NASA CONTRACTOR REPORT 177448

ALGAL CULTURE STUDIES FOR CELSS

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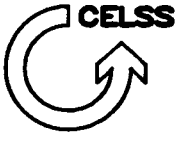

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ABSTRACT

Microalgae are well-suited as a component of a Closed Environmental Life Support System (CELSS), since they can couple the closely related functions of food production and atmosphere regeneration. The objective of this study was to provide a basis for predicting the response of CELSS algal cultures, and thus the food supply and air regeneration system, to changes in culture parameters (cell concentration, light intensity, carbon dioxide concentration, and temperature).

1) *Scenedesmus* growth was measured as a function of light intensity and the spectral dependence of light absorption by the algae and algal respiration in the light were determined as a function of cell concentration. These results were used to test and confirm a mathematical model that describes the productivity of an algal culture in terms of the competing processes of photosynthesis and respiration. According to this model the productivity of an algal culture is given by the relation $Y = EmIoAK(1 - e^{-\alpha cl}) - GRcV$, where Y = yield (g cells/hr), Em = the maximum attainable photosynthetic conversion on an energy basis (%), A = illuminated area (m^2), K = the energy equivalent of the algae (g cells/hr watt), Io = light intensity (watts/m^2), α = extinction coefficient (l/cm g), c = cell concentration (g/l), l = light path length (cm), R = respiration rate (g carbon/g cells hr), V = culture volume (l), and G = cell/carbon mass ratio (g cells/g carbon).

2) The relationship of algal productivity to cell concentration was determined at different carbon dioxide concentrations, temperatures and light intensities. At low cell concentrations (less than 0.1 g/l dry weight) the productivity of a *Scenedesmus* continuous culture was the same regardless of whether the gas stream was air or 2% CO_2 in air. At higher cell concentrations the productivity of the air-grown culture was consistently lower than the productivity of a 2% grown culture, suggesting that at these cell concentrations the CO_2 content of air was limiting the productivity of the culture. The maximum productivity achieved by an air-grown culture was found to be within 10% of the computed maximum productivity, indicating that CO_2 was very efficiently removed from the gas stream by the algal culture. The response of productivity to changes in cell concentration for 2% CO_2 and air-grown cultures were similar at both 32 °C and 25 °C; however, at 20 °C the productivity of a 2% CO_2 culture was reduced relative to the other temperatures. Measurements of biomass productivity as a function of cell concentration at different light intensities indicated that at 21 W/m^2 both the productivity and efficiency of light utilization were greater than at 12.9 W/m^2. These results are consistent with the proposed model of algal productivity, since the ratio of usable input energy to maintenance energy is increased at the higher light intensity.

3) *Scenedesmus* was grown in the light and at the expense of an added carbon source, which either replaced or supplemented the actinic light. Hydrolyzed waste *Scenedesmus* biomass was also tested as a carbon source to determine whether the algae themselves could be made part of the biological recycling process. Results indicate that hydrolyzed algal biomass can serve as carbon and energy sources for the growth of algae, suggesting that the efficiency of the closed system could be significantly improved using this recycling process.

4) Approximately two kilograms of *Scenedesmus* paste was produced under controlled culturing conditions. This biomass was shipped to Dr. M. Karel at MIT for use in his work to determine the nutritional value of algae as a component for a space crew diet.

I. INTRODUCTION

A Controlled Environment Life Support System (CELSS) is envisioned as a nearly self-contained system that will provide life support for a space crew. A CELSS must perform several basic functions, such as: 1) regenerating the atmosphere (i.e. converting CO_2 respired by the crew into O_2), 2) providing a food source for the crew, and 3) recycling waste products to provide the necessary raw materials for atmosphere regeneration and food production. Ideally this system will be self-supporting, with the only input being energy. A renewable life support system (such as a CELSS) will be required for long duration space flight since the volume and weight of nonrenewable supplies (food and oxygen) necessary to sustain the crew would be prohibitively large.

Photosynthetic microalgae would be an ideal component of a CELSS since they can couple the closely related functions of air regeneration and food production. Algae can use (sun)light as an energy source to convert CO_2 released by the crew into organic algal biomass (which can serve as a food component for the crew) and release CO_2 as a byproduct, thereby regenerating the atmosphere of the spacecraft.

Algae also have several distinct culturing characteristics that make them well-suited as a component of a CELSS. 1) They grow as a uniform, homogeneous culture that can easily be handled and manipulated (more like chemical reagents than plants); 2) they grow rapidly with a generation time of only a few hours; and 3) although algae are not necessarily intrinsically more efficient than higher plants, the culturing techniques that are available result in much higher efficiencies than are usually attainable with higher plants.

Although algae are an attractive component for a CELSS, further research is needed before an algal growth system can be successfully integrated into a CELSS. The objectives of this project are to: 1) conduct lab-scale studies on continuous algal culture systems to determine the effect of changing culture parameters on algal productivity, and 2) use the continuous culture systems to supply well-characterized algal biomass for use in determining the suitability of algal biomass as a food component for a space crew. This work involves studying aspects of algal metabolism directly related to the utilization of carbon and light, and focuses on the functional relationships of biomass productivity, light utilization and CO_2 utilization efficiency to the inputs of CO_2 and light at a given temperature.

This report contains the results of a two year project. Over the course of this project we have accomplished the above-stated objectives, and thus we have a better understanding of the physiological parameters that are important in order to integrate an algal growth system into a functional CELSS. The specific accomplishments of this project included: 1) developing and testing a mathematical model that accurately predicts the productivity of an algal continuous culture as a function of the cell concentration of the culture. A manuscript describing this model has recently been published in **Biotechnology and Bioengineering** (1, and see Appendix); 2) accumulating basic data concerning the effect of different CO_2 concentrations, temperatures, and light intensities on algal productivity and the efficiency of light utilization; 3) supplying quantities of algal biomass to Dr. M. Karel and his colleagues at MIT for use in

studies on the utilization of algae for human consumption; 4) developing larger algal continuous culture vessels necessary to satisfy the expected increase in demand for algal biomass for food processing studies; and 5) conducting preliminary studies concerning the use of waste algal biomass as a carbon and energy source for further algal growth, thereby potentially improving the overall efficiency of a CELSS algal culture system.

II. MATERIALS AND METHODS

Organisms and Growth Conditions

The two organisms that have been used in these studies are *Scenedesmus obliquus* (Gaffron strain D3) and *Spirulina platensis* (Nord.) Geitler. The *Scenedesmus* has been used by us in-house for years; the *Spirulina* was acquired from the University of Texas Culture Collection.

Scenedesmus was cultured in medium containing 1.0 g/l $MgSO_4.7H_2O$, 0.02 g/l $CaCl_2.2H_2O$, 0.38 g/l K_2HPO_4, 0.15 g/l KH_2PO_4, 6.0 g/l KNO_3 and 2 ml/l micronutrient solution (5.0 g $FeSO_4.7H_2O$, 4.0 g Na_2EDTA, 2.86 g H_3BO_3, 1.81 g $MnCl_2.4H_2O$, 0.22 g $ZnSO_4.7H_2O$, 0.39 g $Na_2MoO_4.2H_2O$, 0.08 g $CuSO_4.5H_2O$, 0.05 g $Co(NO_3)_2.6H_2O$ dissolved in one liter of distilled water). *Spirulina* was cultured in medium that contained 0.2 g/l $MgSO_4.7H_2O$, 0.04 g/l $CaCl_2.2H_2O$, 3.0 g/l $NaNO_3$, 4.0 g/l Na_2CO_3, 16.8 g/l $NaHCO_3$, 0.5 g/l Na_2HPO_4, 2.0 g/l NaCl, 1.0 g/l K_2SO_4, 0.04 mg/l vitamin B_12, 0.04 g/l Na_2EDTA, 0.05 g/l $FeSO_4.7H_2O$ and 1 ml/l of micronutrient solution (5.72 g H_3BO_3, 3.62 g $MnCl_2.4H_2O$, 0.44 g $ZnSO_4.7H_2O$, 0.78 g $Na_2MoO_4.2H_2O$, 0.16 g $CuSO_4.5H_2O$, 0.14 g $Co(NO_3)2.6H_2O$, 0.023 g NH_4VO_3, 0.096 g $K_2Cr_2(SO_4)4.24H_2O$, 0.048 g $NiSO_4.7H_2O$, 0.018 g $NaWO_4.7H_2O$, 0.040 g $Ti(SO_4)_3$ dissolved in one liter of distilled water).

Stock cultures of *Scenedesmus* were maintained in 1000 ml Roux bottles (containing 500 ml of culture) kept in an environmental chamber. The cultures were continuously bubbled with a gas mixture of 2% CO_2 in air. Prior to introduction to the cultures, the gas mixture was humidified by bubbling it through a water reservoir maintained at the same temperature as the cultures. *Spirulina* stock cultures were maintained in an environmental chamber in glass test tubes containing approximately 10 ml of culture. Both *Scenedesmus* and *Spirulina* stock cultures were maintained at 29 $\pm$ 2 °C and a photon flux of 40-90 uE/m^2/sec. Light intensity was supplied by high output, cool white fluorescent bulbs. All cultures were handled and sampled using aseptic techniques.

Growth Rate Determinations of *Scenedesmus*

Experiments to determine the growth rate of *Scenedesmus* as a function of photon flux were performed using Roux bottles that were continuously illuminated and bubbled with 2% CO_2 in air. Each Roux bottle was placed in a black cardboard box that had one side removed. The box was placed directly against the light source (two high output, cool white fluorescent bulbs) with the open side of the box facing the light. Photon flux was measured on the back of the bottle using a LiCor 190SB Quantum Sensor. This arrangement eliminated stray light,

thereby permitting a very accurate measurement of the photon flux incident on the culture. The temperature of the experimental bottle was determined by placing a thermometer in a Roux bottle that was maintained under identical conditions, with the exception that it did not contain algae. At time zero, the experimental bottle was inoculated at a very dilute cell concentration using cells from an exponentially growing stock culture that had been maintained at a temperature and light level similar to the experimental conditions. Twice per day a small sample (10 ml) of the culture was removed, and the cell concentration (cells/ml) determined using a Coulter Counter Model TAII with PCA II population accessory. The temperature and photon flux for each bottle were recorded immediately after each sampling. When the culture reached a density at which the transmitted photon flux was significantly lower than the initial measurements (i.e. when the culture was dense enough to provide a faint trace of color to the bottle), subsequent measurements of the cell concentration were not used in determining the growth rate.

Description of the Continuous Culture Apparatus

The continuous culture apparatus used in this work is similar to the device described by Myers and Clark (2). The apparatus is made of glass and consists of three concentric, cylindrical chambers (Figure 1). The outermost chamber is a temperature regulated water jacket, the middle chamber contains the algal culture, and the inner chamber can house a fluorescent light source. The middle (culture) chamber has a volume of 1200 ml and a path length of 1.25 cm. The algal culture is continuously illuminated by a high output, cool white fluorescent lamp located in the inner light chamber (average light output from this lamp is approximately 12.9 W/m^2). Except as noted, this light source was used for all continuous culture experiments. Cell density is maintained by monitoring the light transmission from this bulb through the culture using a photocell (Clairex CL604L) whose output is fed directly to a microcomputer (Sym-1, Synertek Corp.). When the output of the photocell exceeds a preset reference value (i.e. when the culture grows and absorbs more light) the microcomputer activates a peristaltic pump for a preset period of time and fresh medium is added to the culture. A corresponding volume of algal culture exits the vessel via the overflow tube. After a latent time of 60 sec. to allow for mixing of any newly added medium, the monitoring cycle is reinitiated.

A gas mixture of CO_2 in air was admitted through the fritted bottom of the culture chamber. The gas composition and flow rate depended on the particular experiment (see below). A flow rate >150 ml/min was sufficient to provide relatively rapid mixing of the culture and to prevent the cells from settling.

Higher light intensity was supplied by a very high output, cool white fluorescent lamp placed in the center light chamber of the continuous culture apparatus. This bulb supplied approximately 50% greater photon flux to the culture than the high output bulb. The CO_2 content of the gas stream was controlled by mixing air and CO_2 in a gas proportioner (according to standard calibration curves) before being admitted to the culture. The CO_2 concentration of the gas mixture was verified by use of an infrared CO_2 analyzer (calibrated with certified reference standards).

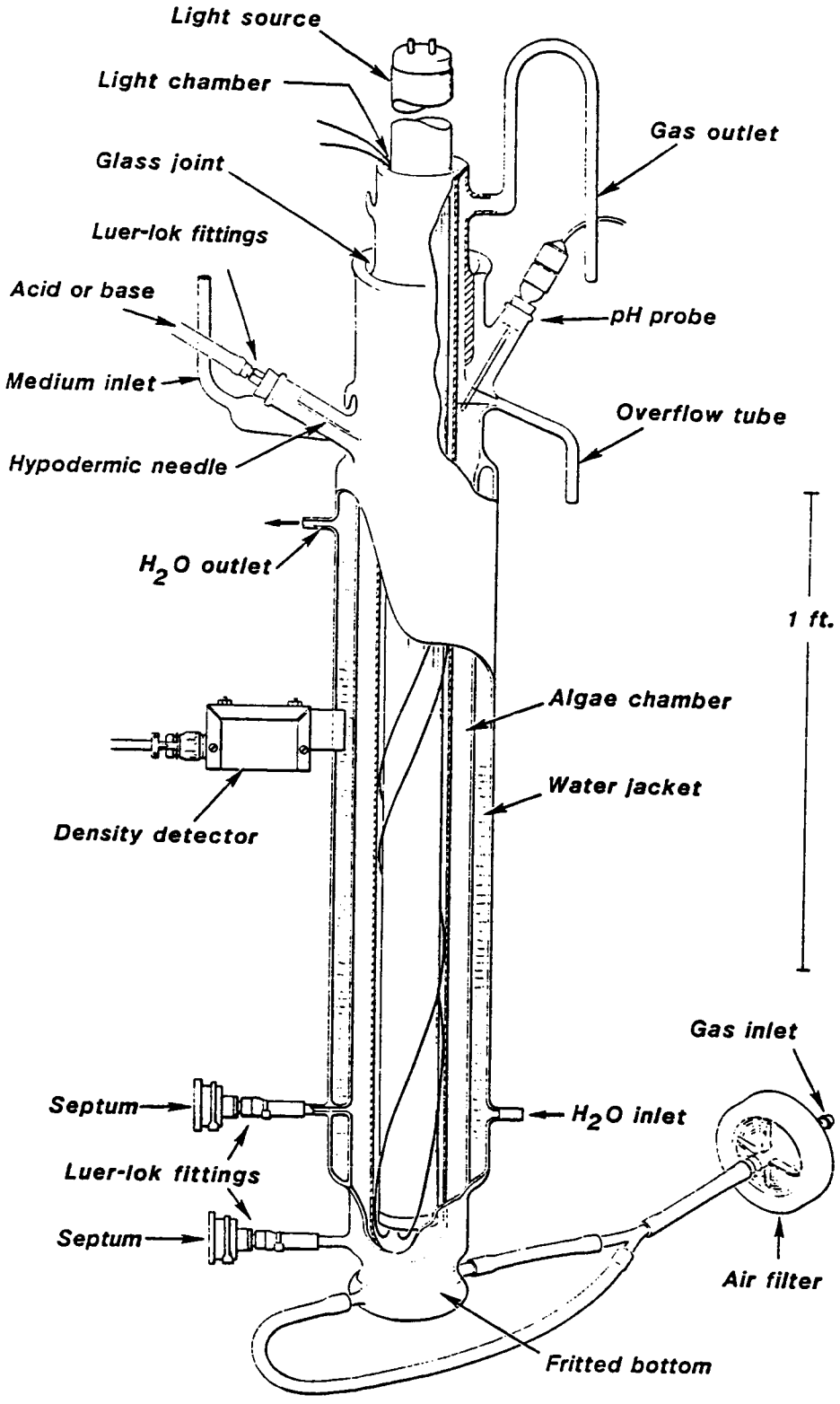


Figure 1. Algal Continuous Culture Apparatus.

Analytical Chemical Methods

The chlorophyll concentration of *Scenedesmus* was determined by adding an aliquot of culture to a 1:1 mixture of Triton X-100 and 5% KOH in methanol, heating at 63 °C for three minutes, and centrifuging. Optical density was determined at 645 nm. The temperature of the continuous cultures was monitored daily by use of a thermocouple immersed in the culture. Dry weight determinations involved filtering a 10 ml aliquot of culture through a glass fiber filter (approximate retention 2.6 μm), rinsing thoroughly with distilled water, drying at 105 °C overnight, and cooling to room temperature in a dessicator. (The filter paper was previously dried at 105 °C before beginning the procedure). The doubling time of a continuous culture was taken as the ratio of the overflow rate (ml/hr) and the total volume of the culture vessel (1200 ml); the biomass productivity (mg dry weight/hr) was taken as the product of the cell concentration (mg dry weight/ml) and the overflow rate.

Light Measurements

Measurements of photon flux (μE/m^2/sec) were performed with a LiCor 190SB Quantum Sensor that integrated the photon flux over the range of 400-700 nm. Light intensity (μW/cm^2-/nm) was measured with an ISCO Model SR spectroradiometer. Measurements were made over the range of 400-700 nm at 25 nm intervals, and the measurements integrated to obtain the light intensity (μW/cm^2).

The extinction coefficient of an algal culture of a particular cell concentration was calculated using the Beer-Lambert Law ($I = Ioe^{-\alpha cl}$), where I is the transmitted light, Io is the incident light, α is the absorption coefficient, c is the cell concentration, and l is the light path length). I and Io were measured using a large glass cuvette (path length of 0.95 cm) that was illuminated by a high output, cool white fluorescent lamp. The light crossing the cuvette in the presence and absence of cells was measured with the quantum sensor, and these values were taken as I and Io, respectively.

The efficiency of light utilization was determined by measuring the light intensity (with the spectroradiometer) that exited the continuous culture apparatus in the presence and absence of the culture. Light measurements were made at eight points around the culture apparatus, and the values were averaged to correct for any lack of symmetry in the apparatus or light field. The difference in light intensity was taken as a measure of the amount of light absorbed by a culture at a particular cell concentration. The light utilization efficiency was calculated as the ratio of the calories/hr of biomass produced and the calories/hr of light energy absorbed. Calories/hr of biomass was converted from the productivity data using a conversion of 1 g *Scenedesmus* = 5500 calories (determined by Galbraith Laboratories, Knoxville, TN).

Oxygen Measurements

Net oxygen evolution and uptake were measured polarographically. The polarograph vessel was surrounded by a water jacket that was maintained at 32 °C. Illumination (800 μE/m^2/sec incident light intensity) was provided by a 1000 W projector bulb filtered through 4 cm of water,

two KG2 neutral density filters, and an OG590 cutoff filter (Schott). Net O_2 was calculated using the concentration of dissolved oxygen in air saturated water as a standard.

Oxygen exchange (O_2 evolution and uptake) measurements were performed with a quadrupole mass spectrometer using isotopically labeled O_2 (98 atom% $^{18}O_2$ Cambridge Isotope Laboratories). The instrumentation and methods for data analysis and calibration were the same as described previously (3), with the exception that the data handling system was a Synertek Sym-1 microcomputer, and the data was stored on magnetic tape. All experiments were done at 32 °C. Illumination was either broad band, saturating orange-red light (Schott OG530 and KG3 heat filters) or fluorescent light having a photon flux comparable to that used to maintain the continuous cultures.

Preparation of Algal Extracts

A water soluble extract of algal biomass was prepared by the following procedure. Dried algal cells (20 g) were thoroughly homogenized in 200 ml of chloroform/methanol (1:1, v/v) and extracted for 24 hr to remove lipid and pigments. The organic extraction was repeated twice. The extracted biomass was collected by centrifugation, dried, and resuspended in 100 ml of water. Cellulase was added to a final concentration of 0.1 mg/ml and the mixture was incubated at 25 °C, with stirring, for 24 hrs. Particulate material was removed by centrifugation and the remaining water soluble extract was heated in a boiling water bath for 20 min to inactivate the cellulase. This heating step also reduced the volume of extract to approximately 50 ml. The extract was sterilized by filtration through 0.25m filters prior to use. No attempt was made to quantitate the level of organic material, or specifically glucose, in the extract. Where appropriate the extract was included in the growth medium at a level of 2%, v/v.

III. MATHEMATICAL MODEL OF ALGAL PRODUCTIVITY

The objective of this series of experiments was to derive and test a mathematical model that would describe the productivity of an algal culture as a function of cell concentration for a light-limited culture in which all other inputs are in excess. This model would predict the yield of an algal culture under a defined set of operational parameters, or conversely, given a particular yield, would define the values of the input parameters necessary to achieve that yield.

During the past contract period we developed such a model and found that it very closely described the experimental test data. Although this model has only been verified for the green alga *Scenedesmus obliquus*, we expect that it will be applicable to other algae. A manuscript describing this work is being published in **Biotechnology and Bioengineering** (see Appendix).

This model is based on the assumption that the productivity (Y) of an algal culture is equal to its rate of photosynthesis minus its rate of respiration. Ideally, for a fixed incident light intensity the total photosynthetic yield of the culture would increase asymptotically with increasing cell concentration. However, this increase in photosynthesis with increasing cell concentration would be counterbalanced by an increase in the total respiration of the culture.

Therefore, the overall productivity of the culture as a function of cell concentration will go through a maximum.

Under conditions in which the photosynthetic apparatus is not light saturated, the total rate of photosynthesis of the culture will be equal to the maximum theoretical rate of photosynthesis minus a term that describes the amount of light transmitted (not absorbed) by the culture. The expression describing the amount of light transmitted is a form of the Beer-Lambert Law, in which the transmitted light intensity I, is equal to $Ioe^{-\alpha cl}$, where α is the absorption coefficient, c is the cell concentration, and l is the path length. Therefore, the rate of photosynthesis of the culture is given by:

$$P = EmIoAK - EmIoAKe^{-\alpha cl}$$

where Em is the maximum attainable photosynthetic conversion on an energy basis (20%, see reference 4), Io is the incident light intensity, A is the illuminated area, and K is the energy equivalent of the algae (0.156 g cells/hr/W, determined calorimetrically). The respiration rate of the culture is the product of the cellular respiration rate (R), the cell concentration (c), the total culture volume (V), and G, a term related to the amount of carbon per cell (2.04 g cells/g carbon for *Scenedesmus*). Therefore, the productivity (yield) of an algal culture is given by the expression:

$$Y = EmIoAK(1 - e^{-\alpha cl}) - GRcV$$

For the culture apparatus described in Figure 1, the illuminated area is 0.15 m^2, the culture volume is 1.2 l, and the average light intensity is 12.9 W/m^2 (59.6 $\mu E/m^2/sec$).

This model only holds for the special condition in which photosynthesis is proportional to the amount of absorbed light, i.e. light is not saturating. Therefore, the initial experiment was to determine the relationship between the growth rate of *Scenedesmus* and photon flux to verify that at a photon flux of 59.6 $\mu E/m^2/sec$ photosynthesis was directly proportional to the light. Figure 2 shows the results of such an experiment using either Roux bottles (closed circles) or the continuous culture apparatus (open circles). The doublings per day increase linearly with photon flux up to 80-100 $\mu E/m^2/sec$. At the highest photon flux of the continuous culture system (approx. 75 $\mu E/m^2/sec$), the growth rate is still on the linear portion of the curve, indicating that the algae in the continuous culture system are not light saturated and that photosynthesis is proportional to the amount of absorbed quanta. Therefore, a necessary condition of the model is satisfied.

To test this model it was also necessary to determine the relationship between the absorption coefficient of the algal culture and the cell concentration. Figure 3 shows the relationship between the integrated (400-700 nm) average absorption coefficient and cell concentration in the continuous culture apparatus. This relationship is neither linear nor constant, reflecting the fact that at high cell concentrations the predominant green light is only poorly absorbed.

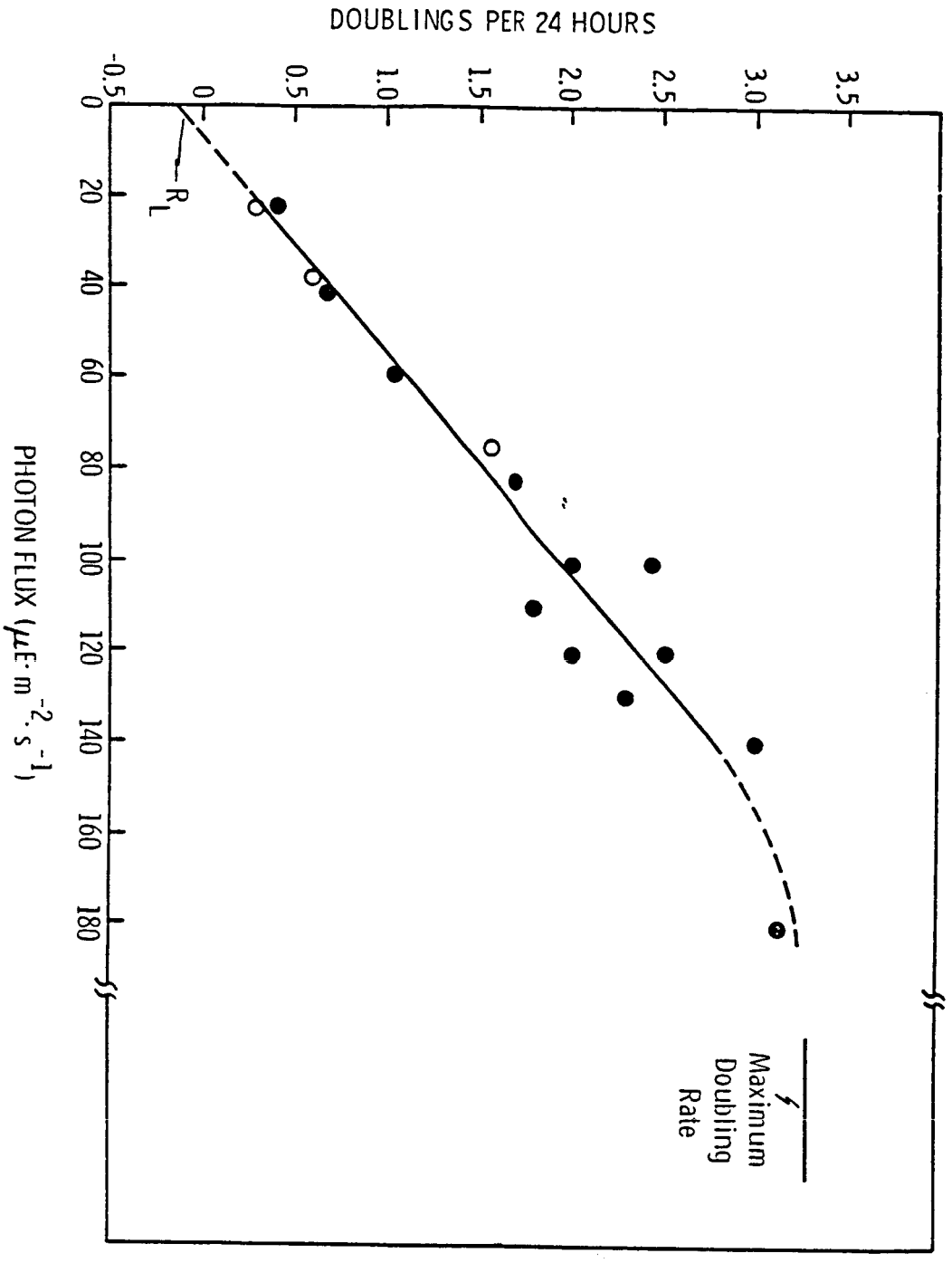


Figure 2. Doublings per day versus photon flux for *Scenedesmus*.

This phenomenon can be quantitatively rationalized in terms of the Beer-Lambert Law, which is strictly valid only for monochromatic light.

Three different approaches were used to measure cellular respiration in the photosynthesizing algal culture. In the first approach, the respiration rate was approximated by extrapolating the data from Figure 2 to zero photon flux. The extrapolated value is -0.15 doublings per day (see RL in Figure 2) and corresponds to a respiration rate of 0.003 g carbon/g cells/hr (assuming the cells are 49% carbon).

The second approach involved measuring oxygen consumption by a darkened culture using an oxygen polarograph. Thoroughly dark-adapted cells had a low respiration rate of 2.8 μmoles O_2 consumed/mg chl/hr or 0.0016 g carbon/g cells/hr (Figure 4). Upon illumination, the respiration rate was masked by a high rate of photosynthetic oxygen evolution. Immediately after the cessation of illumination, there was a high rate of respiration (13.9 μmoles O_2 consumed/mg chl/hr or 0.0081 g carbon/g cells/hr) which, after ten minutes in the dark declined to about the same rate as before illumination. Although these observations are not new (5), these values provide limits for the respiration rate needed for the mathematical model.

The third approach for determining the respiration rate involved the use of the mass spectrometer system described earlier to simultaneously monitor oxygen evolution and oxygen uptake during illumination. The experiment in Figure 5 was performed under the same conditions used for the continuous culture apparatus; thus, the oxygen exchange (evolution and uptake) should closely mimic that occurring in the culture system. Upon illumination there was an initial burst of oxygen uptake that subsequently leveled off and reached a steady state that corresponds to 8.5 μmoles O_2 consumed/mg chl/hr (0.0049 g carbon/g cells/hr). Concurrently, there was a sustained light-dependent rate of O_2 evolution (60 μmoles O_2 evolved/mg chl/hr), a value consistent with the calculated growth rate based on the productivity of the culture. Figure 6 is a compilation of a series of experiments shown in Figure 5 carried out at varying cell concentrations. The rate of O_2 uptake decreased markedly as the cell concentration increased to one g/l dry weight. Above one g/l dry weight, O_2 uptake was relatively constant with cell concentration. The high rate of O_2 uptake at low cell concentrations may be due to the ability of these algae to reduce O_2 using photosynthetically-derived reducing equivalents (6), and not due to "dark" respiration. Whatever its ultimate basis, respiration rates at low cell concentrations have minimal effects on the predicted productivities in the model being tested.

The mass spectrometer approach to determining the respiration rate is probably the most relevant approach since it provides an estimate of the respiration rate during illumination. Furthermore, the respiration rate determined from the mass spectrometer experiments (0.0029 g carbon/g cells/hr) is within the range of values determined by the other two approaches. Therefore, for the purposes of the model, the respiration rate was taken to be approximately 0.0029 g carbon/g cells/hr.

Figure 7 illustrates the observed and predicted relationships between culture productivity and cell concentration. The data points were obtained in a series of experiments in which *Scenedesmus* was maintained in the continuous culture apparatus. The solid line is the re-

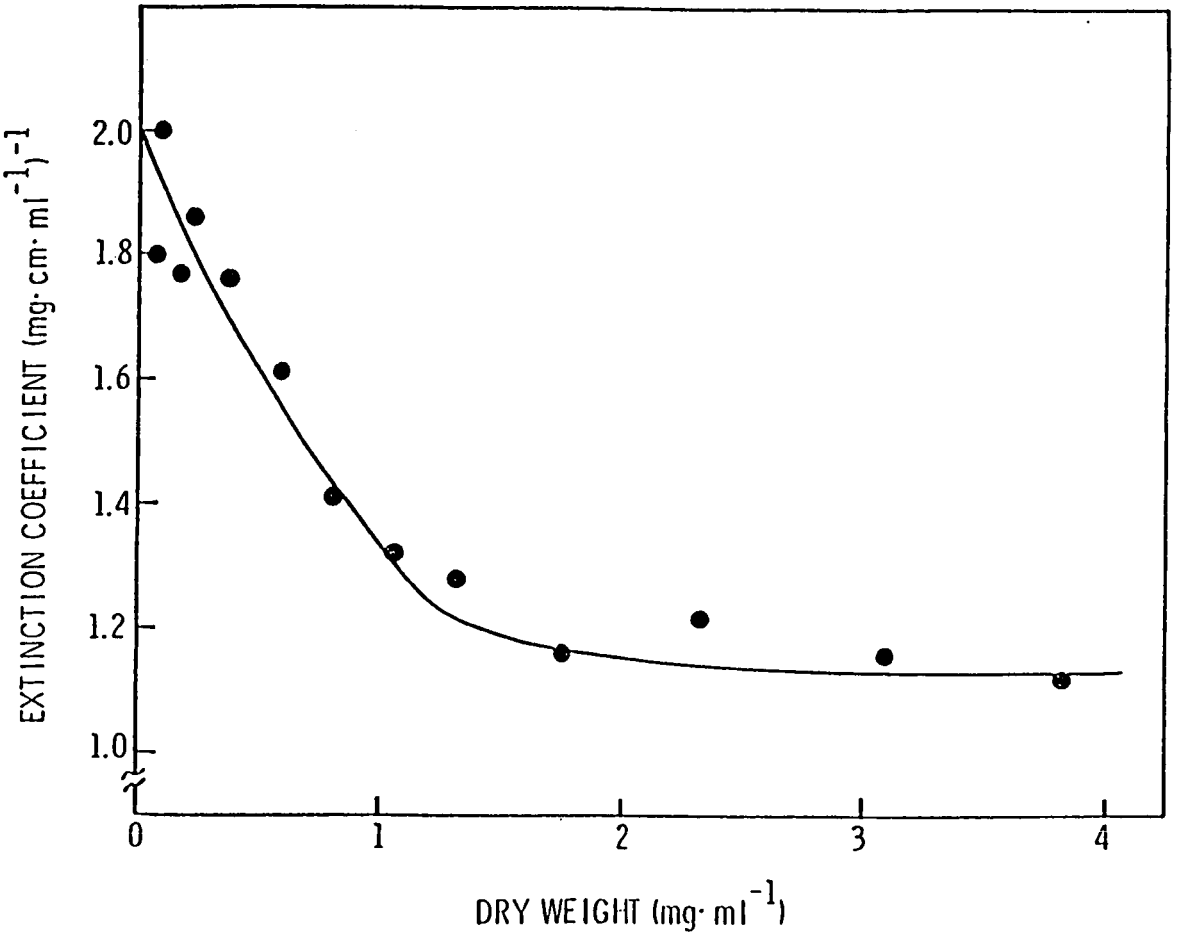


Figure 3. Absorption coefficient of *Scenedemus* as a function of cell concentration.

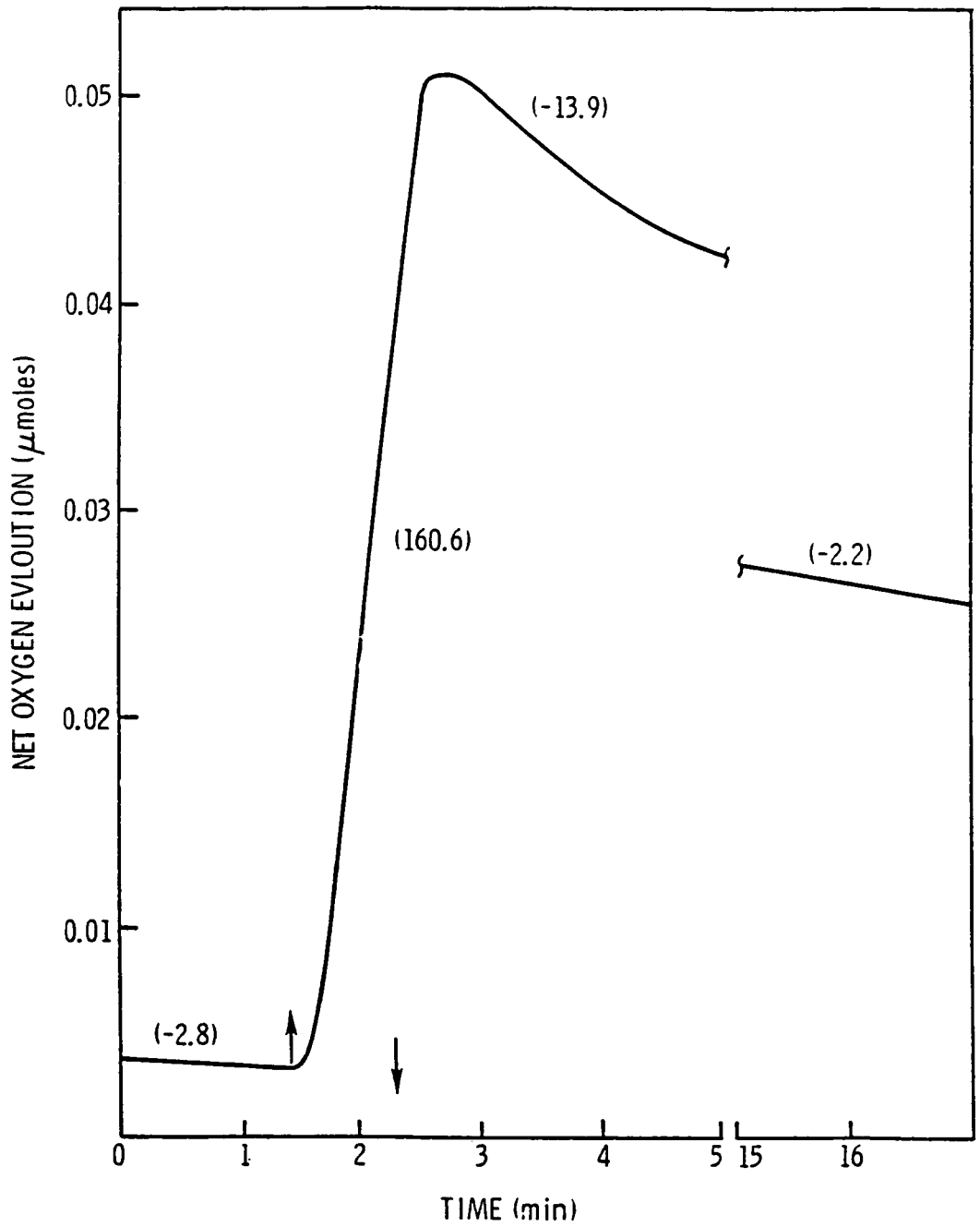


Figure 4. Polarograph trace of dark adapted *Scenedesmus*. Arrows indicate light on and off.

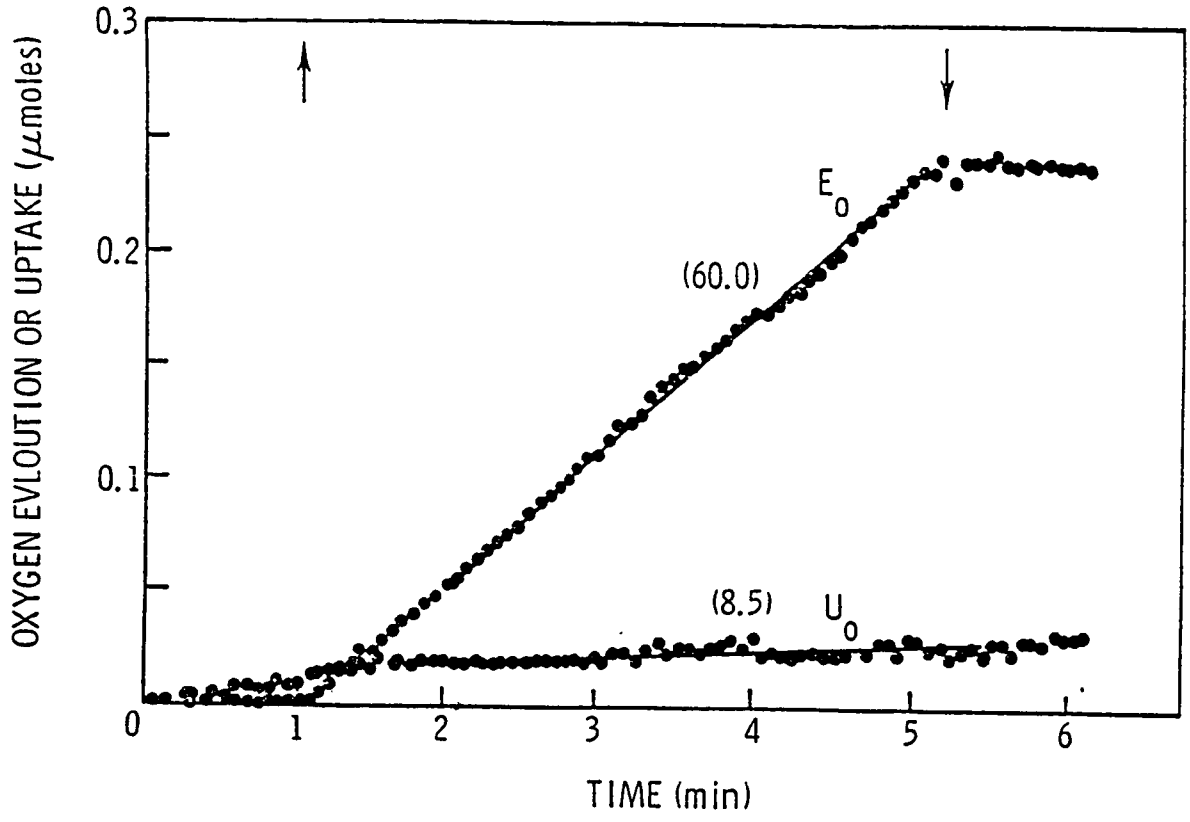


Figure 5. Computed time course of O_2 evolution (E_0) and O_2 uptake (U_0). Arrow indicate light on and light off.

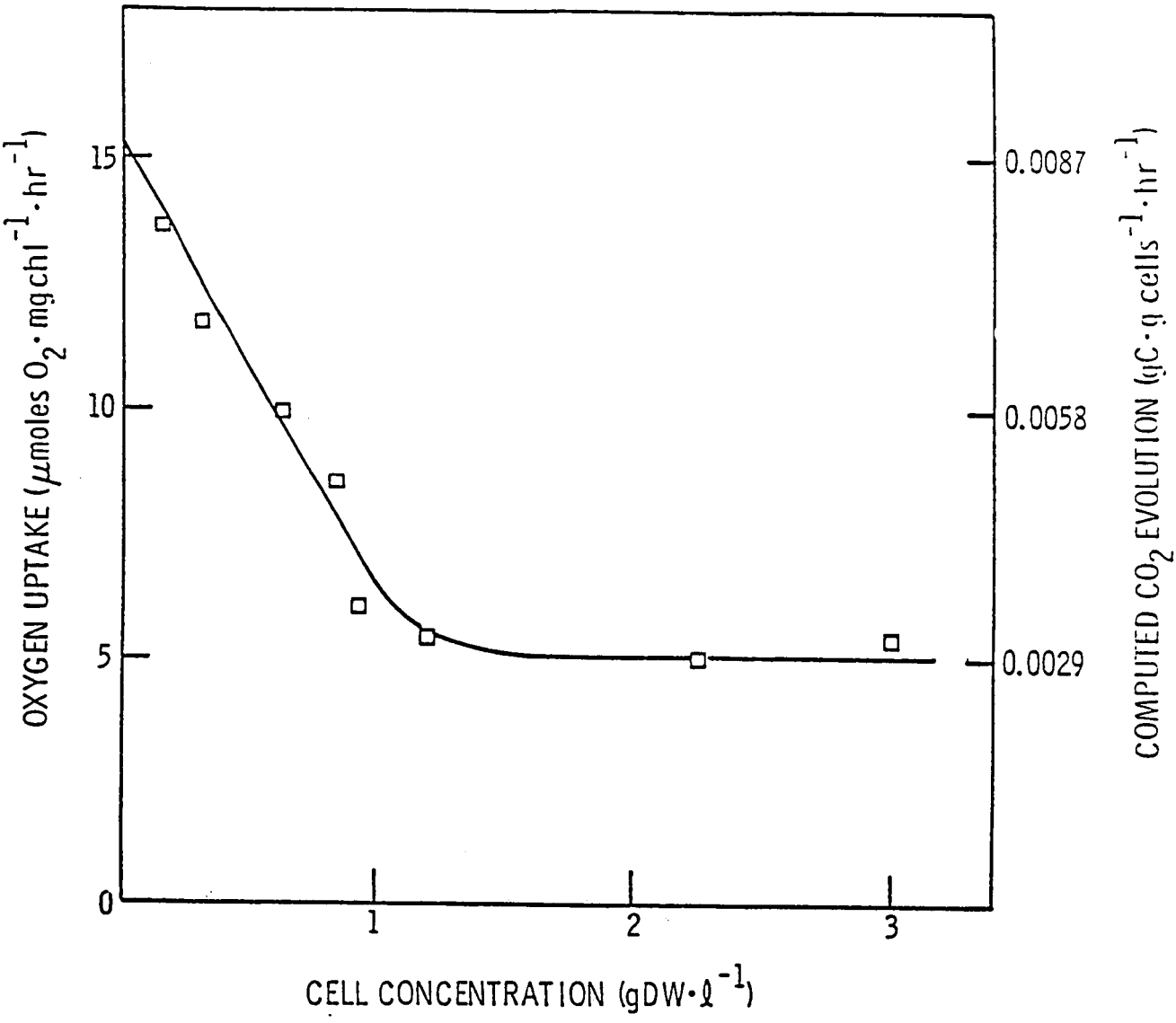


Figure 6. Oxygen uptake and computed CO_2 evolution as a function of cell concentration.

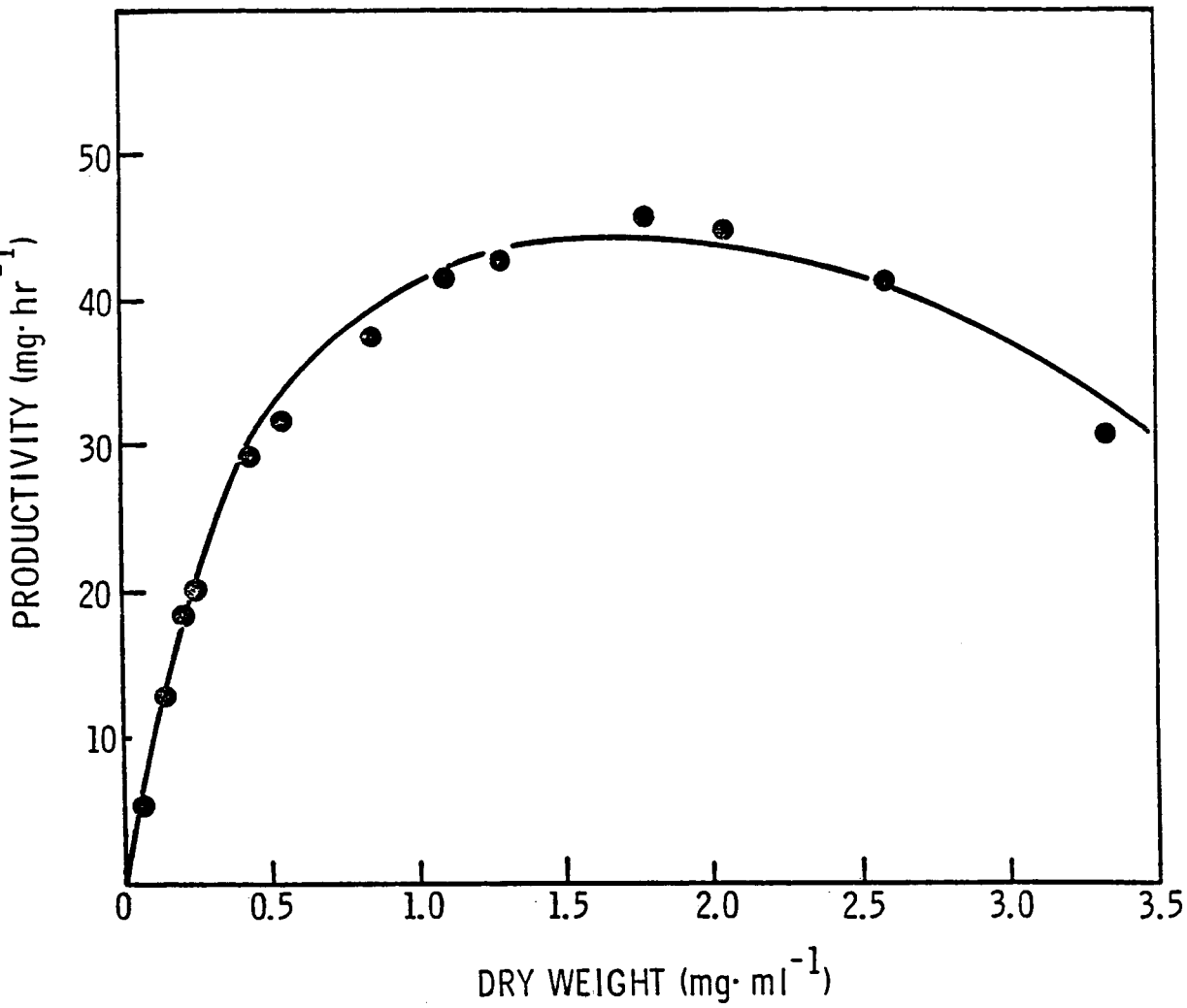


Figure 7. Relationship of culture productivity to cell concentration.

lationship predicted using the values derived above. The excellent agreement between the observed and predicted values suggests that the model accurately describes the culture system in terms of physiological parameters.

At cell concentrations greater than about 1.5 g/l dry weight, both the absorption coefficient and the respiration rate are relatively constant as a function of cell concentration (see Figures 3 and 6). Thus, by differentiating the model equation an expression is obtained for calculating the cell concentration of maximum productivity:

$$Cmp = \frac{1}{\alpha l} \ln \frac{\alpha EmIoAK}{GRV}$$

where Cmp is the cell concentration at maximum productivity. Using the values above, Cmp was calculated to be 1.73 mg/ml dry weight, which agrees well with the data of Figure 7.

IV. THE EFFECT OF CO_2 CONCENTRATION ON ALGAL PRODUCTIVITY

Algae have the ability to adapt to and grow under widely varying CO_2 conditions, and the physiology of algae grown under different CO_2 concentrations may be quite different. The specific objectives of these studies were to 1) determine the efficiency of CO_2 transfer from the gas stream that bubbles through the culture to the liquid phase of the culture, and 2) determine the productivity of an algal culture as a function of the input of CO_2.

Physiochemical CO_2 Removal

The objective of these experiments was to determine the maximum efficiency for the transfer of CO_2 from the gas phase to the liquid phase in the continuous culture apparatus. The extent of CO_2 removal from the gas stream will depend on factors such as the size of the gas bubbles and the length of time the bubbles are in contact with the liquid.

For these determinations the continuous culture apparatus was filled with a 1 N KOH solution and bubbled with a mixture of 2% CO_2 in air at a total gas flow rate of 1000 ml/min. (The presence of a strong base solution will favor the formation of $HCO3^-$, and as a result provide a means to very efficiently trap CO_2 that is removed from the gas stream.) The CO_2 content of the exhaust gas from the apparatus was measured and compared to the CO_2 content of the input gas using an infrared CO_2 analyzer. We found that there was a rapid (within the 5 min response time of the instrument) removal of essentially all of the CO_2 in the gas stream. This result indicates that, for our apparatus under these conditions, the bubble size and residence time of the CO_2 are sufficient for a virtually complete transfer of CO_2 from the gas to the liquid. Furthermore, as little as 28 mls of KOH solution in the apparatus (2.5% of the total apparatus volume) resulted in the rapid removal of 99% of the CO_2 in the gas stream. This indicates that the residence time for CO_2 in the total apparatus is approximately 40 times longer than is necessary for complete CO_2 removal. Over a period of time, the amount of CO_2 removed from the gas stream began to decrease, and eventually CO_2 was no longer

removed from the gas stream, presumably due to saturation of the solution with bicarbonate. The length of time before CO_2 removal declined to one-half of the maximum depended on the volume of KOH solution in the apparatus. These results indicate that with our continuous culture apparatus, CO_2 is very efficiently transferred from the gas stream into the liquid phase of the culture.

Algal Productivity as a Function of CO_2 Concentration

The objective of these experiments was to determine the relationship of algal productivity to the CO_2 concentration of the gas stream. These experiments were done using a continuous culture system set at a cell concentration (approximately 1.5 g/l dry weight) that yielded maximum productivity under conditions of saturating CO_2 and limiting light intensity (see Figure 7). The CO_2 concentration of the gas stream was then systematically changed and the biomass productivity and efficiency of light utilization were determined. The CO_2 concentration of the input gas and the exhaust gas from the culture were determined with the infrared CO_2 analyzer.

As shown in Figure 8, at CO_2 concentrations below 0.5% both the productivity and light efficiency increased with an increase in CO_2 concentration, while at CO_2 concentrations between 0.5% to 2.0% there was no significant difference in the productivity or efficiency of light utilization. The experimentally observed productivity (crosses) and the productivity calculated from the CO_2 consumed by the culture (diamonds) agreed over the entire range of CO_2 concentrations used. These results indicate that under these experimental conditions productivity is proportional to the CO_2 concentration up to approximately 0.5% CO_2.

Based on the results in Figure 8, the relationship of algal productivity to cell concentration was determined at a CO_2 concentration that was not saturating (i.e. less than 0.5%). (It is important to determine the biomass productivity from a culture that is not CO_2 saturated, since a CELSS algal culture may operate under limiting CO_2 concentrations.) At a CO_2 concentration that is not saturating at 1.5 g/l dry weight (i.e., less than 0.5% CO_2, see Figure 8) we expect that: 1) at very low cell concentrations the productivity of the culture will be similar to that of a culture maintained at 2% CO_2, 2) the maximum productivity of the culture will be lower than the maximum productivity of a 2% CO_2 culture, and 3) the light efficiency will extrapolate to approximately 18–19% at very low cell concentrations, but the decline in efficiency with increasing cell concentration will be more rapid than for a 2% CO_2 culture.

A continuous culture of *Scenedesmus* was maintained on either air (approx. 0.03% CO_2) or 0.13% CO_2 in air (total gas flow rate in each case of 1000 ml/min), and the response of productivity to changes in cell concentration was determined. Figure 9 shows the results of this experiment; for comparison the results from the experiment at 2% CO_2 are included (see Figure 7). At very low cell concentrations (<0.1 g/l dry weight) the three curves are similar, indicating that at these cell concentrations the biomass productivity is relatively independent of the CO_2 concentration of the gas stream. At higher cell concentrations the observed productivity for the air-grown culture is lower than the productivity of either the 2% CO_2 or 0.13% CO_2 grown

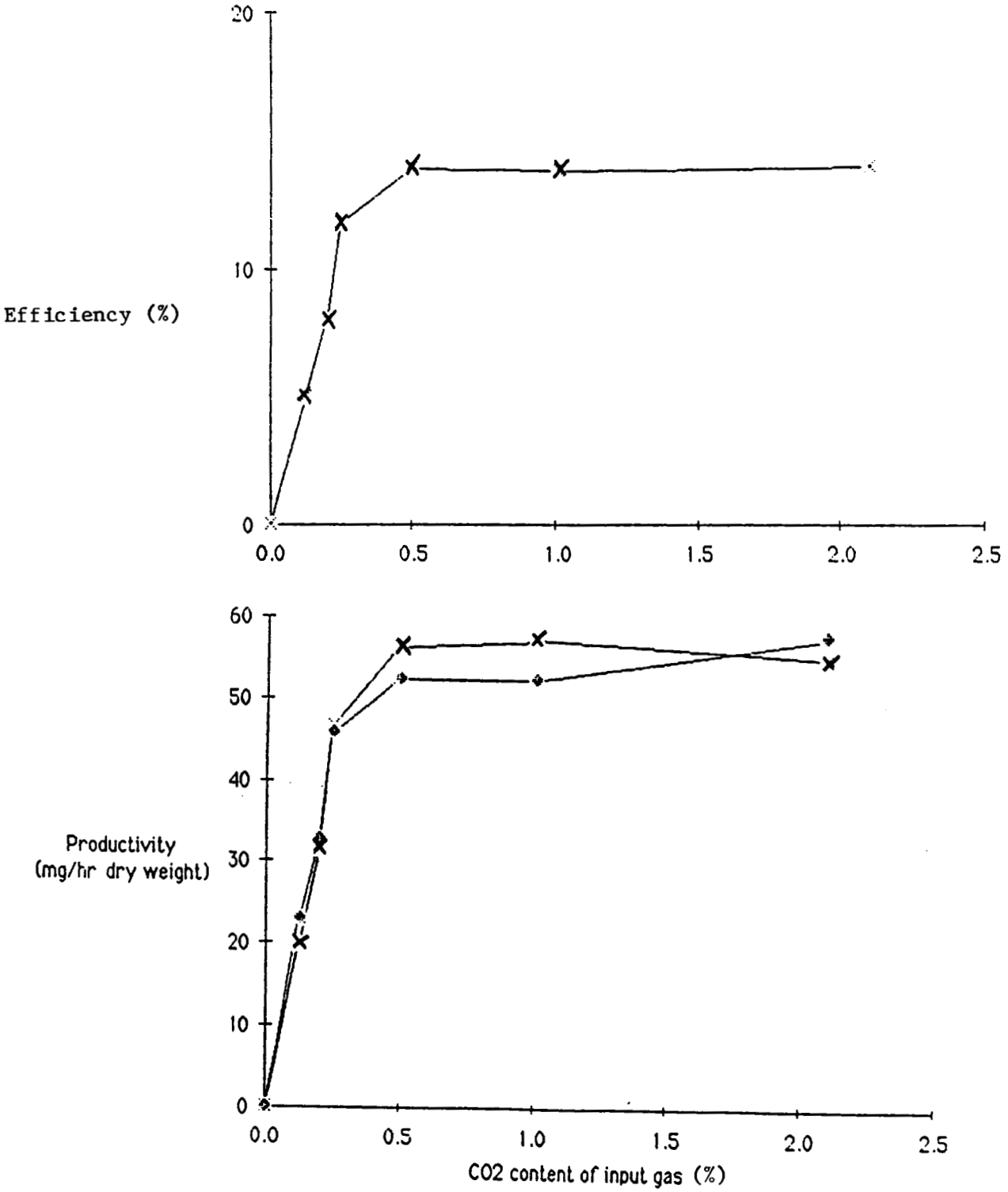


Figure 8. Culture productivity and light efficiency as a function of the CO_2 concentration of the input gas stream.

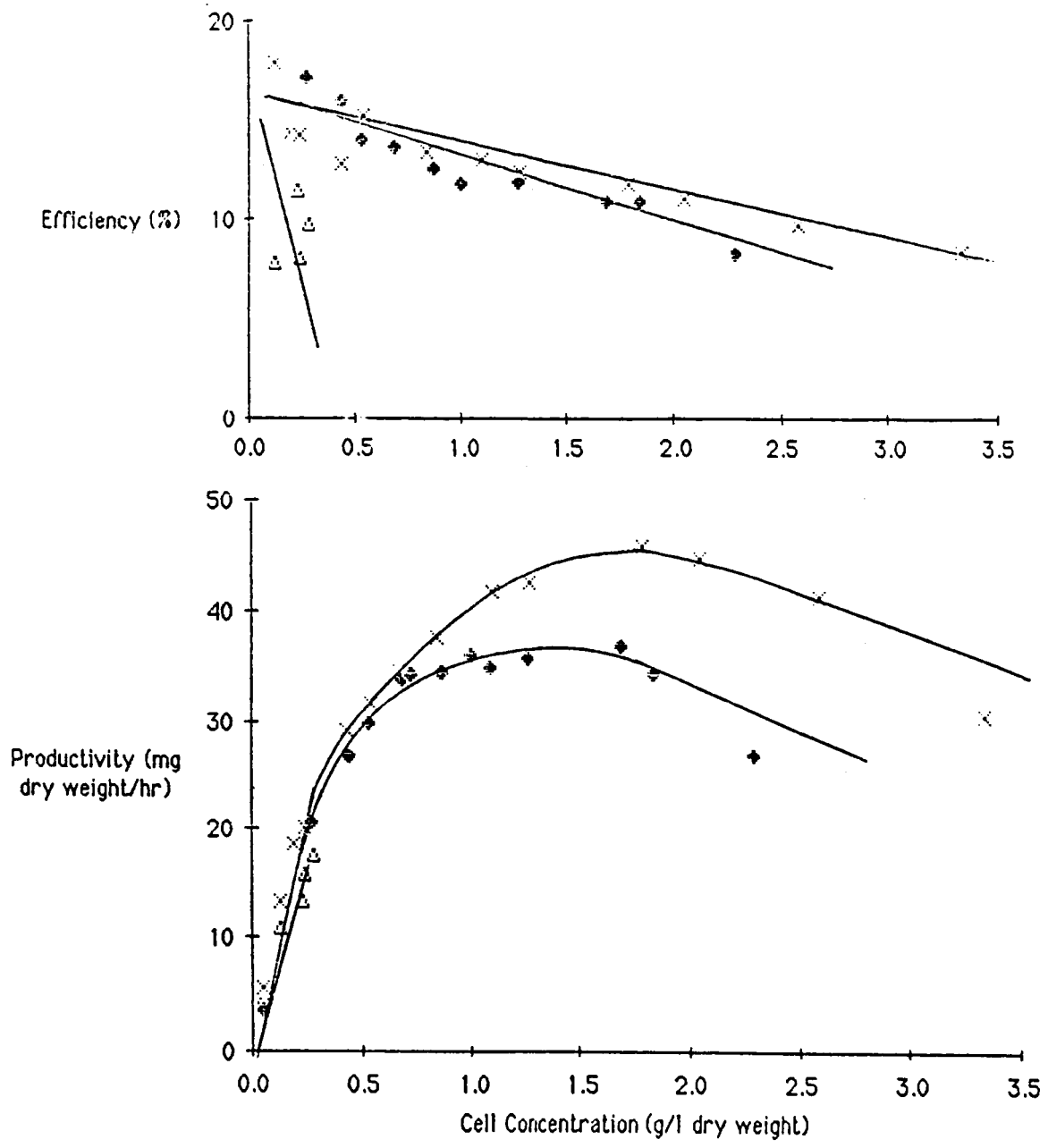


Figure 9. Productivity and light efficiency as a function of cell concentration for a 2% CO_2-grown culture (crosses), a 0.13% CO_2-grown culture (diamonds), and an air-grown culture (triangles) at 32 °C.

culture. At cell concentrations greater than about 0.3 g/l dry weight a stable continuous culture could not be maintained on air, presumably due to the fact that under these conditions the culture is severely CO_2 limited. At these higher cell concentrations the productivity of the culture maintained on 0.13% CO_2 is similar to that of the culture on 2% CO_2. However, at cell concentrations greater than 0.75 g/l dry weight, the productivity of the 0.13% CO_2 culture is consistently less than the productivity of the 2% CO_2 culture, indicating that under these conditions CO_2 is limiting. The upper portion of Figure 9 shows the efficiencies of light utilization for a 2% CO_2-grown, 0.13% CO_2-grown, and an air-grown culture. Although there is a considerable amount of scatter in the data, the light efficiencies extrapolate to approximately the same value (16–18%) at very low cell concentrations. The decline in light efficiency with increasing cell concentration appears to be more rapid for the air-grown culture than for either of the CO_2-amended cultures.

The inability to maintain a continuous culture on air at cell concentrations greater than 0.3 g/l dry weight might be explained by the fact that under conditions of near CO_2 depletion *Scenedesmus* will cycle reducing equivalents to O_2 at rates approaching the maximum rate of CO_2 fixation (4). Such sustained high rates of O_2 cycling may be deleterious to the cells and could lead to a decline of the culture at cell concentrations beyond those required to obtain the maximum productivity.

If all the CO_2 in the gas stream of the air-grown culture (1000 ml/min, 0.03% CO_2) were to be removed by the cells, then this input of CO_2 could at most support a biomass productivity of 19.7 mg/hr dry weight (given that these cells are 49% carbon). The maximum observed productivity (17.7 mg/hr dry weight) is within 10% of this computed maximum productivity. These results indicate that a continuous culture of *Scenedesmus* maintained on air can very efficiently remove CO_2 from the gas stream.

Temperature Effects on Algal Productivity

The objective of this series of experiments was to determine the response of culture productivity to changes in cell concentration at two different temperatures (20 and 25 °C) at saturating and limiting CO_2 concentrations. These results, along with the comparable experiment conducted at 32 °C (see Figure 9), will provide information on the effect of temperature on biomass productivity and the efficiency of light utilization.

The results of these experiments are shown in Figures 10 and 11. At 25 °C, the productivity curve at 2% CO_2 is very similar to that of a 32 °C culture at 2% CO_2, suggesting that over the temperature range of 25 to 32 °C the productivity of a continuous culture is relatively independent of temperature. The productivity of a 25 °C culture maintained on air is similar to that of a 2% CO_2 culture at very low cell concentrations. However, at cell concentrations above 0.1 g/l dry weight the productivity of the air-grown culture is consistently less than that of the 2% CO_2 culture. The maximum productivity obtained at 25 °C for the air-grown culture (18.3 mg/hr) is similar to the maximum productivity of the 32 °C air-grown culture; thus at 25 °C (as at 32 °C) the culture can very efficiently remove CO_2 from the gas stream. The upper panel of Figure 10 shows the efficiencies of light utilization for the 2% CO_2-grown

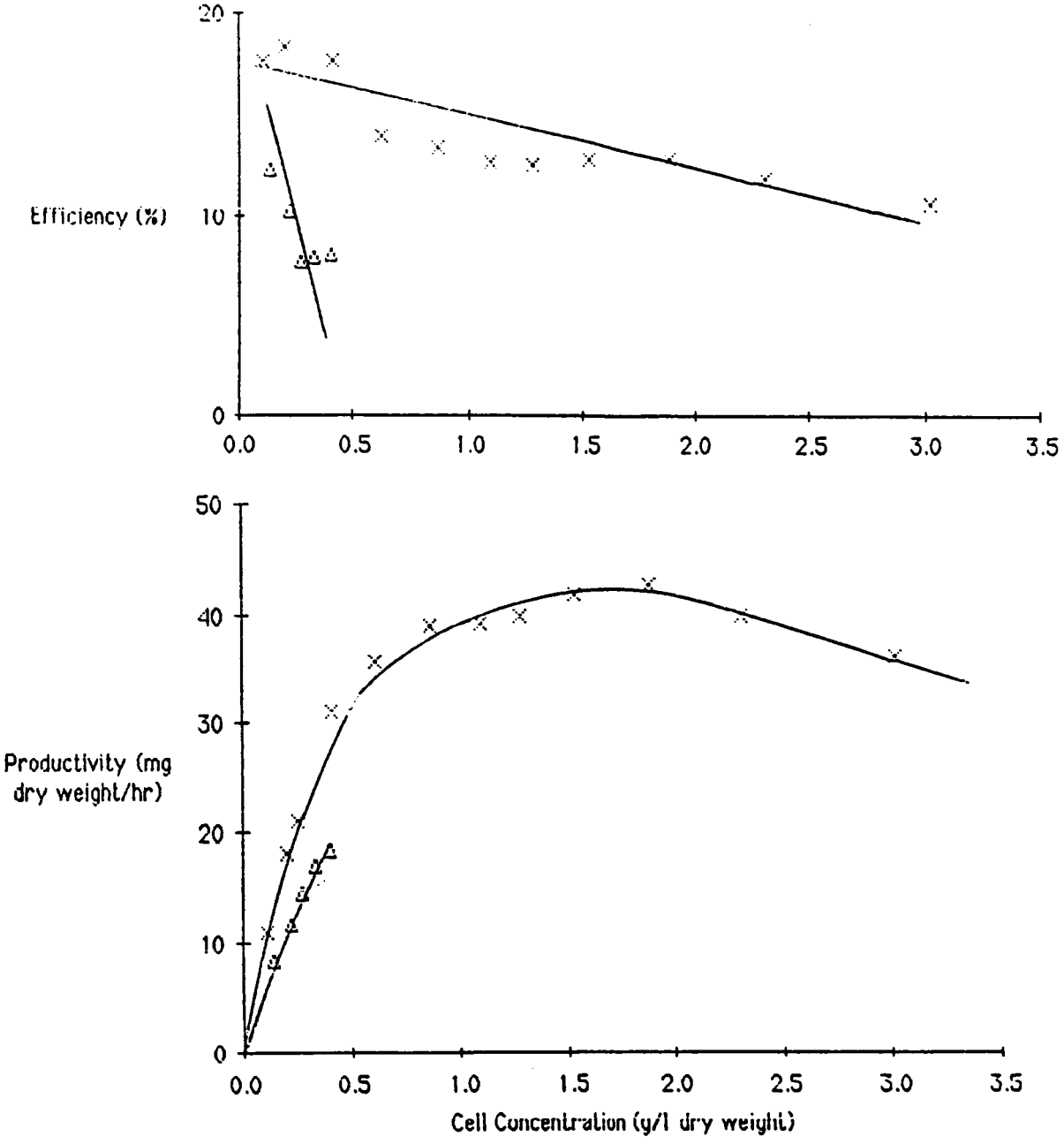


Figure 10. Productivity and light utilization efficiency as a function of cell concentration for a 2% CO_2-grown culture (crosses) and an air-grown culture (triangles) at 25 °C.

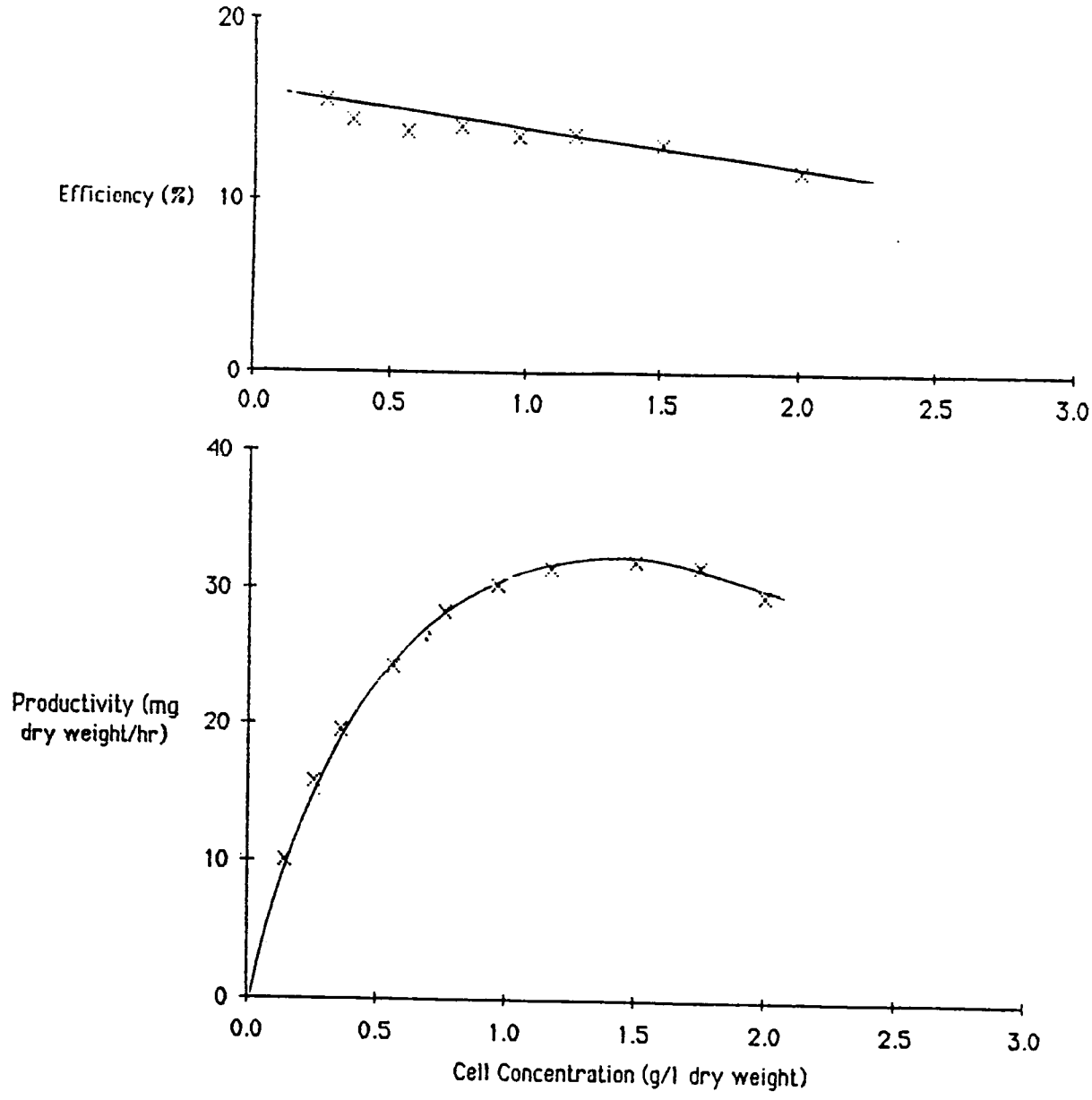


Figure 11. Productivity and light utilization efficiency as a function of cell concentration for a 2% CO_2-grown culture at 20 °C.

and air-grown cultures. In both cases the efficiencies extrapolate to approximately 16–18% at very low cell concentrations; the decline in efficiency with increasing cell concentration is similar to that seen for the corresponding experiments at 32 °C (see Figure 9).

Figure 11 shows the response of biomass productivity and light utilization efficiency to changes in cell concentration at 20 °C for a 2% CO_2-grown culture. Note that at this temperature the productivity and light utilization efficiency are lower than for either a 25 or 32 °C culture grown at the same CO_2 concentration, indicating that 20 °C is a less-than-optimum temperature for a *Scenedesmus* continuous culture.

V. LIGHT INTENSITY EXPERIMENTS

The objective of these experiments was to determine the effects of different light intensities on the biomass productivity and light utilization efficiency of a continuous algal culture. Experiments and determinations of this type could prove to be very significant, since the biomass productivity of an algal culture per unit weight or volume may be more important for a CELSS than highly efficient light (=energy) utilization.

The approach for these experiments was to establish a continuous culture of *Scenedesmus* at a light intensity higher than that used in the "reference experiments" described in earlier sections. This was accomplished by inserting a very high output, cool white fluorescent lamp into the central light chamber of the continuous culture apparatus. This increased the light intensity supplied to the culture by approximately 60%, i. e., to 21 W/m^2 (or 97.7 $\mu E/m^2/sec$), an intensity that is still on the linear portion of the photon flux curve (see Figure 2). The cell concentration of the culture at this higher light intensity was varied, and the resulting biomass productivity and light efficiency determined. At a higher (but not saturating) light intensity and a saturating CO_2 concentration the model of algal productivity described in Section III predicts that: 1) at very low cell concentrations the slope of the productivity curve will increase, 2) the maximum attainable cell concentration and the cell concentration of maximum productivity will be greater at the higher light intensity, 3) the efficiency of light utilization by the culture will be greater at a particular cell concentration than a culture at lower light intensity, and 4) as in the lower-intensity case, the efficiency of light utilization will decrease as the cell concentration increases.

Figure 12 shows the results of a continuous culture experiment conducted at a light intensity of approximately 21 W/m^2 and a temperature of 32 °C, for both a 2% CO_2-grown and an air-grown culture. Note that for the 2% CO_2-grown culture the productivity at every cell concentration is greater than the productivity of a culture maintained at a lower light intensity (see Figure 9). Furthermore, at the higher light intensity, the cell concentration of maximum productivity is higher than at low light intensity (2.1 g/l compared to 1.7 g/l; see Figure 9). The line through the 2% CO_2 points in Figure 12 was calculated using the model described in Section III. All the parameters in the model are the same as described in the previous section with the exception of the light intensity (Io is 21 W/m^2). At the higher light intensity (as at the lower intensity) the model accurately describes the experimental data. The triangles in Figure 12 show the response of productivity to changes in cell concentration for a culture maintained

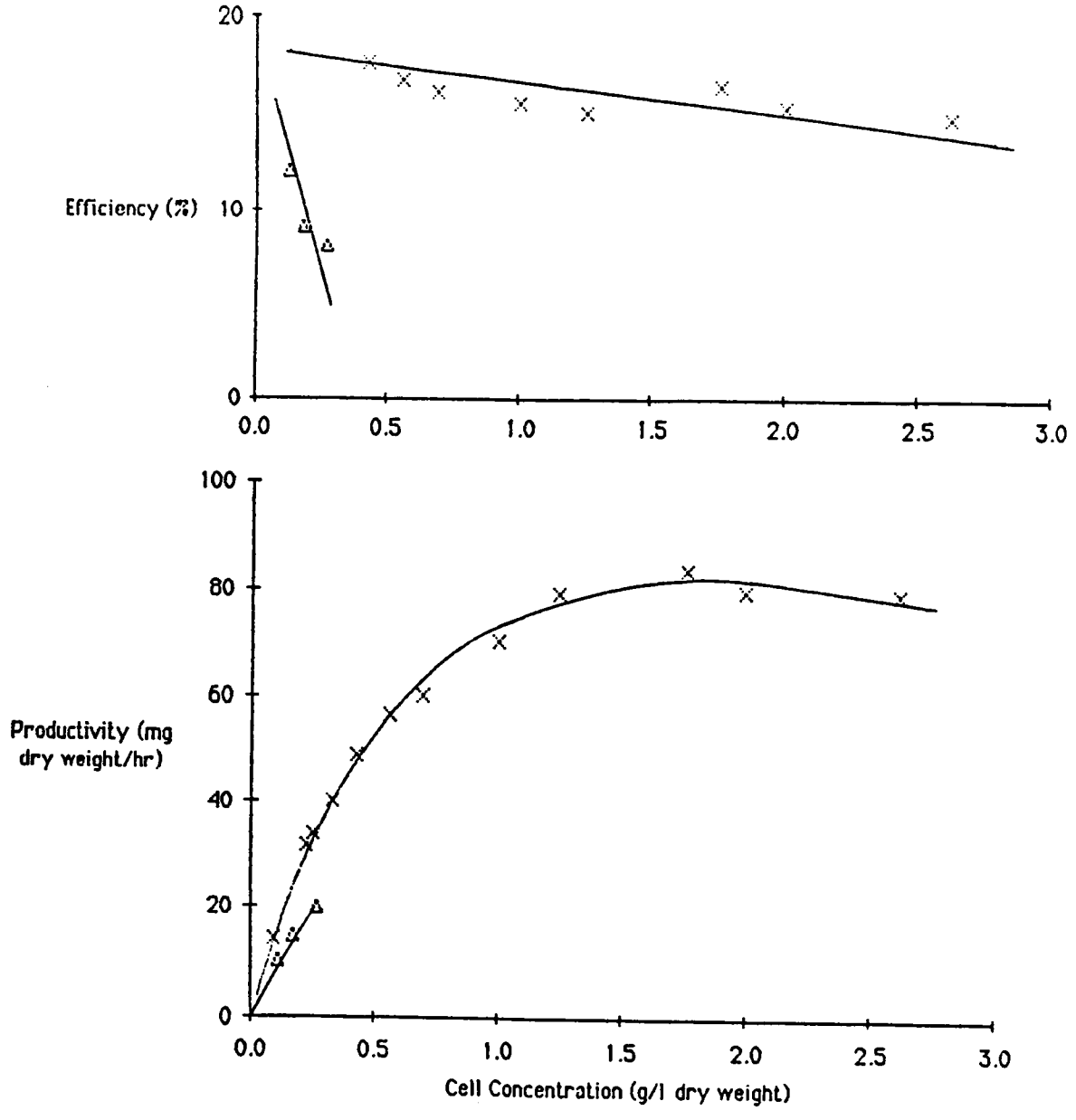


Figure 12. Productivity and light efficiency as a function of cell concentration for a 2% CO_2-grown culture (crosses) and an air-grown culture (triangles) at a light intensity of 21W/m^2.

on air at 21 W/m^2. As in the case of the lower light intensity experiments (Figures 9 and 10), the productivity of an air-grown culture is similar to that of a 2% CO_2-grown culture at very low cell concentrations; at higher cell concentrations the air-grown culture is less productive than the 2% CO_2-grown culture. As in the earlier case, the maximum productivity of the air-grown culture indicates that CO_2 is very efficiently removed from the gas stream. The upper portion of Figure 12 shows the response of the light utilization efficiency to changes in cell concentration for both the 2% CO_2-grown and air-grown cultures. For the 2% CO_2-grown culture the efficiency of light utilization is consistently higher than the efficiency at low CO_2. Also, as expected, the cell concentration of light compensation extrapolates to a higher value at the higher light intensity (13.8 g/l compared to 8.5 g/l).

In summary, the experiments at a higher (but not saturating) light intensity indicated that both the biomass productivity and efficiency of light utilization are greater than at lower light intensities. These results are in agreement with the predictions made by the model of algal productivity, since the ratio of usable input energy to maintenance energy (i.e., respiration) is increased.

VI. ALGAL BIOMASS FOR FOOD PROCESSING STUDIES

Green algal biomass generally contains a high percentage of protein (7), and therefore the production of this biomass for gas regeneration purposes represents a potentially large source of protein for human consumption. Dr. Marcus Karel of the Massachusetts Institute of Technology is currently working on a NASA-sponsored project to determine the feasibility of using algal biomass as a food component of a CELSS. To this end Karel and his colleagues are applying state-of-the-art food technology principles to the development of human nutritional sources from algal biomass. Our contribution to this work has been to supply Dr. Karel with well-characterized algal biomass to be used as the feedstock for these food processing studies.

During the past two years approximately two kilograms of *Scenedesmus* paste has been produced and shipped to Dr. Karel. *Scenedesmus* was continuously cultured in the standard medium (see Materials and Methods) in the large "reaction vessel" continuous culture apparatus that has been described in previous reports (8). The culture was maintained at 32 °C and bubbled with a gas mixture of 2% CO_2 in air. The overflow from the culture vessel ran directly into a storage container that was kept in a refrigerator to prevent decay of the biomass prior to harvesting. When approximately 20 liters of overflow was obtained (60-90 g algal paste), the biomass was harvested by centrifugation. The algal pellet was washed three times with distilled water to remove the culture medium from the biomass. The final algal paste was packed in ice and sent by overnight package delivery to Dr. Karel.

To date, we have produced sufficient biomass for Dr. Karel using our small scale continuous culture apparatuses (largest apparatus is 2 liters); however, this is very labor intensive, and large quantities of biomass could be more efficiently produced with a larger continuous culture apparatus. We have used our own resources to design and construct a prototype of a large continuous culture vessel that operates on a different principle than our present apparatuses (Figure 1). The prototype vessel is 60 cm x 45 cm x 5 cm (outer dimensions) and has a

working volume of approximately 7 liters. The sides of the vessel (the 60 cm x 45 cm faces) are made of glass that is held in an aluminum frame. Illumination is supplied from each side by cool white fluorescent lamps that are spaced on 5 cm centers and mounted in a wooden frame that can be placed within a few centimeters of the side of the tank. The gas stream is admitted through a porous tube that runs near the bottom of the vessel. A unique air-lift design around a central glass plenum provides good circulation of the algal culture, and therefore provides good illumination of the algae.

Figure 13 shows the result of an experiment in which the increase in biomass density (monitored as dry weight) of a *Scenedesmus* culture was determined as a function of time in this 7 liter vessel. The maximum biomass density attained, ca. 8 g/l dry weight, is about three-fold higher than we have achieved using any other vessel. This higher density, as well as the larger volume of the vessel, will allow us to increase by at least five-fold the amount of biomass that we will be able to generate for food processing studies. Currently, the necessary electronics are being assembled to enable this vessel to operate as a continuous culture using the density detector and computer system described in the Materials and Methods.

VII. STUDIES WITH *Spirulina platensis*

Spirulina platensis has received attention for use in a CELSS primarily because of its potential nutritional value. In fact, unprocessed *Spirulina* has a protein efficiency ratio nearly equal to that of casein (7). However, due to its unusual and distinct morphology, *Spirulina* has proven more difficult to culture than *Scenedesmus*. *Spirulina* tends to clump during culture, which ultimately led to the design of the so-called "reaction pot" that is currently used for biomass production (8). Our objectives with *Spirulina* have been: 1) to attempt to find a medium composition that might reduce the tendency of this filamentous alga to "clump", and 2) to devise an alternate continuous culture system for this organism for the production of large quantities of biomass.

Our previous work has indicated that increasing the NaCl concentration of the medium from 1 g/l to 2 g/l reduced the tendency of *Spirulina* to clump. However, even at 2 g/l NaCl, this alga will clump at high cell concentrations or when the culture is not continuously agitated (stirred or bubbled). If a NaCl concentration can be found that further reduces clumping, then higher cell concentrations of *Spirulina* could be maintained in continuous culture, which in turn would reduce the weight and volume of culture needed to generate a particular quantity of biomass (a distinct advantage in terms of reducing the payload weight of an algal culture). We have cultured *Spirulina* in standard medium that was modified to contain either 2, 10, or 30 g/l NaCl. To be certain that the cells were adapted to the particular medium, an aliquot of cells from each type of medium was transferred into a second culture vessel of the same type of medium. None of these modified media significantly reduced the tendency of *Spirulina* to clump.

As described above, *Spirulina* has previously been maintained in continuous culture using the reaction pot, whose geometry is not easily amenable to scale-up. Preliminary data obtained

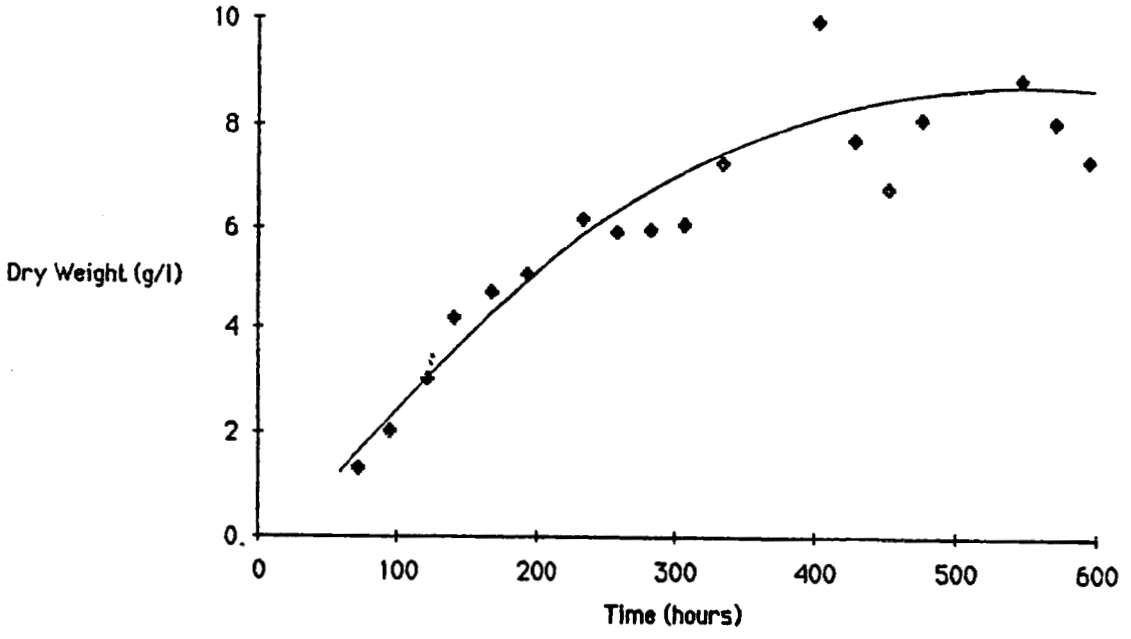


Figure 13. Time course of *Scenedesmus* growth in a 7 liter culture vessel.

using the larger system described in Section VI indicates that the biomass productivity of *Spirulina* in this new photobioreactor is similar to that obtained with the reaction pot.

VIII. RECYCLING ALGAL BIOMASS

The operation and utility of a CELSS, a biologically driven and maintained life-support system that can be considered as a small biosphere, will be subject to the same rules and limitations as any other photosynthetically driven system. This is particularly true of energy flow. Since energy will undoubtedly be one of the limiting commodities in any long-term space-flight mission, the use and reuse of energy during the course of the mission will be of fundamental importance.

The efficiency of energy utilization in a photosynthetically-driven system has two fundamental limitations: 1) photosynthetic systems can harvest at most 20% of the (white) light energy that is absorbed by the photosynthetic tissues, and 2) only a relatively small fraction of the photosynthetic product will generally be useful as food for the crew, the remainder being discarded by the crew in the course of food preparation. The losses occurring due to the first limitation may be immutable, whereas the losses due to the second limitation may potentially be reduced.

Approximately 50% of *Scenedesmus* biomass is in the form of protein that is beneficial to humans. If these algae were produced and harvested only for their protein, then about one-half of the energy captured by the algae and converted into biomass would be degraded via the waste handling system of a CELSS. One possible means for surmounting this problem would be to recycle this waste biomass; i.e., use the energy content and associated carbon skeleton structures of the waste biomass to produce new useful biomass. This would minimize the intervening oxidative (waste processing) steps, which basically degrade high-grade light energy to low-grade heat energy without the extraction of useful metabolic energy.

Several groups of organisms possess the potential to grow on waste biomass, including yeast, bacteria and algae. (It should be noted that higher plants are not capable of heterotrophic growth.) The objective of our preliminary experiments on this topic was to determine the feasibility of using waste algal biomass as a carbon and energy source to either supplement or replace CO_2 and light, thus enabling the extraction of useful "work" from otherwise unusable biomass.

Table 1 summarizes the results of a series of experiments in which we tested the ability of various carbon sources to support or supplement the growth of *Scenedesmus*. In these experiments the organisms were grown either in the dark or under limiting light (25 $\mu E/m^2/sec$; see Figure 2), and the growth medium was supplemented with various carbon sources; i.e. carbon dioxide (either 0.03% or air amended with 2% CO_2), glucose (0.5%, w/v), or algal extract (2%, v/v). For each growth condition cell number was monitored as a function of time. These data were graphed, and the minimum doubling time for each culture was determined from the graph. The maximum growth rate (5) was then calculated from the doubling times. Each value in Table 1 represents the mean of two separate experiments.

Table 1. Growth rate and doubling time of *Scenedesmus* as a function of available energy source.

Growth Condition	Carbon Source	Doubling Time (hr)	Growth Rate (μ)
1a. light/autotrophic	air (CO_2)	50.7	0.014
b. light/autotrophic	2% CO_2	28.8	0.024
c. dark/autotrophic	2% CO_2	n.d.	0.0
2a. light/mixotrophic	glu + air (CO_2)	16.1	0.043
b. light/mixotrophic	glu + 2% CO_2	15.6	0.044
c. heterotrophic (dark)	glu + 2% CO_2	18.9	0.037
3a. light/mixotrophic	extract + air (CO_2)	23.5	0.029
b. light/mixotrophic	extract + 2% CO_2	23.0	0.030
c. heterotrophic (dark)	extract + 2% CO_2	24.2	0.029

The first two items in Table 1 (items 1a and 1b) show the growth rates and doubling times observed when *Scenedesmus* was cultured under limiting light (25 $\mu E/m^2/sec$) with unamended air (0.03% CO_2) and air amended with 2% CO_2. Note that even under these limiting light conditions the growth rate was increased substantially by increasing the CO_2. We should also note that the maximum rate observed in these experiments (μ=0.024) is about a factor of four less than the maximum rate observed with these organisms in our hands under saturating light and CO_2. Item 1c is the "dark control".

Items 2a–2c show the effect of added glucose on the growth rates. Under these low-light conditions the growth rate of *Scenedesmus* in the presence of added glucose was almost double the rate observed in autotrophic cultures at the same light intensity. Note that there was very little effect of added CO_2; the observed rates at 0.03% CO_2 and 2% CO_2 were almost identical. This observation is confirmed by the results shown in item 1c: the glucose-supported growth rate in the absence of light was substantially higher than the autotrophic rate, and was within ca. 20% of the mixotrophic rate. Thus under these conditions, the glucose-supported heterotrophic rate is higher than the CO_2-supported autotrophic rate and is within a factor of two to three of the maximum observed rate under saturating light and CO_2.

As shown in items 3a–3c, algal extract also supported a higher rate than that observed in autotrophic cultures, though the observed effect was less than with glucose. Again, as in items 2a and 2b above, there was no appreciable difference between the rates observed with or without added CO_2, and indeed, no significant effect of light at all. Although we have no ready explanation for this observation, these results do indicate that an algal extract prepared from *Scenedesmus* can support the growth, either heterotrophically or mixotrophically, of this same organism.

Our results show that hydrolyzed *Scenedesmus* biomass can serve as a carbon and energy source for the growth of these same algae. This finding suggests that the efficiency of biomass production in a CELSS might be significantly increased by recycling unusable waste biomass,

generated from the production of food from algal and plant biomass, through the algal system for the production of additional whole algal material.

A report of these preliminary experiments was presented at the 1986 COSPAR Meetings, and will be published in the Proceedings of these meetings.

IX. OTHER PROJECTS RELEVANT TO A CELSS

In addition to the stated objectives of our contract, we have also been studying several other issues that are relevant to a CELSS, including: 1) closure of the water cycle within an algal growth system, and 2) a consideration of the tradeoffs in energy efficiency versus weight and volume for the algal component of a CELSS. Although not directly related to our present objectives, these issues are important considerations of the suitability of an algal system as a component of a CELSS.

Water Recovery From A Closed Algal Culture System

Any attempt to derive a stable closed algal culture system will have to deal with the problem of water loss from the system through two main routes, evaporation and cell handling during harvesting. Of these two paths, the losses during cell harvesting appear to be the most important.

In order to estimate the magnitude of water loss from such a system we can examine a hypothetical model. We will assume that the culture system is completely air- and water-tight so that we can ignore any losses through leakage. The model would consist of a chamber for the liquid algal culture and an air reservoir along with a peristaltic pump to circulate air between the two chambers. These three components would form a loop so that air and liquid are neither introduced nor lost from the system. Assume that the liquid volume of the culture is one liter and the total air volume (reservoir and culture chambers combined) is ten liters. The "air" will be enriched with 2% carbon dioxide.

In this example, *Scenedesmus* will be used as the alga to be cultured in the vessel. The carbon content of *Scenedesmus* (as in most algal cells) is approximately 50% of the dry weight. If the productivity of the culture is 50 milligrams of dry weight per hour, then the cells will consume 50 milliliters of carbon dioxide per hour for conversion to cell carbon. At this rate the 200 ml of carbon dioxide contained in the closed system would be depleted after 4 hours of growth. We could then spike the system through an air tight fitting with one liter of a 4:1 mixture of nitrogen/carbon dioxide. This would replace the 200 ml of carbon dioxide consumed by the cells. It would also displace one liter of spent air from the system, carrying with it 30 microliters of water. Most of this water (probably 90% or more) could be recovered from the spent air by releasing the air from the system through a cold finger to condense the water. The end result would be an evaporate loss of only 3 microliters or less for every 200 milligrams of cell dry weight produced.

If the cells are harvested by centrifugation then 95% or more of the water removed from

the system for harvesting can be recovered as supernatant and recycled directly back into the culture (after the addition of the appropriate salts and minerals). However, dry cellular biomass typically comprises only 25% or less of the wet cell pellet; the rest of the weight is intercellular and interstitial water. Therefore, for every 200 milligrams of dry weight collected, 600 milligrams of water would be contained in the wet pellet. This water could be drawn off from the cells and recovered by lyophilization of the pellet and collection of the water in a trap flask. In a preliminary experiment using a trap flask submerged in a dry ice/acetone bath 93% of the wet weight of the pellet was recovered. This recovery could be increased by the use of a liquid nitrogen bath and the construction of a baffled flask to increase the surface area for condensation. Even without any improvements the combined water loss through evaporation and cell harvesting should be less than 60 microliters for every 200 milligrams of dry biomass collected.

Culture System Tradeoffs: Light Energy vs. Weight and Volume

The maximum light energy utilization for algal growth occurs at rather low incident light intensities (see Figure 2). Note that photosynthetic productivity (i.e., growth) vs. intensity is linear up to about 100 $\mu E/m^2/sec$; this is the intensity regime of maximum photosynthetic efficiency. Above ca. 120 $\mu E/m^2/sec$ growth vs intensity drops off significantly. The difference between the solid curve and an extrapolation of the linear portion of the graph is the energy loss due to light saturation.

A fundamental question concerning a CELSS algal culture is the following: Under what circumstances might it pay to increase the energy density (=light intensity) beyond the linear range? Stated another way, What is the tradeoff between energy efficiency and volume?

Figure 14 was constructed using the data of Fig. 2 and the model described in the previous sections. This figure shows the productivity as a function of cell density at different light intensities. We should note that the algal culture should always be operated at Cmp, the value of which is determined only by the light intensity (I) and the path length of the culture system (l). Note that this value increases for the cases shown in Fig.14 from ca. 1.5 g/l (at I=60) to ca. 2.5 g/l (at I=162), which corresponds to the maximum theoretical value for this particular path length.

We assume that, to a first approximation, the weight of the system is proportional to its volume (i.e., the culture weight is primarily attributable to the volume of water it contains). One might expect that there could be a tradeoff between minimum energy consumption and minimum weight. If we let C=total cost, E=energy cost, and W=launch-weight cost, then we would want to minimize C=aE+bW. To get these parameters into common units, we would make the computation in dollars.

Size of Culture Required to Support One Astronaut

In an effort to determine the approximate physical size and weight of an algal culture capable of supporting an astronaut, we will consider a configuration of the type shown in Figure 15.

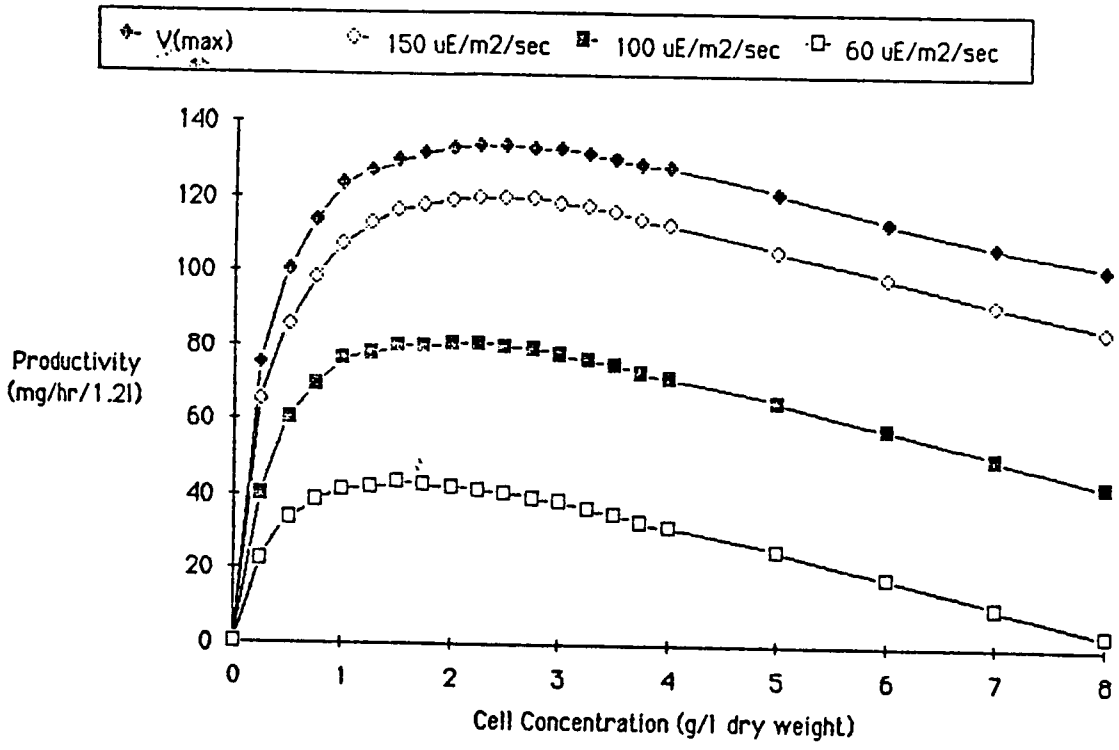


Figure 14. Productivity as a function of cell concentration for four light intensities.

The primary functional unit of this culture system is a "photobioreactor", which consists of a long thin tank (dimensions of ca. 125 cm high x 250 cm long x 10 cm thick – approximately 4 ft x 8 ft x 4 in – working volume of ca. 275 liters) filled with algal suspension. These reactors would have a unique air-lift design that allows a highly efficient circulation of the algal cells and good culture temperature control.

An algal growth unit would consist of a series of these photobioreactors interleaved with 2 kilowatt lighting units oriented to allow for equal and uniform illumination of the two adjacent reactor faces (c.f. Figure 15). The lighting units themselves would contain 50 40-watt fluorescent bulbs (or 5 400-watt high-pressure bulbs). The principal advantages of this modular design are:

1. Accessibility to both bulbs and tanks;
2. Control of productivity (the number of reactors in service can be continuously varied);
3. Good response to contamination and equipment failure (faulty modules can be repaired and/or replaced without shutting down the entire growth unit). The support equipment for these growth units would function to control cell density, culture temperature and gas flow. All of these control functions are well within the current state-of-the-art and are presently utilized in smaller scale units in operation in our laboratory.

The theoretical limitation on the production of algae using artificial lighting can be ascertained as follows:

1. Green algae such as *Chlorella* and *Scenedesmus* have an energy content of ca. 5.8 Kcal per gram dry weight;
2. The maximum energy efficiency for photosynthetic growth is 20%;
3. The maximum conversion efficiency of fluorescent lights is 20%;

Therefore, >75 kW.hrs of electrical power are required to produce one pound (dry weight) of these algae. One 40-watt bulb consumes ca. one kW.hr per day, which corresponds to ca. 6 grams per day per bulb; thus 50 bulbs will produce < 300 grams of dry algae per day.

An astronaut requires ca. 600 grams of food per day (ca. 1.3 pounds). We can therefore derive the following minima:

Number of modules: >2
Size: >4 ft x 4 ft x 1 ft
Volume: >550 liters (150 gallons)
Weight: >550 kg
Power: >4 kW

We should note that some of these parameters will be a function of the chosen reactor design. Others, however, such as the amount of power required, are set by physical considerations and are not amenable to improvement.

A major part of our task in this program will be to develop systems that can approach these theoretical limitations. To the extent that these limits cannot be met, we may need to make use of the tradeoffs described in the previous section.

Algal Growth Unit

Each unit consists of 50 tanks with lighting units between each tank. Each tank is 2.5 x 1.2 x 0.1 m with a volume of about 275 liters. Lighting units each contain 50 fluorescent "cool white" bulbs (40 W each).

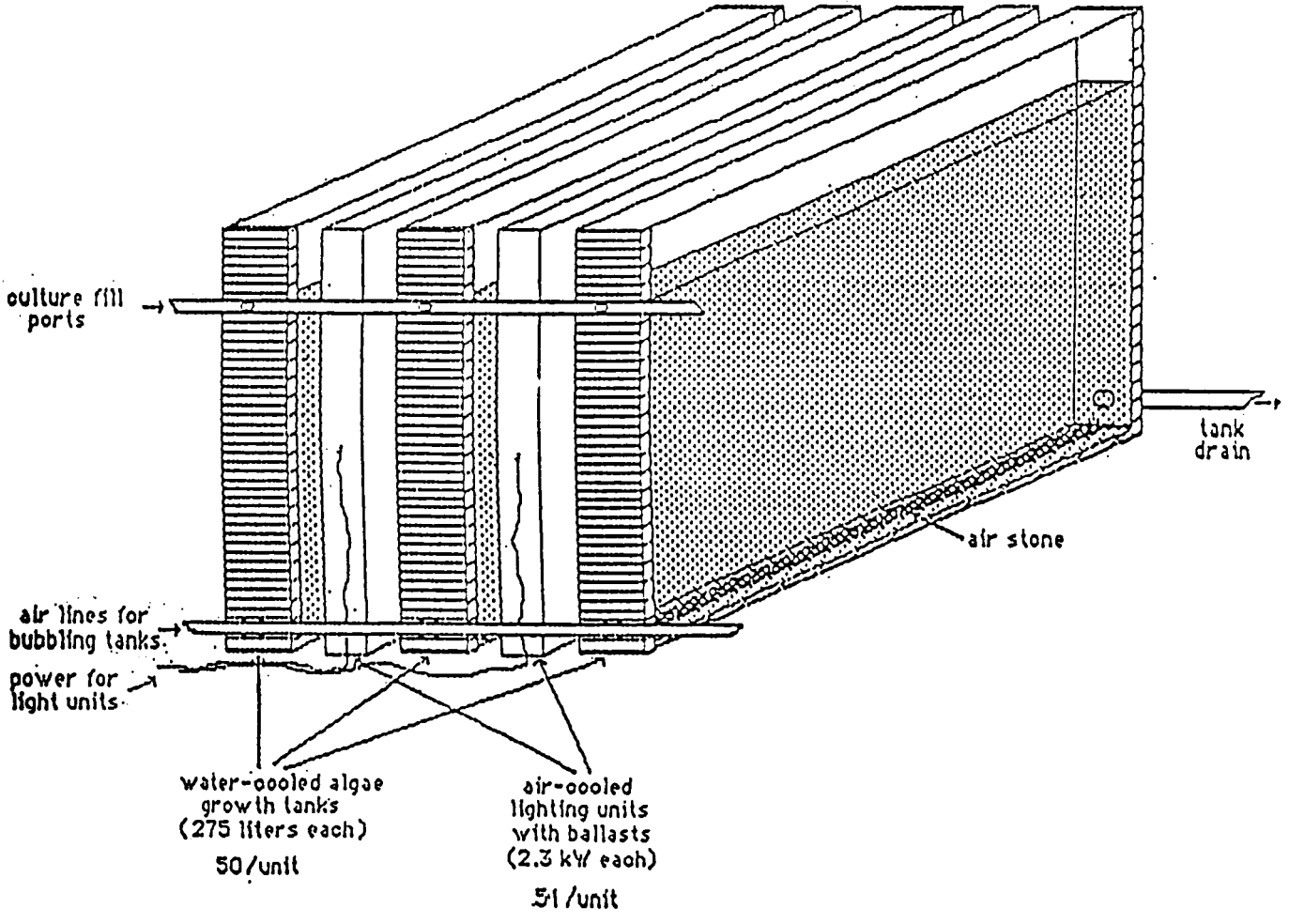


Figure 15

X. REFERENCES

1. Radmer, R., P. Behrens, and K. Arnett, **Biotechnology and Bioengineering** (in press), "Analysis of the productivity of a continuous algal culture system".

2. Myers, J. and L. B. Clark, J. Gen. Physiol. 28: 103-112 (1944). Culture conditions and the development of the photosynthetic mechanism. II. An apparatus for the continuous culture of *Chlorella*.

3. Radmer, R. and O. Ollinger, Methods in Enzymology Vol. 69, A. San Pietro, ed., Academic Press, p. 546-560 (1980). Measurement of the oxygen cycle: the mass spectrometric analysis of gases dissolved in a liquid phase.

4. Radmer, R. and B. Kok, In: Encyclopedia of Plant Physiology Vol 5, A. Trebst and M. Avron, eds. Springer-Verlag, p. 125-135 (1977). Light conversion efficiency in photosynthesis.

5. Decker, J. P. Plant Physiol. 34: 100-102 (1959). Comparative responses of carbon dioxide outburst and uptake in tobacco.

6. Radmer, R. and B. Kok, Plant Physiol. 58: 336-340 (1976). Photoreduction of O_2 primes and replaces CO_2 assimilation.

7. Waslien, C., J. Myers, B. Kok and W. Oswald, In: Protein Resources and Technology: Status and Research Needs, M. Milner, N. Scrimshaw, D. Wang, eds., p. 522-542, Avi Publishing Co. (1978).

8. Radmer, R., P. Behrens, E. Fernandez, O. Ollinger, C. Howell, A. Venables, D. Huggins, and R. Gladue, NASA Contract No. NAS2-10969, Final Contract Report (1984). Algal culture studies related to a Closed Ecological Life Support System (CELSS).

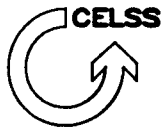
XI. PUBLICATIONS AND REPORTS

1. Radmer, R., P. Behrens, E. Fernandez, and K. Arnett, In: Controlled Ecological Life Support Systems: CELSS '85 Workshop, National Aeronautics and Space Administration, R. D. MacElroy, N. V. Martella, D. T. Smernoff, eds., NASA TM 88215, 1985. An Analysis of the Productivity of a CELSS Continuous Algal Culture System.

2. Radmer, R., J. Cox, D. Lieberman, P. Behrens, and K. Arnett, In: Proceedings of the 1986 COSPAR Meeting, 1986. Biomass Recycle as a Means to Improve the Energy Efficiency of CELSS Algal Culture Systems.

3. Radmer, R., P. Behrens, and K. Arnett, **Biotechnology and Bioengineering** (in press). Analysis of the Productivity of a Continuous Algal Culture System.

4. Radmer, R., P. Behrens, K. Arnett, R. Gladue, J. Cox, and D. Lieberman, NASA Contract Number NAS2-12115, Annual Contract Report, 1986. Algal Culture Studies for CELSS.



Controlled Ecological Life Support Systems (CELSS)
A Bibliography of CELSS Documents Published as NASA Reports

1. Johnson, Emmett J.: Genetic Engineering Possibilities for CELSS: A Bibliography and Summary of Techniques. NASA CR-166306, March 1982.

2. Hornberger, G.M. and Rastetter, E.B.: Sensitivity Analysis as an Aid in Modelling and Control of (Poorly-Defined) Ecological Systems. NASA CR-166308, March 1982.

3. Tibbitts, T.W. and Alford, D.K.: Controlled Ecological life Support System: Use of Higher Plants. NASA CP-2231, May 1982.

4. Mason, R.M. and Carden, J.L.: Controlled Ecological Life Support System: Research and Development Guidelines. NASA CP-2232, May 1982.

5. Moore, B. and MacElroy, R.D.: Controlled Ecological Life Support System: Biological Problems. NASA CP-2233, May 1982.

6. Aroeste, H.: Application of Guided Inquiry System Technique (GIST) to Controlled Ecological Life Support Systems (CELSS). NASA CR-166312, January 1982.

7. Mason, R.M.: CELSS Scenario Analysis: Breakeven Calculation. NASA CR-166319, April 1980.

8. Hoff, J.E., Howe, J.M. and Mitchell, C.A.: Nutritional and Cultural Aspects of Plant Species Selection for a Controlled Ecological Life Support System. NASA CR-166324, March 1982.

9. Averner, M.: An Approach to the Mathematical Modelling of a Controlled Ecological Life Support System. NASA CR-166331, August 1981.

10. Maguire, B.: Literature Review of Human Carried Microbes' Interaction with Plants. NASA CR-166330, August 1980.

11. Howe, J.M. and Hoff, J.E.: Plant Diversity to Support Humans in a CELSS Ground-Based Demonstrator. NASA CR-166357, June 1982.

12. Young, G.: A Design Methodology for Nonlinear Systems Containing Parameter Uncertainty: Application to Nonlinear Controller Design. NASA CR-166358, May 1982.

13. Karel, M.: Evaluation of Engineering Foods for Controlled Ecological Life Support Systems (CELSS). NASA CR-166359, June 1982.

14. Stahr, J.D., Auslander, D.M., Spear, R.C. and Young, G.E.: An Approach to the Preliminary Evaluation of Closed-Ecological Life Support System (CELSS) Scenarios and Control Strategies. NASA CR-166368, July 1982.

15. Radmer, R., Ollinger, O., Venables, A. and Fernandez, E.: Algal Culture Studies Related to a Closed Ecological Life Support System (CELSS). NASA CR-166375, July 1982.

16. Auslander, D.M., Spear, R.C. and Young, G.E.: Application of Control Theory to Dynamic Systems Simulation. NASA CR-166383, August 1982.

17. Fong, F. and Funkhouser, E.A.: Air Pollutant Production by Algal Cell Cultures. NASA CR-166384, August 1982.

18. Ballou, E. V.: Mineral Separation and Recycle in a Controlled Ecological Life Support System (CELSS). NASA CR-166388, March 1982.

19. Moore, B., III, Wharton, R. A., Jr., and MacElroy, R.D.: Controlled Ecological Life Support System: First Principal Investigators Meeting. NASA CP-2247, December 1982.

20. Carden, J. L. and Browner, R.: Preparation and Analysis of Standardized Waste Samples for Controlled Ecological Life Support Systems (CELSS). NASA CR-166392, August 1982.

21. Huffaker, R. C., Rains, D. W. and Qualset, C. O.: Utilization of Urea, Ammonia, Nitrite, and Nitrate by Crop Plants in a Controlled Ecological Life Support System (CELSS), NASA-CR 166417, October 1982.

22. Gustan, E. and Vinopal, T.: Controlled Ecological Life Support System: Transportation Analysis. NASA CR-166420, November 1982.

23. Raper, C. David, Jr.: Plant Growth in Controlled Environments in Response to Characteristics of Nutrient Solutions. NASA CR-166431, November 1982.

24. Wydeven, T.: Composition and Analysis of a Model Waste for a CELSS. NASA Technical Memorandum 84368, September 1983.

25. Averner, M., Karel, M., and Radmer, R.: Problems Associated with the use of Algae in Bioregenerative Life Support Systems. NASA CR-166615, November 1984.

26. Radmer, R., Behrens, P., Fernandez, E., Ollinger, O., Howell, C., Venables, A., Huggins, D. and Gladue, R.: Algal Culture Studies Related to a Closed Ecological Life Support System (CELSS). NASA CR-177322, October 1984.

27. Wheeler, R. and Tibbitts, T.: Controlled Ecological Life Support System: Higher Plant Flight Experiments. NASA CR-177323, November 1984.

28. Auslander, D., Spear, R., Babcock, P. and Nadel, M.: Control and Modeling of a CELSS (Controlled Ecological Life Support System). NASA CR-177324, November 1984.

29. Karel, M. and Kamarei, A.R.: Feasibility of Producing a Range of Food Products from a Limited Range of Undifferentiated Major Food Components. NASA CR-177329, April 1984.

30. MacElroy, R.D., Smernoff, D.T., and Klein, H.: Life Support Systems in Space Travel. (Topical Session of XXVth COSPAR meeting, Graz, Austria) NASA CP-2378, May 1985.

31. MacElroy, R.D., Martello, N.V., Smernoff, D.T.: Controlled Ecological Life Support Systems: CELSS '85 Workshop, NASA TM-88215, January 1986.

32. Tibbitts, T.W.:Controlled Environment Life Support System: Calcium-Related Leaf Injuries on Plants. NASA CR-177399, March 1986.

33. Tibbitts, T.W., Wheeler, R.M.: Controlled Environment Life Support System: Growth Studies with Potatoes, NASA CR-177400, March 1986.

34. Babcock, P.S.: Nonlinear System Controller Design Based on Domain of Attraction: An Application to CELSS Analysis and Control, NASA CR-177401, March 1986.

35. Smernoff, D.T.: Atmosphere Stabilization and Element Recycle in an Experimental Mouse-Algal System, NASA CR-177402, March 1986.

36. Oleson, M., Olson, R.L.: Controlled Ecological Life Support Systems (CELSS): Conceptual Design Option Study, NASA CR-177421, August 1986.

37. Oleson, M., Slavin, F., Liening, R., Olson, R.: Controlled Ecological Life Support Systems (CELSS): Physiochemical Waste Management Systems Evaluation, NASA CR-177422, August 1986.

38. Knox, J.: A Method of Variable Spacing for Controlled Plant Growth Systems in Spaceflight and Terrestrial Agriculture Applications, NASA CR-177447, February 1987.

39. Radmer, P., Arnett, K., Gladue, R., Cox, J., Lieberman, D.: Algal Culture Studies for CELSS, NASA CR-177448, February 1987.

40. Karel, M., Nakhost, Z.: Utilization of Non-Conventional Systems for Conversion of Biomass to Food Components, NASA CR-177449, February 1987.

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16. Abstract

Microalgae are well-suited as a component of a Closed Environmental Life Support System (CELSS), since they can couple the closely related functions of food production and atmosphere regeneration. The objective of this study was to provide a basis for predicting the response of CELSS algal cultures, and thus the food supply and air regeneration system, to changes in culture parameters (cell concentration, light intensity, carbon dioxide concentration and temperature). *Scenedesmus* growth was measured as a function of light intensity, and the spectral dependence of light absorption by the algae as well as algal respiration in the light were determined as a function of cell concentration. These results were used to test and confirm a mathematical model that describes the productivity of an algal culture in terms of the competing processes of photosynthesis and respiration. The relationship of algal productivity to cell concentration was determined at different carbon dioxide concentrations, temperatures, and light intensities. The maximum productivity achieved by an air-grown culture was found to be within 10% of the computed maximum productivity, indicating that CO_2 was very efficiently removed from the gas stream by the algal culture. Measurements of biomass productivity as a function of cell concentration at different light intensities indicated that both the productivity and efficiency of light utilization were greater at higher light intensities. These results are consistent with the model of algal productivity, since the ratio of usable input energy to maintenance energy is increased at higher light intensity. *Scenedesmus* was grown in the light and at the expense of an added carbon source, which either replaced or supplemented the actinic light. Hydrolyzed, waste *Scenedesmus* biomass was also tested as a carbon source to determine whether the algae themselves could be made part of the biological recycling process. Results indicate that hydrolyzed algal biomass can serve as carbon and energy sources for the growth of algae, suggesting that the efficiency of the closed system could be significantly improved using this recycling process.

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