. Evolutionary expansion is a step-by-step program away from low Earth orbit. The first step will probably be the establishment of a lunar outpost that will lead to a self-sufficient lunar base. A self-sufficient lunar base will require the utilization of *in situ* resources for construction materials, propellants, life-support systems, etc. The growth of plants at a lunar base will be essential to sustain a self-sufficient human colony, and there are several systems in which to grow plants, e.g., hydroponics, aeroponics, and solid-support substrates. Most of the plant-growth research in controlled ecological life support systems (CELSS) has been aimed toward

hydroponic systems. Soils may also be viable plant-growth systems, however, our knowledge of how lunar materials will react as soil is virtually unknown.

BACKGROUND

Controlled Ecological Life Support Systems (CELSS)

A bioregenerative Controlled Ecological Life Support System (CELSS) is a system where the crew is supplied with water, food and oxygen that is produced by photosynthesis of living plants. The photosynthetic organisms (primarily plants and algae) consume carbon dioxide (CO₂), the major metabolic human waste, and combine it with water converting these materials into food as well as producing the essential gas oxygen. Development of such a CELSS is needed not only to allow for self sufficiency but to reduce the transportation costs of resupply when the numbers of crew members rise above those required for an initial outpost.

Growing plants at a lunar base has other attractive features beyond supply of food to lunar crews. Instead of re-supplying food to Space Station Freedom (or other space stations) from Earth, resupplying through lunar base agricultural production could reduce the transportation costs. Furthermore, industrial and human wastes from space stations could be transported to the lunar surface where they could be re-cycled; thus they become a resource available to lunar base expansion rather than a liability for Shuttle to return to Earth. Manned interplanetary spacecraft will be extremely large vehicles requiring on-orbit assembly and supply due to their long-duration missions. Initial supplies of food could be provided from lunar base agricultural production. Waste products from the returning manned interplanetary spacecraft could be sent to the lunar surface for renovation and use.

In fact, a true bioregenerative CELSS will have its greatest application at a lunar base rather than a space station or other space vehicle simply because of the tight mass, volume, power, and thermal requirements associated with space vehicles. The Moon also offers a gravity gradient which will make the engineering of systems involving fluid flow much easier. The 1/6th g of the moon might also be ideal for breeding varieties of food plants to maximize fruit production over stem rigidity, for example.

Assuming the growth of plants at a lunar base is inevitable, the question of the required technology arises. Terrestrial agricultural practices are well developed, and it seems fitting that as humans seek to establish a colony on the Moon that they take a familiar practice with them.

Terrestrial Soil

On Earth, in addition to the absence of plant disease and insect pests, there are six external factors that affect the growth of all plants. These factors are: (1) light, (2) mechanical support, (3) heat, (4) air, (5) water, and (6) nutrients [1]. Soil is integral to each of these factors except light. Robust plant growth is dependent on

a favorable combination of these factors; and if one is out of balance, plant growth can be restricted or even totally inhibited. Furthermore, whichever factor is least optimum determines the maximum level of plant growth; this is especially true with respect to nutrients. Five of these six plant growth factors are influenced to some degree by both physical and chemical properties of the soil. For example, soil texture and structure influence air, water and heat gradients within the soil profile. Soil solution pH, ion concentration, ion balance, and activity are important chemical properties.

The sixteen essential nutritive elements needed by plants and their sources are listed in Table 1. Of the elements obtained from the soil by plants, six are used in relatively large quantities (macronutrients): nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur. Plant growth can be limited by a deficiency of any one or a combination of these elements in the soil. Each nutrient must exist in a form available to the plant; and each nutrient must be balanced with respect to the others. The remaining nutrients, while essential to plant growth, are used in much smaller quantities, and are called micronutrients or trace elements (Table 1).

Four areas must be examined with respect to plant growth nutrients and their potential deficiencies. They are: (1) the macronutrient content of the mineral portion of the soil, (2) the ionic form of the nutrient in soil solution, (3) the process by which nutrients become available to plants, and (4) the soil solution and its pH. The mineral portion is established geologically and weathers to yield the various compounds and ions. The ionic form of the plant growth nutrients that are in the soil solution or on the soil colloids (Table 2) are important from the standpoint of being available for uptake into the plant. Some ionic forms of the elements are preferentially absorbed by plants. In addition to physical weathering of soil minerals and those derived from the atmosphere, plant growth nutrients are supplied by microbiological degradation of organic matter (nutrients are also added via commercial fertilizers).

TABLE 1. Essential plant growth elements and their sources [1].

Elements Used in Relatively Large Amounts		Elements Used in Relatively Small Amounts	
Mostly from			
<u>Air and Water</u>	<u>Soil Solids</u>	<u>Soil Solids</u>	
Carbon	Nitrogen	Iron	Copper
Hydrogen	Phosphorous	Manganese	Zinc
Oxygen	Potassium	Boron	Chlorine
	Calcium	Molybdenum	
	Magnesium		
	Sulfur		

TABLE 2. Important ions present in the soil solution or on the soil colloids [1].

Element	Solution Species	Element	Solution Species
N	NH_4^+ , NO_2^- , NO_3^-	Ca	Ca^{2+}
P	$\text{H}_2\text{PO}_4^{2-}$, H_2PO_4^-	Mg	Mg^{2+}
K	K^+	S	SO_3^{2-} , SO_4^{2-}
Fe	Fe^{2+} , Fe^{3+}	Zn	Zn^{2+}
Mo	MoO_4^{2-}	B	H_2BO_3^- , H_3BO_3
Mn	Mn^{4+} , Mn^{2+}	Cl	Cl^-
Cu	Cu^+ , Cu^{2+}	water	H^+ , OH^-

The soil solution is the water in which the dissolved ionic forms of plant nutrients reside. The concentration of ions in the soil solution changes as the volume of water changes and also as the nutrients are added or removed. Another important aspect of the soil solution is its pH. Soil pH ranges from 5 to 7 in humid region soils, and 7 to 9 in arid region soils with extremes near 3.5 to over 10. The importance of pH rests with its effect on the availability of several of the essential nutrients as well as the solubility of certain elements toxic to plant growth. Iron, manganese, and zinc tend to become less available as the pH rises from 5.0 to 8.0. Conversely, molybdenum is increasingly available at these higher pH's. At pH values below approximately 5.0, aluminum, chromium, iron, and manganese are often soluble in quantities sufficient to be toxic to the growth of some plants.

Lunar Regolith

Both the lunar regolith and its parent bedrock are composed (like Earth rocks) mainly of small crystals (minerals) that are chemical compounds formed by the combination of metal elements with oxygen (see Table 3). These minerals are of two principal types: (1) silicates in which various metals (calcium, iron, aluminum, magnesium) are combined with networks of oxygen and silicon atoms; and, (2) oxides in which metal (titanium and iron, primarily) are combined directly with oxygen atoms. The lunar regolith also contains free iron metal (0.1 to 1.0 percent) and iron sulfides. These substances occur as small particles derived from both the lunar bedrock and from impacting meteorites. This metal also contains significant amounts of nickel, cobalt and chromium.

The most common components of the lunar regolith are minerals (and glasses) made of silicates (see Table 3). These minerals are identified as: pyroxene (calcium-iron-magnesium aluminosilicates, e.g., enstatite,

TABLE 3. Lunar minerals [2].

Major Minerals ^a	Minor Minerals ^b
Olivine (Mg,Fe) ₂ SiO ₄	Spinel (Fe,Mg,Al,Cr,Ti)O ₄
Pyroxene (Ca,Mg,Fe)SiO ₃	Armstrongite (Fe ₂ TiO ₅)
Plagioclase feldspars (Ca,Na)Al ₂ Si ₂ O ₈	Silica (quartz, tridymite cristobalite) SiO ₂
	Iron Fe (variable amounts of Ni and Co)
	Troilite FeS
	Ilmenite FeTiO ₃
Trace Minerals ^c	
Phosphates	Oxides
^d Apatite Ca ₅ (PO ₄) ₃ (F,Cl) ₃	Rutile TiO ₂
^d Whitlockite Ca ₉ (Mg,Fe)(PO ₄) ₇ (F,Cl)	Corrundum (?) Al ₂ O ₃
	Hematite (?) Fe ₂ O ₃
	Magnetite Fe ₃ O ₄
	Goethite (?) FeO(OH)
Zr Minerals	Metals
^d Zircon ZrSiO ₄	Copper (?) Cu
Baddeleyite ZrO ₂	Brass (?)
	Tin (?) Sn
Silicates	Zr-rich Minerals
Pyroxferroite (Fe,Mg,Ca)SiO ₃	^d Zirkelite or zirconolite
Amphibole (Ca,Mg,Fe)(Si,Al) ₈ O ₂₂ F	CuZrTi ₂ O ₇
Garnet (?)	Meteoritic Minerals
^d Tranquillityite Fe ₈ Zr ₂ Ti ₃ Si ₃ O ₄	Schreibernite (Fe,Ni) ₃ P
	Cohenite (Fe,Ni,Co),C
Sulfides	Ninningerite (Mg,Fe,Mn)S
Mackinawite (Fe,Ni) ₉ S ₈	Lawrencite (?) (Fe,Ni)Cl ₂
Pentlandite (Fe,Ni) ₉ S ₈	
Chalcopyrite CuFeS ₂	
Cubanite CuFe ₂ S ₃	
Sphalerite (Zn,Fe)S	

^a Major minerals may occur in concentrations up to 100%.

^b Minor minerals generally occur at less than 2 percent.

^c Trace minerals never exceed a few tenths of a percent.

^d These minerals are known to exhibit complete substitution, particularly of elements like Y, Nb, Hf, U, and the rare earth elements that are concentrated in these minerals.

? Controversial with respect to indigenous lunar origin.

wollastonite, ferrosilite), feldspar (calcium aluminosilicates, anorthite or plagioclase), and olivine (iron-magnesium aluminosilicates, forsterite or fayalite). These different materials occur in the lunar regolith as fine particles. While the regolith does contain boulder, cobble, and chip sized materials, the average grain size of the portion of the lunar regolith that passes through a 1 mm sieve is only about 0.045 to 0.1 millimeters [2].

Most of the particles in the lunar regolith are complex, composed of mixed glasses and mineral fragments. These particles are called agglutinates and are produced by the melting and mixing caused by micrometeorite impacts. Agglutinates are frequently porous. Embedded in them are small quantities of gas (volatiles) implanted by the solar wind. The small size and irregular shape of these agglutinate particles are largely responsible for the strongly cohesive character of the lunar regolith.

Since individual particles in the lunar regolith have chemical compositions that vary more widely than does that of the bulk regolith itself, it may be possible to obtain several chemically different feedstocks by processing the regolith to separate out and concentrate particular components. Such processes may be pre-treatments to the formation of a viable agricultural substrate. A feldspar-rich concentrate would have a higher proportion of calcium and aluminum than would the bulk soil. Similarly, an ilmenite-rich concentrate from a basalt lava would be an improved source of iron for soil development and a source of titanium, as a by-product, for other lunar applications. The traces of iron metal in the soil might be directly concentrated by magnetic methods or other innovative techniques. Agglutinate particles might be concentrated as a source of volatile materials, although their content of solar wind gases is relatively small [2].

On the basis of chemical composition and available tonnages, lunar resources could well form the basis of an extensive manufacturing technology and similarly promote the formation of an agricultural enterprise to support man's endeavors in space. What is now lacking is the technology needed to extract the essential chemical elements for these manufacturing and agricultural undertakings.

Comparison of Terrestrial Soil and Lunar Regolith

A comparison of the concentration of the sixteen essential plant growth nutrients in terrestrial soils, plants, and in the lunar regolith is shown in Table 4. Carbon, hydrogen, and oxygen are obtained by plants from the Earth's atmosphere and water; these primary plant-essential elements are very abundant in the Earth's air and water. Carbon and hydrogen are in very short supply on the Moon; however, there is approximately 40% oxygen by weight in the lunar regolith.

The lunar regolith contains much more Ca, Mg, and S than do terrestrial soils and with exposure to a moist, aerobic environment, dissolution of these macronutrients should take place quite easily and in sufficient quantities for plant growth. High levels of P are contained in the lunar regolith in the mineral apatite; however, this mineral has a low solubility in the near-neutral pH of the lunar soil (pH of 7.38 in deionized water and 6.32 in CO₂ - charged water; [3]) and dissolution rates are likely to be slow. The lunar regolith contains about half as much K as do terrestrial soils and might require the addition of fertilizer K to insure good plant growth. Of

TABLE 4. Comparison of essential plant growth nutrients in terrestrial soils, plants, and lunar regolith [1, 11].

Element	Terrestrial ^a		Plants	Lunar ^b	
	wt. %	kg m ⁻³	wt. %	wt. %	kg m ⁻³
Macronutrients					
Carbon ^c	atm	atm	18	0.011	0.18
Hydrogen ^c	atm	atm	8	0.0055	0.09
Oxygen ^c	atm	atm	70	40	664
Nitrogen	0.14	2.0	0.3	0.01	0.17
Phosphorous	0.06	0.8	0.07	0.4	6.64
Potassium	0.83	11.6	0.3	0.4	6.64
Calcium	1.4	20	0.5	9.0	149
Magnesium	0.5	7	0.04	6.0	99.6
Sulfur	0.07	1	0.07	0.5	8.3
Micronutrients					
	ppm	kg m ⁻³	ppm	ppm	kg m ⁻³
Iron	38000	53	100	90000	149
Manganese	800	1.1	10	2000	3.3
Boron	10	0.014	10	20	0.03
Molybdenum	3	0.004	0.1	1	0.002
Copper	20	0.03	2	13	0.022
Zinc	50	0.07	5	28	0.046
Chlorine	100	0.14	200	26	0.043

^aAverage density of cropped soils taken to be 1.4 g cm⁻³

^bAverage density of lunar regolith in the 0-60 cm depth taken to be 1.66 g cm⁻³

^cPlants obtain these nutrients mainly from air and water; the other nutrients are obtained from the soil.

the micronutrients, only Cl is present in appreciably lower concentrations on the Moon than in terrestrial soils but since it is one of the micronutrients required by plants in quite small quantities it should not represent a serious problem.

Factors such as pH, Eh, and mineralogical composition will affect concentration and ionic species of elements potentially toxic to plants. Table 5 lists thirteen elements that have been shown to be toxic to plants and their total soil and soil solution concentrations. A high total concentration does not necessarily mean that

the element will be toxic. Other factors such as mineral or ionic form of the element and the plant species grown, along with soil factors such as composition, pH, Eh, and moisture status all affect the element's ability to be absorbed by the plant [4].

Of the toxic elements listed in Table 5, only Cr and Ni are present in the lunar regolith in concentrations appreciably higher than in terrestrial soils. Terrestrial concentrations of Cr and Ni are approximately 20 and 40 ppm (or mg/kg), respectively, while lunar concentrations of Cr range from 479 to 6705 ppm and Ni from 55 to 720 ppm [5]. Within the normal range of pH and Eh in terrestrial soils, chromium can exist as the Cr^{3+} cation and the CrO^- anion, and two hexavalent states, the $\text{Cr}_2\text{O}_7^{2-}$ and CrO_4^{2-} anions [6]. The hexavalent state appears to be more toxic to plants than the trivalent state [7]. Trivalent chromium is more common in soils than is Cr(VI) since chromium is quite easily reduced; however, Bartlett and James [8] have shown that oxidation of Cr(III) to Cr(VI) does take place in aerobic, non-acid soils. According to Bohn et al. [9], Cr(VI) stability increases with increasing pH. Since the chromium content is high in the lunar regolith and the pH is near neutral, chromium toxicity will have to be addressed.

Nickel toxicity in lunar soils is less likely than chromium toxicity, but the potential exists in those lunar soils containing the higher levels of nickel. In some areas nickel toxicity has been shown where concentrations as high as 8000 ppm total Ni have been measured. Slingsbury and Brown [10] have suggested that increasing

TABLE 5. Concentrations of plant toxic elements in terrestrial soils and plants [9].

Element	Total Soil		Soil Solution	Plants Range
	Typical Value	Range		
	mg kg ⁻¹	mg kg ⁻¹	mg L ⁻¹	mg kg ⁻¹
Al	50,000	10,000-200,000	0.1-0.6	---
As	5	1-50	0.1	---
Be	1	0.2-10	0.001	---
Cd	0.06	0.01-7	0.001	0.1-0.8
Cr	20	5-1000	0.001	---
Co	8	1-40	0.01	0.05-0.5
Cu	20	2-100	0.03-0.3	4-15
Pb	10	2-200	0.001	0.1-10
Mn	850	100-4000	0.1-10	15-100
Hg	0.05	0.02-0.2	0.001	---
Ni	40	10-1000	0.05	1
Se	0.5	0.1-2.0	0.001-0.01	---
Zn	50	10-300	<0.005	8-15

the soil pH and organic matter content might reduce the nickel availability to plants. With respect to use of lunar regolith as a plant growth medium, nickel toxicity may be a problem only if the pH of the soils decreases over time and nickel concentrations are high.

SOLID-SUPPORT SUBSTRATES

Native Lunar "Soils"

Weathering is the action of the elements in altering the color, texture, composition, or form of exposed objects; specifically, the physical disintegration and chemical decomposition of Earth materials at or near the Earth's surface. Weathering is basically a combination of destruction and synthesis. Rock fragments and the minerals contained in them are attacked by weathering forces and are changed into new minerals (secondary minerals) either by minor modifications (alterations) or by complete chemical changes [1]. These changes are accompanied by a release of soluble constituents into the soil solution. The minerals which are synthesized are in two groups: 1) the silicate clays, and 2) the very resistant end products, including iron and aluminum oxides. Two basic processes are involved in the weathering process: mechanical and chemical. Mechanical or disintegration takes place as a result of temperature fluctuations (differential expansion, frost action, and exfoliation), erosion and deposition by water or wind, and plant or animal influences. Chemical processes include hydrolysis, hydration, carbonation and related acidity processes, oxidation, and solution.

Mechanical forces of major impact events were once very active on the moon. Micrometeorite impacts still play an important role in mechanically altering lunar surface materials. Since the Moon is essentially devoid of water, the chemical processes of terrestrial weathering are not at work. However, when we establish an Earth-like environment we can introduce and control an environment of our choosing. This presents a host of questions concerning the lunar regolith's response to such Earth-like environments which we can impose, control and change. Parameters such as temperature, atmospheric composition and pressure, and weathering solvents can be controlled as a function of time. Specific environments can be introduced to perform certain functions and then changed to perform another function; eventually getting to the point of having a true lunar "soil" capable of growing plants.

In work currently underway at the Johnson Space Center, experiments are being conducted to examine the effects of various solvents on simulated lunar regolith as a function of time. Solvents range from water to solvents representing a range of complexing abilities and include humic and fulvic acids extracted from terrestrial soil samples. Humic and fulvic acids are complex organic acids common in the root zone environment of plants and promote good soil structure, increase cation exchange capacity, improve pH buffering, and increase water holding capacities [11]. Since hydrolysis, the reaction of a substance with dissociated ions of water (H^+ and OH^-), is the most important terrestrial weathering reaction, special emphasis is being placed on it.

Simulated lunar material (and later actual lunar material) is being subjected to a complement of weathering environments ranging from relatively mild conditions such as those under a growing crop to much harsher conditions of low pH, high temperature, and increased pressures. Weathering products and the leachates are being analyzed to determine modifications induced by such environments.

Workshop

A workshop entitled "Lunar Derived 'Soils' for the Growth of Higher Plants" was held at the Johnson Space Center in Houston, Texas on June 1-2, 1987. The workshop was sponsored by the Solar System Exploration Division at JSC and the NASA-Headquarters CELSS Program within the Life Sciences Division. The more than 100 participants represented over 25 different universities, companies and federal agencies with a diverse range of agricultural and related disciplines. Soil and crop science, microbiology, biology, geology and lunar science, and engineering disciplines were well represented. A book describing the results of the workshop is currently in preparation and will be published by the American Society of Agronomy in the summer of 1989 [12]. Soil scientists were in general agreement that lunar material "has the potential to be an excellent medium for the growth of higher plants" and that the lunar regolith "when exposed to a moist, aerobic, Earth-like environment, can be the source for many of the plant essential nutrients" [13]. One reservation that both the soil scientists and soil microbiologists had was potential plant toxicities due to chromium and nickel. Microbiologists were of the opinion that microbial species could play an important role in beneficiation of the regolith. Recovery of useful constituents (ranging from oxygen and hydrogen to chromium and nickel), removal of materials potentially toxic to plants and other microbial species, and development of a highly productive soil for food production were discussed [14].

Only one major unanticipated item surfaced during the workshop; the emphasis placed on the need for simulated lunar regolith. The shared opinion was that a set of lunar simulants with varying degrees of fidelity was needed in order to carry out this complex line of research. Lunar samples returned during the Apollo missions are an extremely valuable resource and can only be expected to be made available in extremely small amounts for specific experiments after the experimental procedure has been fully verified. Thus, initial experiments and experiments requiring relatively large amounts of material (say, larger than 5 grams) must be conducted with simulated lunar material.

Lunar "Soil" Simulants

The lunar samples returned during the Apollo program represent an extremely valuable resource and are made available to researchers only in very small quantities. Even then, researchers are usually required to fully verify their experimental procedures with some substitute material before using actual lunar material. Typical allocations of lunar sample are in the tens of milligrams quantities. Thus, experiments requiring sample

material of greater than say 5 grams are forced to resort to use of some sort of simulated material. At this time, the only known source of simulated lunar material is from Drs. Paul Weiblen and Ken Reid of the University of Minnesota [15]. They have been preparing relatively large amounts of lunar soil simulant from a fine-grained basaltic rock that closely resembles the Apollo 11 high-titanium basalts in mineralogy and bulk chemistry. The crushed and ground material has the size distribution of the Apollo 11 soil samples. They have also successfully produced agglutinate-like material using a plasma arc melting technique. At present, this is the only source of any type of lunar simulant.

There are limitations to the degree of fidelity any simulant can achieve. Even the returned Apollo samples were altered when they were removed from the lunar surface; most notably, in terms of the *in situ* characteristics of bulk density and stratigraphy. The least physically disturbing method of sampling the lunar soil was with the large diameter core tubes used on Apollo 15, 16, and 17 [16]. The lunar samples undergo further changes in the laboratory since the lunar conditions of hard vacuum and freedom of water molecules and other atmospheric gases cannot be maintained on Earth. In the lunar sample curatorial facility 'pristine' samples are stored and handled only under dry nitrogen. Despite this, small amounts of water and other gases are probably adsorbed on the highly reactive surfaces of lunar soil grains.

Simulating the lunar soil for laboratory experimentation is approached from three aspects: soil grain size distribution, soil particle type distribution, and particle chemistry [17]. Grain size distribution curves have been determined for most Apollo soils [18]. The grain size distribution of simulants should be prepared with the fewest sieve sizes that adequately characterize the grain size distribution curve and yet are practical to use. For example, simulant composition should be defined as 90 percent finer than 1 mm, 75 percent finer than 0.25 mm, and 50 percent finer than 0.075 mm.

Most particles comprising the lunar soil are igneous or breccia lithic grains, mineral grains, glass fragments, and agglutinates. A simulant could be prepared with crushed basalt or minerals to substitute for the lithic and mineral fragments and by using crushed glass to substitute for the glass fragments and agglutinates. Glasseous basaltic melts have been prepared in small quantities in the laboratory using a formula or recipe developed by Williams (personal communication, 1987). These glasses are difficult and time consuming to prepare and can be made only in small quantities (20 - 30 grams) since high temperature furnaces and Platinum crucibles must be used. Large quantities of glass can be made by commercial glass companies. Approximately 90 kilograms of 11-component glass can be produced under controlled conditions in one company's laboratory. The 11 lunar components are: silicon, iron, aluminum, calcium, magnesium, titanium, sodium, phosphorous, manganese, potassium, and chromium. Each will be added as the oxide in the correct proportions based on actual analyses of selected lunar glasses. Two glass starting materials have been prepared by a commercial glass company for our lunar "soil" dissolution studies at JSC. Chemical compositions of the glass (see Table 6) prepared by the glass company have not yet achieved the analogous compositions of lunar glasses; however, we are working with the company to improve the fidelity of the lunar simulant.

For lunar soil dissolution studies, we are using the fine-grained basaltic rock simulant (along with the agglutinate-like melt which will be obtained from the University of Minnesota) and the 11-component glassy melt obtained from the research laboratory of the commercial glass company. The material has been ground to a selected particle size distribution (one matching an actual measured lunar size distribution) and mixed in the appropriate proportions. This mixture will represent the 'baseline simulant' for current and future research and will allow for comparability of results between experiments. Simulants representing other lunar samples and other degrees of fidelity (such as solar wind implanted ions) will be addressed on an as-needed basis.

TABLE 6. Chemical compositions of lunar analog glasses prepared by commercial glass company. Lunar glass simulants B-1, B-2D, and B-2P are being stored at the Johnson Space Center in environmental glove boxes.

Oxides	Requested ^a	B-1	B-2D	B-2P
----- wt. % -----				
SiO ₂	42.1	45.75	47.93	50.47
Al ₂ O ₃	13.9	10.18	12.99	12.35
TiO ₂	7.52	5.37	6.62	6.43
FeO	15.7	22.84	12.81	12.09
MnO	0.20	0.16	0.16	0.14
MgO	7.92	5.78	6.89	6.51
CaO	12.1	8.57	10.39	9.94
Na ₂ O	0	0.04	0.03	0.03
K ₂ O	0	0.03	0.05	0.04
Cr ₂ O ₃	0.30	0.21	0.22	0.27
P ₂ O ₅	0.10	0.12	0.15	0.15
Total	99.84	99.05	98.24	98.36

^aOxide compositions requested by JSC to commercial glass company. Chemical compositions were determined by electron microprobe analysis.

Synthetic or Manufactured "Soils" at a Lunar Base

Lunar materials as they now exist may not provide an adequate growth medium for higher plants for several reasons. The poor physical structure of lunar regolith may prevent proper aeration and water movement through the lunar materials. Minerals and materials that comprise the lunar surface may have little nutrient supplying power or retention capacity for plant-essential elements. Also, the toxicity of lunar materials to plants is not well known. For example, high Cr and Ni contents in lunar materials may be toxic to plants. It may be necessary therefore to alter lunar materials or prepare "soils" that will be productive for plant growth.

Advantages and Disadvantages of a Synthetic "soil"

Synthetic soils are attractive because the physical, chemical, and mineralogical properties of lunar materials may be altered to best suit plant growth. Lunar regolith is a fine-grained deposit, which is primarily a result of meteorite impacts on the surface. The mean grain size of lunar "soils" ranges between 0.04 and 8 mm, however, the majority of the particles fall between 0.045 and 0.1 mm (*vide supra*). The regolith is poorly sorted with no "soil" structure and average bulk densities range from 1.50 to 1.74 Mg m⁻³. These "soil" physical conditions may not be suited for plant growth due to the high bulk density of the regolith. A synthetic or manufactured soil could be sized and prepared to best suit the physical and chemical properties necessary to maximize plant growth.

Lunar regolith consists of mineral (e.g., plagioclase feldspar, pyroxene, olivine, ilmenite) and glass phases. The glass phases have formed primarily by meteorite impact melting and should be the most reactive regolith phases in water. Elements toxic to plants may exist in high enough concentrations in glasses to be detrimental to plant growth. Lunar materials may be found that do not contain high levels of plant-toxic elements on the surface. However, the easiest way to avoid toxicity problems may be to synthesize a soil without these elements. One of the first resources to be extracted lunar from materials will be oxygen, which will be used for rocket propellant and in life-support systems. A number of by-products will be produced during oxygen production that may not contain plant-toxic elements; therefore, these by-products may be desirable materials to use as a solid-support substrates for plant growth.

Minerals that exhibit cation exchange as a result of isomorphic substitution (e.g., phyllosilicates) do not exist on the Moon. The lunar regolith therefore lacks retention for plant-essential cations. It may be possible to alter regolith glasses to form minerals with cation exchange capacities (CECs). With this problem in mind, Ming and Lofgren [19] have synthesized minerals with CECs (e.g., smectites, zeolites, tobermorites) from lunar-analog glasses (Fig. 1). These reactive minerals may be added to other regolith materials to create a soil that will have a retention capacity for plant-essential elements.

There are drawbacks to manufacturing a soil at a lunar base. Special equipment to synthesize soils may need to be shipped to the moon. Initially, the cost of shipping equipment and resources required to produce a

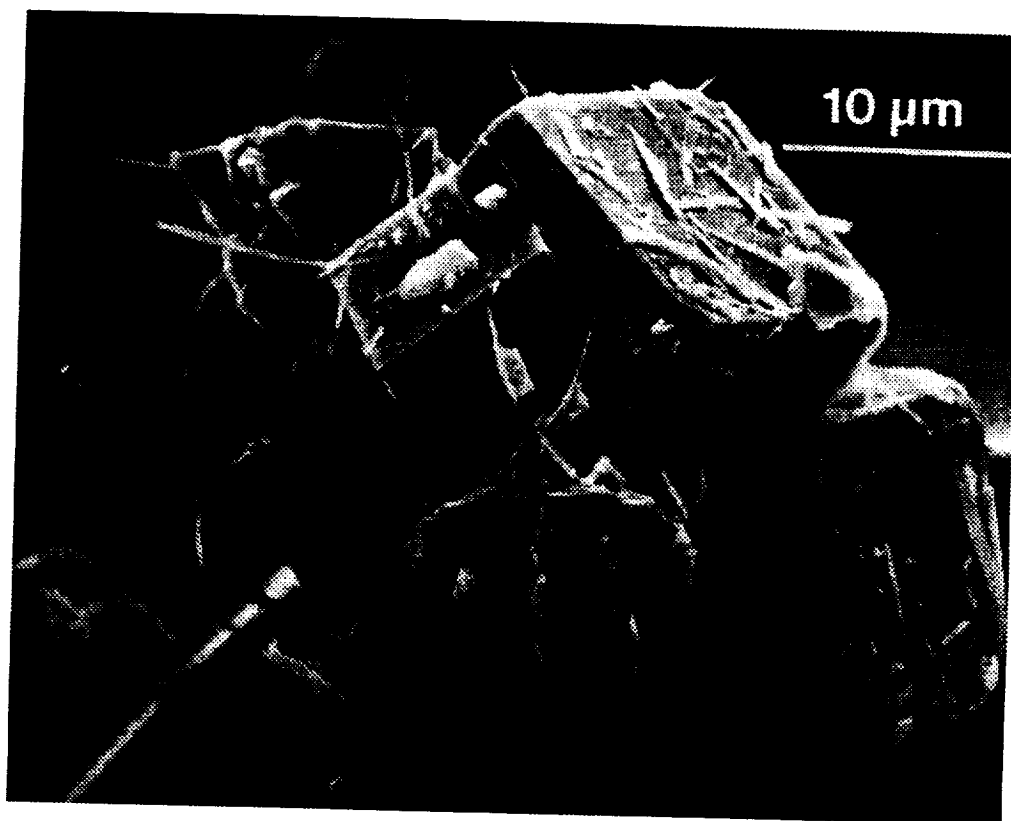


FIGURE 1. Scanning electron micrograph of Zeolite A (twinned cubes in upper center of micrograph) and Zeolite P_t (bipyramidal-like crystals in lower center of micrograph). These zeolites were synthesized under mild hydrothermal conditions from synthetic basaltic glass with chemical compositions analogous to basaltic glass of the lunar highlands. (Hydrothermal conditions: temp. = 150 C, solution = 0.1 M Na₂SiO₃·5H₂O, duration = 76 h, press. = ~0.3 MPa, glass/solution mass ratio = 0.015). See Ming and Lofgren [19] for experimental procedures.

soil may not be economically feasible. The synthesis of most minerals that exhibit CECs will require water as a solvent; however, there are potential methods to synthesize minerals that exhibit CEC without water as a solvent (*vide infra*). Because the moon is devoid of water, materials to make water (e.g., H) may have to be shipped to the moon; or water will have to be made on the lunar surface from regolith oxygen and solar wind implanted hydrogen. Oxygen production on the lunar surface should not be a problem; however, small concentrations of hydrogen in lunar materials (generally < 50 ppm) could preclude the economical production of water using solar-implanted hydrogen.

No doubt, the samples returned by the Apollo missions have provided us with a wealth of information on the physical, chemical, and mineralogical properties of lunar materials. However, finding the starting materials best suited for synthetic soils may have to wait until we go back to the Moon and conduct a thorough

survey of lunar resources. Until then, we will have to use existing chemical and mineralogical data on lunar materials to design synthetic soil systems.

Synthetic "Soils"

Four primary functions of a root media should be considered when developing a synthetic lunar soil--nutrient retention, aeration, moisture retention, and mechanical support. Most terrestrial potting media have organic matter that provides nutrient and water retention. The production of organic components for a root media at a lunar base will be difficult because of the very small quantities of organic compounds present in the regolith. Therefore, several hypothetical, plant-growth systems in inorganic, solid-support substrates have been proposed by the authors in this paper; however, a considerable amount of basic research is necessary before these systems can be used to their fullest potential at a lunar base or even in terrestrial applications.

Zeoponics

The term "zeoponics" was first used by Parham [20] to describe an artificial soil that consisted of zeolites, peat, and vermiculite used by Bulgarian researchers. Zeolites are hydrated aluminosilicates of alkali and alkaline-earth cations (e.g., K^+ , Na^+ , Ca^{2+} , Mg^{2+}) that possess infinite, three-dimensional crystal structures (i.e., tektosilicates). The primary building units of the zeolite crystal structure are $(Al,Si)O_4$ tetrahedra. When Al^{3+} and sometimes Fe^{3+} substitute for Si^{4+} in the central cation position of the tetrahedron, a net-negative charge is generated. This negative charge is counterbalanced primarily by monovalent and divalent cations (generally called "exchange cations"). Zeolites have the ability to exchange most of their constituent exchange cations as well as hydrate/dehydrate without major change of the structural framework. There have been about 50 zeolites found in nature, and several hundred synthetic species have been made in the laboratory. Natural zeolites can have CECs of 200 to 300 $cmol_c kg^{-1}$, whereas some synthetic zeolites have CECs as high as 600 $cmol_c kg^{-1}$ (see Table 7). The chemical and mineralogical properties of zeolites have been reviewed by Ming and Mumpton [21], Gottardi and Galli [22], and Breck [23].

Most zeolites have large channels and/or cages which allow easy access of exchange cations, including plant-essential cations, to sites of charge (Fig. 2). Zeolites have unique cation selectivities that depend upon a number of factors: 1) framework topology (channel configuration and dimensions), 2) size and shape of the exchange ion(s), 3) charge density in the channels and cages, 4) valence and charge density of the exchange ion(s), and 5) electrolyte composition and concentration in the external solution [24].

Based upon these unique properties of natural and synthetic zeolites, a number of agricultural scientists have examined potential uses of zeolites, including 1) slow-release fertilizers (see Table 8), 2) traps for heavy metal ions (e.g., Cd, Pb, Zn) in soils [25, 26, 27, 28, 29], 3) dietary supplements in animal nutrition [30, 31];

TABLE 7. Representative unit cell formulae and selected physical and chemical properties of minerals that exhibit cation exchange.

Special Purpose Minerals	Representative Unit-cell Formulae ^a	Typical Void Volume (%)	Theoretical Cation Exchange Capacity (cmol _c kg ⁻¹)
Zeolites			
Analcime	$\text{Na}_{16}\{\text{Al}_{16}\text{Si}_{32}\text{O}_{96}\}\cdot 16\text{H}_2\text{O}$	18	460
Chabazite	$(\text{Na}_2,\text{Ca})_6\{\text{Al}_{12}\text{Si}_{24}\text{O}_{72}\}\cdot 40\text{H}_2\text{O}$	47	420
Clinoptilolite	$(\text{Na}_3\text{K}_3)\{\text{Al}_6\text{Si}_{30}\text{O}_{72}\}\cdot 24\text{H}_2\text{O}$	34	220
Mordenite	$\text{Na}_8\{\text{Al}_8\text{Si}_{40}\text{O}_{96}\}\cdot 24\text{H}_2\text{O}$	28	220
Phillipsite	$(\text{Na},\text{K})_5\{\text{Al}_5\text{Si}_{11}\text{O}_{32}\}\cdot 20\text{H}_2\text{O}$	31	380
Linde Type A	$\text{Na}_{96}\{\text{Al}_{96}\text{Si}_{96}\text{O}_{384}\}\cdot 216\text{H}_2\text{O}$	47	540
Linde Type X	$\text{Na}_{86}\{\text{Al}_{86}\text{Si}_{106}\text{O}_{384}\}\cdot 264\text{H}_2\text{O}$	50	470
Phyllosilicates			
Vermiculite	$\text{Mg}_{0.4}(\text{Mg},\text{Fe}^{2+})_3(\text{Al}_{0.8}\text{Si}_{3.2})\text{O}_{10}(\text{OH})_2\cdot n\text{H}_2\text{O}$	--	160 ^c
Smectite ^b	$\text{Ca}_{0.25}(\text{Al}_{1.5}\text{Mg}_{0.5})\text{Si}_4\text{O}_{10}(\text{OH})_2\cdot n\text{H}_2\text{O}$	--	110 ^c

^aTaken mainly from Breck [23]

^bMontmorillonite

^cAlexaides and Jackson [56]

4) nutrient retention and odor control in animal manures [32, 33; P. van Straaten, 1985, personal communication]; and 5) other agricultural uses [31, 34].

Zeolites were first suggested as major components of soils in the mid-1800s. This misconception was based upon the similar exchange behavior of zeolites and soils. In fact, Breazeale [35] showed how K-zeolites and soil exchange complexes behaved in a similar fashion. Breazeale's experiments were probably the first conducted on plant growth in a zeoponic system. However, the introduction of modern X-ray diffraction in the late 1920s disproved the hypothesis that zeolites were major components of soils and, in fact, proved that phyllosilicates, primarily smectites, were responsible for the CEC in soils. Plant growth in zeolite substrates was essentially ignored until the 1980s. Recently, several studies have investigated the potential use of zeolites as slow-release fertilizers or in-soil reservoirs for NH_4^+ and K^+ (see Table 8); however, little has been done

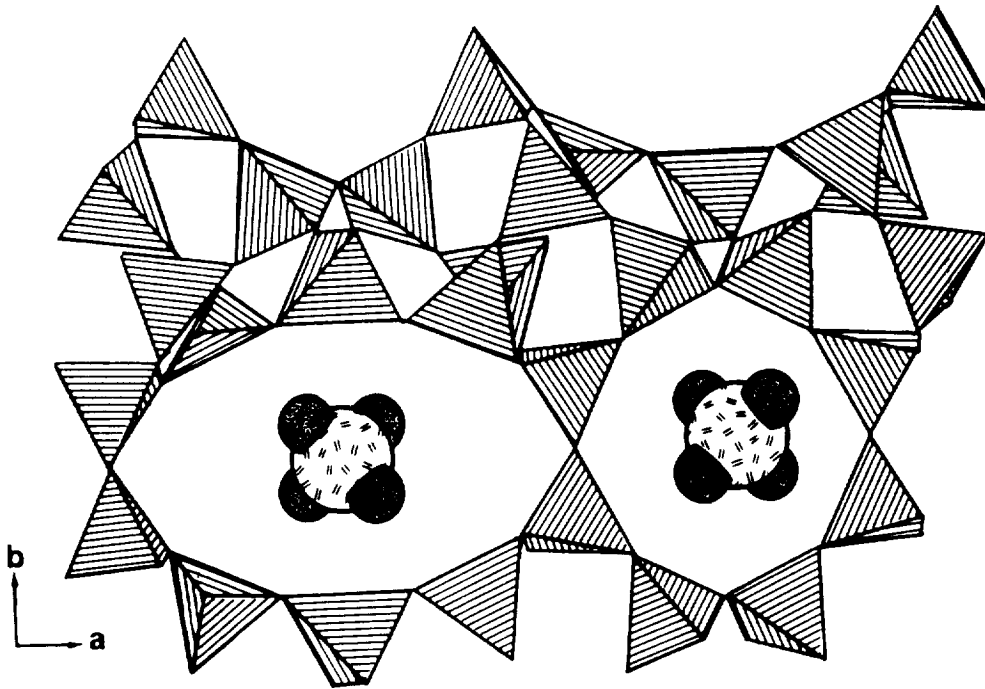
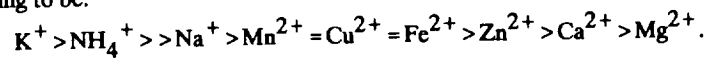


FIGURE 2. Clinoptilolite structure showing 10- and 8-ring channels (modified from Koyama and Takeuchi, [67]). Ammonium ions readily access channels.

using zeolites as a substrate by themselves. Although little information is available, Bulgarian researchers [36] reported the use of clinoptilolite (a highly-siliceous natural zeolite with a CEC around $200 \text{ cmol}_c \text{ kg}^{-1}$) as a raw material for plant substrates. The zeolitic substrate was found to act as a reservoir for nutrient cations, to have desirable strength and other physical properties, to be sterile with respect to pathogenic microorganisms, and to be aesthetically pleasing. Depending on the plant variety, 20-150% increases in yields over control plots were observed for tomatoes, strawberries, peppers, and rice. Also, the ripening of rice, cotton, and tomatoes was accelerated in the zeolite substrate.

Zeoponics is only in its developmental stages at the NASA Johnson Space Center. In this work, zeoponics has been defined as the cultivation of plants in zeolite substrates that 1) contain essential, plant-growth cations on their exchange sites, and 2) have minor amounts of mineral phases (e.g., apatite) and/or anion-exchange resins (e.g., activated aluminum resins) that supply essential, plant-growth anions (e.g., H_2PO_4^-). A zeoponics system is illustrated in Fig. 3. It is possible to saturate partially or wholly plant-essential cations (e.g., NH_4^+ , K^+ , Mn^{2+} , Cu^{2+} , Fe^{2+} , Zn^{2+} , Ca^{2+} , Mg^{2+}) on clinoptilolite. Ming et al. [37] have determined the apparent selectivity of plant-essential cations and Na for native cations in clinoptilolite from Oregon and Wyoming to be:



The dissolution of apatite is accelerated in a zeolite system [38, 39, 40, 41]. Apparently, the zeolitic exchange sites act as sinks for Ca^{2+} released into solution by the dissolution of apatite. Once Ca^{2+} is removed

TABLE 8. Recent studies using zeolites as slow release fertilizers or in-soil reservoirs for several plant essential cations.

Cation(s)/Zeolite-Mineral	Reference
NH ₄ /clinoptilolite	Pirela et al. [57] Mackown and Tucker [58] Ferguson et al. [59] Ferguson and Pepper [60] Bartz and Jones [61] Lewis et al. [62] Weber et al. [63]
NH ₄ ,K/clinoptilolite	Iskenderov and Mamedova [64]
K/zeolites	Hershey et al. [65]
NH ₄ ,K,Zn/clinoptilolite	Lewis [66]
NH ₄ /clinoptilolite-apatite	Lai and Eberl [38], Barbarick et al. [39]
NH ₄ /zeolites-apatite	Chesworth et al. [40]
K,NH ₄ /clinoptilolite-apatite	Allen and Hossner [41]
NH ₄ ,K,Mn,Cu,Fe,Zn,Ca,Mg/clinoptilolite	Ming et al. [37]
Zeolitic substrates	Stoilov and Popov [36]

from solution by the zeolite, the dissolution of apatite proceeds, thereby causing the release of phosphate into solution. In zeoponic systems, Ca²⁺ released from apatite should compete with plant-essential cations located at zeolitic sites. Once released into solutions, these plant-essential cations become available for plant uptake.

A NASA Graduate Student Fellow at Texas A&M University has been conducting research in our laboratories at JSC on the zeolite/apatite system [41]. Chemical equilibrium relationships between the zeolite clinoptilolite and apatite have been investigated using batch equilibrium experiments. Sufficient levels of N, P, K, and Ca were supplied to solution in the zeolite/apatite system. The concentrations of nutrients can be adjusted by changing the type of apatite, varying the ratio of clinoptilolite to apatite, or by varying the ratio of exchangeable K⁺ to exchangeable NH₄⁺ on the zeolitic exchange sites. The equilibrium solution P concentrations were from 1 to 2 orders of magnitude greater for the zeolite/apatite mixtures than for apatite alone, indicating that the addition of the zeolite increased the solubility of apatite (see Fig. 4).

Since most sulfur compounds are more soluble than phosphorus compounds, it should not be difficult to find a sulfur-containing material to supply necessary sulfur for plant growth in a zeoponic system.

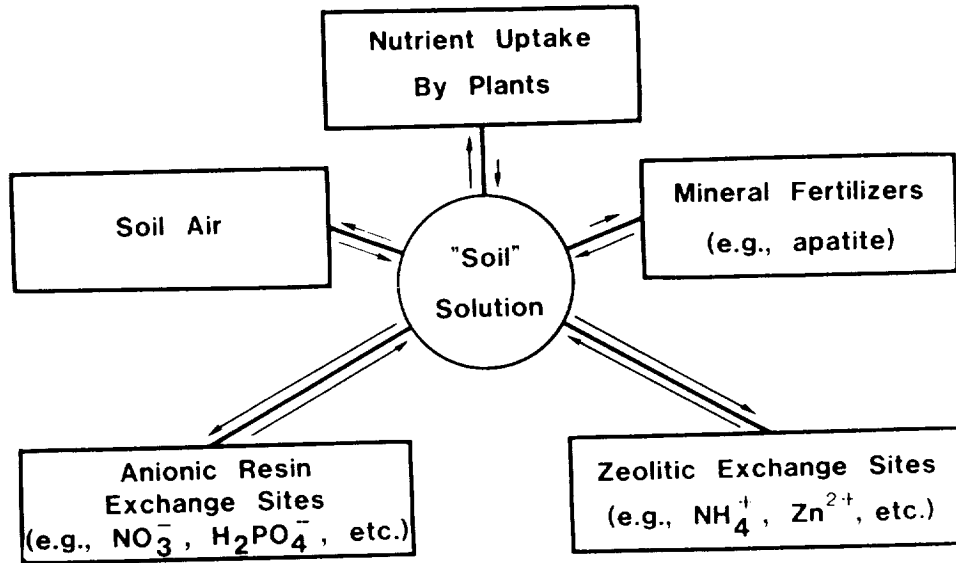


FIGURE 3. Dynamic equilibria for a zeoionics system. The reactions in soil solution should (theoretically) be driven towards the root-soil interface by the uptake of nutrients by the plant.

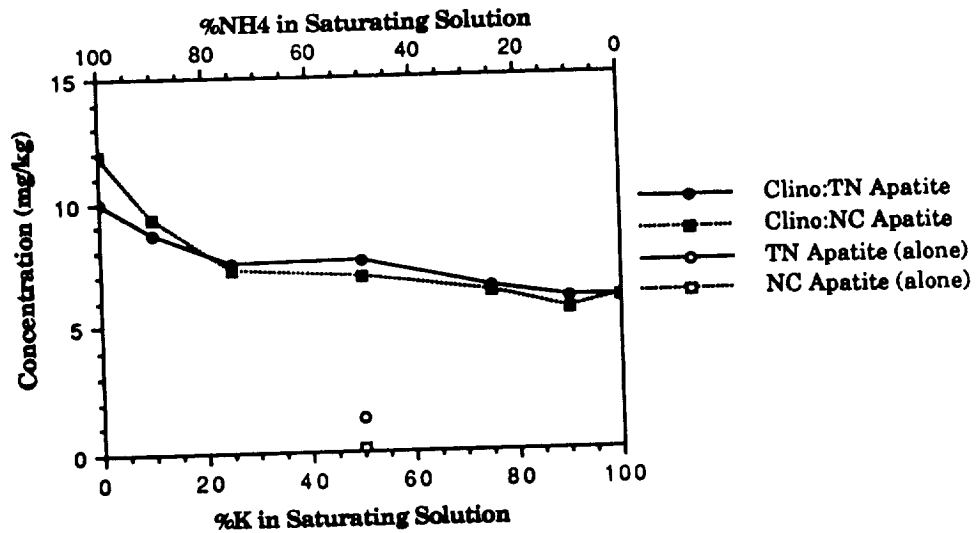


FIGURE 4. Solution Phosphorus concentrations as a function of the composition of the saturating solution for a clinoptilolite (zeolite)/apatite system (Clino = clinoptilolite; TN = Tennessee, NC = North Carolina).

Undoubtedly, the redox potential of the system will have an important role in selecting the sulfur compound best suited for a zeoponic system.

Alternatively, anion-exchange materials may be used to supply plant essential anionic elements (e.g., H_2PO_4^- , SO_4^{2-} , NO_3^-). For example, Checkai et al. [42] developed a mixed-resin hydroponic system that buffered the activities of cations and phosphate in solution without appreciably affecting pH or ionic strength. The P content was controlled by a cation-exchange resin (Dowex 50W-X4) containing adsorbed polynuclear hydroxylaluminum. Even though this resin was designed for hydroponic systems, similar resins may provide anion sinks in solid-support substrates.

Zeoponic systems have the potential to be regenerated with plant-essential nutrients and reused over and over. Nutrient solutions that contain experimentally-determined concentrations of the plant-growth elements will be passed through zeoponic materials until the desired type and amount of each nutrient has been adsorbed on zeolitic-exchange sites and anion-exchange resins.

Several problems may develop in zeoponic systems. First, the plant requirements for essential, plant-growth elements may not be completely satisfied by a single ionic phase. For example, plants are known to take up N in the form of NH_4^+ as well as NO_3^- . However, it is not well known to what extent plants will take up and utilize NH_4^+ and/or NO_3^- . Because the zeolite substrate will supply $\text{NH}_4\text{-N}$, it may be necessary to amend the zeoponic system with nitrates (possibly by anion-exchange resins saturated with NO_3^-) to facilitate maximum yields. Another problem may be in establishing a buffered pH that will best suit the growth of a particular plant species. Because of the abundance of exchange reactions occurring in a zeoponic system, it may be difficult to maintain a constant pH and ionic strength of solution; however, the addition of a mineral phase (e.g., apatite) will help buffer the solution pH and ionic strength.

It will be essential to understand the exchange behavior for zeolites and/or anion-exchange resins used in zeoponic systems; therefore, it will be necessary to establish ion-exchange isotherms for these exchange resins at specific electrolyte concentrations. Zeoponics must have the ability to buffer ionic strength and pH. Once the exchange behavior of plant-essential elements for the synthetic or natural zeolites and anion exchange materials is understood, plant-growth experiments must be conducted to determine economic feasibility and how plant production in zeoponics compares to other plant-growth systems (e.g., hydroponics).

Preparation of zeoponic systems at a lunar base will not be an easy task. Raw materials will have to be found on the lunar surface that will be best suited for the syntheses of zeolites and other exchange resins. However, Ming and Lofgren [19] have shown that zeolites can be synthesized from lunar analog glass subjected to mild hydrothermal conditions. Plant-essential elements will have to be extracted from the regolith or transported from Earth as may be the case for N. Because small traces of apatite and metal sulfides occur in the regolith [2], the extraction of PO_4 and S should not be a major problem.

The use of synthetic zeolites at lunar bases should not be limited to agricultural purposes. Based on their unique adsorption, hydration/dehydration, molecular-sieving, ion-exchange, and catalytic properties, synthetic zeolites may be used 1) as adsorption media for the separation of various gases, 2) as catalysts, 3) as

molecular sieves, and 4) as cation exchangers in sewage-effluent treatment, in radioactive-waste disposal, and in pollution control [43].

Other Cation-Exchange Materials

Smectite, vermiculite, and organic matter are a few of the materials used in terrestrial greenhouses to increase the cation exchange capacity and nutrient retention in plant-growth substrates. The addition of organic matter to a lunar soil will be nearly impossible due to the low abundance of organic molecules in the regolith. However, wastes from various processes (e.g., crop by-products, composted garbage) may be used as organic additives to lunar soils. A more likely candidate for increasing the CEC of these lunar soils will be the addition of inorganic phases which exhibit cation exchange.

Expanded vermiculites are in wide spread use as terrestrial potting media. Water between vermiculite particles (or quasi-crystals) causes permanent expansion between particles upon heating. The expanded volume of the vermiculite can be up to 16 times larger than the original material. Expanded vermiculites are desirable solid-support substrates for plant growth because of their nutrient and water retention, good root aeration, and low bulk densities. Lunar regolith amended with expanded vermiculite could act as excellent soils for plant growth at a lunar base; however, it may be difficult to synthesize vermiculites from lunar materials. In nature, vermiculites are thought to be alteration products of micas [44]. In the laboratory, vermiculization of chlorite can be readily achieved by thermal treatments of chlorite [45, 46, 47]. The direct synthesis of vermiculite from solution however is rather difficult to achieve in the laboratory. However, it may be possible to synthesize high-charged vermiculite-type silicates in the absence of water by heating lunar starting materials (e.g., pyroxenes) and NaF, MgF₂, or CaF₂, then allow the melt to slowly cool to promote crystallization. Gregorkiewitz and Rausell-Colom [48] synthesized a high-charged, mica-type silicate from the reaction of augite in NaF-MgF₂ melts, which were allowed to slowly cool. The interlayer of the mica-type silicate, Na_{4.0}(Mg_{6.0}Ti_{0.05})[Fe_{0.1}Al_{3.4}Si_{4.5}O_{20.7}F_{3.3}], readily hydrated and Na⁺ in the interlayer was easily replaced by K⁺ in solution, indicating that the product exhibited cation-exchange properties.

Smectites, which are responsible for a large portion of the CEC in terrestrial soils [49], may be a more realistic material to amend lunar soils than vermiculites in order to increase the CEC. Smectites may be easier to synthesize from lunar regolith than chlorites and micas. Ming and Lofgren [19] have synthesized smectites from hydrothermally-altered glass, which has a chemical composition similar to lunar basaltic glasses. In terrestrial soils, smectitic cation-exchange sites create sinks to hold fertilizer cations such as K⁺, NH₄⁺, Ca²⁺, Mg²⁺, Zn²⁺, and Fe²⁺. The addition of smectite to lunar regolith will increase the nutrient and water-retention capacity of the manufactured soil. Calcined smectites (i.e., aggregates of smectite particles heated to high temperatures) forms hardened particles, and when mixed with other components (e.g., quartz sand, soil, peat moss) make a productive terrestrial root medium. The irregular shape of particles creates large pores for aeration and drainage. Calcined smectites also have sizable CECs (e.g., as high as 25 cmol_c kg⁻¹),

which results in good nutrient retention. A productive lunar soil could consist of calcined smectite combined with lunar materials (e.g., sand-sized feldspar).

As with zeolites, the use of synthetic smectites at lunar bases should not be limited to agricultural purposes. Smectites could be used as 1) adsorption media for waste renovation, 2) cation exchangers, and 3) adsorption media for organic molecules (Ming, 1989).

Nutriculture

Nutriculture is the cultivation of plants in inert substrates (e.g., water, sand, air). Hydroponics (cultivation of plants in water) has been considered by plant physiologists as a leading candidate for plant-growth systems in controlled ecological life support systems (CELSS). Since hydroponic systems for CELSS have been discussed elsewhere [50, 51, 52, 53], only solid-support substrates used in nutriculture will be discussed.

Nutriculture systems using a solid-support substrate have been suggested as viable growth system for potatoes in CELSS. Tibbitts and Wheeler [54] found that tuberization was normal when recirculating nutrient solutions were passed through calcined clay particles or sphagnum moss; however, when solution was recirculated through containers filled with nutrient solution and the plant roots immersed, tuberization was delayed and the plants failed to tuberize normally. If potatoes and other edible tubers are to be grown in CELSS, it may be necessary to have a solid-support substrate to promote tuberization.

Several inorganic materials and plastics are used terrestrially as inert, solid-support substrates, including perlite (crushed siliceous volcanic rock that expands to a lightweight cellular material 10 to 20 times the original volume when heated to high temperatures), rockwool (mass of fine, intertwined fibers formed by passing molten coke, basalt, limestone, and possibly slag through a high-speed rotor), and polystyrenes (plastic made by polymerization of the hydrocarbon styrene). These materials, unless they are chemically altered, e.g., chloromethylation of polystyrene, should not contribute to or alter plant nutrients. Unfortunately, these materials do not exist on the Moon, or could be very difficult to synthesize on the lunar surface; the synthesis of polystyrene would be difficult because of the lack of organic molecules in lunar materials. However, other materials produced from lunar materials may serve as excellent, inert, solid-support substrates for nutriculture.

A resource-processing pilot plant will probably be the first industry to be built on the Moon that will utilize *in situ* resources [55]. The primary products of a lunar processing plant will include 1) oxygen for rocket propellant and life-support systems, 2) construction materials (e.g. concrete, ceramics, glasses), 3) volatiles (e.g., H₂, CO₂) for life-support systems, and 4) metals for construction. Undoubtedly, a number of useful by-products will be produced by a resource-processing pilot plant. Some of these products and by-products (e.g., ceramics, concrete) may provide excellent solid-support substrates for plant growth. For nutriculture systems, special attention will have to be given to the particle size and the reactivity of the substrate. Ideally, the substrate in nutriculture systems should be inert and have a particle size that allows proper root aeration and

drainage between the addition of the nutrient solution. With the addition of a nutrient solution (e.g., Hoaglands solution), plant-growth systems using inert, solid-support substrates are productive and fairly well understood; thereby, these systems should become attractive for use as solid-support substrates for plant growth at a lunar base.

SUMMARY

It appears that a "soil" capable of growing plants can be produced from lunar materials. However, the synthesis of a lunar soil is only the beginning of a very complex and advanced research effort. Many factors (e.g., source of water, source of plant nutrients, growth modules, effects of radiation, plant varieties, microbial populations, reduced gravity) must be thoroughly examined before plants can be grown in lunar materials.

There are a variety of materials that could be used as solid-support substrates at lunar bases; some of the more likely candidates include 1) native lunar "soils", 2) sized lunar regolith amended with synthetic materials (e.g., zeolites, smectites) that provide nutrient and water retention, 3) synthetic, inorganic, highly-reactive substrates (e.g., zeoponics), and 4) sized lunar materials or industrial by-products used as inert, solid-support substrates with nutriculture systems. The design of new, plant-growth substrates could have tremendous terrestrial applications; for example, zeoponic systems could be used in commercial greenhouses. These systems are only in their research stages and will require much more research before they can be used to their fullest potential.

The success of a permanently human-occupied lunar base will be insured if it is viewed as an integral part of the Earth's space infrastructure. That is, it will represent a piece of the overall, long-term efforts of humans to explore our Solar System. Lunar base can become a supplier of many necessary items in this complex scenario while at the same time contributing significantly to answering scientific questions through research conducted at a lunar base.

One of the key long-term technologies to human exploration is the reliable supply of consumables through regeneration of wastes and production of food. Without a reliable and economical supply, the ability of humans to venture very far from Earth will be severely limited. The lunar regolith represents a valuable resource which can supply many things to space explorers, not the least of which is a major role in providing a reliable long term supply of food to sustain those efforts.

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16. Abstract <p>In February 1989, NASA's Controlled Ecological Life Support Systems (CELSS) program assembled a diverse group of investigators in Orlando, Florida, to discuss a variety of topics which must be addressed in order to develop bioregenerative systems for use during piloted missions early in the next century. The meeting was attended by investigators from several NASA centers (Ames, Johnson, JPL, and Kennedy) as well as scientists from universities and private industries from around the United States. These proceedings contain the 25 papers presented during the two days of the conference. Topics concerning the production of edible biomass range from studies on the efficiency of plant growth, to the conversion of inedible plant material to edible food, to the use of plant tissue culture techniques. Models of plant growth, and of whole CELSS systems, are also included. The use of algae to supplement and improve dietary requirements is addressed. Several papers discuss the development of CELSS technology, both ground-based and flight-qualified. Work at Kennedy's Breadboard Facility and Ames' Crop Growth Research Chamber is described. Flight experimentation is covered in topics ranging from a Salad Machine for use on Space Station <i>Freedom</i> to conceptual designs for a Lunar Base CELSS. Control issues are reviewed and recommendations made concerning a strategy for development of a robust control system. Processing of waste materials is covered, including analysis of physical/chemical, biological, and hybrid systems, and how source characterization affects design criteria.</p>					
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