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# A Simple, Mass Balance Model Of Carbon Flow In A Controlled Ecological Life Support System

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## ABSTRACT

Internal cycling of chemical elements is a fundamental aspect of a Controlled Ecological Life Support System (CELSS). Mathematical models are useful tools for evaluating fluxes and reservoirs of elements associated with potential CELSS configurations. A simple mass balance model of carbon flow in CELSS was developed based on data from the CELSS Breadboard project at Kennedy Space Center. All carbon reservoirs and fluxes were calculated based on steady state conditions and modelled using linear, donor-controlled transfer coefficients. The linear expression of photosynthetic flux was replaced with Michaelis-Menten kinetics based on dynamical analysis of the model which found that the latter produced more adequate model output.

Sensitivity analysis of the model indicated that accurate determination of the maximum rate of gross primary production is critical to the development of an accurate model of carbon flow. Atmospheric carbon dioxide was particularly sensitive to changes in photosynthetic rate. The small reservoir of CO<sub>2</sub> relative to large CO<sub>2</sub> fluxes increases the potential for volatility in CO<sub>2</sub> concentration. Feedback control mechanisms regulating CO<sub>2</sub> concentration will probably be necessary in a CELSS to reduce this system instability.

Examination of alternative food production subsystems demonstrated that enzymatic conversion of inedible plant material has the potential to significantly increase human food supply. Aquaculture systems are much less efficient food producers, and should be considered for their qualitative rather than quantitative role in human nutrition.

## INTRODUCTION

Life support is currently a major limitation to man's long term presence in space. The Controlled Ecological Life Support System (CELSS) program is a

long range NASA effort of research and engineering whose goal is the development of an operative regenerative life support system. A CELSS would be closed to all external material supplies except energy (sunlight). The Kennedy Space Center's (KSC) current CELSS Breadboard project involves the study of plant growth in a large sealed unit called the Biomass Production Chamber (BPC). Future efforts at KSC will focus on integrative, manned testing of waste management, biomass processing, and biomass production subsystems.

Carbon is the key element of life. Knowledge of the carbon cycle, therefore, is fundamental in understanding a biologically-based regenerative life support system like CELSS.

A simple mass balance model of carbon flow in CELSS could be used for several purposes in the KSC-CELSS Breadboard project; 1) quantify approximate carbon reservoir and flux values, 2) identify "data poor" areas, 3) identify potential instabilities in the system, and 4) determine the sensitivity of the system to uncertainty in system variables. This knowledge could increase the effectiveness of management decisions involving future research efforts. Resources could be focused on the accurate determination of parameters which have the greatest influence over system behavior. Control mechanisms could be developed to deal with system instabilities. Future engineering projects could be scaled based on pool and flux sizes. For these reasons, a model was developed based on a general conceptualization of a CELSS system with consideration of the specific areas of research in CELSS at KSC.

## METHODS

A box and arrow diagram was made showing the selected carbon reservoirs and flows into and out of each compartment (Figure 1). Each box in

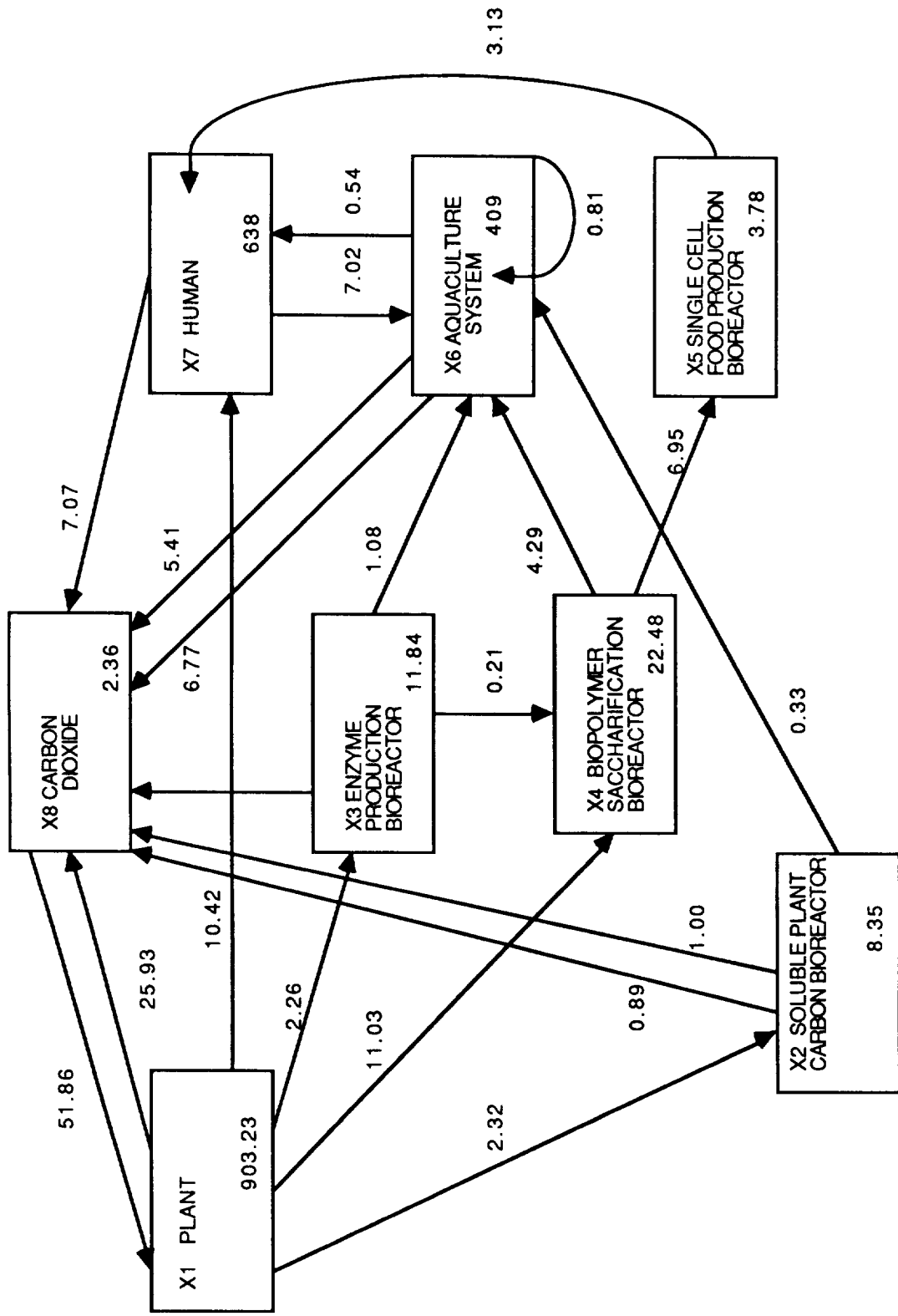


Figure 1. Flow diagram of carbon in a CELSS. Boxes represent reservoir size (g C/sq. meter growing area), arrows represent flux rates (g C/sq. m./day).

the figure represents the reservoir of carbon in that compartment in grams carbon/square meter of plant growth area, and the arrows represent the appropriate carbon flows in gC/m<sup>2</sup>/day.

Values for all reservoirs and fluxes were calculated based on assumed steady state conditions (i.e., - input=output for all reservoirs). The source of these values are described below.

A Fortran computer program was written to describe the changes in carbon reservoirs over time. All energy fluxes were modelled as linear, donor-controlled fluxes. Coefficients for these parameters were calculated by dividing the energy flow for a flux by the reservoir size in the donor box at steady state conditions. Models containing equations describing biological processes within the system would be more flexible than the model described here. For example, equation-based models could be adjusted to account for crop-specific differences in plant growth by changing equation parameters. While the mass balance, donor-controlled nature of this model does not allow for such flexibility, it is the simplest methodology which will serve the purposes outlined in the introduction.

Numerical solutions to the system of differential equations describing reservoir sizes in the system were obtained using the fifth-order Runge-Kutta technique (DVERK program in the IMSL library of the CDC computer at the University of Virginia).

### Model Structure

Vascular plant growth is assumed to be fundamental for food production and gas exchange in this system. Harvested edible plant material is classified into edible, soluble inedible, and insoluble inedible fractions.



Edible material production is assumed to be completely consumed by humans without any losses due to food processing. Food processing is not evaluated in this model for any edible products.

Significant amounts (35%) of the inedible fraction of potential CELSS crops like wheat are readily soluble in water. This fraction contains significant quantities of both organic compounds and inorganic plant nutrients. Recent work at KSC evaluating the direct use of this "leachate" fraction as a source of nutrients for hydroponic plant growth found that biological pretreatment to remove organics significantly increased its effectiveness (Garland et al. 1988). This pretreatment would involve concomitant production of microbial biomass which can be used as feed for aquaculture production systems.

The insoluble fraction of agricultural residues are largely composed of the biopolymers cellulose and hemicellulose (Alexander 1977). Enzymatic conversion of these polymers to their monosaccharide subunits (glucose and xylose/arabinose, respectively) produces a readily available energy source for the production of single cell food suitable for human consumption. Glucose could also be consumed directly by humans, but this model has all monosaccharide production passing into single cell food production.

The common method of enzymatic conversion involves separate enzyme production and hydrolysis steps (KSC-AIBS Biomass Processing Panel Report 1986). A relatively small amount of insoluble material is used for enzyme induction in selected microbial strains which produce significant quantities of extracellular enzyme. The enzyme is then harvested and transferred to a hydrolysis reactor where a large fraction of the insoluble material is converted to monosaccharides.

A key advantage to enzymatic hydrolysis is the decoupling of enzymatic breakdown and microbial production in the decomposition pathway. This

should maximize production of edible material since the monosaccharide can be isolated and used to grow microbial strains which have high production rates, palatability, and nutritional value. At present, the organisms with optimal production of the cellulase and hemicellulase enzymes do not also possess optimal features for food production. Furthermore, separation of the monosaccharide from the residual, non-decomposed plant material allows for a more readily purified single-cell food.

An advantage of enzymatic hydrolysis of polymers compared to chemical methods is the production of high protein "wastes" (microbial biomass from enzyme production phase and spent enzyme) suitable for consumption by intermediate consumer organisms such as fish in the CELSS system.

Tilapia, a native African species, is a suitable candidate for food production in CELSS because of its resistance to poor water quality and disease, tolerance of a wide range of environmental conditions, and ability to utilize organic wastes as food (Balarin and Haller 1982). The potential for space conservation and containment in combined aquaculture/hydroponic systems are added advantages of aquatic versus non-aquatic animal production systems.

## Model Parameters

### Plant Production

Plant standing crop and production values are based on continuous culture of dwarf wheat, *Triticum aestivum* L. cv. Yecora roja, using the nutrient film technique hydroponic system in the BPC at KSC. The BPC contains 64 tray positions (0.24 m<sup>2</sup>/tray) for nutrient film technique (NFT) culture. Since the life cycle of Y. roja is approximately 64 days it was assumed that one tray would be harvested per day. It is estimated that the maximal yield of wheat under BPC

conditions will be 1000 g dry wt/tray (Cheryl Mackowiak, pers. comm.). This production rates corresponds to a daily areal value of 65.10 g dry wt/m<sup>2</sup>/day (1000 g/day/[64 trays x 0.24 m<sup>2</sup>/tray]). If the carbon content of plant material is estimated as 40% (Alexander 1977), then a total of 26.04 gC/m<sup>2</sup>/day will be produced.

The standing crop of plant material was estimated by determining the mean plant wt/tray from plant growth curves for *Y. roja* (Figure 2). The growth curve was generated from experiments performed as part of the microbiological research group at KSC (unpublished data). The average weights for five day intervals in the life cycle were estimated. These values were converted to the percent of the total biomass at harvest and multiplied by the 1000 g/tray estimates. These values were then multiplied by five, summed, and adjusted for growing area and carbon content ( $[(34660 \text{ g}/\{15.35 \text{ m}^2\}) \times 40\% \text{ C}]$ ). This represents the plant standing crop with the system in steady state when one tray is harvested and planted per day.

The rates of carbon fixation (gross primary production [GPP]) and plant respiration (gross primary production - net primary production [NPP]) were estimated based on the calculated daily production of respired C from other sources in the system of 25.89 gC/m<sup>2</sup>/d (see below for details). If the system is in steady state, the rate of carbon fixation must be equivalent to the sum of plant respiration and respiration from other sources (25.89). Net primary production is often estimated as 50% of GPP (Odum 1959), which would make NPP equivalent to 25.89 gC/m<sup>2</sup>/d and GPP equivalent to 2 x 25.89 gC/m<sup>2</sup>/d in this steady state model.

The present state of this model ignores loss of photosynthetically fixed carbon through the roots of plants via exudation. While this is known to represent a significant (12-18% of NPP) flow of carbon (Barber and Martin

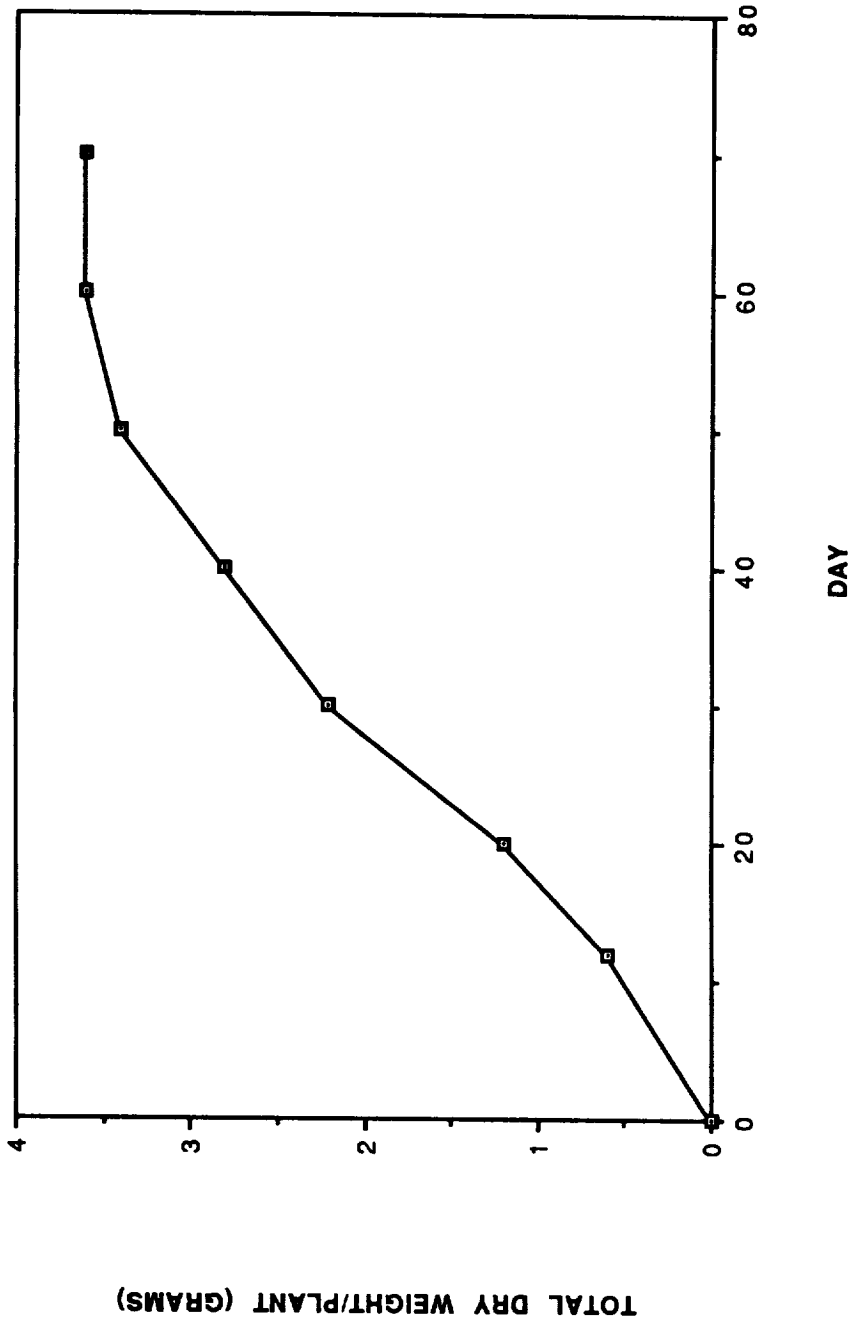


Figure 2. Growth of Yecora rojo in non-recirculating hydroponic systems. (Data from KSC-CELSS Experiment MR3A).

1976) for the purposes of this model, it is assumed that practically all exuded carbon is respired by the rhizosphere microbial community and, therefore, included in the plant respiration value. Future measurements of bacterial production and turnover rates in the hydroponic system would allow for the modelling of carbon associated with plant root exudation.

#### Leachate Bioreactor

Work at KSC has shown that approximately 35% of the dry weight of inedible wheat residues is readily soluble in water. Preliminary studies were performed at densities of 50 g straw/L water. At this concentration, 3 g/L of dissolved organic carbon was measured, indicating that 17% of the leachate is carbon by weight ( $3/[50 \times 0.35]$ ). The harvest yield (edible dry weight/total dry weight) of *Y. roja* is approximately 40%. Therefore, of the 62.10 g dry weight of aerial production of plant material, inedible material constitutes 37.26 g, and the soluble fraction of the inedible material will total 13.04 g. Finally, soluble carbon will comprise 17% of that fraction or 2.22 g/m<sup>2</sup>/d.

Experiments at KSC with bacterial treatment of leachate found that 60% of the soluble carbon was metabolized after four days incubation (Garland, unpubl. data). Recalcitrant carbon production, therefore, would equal 0.888 g/m<sup>2</sup>/d ( $2.22 \times 0.40$ ).

Microbial biomass, as estimated by total viable counts and protein content, reached near maximal levels in the reactor at day 1 and remained constant until day 4 when numbers declined. This was the major criteria for selecting a four day residency time of soluble organic carbon in the bioreactor. Protein content of the particulate material at day 4 was 0.222 mg/ml. If a N content for protein of 20% and a C/N ratio of bacterial cells of 10/1 are assumed (Alexander 1977) the carbon concentration in microbial biomass harvested at

day 4 will be 0.444 mgC/ml. At the substrate concentration of 50 g/L stated above, the daily loading volume (which will be equivalent to the daily harvest volume in a steady state system) will equal  $(37.26/50)$  0.774 L. Daily areal production of microbial carbon, therefore, will be 0.330 gC/m<sup>2</sup>/d (0.444 mg C/ml x 0.744 L/day).

Respiration was calculated from the difference between input of dissolved carbon and output of microbial biomass and recalcitrant carbon.

The changing levels of microbial carbon and dissolved organic carbon were averaged over the four day residency time to determine the reservoir of carbon in the reactor. A constant daily rate of degradation based on 60% reduction in four days (15%/day) was assumed. Average microbial biomass was estimated as half of maximal for day 1 and maximal for days 2, 3, and 4.

Garland et al. (1988) found that half of the remaining carbon is metabolized by bacteria in the hydroponic system after 30 days of plant growth. It is questionable to assume a constant rate of degradation over such a relatively long period of time, especially since the remaining carbon may be increasing in recalcitrance with time. Until more data is available on rates of decay and the residency time of carbon in the hydroponic system (i.e., - how often will the hydroponic system be "cleansed" of its organics?) recalcitrant carbon will be treated as if it is completely combusted at an instantaneous rate.

### Enzyme Production

The remaining inedible carbon production ( $[26.02 \text{ g/m}^2/\text{d} \times 0.60\%]$  -2.32 g/m<sup>2</sup>/d) is 13.29 g/m<sup>2</sup>/d, and will largely consist of cellulose and hemicellulose. Part of this material will be needed to induce the cellulase and hemicellulase enzymes for biopolymer saccharification. A reasonable substrate concentration for optimal enzyme production is 2% (per. obs.). The crude enzyme suspension

produced in this manner can convert approximately 100 g of wheat straw. Therefore, for every 20 g used for enzyme production, 100 g of straw can be hydrolyzed. In a steady state system, then, 17% of insoluble residue production, or 2.26 g/m<sup>2</sup>/d will be used for enzyme production and 83%, or 11.03 g/m<sup>2</sup>/d will be added to the hydrolysis reactor.

The daily input of material on a dry weight basis equals 5.65 g/m<sup>2</sup> (2.26/0.40). Assuming a substrate concentration of 2% for enzyme induction a volume of 282 ml will be needed for the daily input. Enzyme production in *Trichoderma reesei* cultures, typically is maximal after seven days (Mandels and Andreotti 1978), so total volume allocated for enzyme production would be 1.97 L (282 ml x 7 days). Data from Acebal et al. (1986) of the percent weight remaining in *T. reesei* cultures was used to estimate standing crop and loss values.

Day	% remaining	Weight remaining (g C)
1	119	2.69
2	100	2.26
3	76	1.72
4	67	1.51
5	57	1.29
6	57	1.29
7	48	<u>1.08</u>
		11.84

The standing crop of the enzyme production system will be the sum of the carbon content at each of the seven days. Production of waste carbon (undigested wheat straw, fungal mycelium) will 1.08 g/m<sup>2</sup>/d since 48% of the dry weight is left at harvest. These estimates assume that dry weight loss is highly correlated with loss of C. This may not be entirely true since dry weight

remaining at different days contains varying percentages of fungal biomass which may contain a higher proportion of carbon/unit weight than plant material. Direct measurements are needed to determine more accurate values.

Total carbon transfer of  $0.211 \text{ gC/m}^2/\text{d}$  to the hydrolysis reactor was calculated assuming a harvest volume of 282 mL, a protein content of harvest enzyme suspension of 1.5 mg/ml (Mandels and Andreotti 1978), and a 50% carbon content for protein. The latter value was obtained by examining the average carbon content of amino acids.

Since the level of soluble carbon in the enzyme production system is very low compared to the insoluble residue and microbial biomass it was excluded from the calculation of the carbon reservoir.

Respiration was estimated by subtracting the soluble and insoluble loss rates from the input.

#### Biopolymer Saccharification Reactor

A reactor volume of 551 mL was estimated as above by adjusting the carbon input to a dry weight basis and assuming a substrate concentration of 5% ( $[11.03/0.4]/0.05$ ). If enzymatic conversion efficiency is 70%, then 7.72 g of monosaccharide C will be produced on an daily areal basis. This value should be adjusted to account for the addition of water in the hydrolysis reaction by multiplication by 0.9 ( $6.95 \text{ g/m}^2/\text{d}$ ), because conversion efficiency is usually defined on a weight rather than carbon basis. Waste output (spent enzyme, undigested wheat straw) is the difference between inputs and monosaccharide output ( $[11.03+.211]-6.95=4.29$ ). Since the optimal hydrolysis time is approximately two days and no carbon losses through respiration are present over time, the carbon reservoir of the reactor is sum of the daily inputs multiplied by a factor of two.



A 70% conversion efficiency is within the range found in recent work at KSC involving enzymatic conversion of cellulose in wheat straw (Garland and Strayer, in prep).

### Microbial Protein Production

The yield coefficient (g biomass produced/g substrate) of most potentially edible microorganisms grown on monosaccharides range between 0.4 to 0.5 (Gottschalk 1986). Assuming an intermediate yield coefficient of 0.45, daily areal production of microbial C would be 3.13 gC/m<sup>2</sup>/d given the monosaccharide production rate of 6.95 gC/m<sup>2</sup>/d. This direct estimate of microbial carbon from monosaccharide carbon is possible because both monosaccharide and yeast or fungi are approximately 40% carbon by weight.

Respiration was estimated by taking the difference between input and output.

Maximum growth yield in batch culture of many edible fungi, including a patented fungi (U.S. Patent 3,937.654) used for mycoprotein production, is usually reached after 24-36 hr. The average turnover time would be 30 hr, or 1.2 days. Turnover rate, the inverse of turnover time, is equivalent to the production/standing crop ratio. Therefore, standing crop of the single cell food production unit is equal to 3.13/0.833 or 3.75 g/m<sup>2</sup>.

### Aquaculture System

The energy conversions in the aquaculture production system are based on a poikilothermic animal grazing on a variety of food sources, some of low food quality. Production rate was calculated assuming a 50% assimilation rate of ingested food, 80% respiration of assimilation energy, and an edible index (filet wt/total wt) of 40% (Balarin and Haller 1982, Majowski and Warwood 1981,

Patrice Owens, pers. comm.). While these values are in the range of reported values, they are speculative. More research is needed in the physiology of Tilapia fed CELSS-derived materials to more accurately define these values.

It is assumed that Tilapia waste is of little nutritive value since it already has passed through the digestive tract. However, microbial conditioning of this material in a biofilter integrated with the aquaculture system may significantly increase food quality of the waste material. Such a biofilter may be a necessary component of the aquaculture system to reduce ammonia levels. Due to the paucity of available data, however, fish waste is assumed to undergo complete combustion to regenerate CO<sub>2</sub>.

Fish production influences the level of human ingestion, and, correspondingly, human egestion. Since the latter is an input to the aquaculture reservoir, flux values were run through the model with iterative adjustments until equilibrium in all the aquaculture and human fluxes were reached.

Annual production/biomass (P/B) ratio for young of the year sunfish, which should closely approximate Tilapia in growth characteristics, have been estimated in the field at 1.174. (Gerking 1962). This converts to a ratio of 0.0033 on a daily basis. Standing crop biomass therefore, was estimated as  $1.35/0.0033$ , or 409.1 g/m<sup>2</sup>.

## Human

A human egestion rate of 50% was assumed based on a average value of a vertebrate homeotherm eating a vegetable diet (Heal and Maclean 1975). An adult crew is expected in a CELSS system for the near term so all assimilated carbon is respired.

The reservoir of human carbon was estimated by determining the amount of biomass that could be supported by the available energy. Total carbon input to humans is 14.24 g/m<sup>2</sup>/d. Assuming a 40% carbon content, this corresponds to 35.6 g dry wt/m<sup>2</sup>/d. Since a reasonable estimate of caloric content of biomass is 4.5 kcal/g dry weight, this converts to 160.2 kcal/m<sup>2</sup>/d. Total kcal available for the BPC growing area is 2460.7 kcal/d, which is the approximate maintenance caloric value for an active 54.5 kg adult between age 25-34. Adjusted to an areal basis, and assuming an 18% percent carbon content for humans (Starr and Taggart 1988), the reservoir of human biomass is 609 gC/m<sup>2</sup>.

In an actual CELSS system food reserves would probably be used to offset short term energy deficits in humans. This would mean that the human reservoir and related fluxes (i.e., - ingestion, respiration, and egestion rates) would stay constant despite fluctuations in the rest of the system. However, such food supplements are ignored in this model so that the dynamic effects of changes in the system on human carbon can be observed.

#### Atmospheric CO<sub>2</sub>

All respired CO<sub>2</sub> is assumed to completely enter the atmospheric pool, even though dissolved gas and carbonate will represent a separate pool in aquatic subsystems. This pool and related fluxes should be addressed as the model grows in complexity.

Since elevated CO<sub>2</sub> concentrations increase plant growth, it is expected that CO<sub>2</sub> concentrations of 1000 ppm will be maintained in the BPC. The BPC is a cylinder of 3.5 m diameter and 7.5 m height (73 m<sup>3</sup> volume). Additional duct work brings the total chamber volume to 112 m<sup>3</sup> or 112000 L. Assuming an air density of 1.2 g/L, the chamber will contain 134400 g of air CO<sub>2</sub>

concentration of 1000 ppm corresponds to 134.4 g of CO<sub>2</sub>, and, accordingly, 36.39 g C. Adjusted on the growing area basis, 36.29/15.36, or 2.36 gC/m<sup>2</sup>, will be present in the system.

### Changes in Description of Photosynthetic Flux

Donor control flux is a reasonable assumption for all flows in the model except photosynthesis. Donor control implies that photosynthesis is linearly dependent only on available carbon, and ignores the major influences of available radiance and nutrients. An alternative linear model of photosynthetic flux, recipient control, may be a more realistic expression since CO<sub>2</sub> uptake would be directly dependent on plant biomass. Nonlinear Michaelis-Menten kinetics may be an even more realistic description of photosynthetic flux. Results of experiments performed at KSC with soybean (Figure 3) show the asymptotic relationship between photosynthesis and CO<sub>2</sub> predicted by such kinetics.

The difficulty in using the Michaelis-Menten equation arises from the need to quantify coefficients within the expression. The equation states that:  $V = (V_{max} * S)/(K_m + S)$  where V equals reaction velocity, V<sub>max</sub> equals maximum velocity reaction, S is substrate concentration, and K<sub>m</sub> is the substrate concentration at half maximum velocity. The CO<sub>2</sub> concentration is considered the substrate concentration in photosynthesis. Given the model assumption that NPP=1/2GPP, then K<sub>m</sub> is equivalent to the CO<sub>2</sub> concentration when NPP is zero. A graph of NPP versus CO<sub>2</sub> concentration measured under various light levels was developed from measurements taken at KSC (Figure 3). The x intercept on this graph, which is called the CO<sub>2</sub> compensation point and is analogous to K<sub>m</sub> when NPP=1/2GPP, is approximately 50-100 ppm. The approximated mean of 75 ppm is equivalent to 0.177 gC/m<sup>2</sup>. As discussed

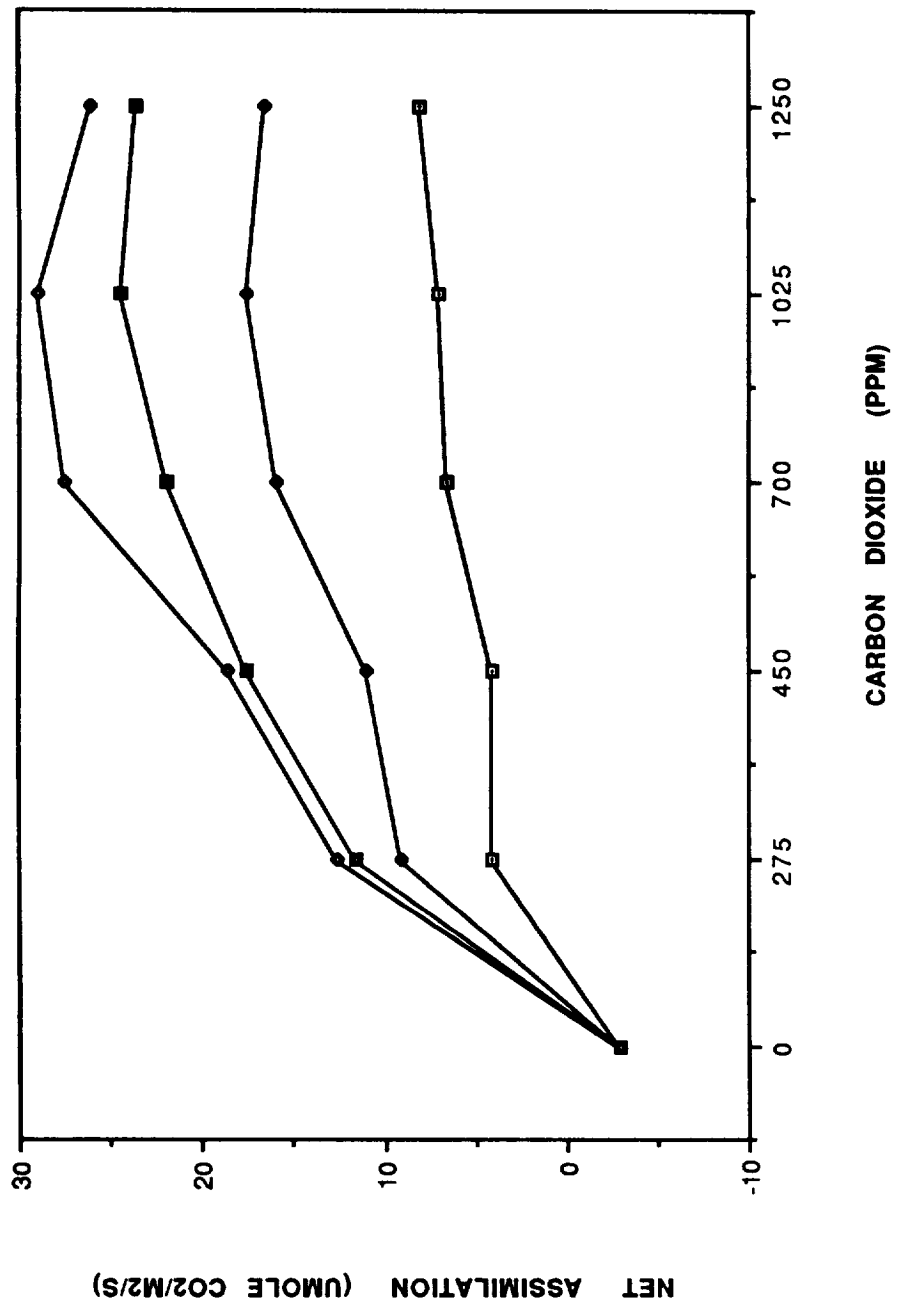


Figure 3. Net carbon digests assimilation by soybeans (data provided by Ray Wheeler).

above, the maximum velocity is the flux at steady state (51.86 gC/m<sup>2</sup>/d) since the model assumed a maximum areal production rate of 1000 g/tray. To maintain steady state mass balance, 51.86 was used as the value for V, and the equation was solved for V<sub>max</sub> using the steady state S value (2.36) and the K<sub>m</sub> value of 0.177. V<sub>max</sub> can be expressed as a constant (A) times the amount of plant biomass. Using the steady state value for plant standing crop of 903.23, A becomes 0.06172.

The response of the model to perturbation from steady state was compared for the three different expressions of photosynthetic flux; donor control, recipient control, and Michaelis-Menten. The perturbation was a 33% reduction in plant standing crop. This simulates in a general sense some type of failure in the plant growth system (i.e., plant disease, pump failure, etc.). The purpose of this exercise, however, was not to accurately model a particular perturbation in the system, but to evaluate the validity of the expression for photosynthetic flux.

### Model Analysis

Model parameters were also adjusted to examine the system's sensitivity to selected functions. It would be useful to know the extent of changes caused in the system by uncertainty in certain system functions. Near term research efforts would be more productive if emphasis was placed on accurate estimation of those parameters which cause the most significant effects in system function.

The sensitivity of the model to changes in the V<sub>max</sub> and K<sub>m</sub> parameters was analyzed to further evaluate the carbon fixation transfer term. Model runs were performed with V<sub>max</sub> values 10% greater than and less than that in the steady state model. Separate model runs were performed assuming CO<sub>2</sub>

compensation points of 100 and 50 ppm, which represents the approximate range found in Figure 3.

The effects of altered enzyme conversion efficiency and fish assimilation rate were also addressed. In these cases, concomitant adjustment in parameters relating the biopolymer saccharification reactor and aquaculture reservoirs were made. For example, a 10% increase in enzyme conversion efficiency would cause a 10% reduction in waste production from the reactor. The original and adjusted values of all parameters altered in these model runs are shown in Table 1.

## RESULTS

### Comparison of Different Models of Photosynthetic Flux

The relative responses of the three models to decreased plant biomass were evaluated with respect to expected system behavior. Changes with time were evaluated for three carbon reservoirs: plant, human, and CO<sub>2</sub>. It is reasonable to assume that plant biomass would return to or near the original steady state value after the perturbation, while human carbon levels would decline in response to lower food availability. Levels of CO<sub>2</sub> would probably rise initially due to the relatively high turnover rate of carbon in the microbial and aquaculture subsystems. Respiratory and waste CO<sub>2</sub> production rates would be based on steady state levels in these reservoirs while CO<sub>2</sub> uptake rate would be significantly reduced due to the lower plant biomass. However, CO<sub>2</sub> levels should return to near steady state values as plant biomass increased.

The responses of the models including donor and recipient control of photosynthesis do not agree with this intuitive system behavior (Figures 4, 5). The donor control model output shows the expected rebound in plant biomass

Table 1. Parameter adjustments used in sensitivity analyses.

Term	Steady State	10% increase	10% Decrease
Vmax	0.06171	0.06789	0.0555
Km	0.177	0.236*	0.118*
Enzyme efficiency			
A45	0.309	0.3447	0.260
(CH <sub>2</sub> O prod)			
A46	0.191	0.1552	0.2399
(waste prod)			
Assimilation efficiency			
A66	0.00198	0.00238	0.00146
(inedible prod)			
A67	0.00132	0.00159	0.000997
(edible prod)			
A68	0.0132	0.015878	0.01058
(respiration)			
A68W	0.0165	0.01323	0.01985
(egestion)			

\* - values were adjusted based on CO<sub>2</sub> compensation point of 100 ppm and 50 ppm.



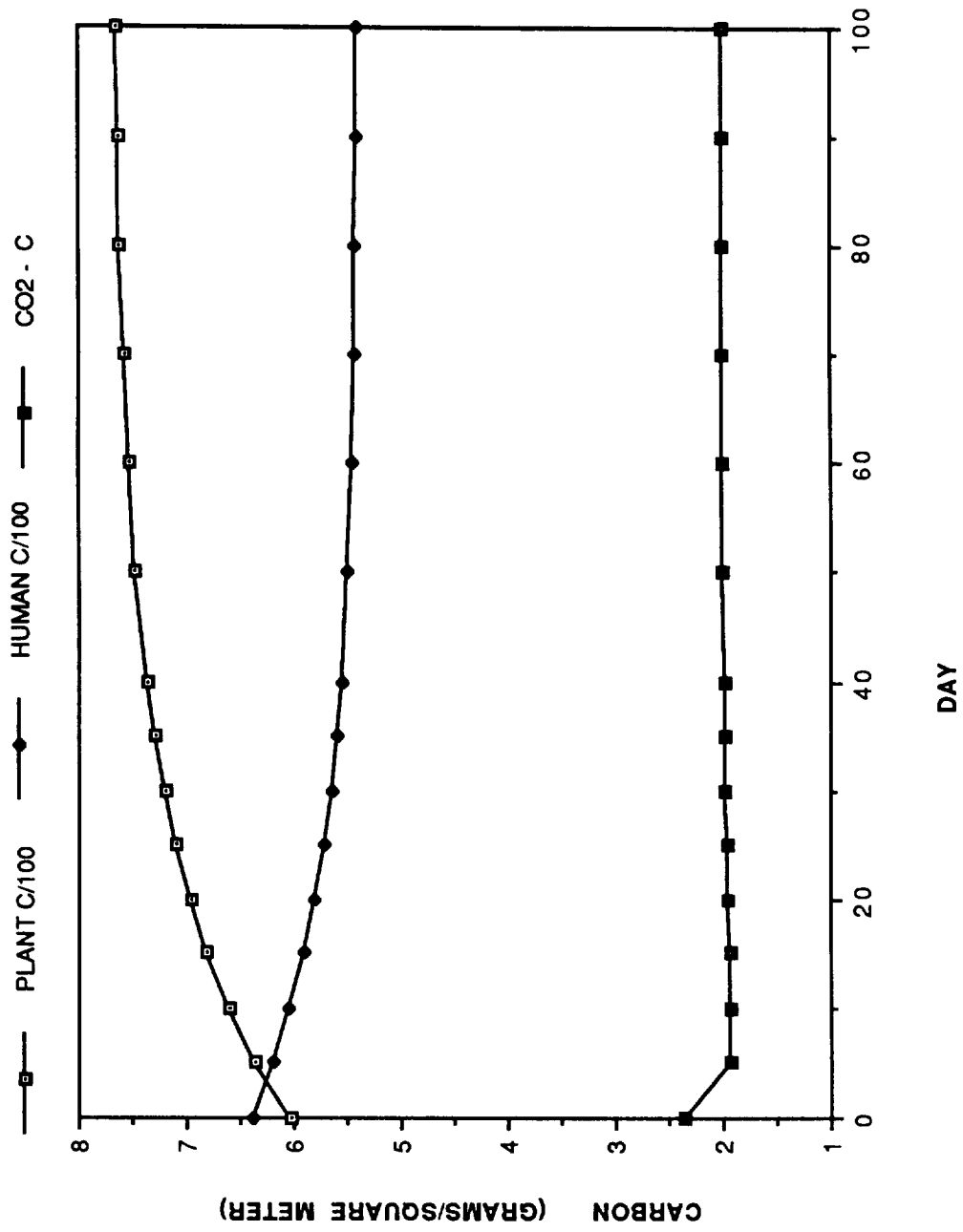


Figure 4. Effects of decreasing initial plant biomass by a third in CELSS model with donor-controlled photosynthetic flux.

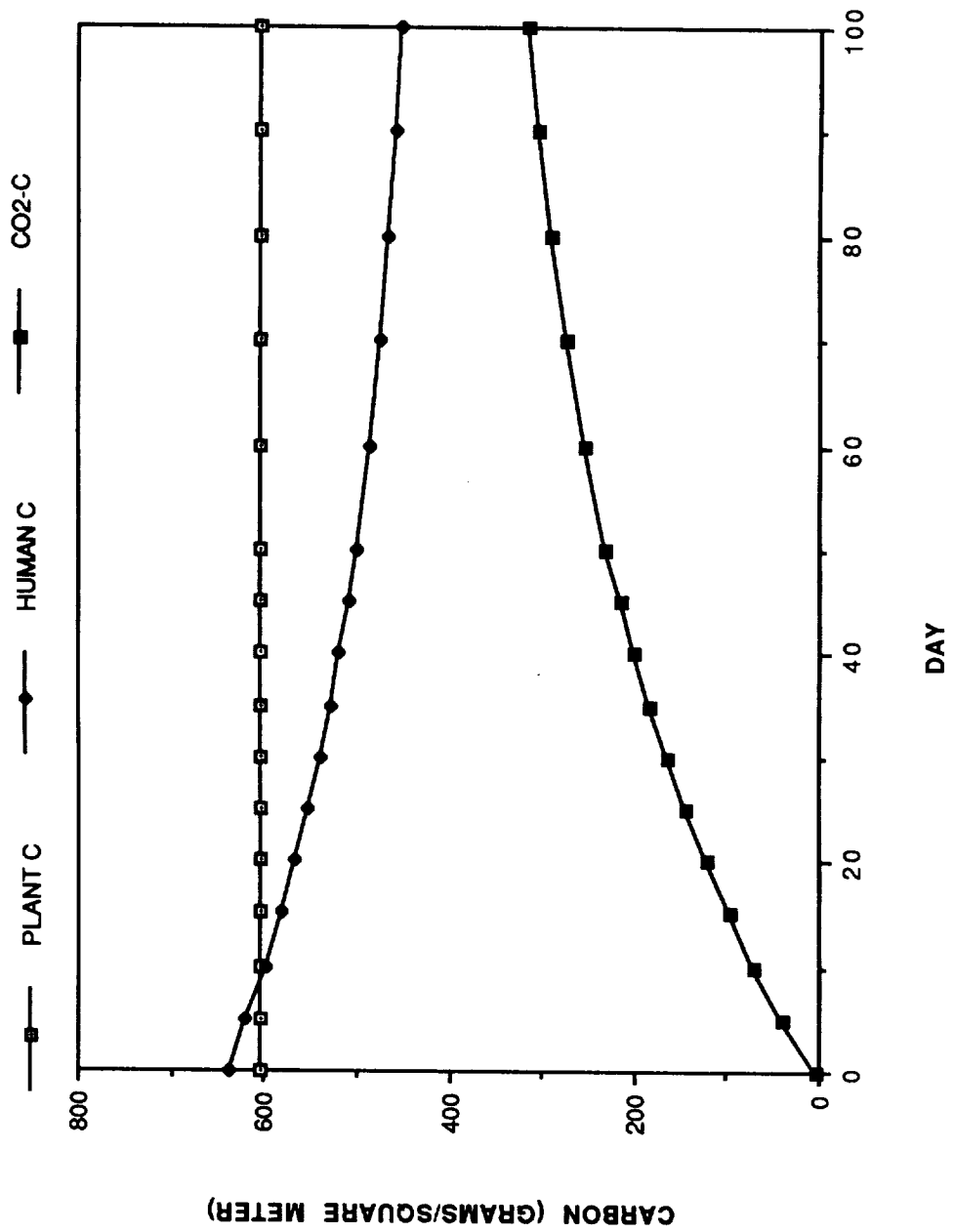


Figure 5. Effects of decreasing initial plant biomass by a third in CELSS model with recipient control photosynthetic flux.

and decline in human biomass, but little change in CO<sub>2</sub>. In the recipient control model human carbon declines, but plant biomass carbon remains constant and CO<sub>2</sub> rises asymptotically. The linear dependence of photosynthetic rate on plant biomass causes the lack of responsiveness in the plant reservoir. Accordingly, CO<sub>2</sub> levels never decrease because photosynthetic rate remains low.

The non-linear model captures the expected changes in all three carbon reservoirs (Figure 6). Carbon dioxide increases 30 fold by day 25 and then declines to the original steady state level by day 70. The new steady state levels of plant and human carbon are lower than those in the original steady state. This is reasonable since 300 g of carbon were removed from the system as part of the perturbation.

This exercise has shown that the non-linear model is probably a more adequate model since it duplicates more of the expected system behavior than the linear models. Adequacy is defined as the fraction of the actual system behavior which is correctly modelled (Mankin et al. 1975). The non-linear model, therefore, was used in the sensitivity analyses described below.

The recovery time of the system to perturbation (i.e., - time to new steady state) is approximately 70 days, or the time it takes wheat to pass through an entire life cycle.

#### Sensitivity of the Model to Changes in V<sub>max</sub> and K<sub>m</sub>

A decrease in V<sub>max</sub> causes significant effects in all carbon reservoirs while an increase does not significantly affect any reservoir except CO<sub>2</sub> (Table 2). This asymmetry is probably a result of the asymptotic nature of the Michaelis-Menten equation. Changes in K<sub>m</sub> cause little effect in any carbon

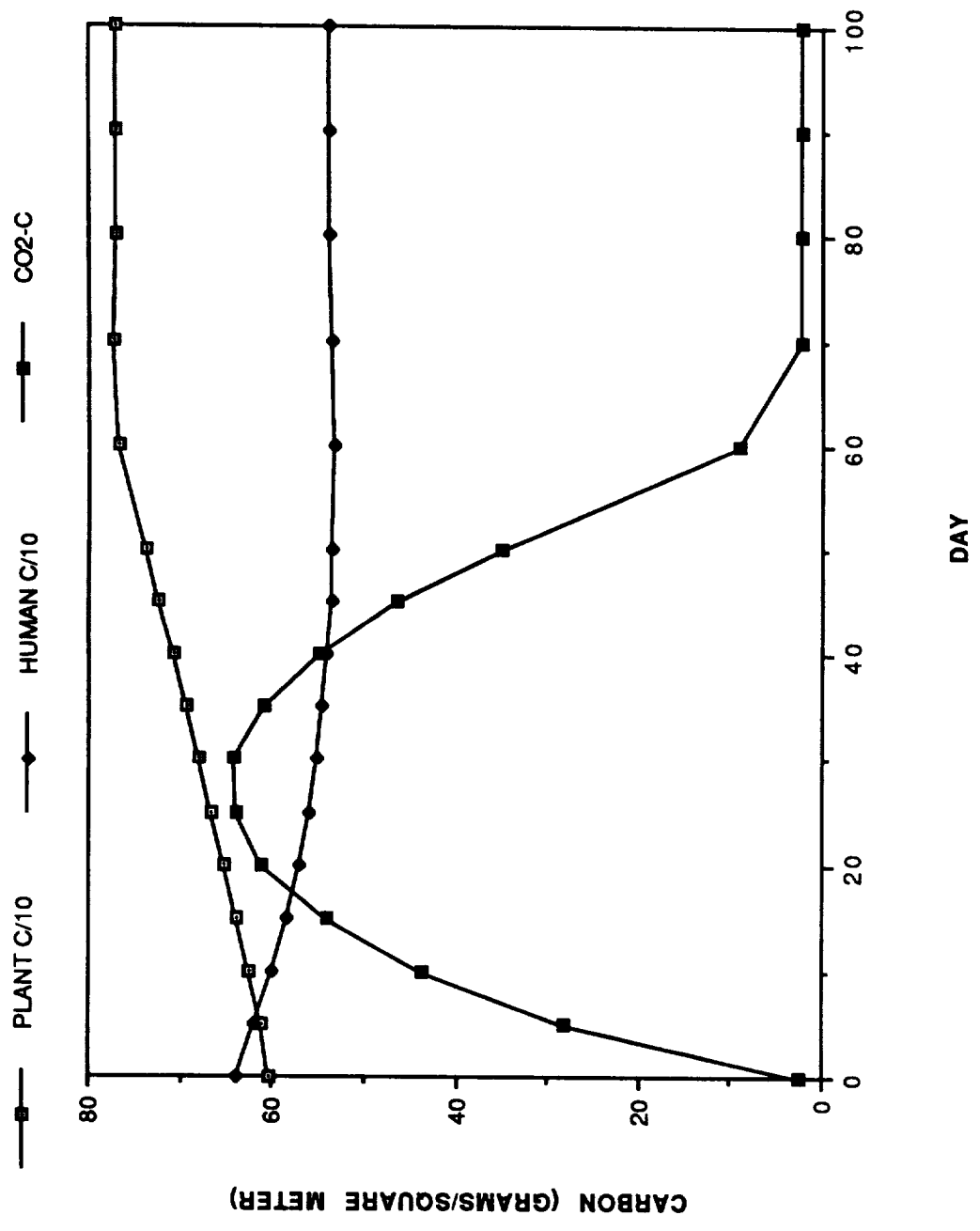


Figure 6. Effects of decreasing initial plant biomass by a third in CELSS model with Michaelis-Menten kinetics describing photosynthesis.

Table 2. Results of sensitivity analysis

Carbon Reservoir	Parameter			
	Km		Vmax	
	+	-	+	-
Plant	0.2+	0.1+	0.1+	32.3-
SPCB	0.0	0.1+	0.1+	32.0-
EPB	0.0	0.08+	0.1+	1.7-
BSB	0.4-	0.04+	0.1+	32.2-
SCFPB	0.0	0.0	0.03	1.0-
Aquaculture System	0.6+	0.25+	0.2+	24.0-
Human	0.2+	0.001-	0.1+	25.9-
CO <sub>2</sub>	31.3+	34.3-	59.3-	24100.0+

SPCB = Soluble Plant Carbon Bioreactor

EPB = Enzyme Production Bioreactor

BSB = Biopolymer Saccharification Bioreactor

SCFPB= Single Cell Food Production Bioreactor

+ = increase in parameter (see test)

- = decrease in parameter (see test)

All values are percent change in carbon reservoir from steady state level after 200 day model run.

reservoir except CO<sub>2</sub>. Since K<sub>m</sub> lies well below the asymptotic region of the Michaelis-Menten curve, the CO<sub>2</sub> response is symmetrical.

These results reveal several important features of the model and, hence, the system. First, the accurate determination of V<sub>max</sub> is critical to the effective modelling of the system. A 10% decline in V<sub>max</sub> causes 30% reductions in most carbon reservoirs. The human reservoir has not reached steady state even after 200 days (Figure 7). Secondly, the CO<sub>2</sub> reservoir is particularly sensitive to changes in photosynthetic rate. Carbon dioxide was the only reservoir to be significantly effected by all changes in K<sub>m</sub> and V<sub>max</sub>.

Concentration of CO<sub>2</sub> increased 24100% to a level of 241000 ppm with a 10% change in V<sub>max</sub>.

The relatively small CO<sub>2</sub> pool and large CO<sub>2</sub> fluxes are the probable cause of this volatility in CO<sub>2</sub> concentration. To prevent large fluctuations in CO<sub>2</sub> concentrations, feedback control of photosynthesis may be necessary. This could require that the baseline plant growth rate is less than the maximum rate so that increasing CO<sub>2</sub> can be corrected for by increasing CO<sub>2</sub> uptake. Alternative CO<sub>2</sub> "trapping" methods may also be effective. Varying the rate at which solid wastes are combusted is one potential alternative, although it represents a much smaller flux than photosynthesis.

#### Sensitivity of the Model to Changes in Enzyme Efficiency

The greatest effects of altered enzyme efficiency are found in the reservoirs directly influenced by products of the biopolymer saccharification reactor; the single cell food production reactor and the aquaculture subsystem (Table 3). The asymmetrical responsiveness of the system described above is also apparent in these results. Decreases in enzyme efficiencies cause almost twice the change in reservoir sizes as do increases. Human carbon decreases

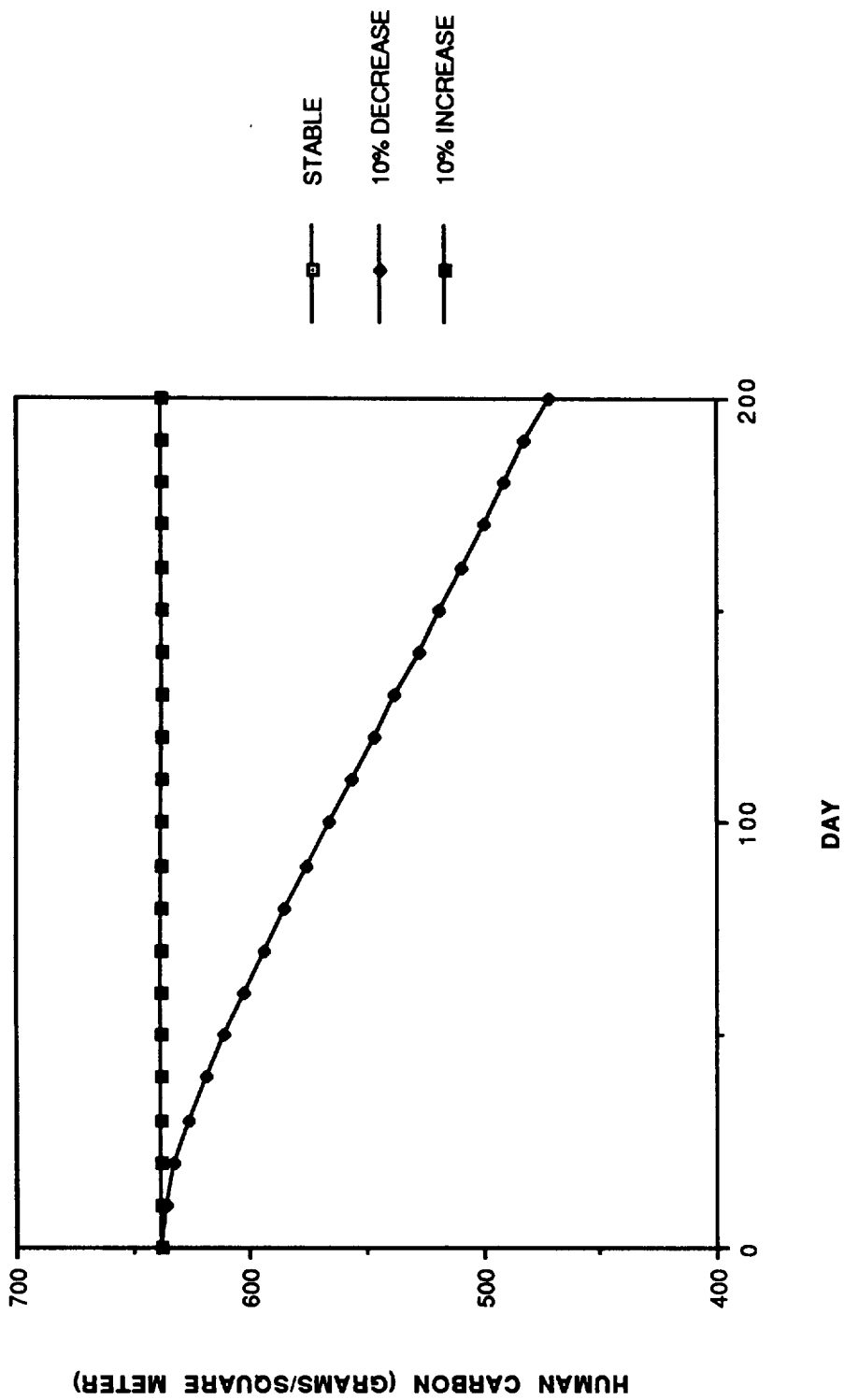


Figure 7. Effects of 10% changes in  $V_{max}$  on steady state model.

Table 3. Results of sensitivity analysis.

Carbon Reservoir	Test				
	Enzyme Efficiency			Fish Assimilation	
	+	-	0	+	-
Plant	0.3+	0.4-	2.1-	0.5-	0.7+
SPCB	0.3+	0.4-	2.0-	0.5-	0.8+
EPB	0.3+	0.4-	2.1-	0.5	0.7+
BSB	0.2+	0.4-	2.1-	0.5-	0.7+
SCFOB	11.9+	22.8-	100.0-	0.5-	0.8+
Aquaculture System	4.6-	9.3+	40.5+	1.0+	1.0-
Human	2.4+	5.2-	22.3-	0.1+	0.5-
CO <sub>2</sub>	1.3-	1.3-	1.2-	1.3+	1.3-

SPCB = Soluble Plant Carbon Bioreactor

EPB = Enzyme Production Bioreactor

BSB = Biopolymer Saccharification Bioreactor

SCFPB= Single Cell Food Production Bioreactor

+ = increase in parameter (see text)

- = decrease in parameter (see text)

0 = parameter set to 0

All values are percent change in carbon reservoir from steady state level after 200 day model run.



by 5.2% and increases by 2.4% with a 10% decrease and increase, respectively, in enzyme efficiency. This asymmetry reflects the dominant role of photosynthetic flux in the system.

The relatively small change in the human reservoir caused by altered enzyme efficiency suggests that enzymatic biomass conversion may not be an efficient means of food production in CELSS. To test this hypothesis a model run was performed with an enzyme efficiency of 0%. This elimination of enzymatic conversion causes a 22% reduction in human carbon (Table 3, Figure 8). Based on these results it can be concluded that enzymatic conversion provides a substantial augmentation of food production for humans. Accurate determination of enzyme conversion efficiency, however, is not of critical importance in modelling the system.

#### Sensitivity of the Model to Changes in Assimilation Efficiency of Fish

The model is very insensitive to changes in fish assimilation efficiency. No reservoir changes by more than 1% with a 10% increase or decrease in efficiency. This not only indicates that accurate determination of flux rates in the aquaculture system are not critical for system modelling, but suggests that the aquaculture system may not be an effective means of significantly increasing the rate of food production. Substantially greater assimilation and respiratory efficiencies could increase production rates, as could utilization of aquaculture waste products. It appears that single cell food production is a much more efficient means of processing inedible plant material. The difference in palatability and nutritional value between single cell food and fish, however, is an important consideration when comparing the separate food production techniques.

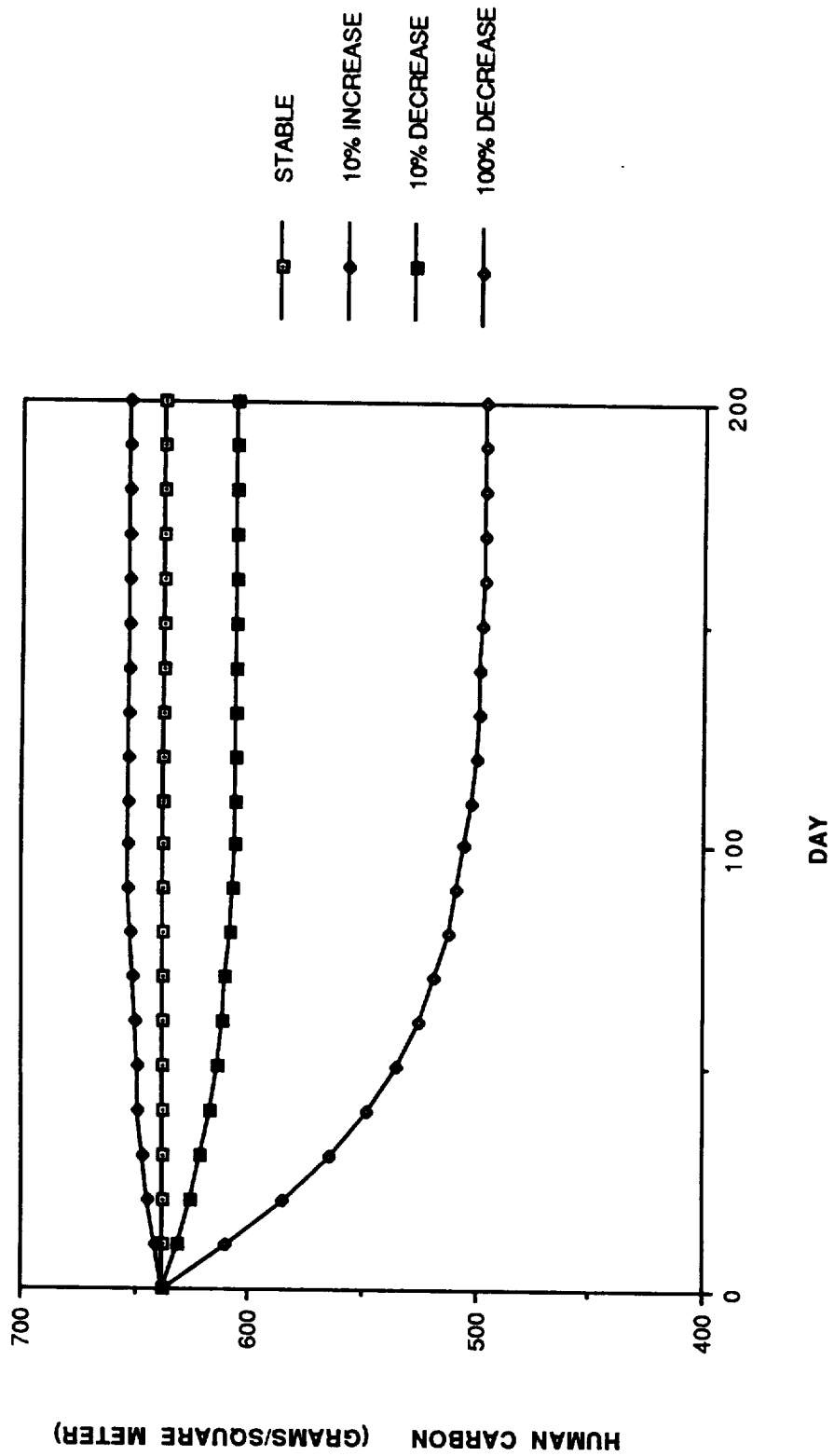


Figure 8. Sensitivity of human carbon reservoir to changes in enzyme conversion efficiency.

## CONCLUSIONS

1) A CELSS model containing a non-linear expression of photosynthetic flux (Michaelis-Menten kinetics) seems to produce more adequate model output compared to models using donor or recipient control of photosynthesis.

2) The accurate determination of the maximum rate of gross primary production ( $V_{max}$  term in Michaelis-Menten equation) is critical for modelling carbon flow in CELSS.

3) Feedback control mechanisms are probably necessary in a CELSS in order to prevent severe fluctuations in atmospheric  $CO_2$  concentrations.

4) Enzymatic conversion of inedible material has the potential to significantly increase the amount of food energy available for human consumption. Near-term research efforts in accurate determination of enzyme conversion efficiency, however, are not critical.

5) Aquaculture does not appear to be an efficient means of producing food energy unless fish are substantially more efficient energy converters than assumed in this model.

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# Report Documentation Page

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16. Abstract Internal cycling of chemical elements is a fundamental aspect of a Controlled Ecological Life Support System (CELSS). Mathematical models are useful tools for evaluating fluxes and reservoirs of elements associated with potential CELSS configurations. A simple mass balance model of carbon flow in CELSS was developed based on data from the CELSS Breadboard project at Kennedy Space Center. All carbon reservoirs and fluxes were calculated based on steady state conditions and modelled using linear, donor-controlled transfer coefficients. The linear expression of photosynthetic flux was replaced with Michaelis-Menten kinetics based on dynamical analysis of the model which found that the latter produced more adequate model output. Sensitivity analysis of the model indicated that accurate determination of the maximum rate of gross primary production is critical to the development of an accurate model of carbon flow. Atmospheric carbon dioxide was particularly sensitive to changes in photosynthetic rate. The small reservoir of CO <sub>2</sub> relative to large CO <sub>2</sub> fluxes increases the potential for volatility in CO <sub>2</sub> concentration. Feedback control mechanisms regulating CO <sub>2</sub> concentration will probably be necessary in a CELSS to reduce this system instability.			
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16. Abstract (continued)

Examination of alternative food production subsystems demonstrated that enzymatic conversion of inedible plant material has the potential to significantly increase human food supply. Aquaculture systems are much less efficient food producers, and should be considered for their qualitative rather than quantitative role in human nutrition.