BIOMASS RECYCLE AS A MEANS TO IMPROVE THE ENERGY EFFICIENCY OF CELSS ALGAL CULTURE SYSTEMS

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ABSTRACT

Algal cultures can be very rapid and efficient means to generate biomass and regenerate the atmosphere for closed environmental life support systems. However, as in the case of most higher plants, a significant fraction of the biomass produced by most algae cannot be directly converted to a useful food product by standard food technology procedures. This waste biomass will serve as an energy drain on the overall system unless it can be efficiently recycled without a significant loss of its energy content.

We report experiments in which cultures of the alga Scenedesmus obliquus were grown in the light and at the expense of an added carbon source, which either replaced or supplemented the actinic light. As part of these experiments we tested hydrolyzed waste biomass from these same algae to determine whether the algae themselves could be made part of the biological recycling process. Results indicate that hydrolyzed algal (and plant) biomass can serve as carbon and energy sources for the growth of these algae, suggesting that the efficiency of the closed system could be significantly improved using this recycling process.

INTRODUCTION

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 photosynthetically-driven systems -- be they alga-based, higher-plant-based, or as is most likely, a combination of the two -- has two fundamental limitations with respect to energy utilization and conservation:

1) Photosynthetic systems can harvest at most 20% of the (white) light energy that is absorbed by the photosynthetic tissue. (This value can be increased to ca. 30% if relative monochromatic red light can be efficiently supplied, although this may have deleterious secondary consequences in the case of many higher plants.)/1/

2) Only a relatively small fraction of the plant or algal product will generally be useful as food for the crew. In most cases only part of the plant or alga will be consumed by the crew, the remainder being discarded during the course of preparation.

The losses occuring due to the considerations of item #1 may be immutable (at least in the relatively near term) and thus will not be considered further in this communication. Instead, we will concentrate our efforts on item #2, and explore means by which this energy drain could be alleviated or eliminated.

The yield potential of agronomic crops has been substantially increased through breeding programs whose goal was the development of plants that provide a greater percentage of useful biomass (e.g. seeds, edible stems, tubers, etc.) to total biomass. This proportion, called the harvest index, is a measure of how the products of photosysthesis (and indirectly light energy) are partitioned. Table 1 is a compilation of the harvest index of a number of crop plants, some of which are currently being considered for a CELSS. Note that this value is generally below 50%, indicating that for most plants more than one-half of the energy photosynthetically fixed would be unavailable to the CELSS biosystem. A similar, though less clear-cut case can be made for the case of algae, particularly Scenedesmus, Chlorella and Spirulina (Table 2). In this instance roughly 50% of the biomass is in the form of protein that is beneficial to humans. If these algae were to be produced and harvested only for their protein content, the remainder would be waste biomass.

These data suggest that, without special provisions, about one-half of the captured energy of the food plants of a CELSS would be degraded via the waste-handling system. 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. In this way, one would be able to 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. In essence, we would attempt to extract useful "work" from otherwise

unusable biomass.

TABLE 1. Harvest index of some crop species. The harvest index in the percentage of total aerial dry weight at maturity that represents economic yield (grain or seed). Adapted from ref. 2.

Crop	Harvest Index (%)	
	Average	Range (different species)
Maize, hybrids	42	38 to 47
Sorghum	41	40 to 41
Rice	51	43 to 57
Barley	48	35 to 52
Wheat	35	23 to 46
Rye	27	27 to 29
Dry bean	59	53 to 67
Soybean	32	29 to 36

TABLE 2. Chemical Composition of Different Algae (% dry matter) Adapted from ref 3.

Component	Scenedesmus	Spirulina	Chlorella
Crude Protein	50 to 55	55 to 65	40 to 55
Lipids	8 to 12	2 to 6	10 to 15
Carbohydrates	10 to 15	10 to 15	10 to 15
Crude fiber	5 to 12	1 to 4	5 to 10
Ash	8 to 12	5 to 12	5 to 10
Moisture	5 to 10	5 to 10	5 to 10

There are several options available for the heterotrophic component this recycle system: yeast, which have a long history of useful controlled fermentative growth, some bacteria, and some algae. [We should note that higher (crop) plants cannot function in this role.] In this communication we will concentrate on the use of algae.

Under normal conditions, most algae grow photosynthetically, but some strains have the ability to use alternate growth modes (see e.g. ref 4). This photosynthetic growth mode, in which cell carbon is obtained from the carbon dioxide (CO_2) in the gas phase and metabolic energy is obtained from sunlight, is often referred to as photoautotrophy. At the opposite extreme, so-called chemoheterotrophy, cell carbon and metabolic energy are both derived from organic compounds (nutrients). Other, middle-ground, growth modes also are observed in some algae. In the case of photoheterotrophy, growth is maintained at the expense of organic compounds which are taken up, or photoassimilated, at the expense of light energy. In another mode of growth, so-called mixotrophy, there is a simultaneous assimilation of organic carbon sources and CO_2 in the light in amounts that vary with culture conditions.

In the present communication we will describe some experiments in which we attempt to use some of these alternate modes of algal growth to produce the green alga Scenedesmus obliquus. Our results suggest that this organism can be produced in this manner, and that these alternate modes of growth could provide the means to significantly increase the overall energy utilization efficiency of a CELSS.

MATERIALS AND METHODS

Culture Conditions and Sampling Techniques.

Growth of S.obliquus was achieved in a mineral salts medium, supplemented where appropriate with heterotrophic carbon sources. The Basal Medium contained per liter: KNO_3, 2.0g; K_2HPO_4, 0.19g; KH_2PO_4, 0.075g; $MgSO_4.7H_2O$, 0.5g; $CaCl_2.2H_2O$, 0.01g; Hutner's Mineral Salts /5/, 1.0 ml. The carbon source for this medium was provided by bubbling with 2% CO_2 in air. If required, solid medium for growth of S.obliquus was prepared by addition of glucose (0.5%, w/v) and Bacto agar (1%, w/v) to the above medium.

Small scale cultures were maintained in 15 ml capped test tubes containing 3 ml of Basal Medium plus glucose and incubated on a rotary drum at 28°C and low light (40μE m^{-2} sec^{-1}). Larger scale cultures, for growth experiments, were grown in Roux bottles containing 700 ml of Basal Medium. Mixing was achieved by bubbling with CO_2 or air. The culture bottles were enclosed in a blackened box that had only one side open to the light. This served two purposes: (i) it minimized the amount of reflected light reaching the culture, and (ii) it allowed the level of incident light to be varied by use of cheesecloth screens over the open side of the box.

To facilitate intermittent sampling, the Roux bottles were sealed with a rubber stopper pierced by two stainless steel sparging needles and a vent opening. A sterile 20 ml syringe was attached to one of the needles and CO_2/air was admitted to the culture via the other. To remove a sample, the vent was clamped shut, whereupon the mounting air pressure inside the vessel forced algal suspension into the removable syringe.

Preparation of Algal Extracts.
A water soluble extract of algal biomass was prepared in the following way. Dried algal cells (20 g) were homogenized thoroughly in 200 ml of chloroform/methanol (1:1, v/v) and extracted for 24 h 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 h. 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 0.2µm filters prior to use.

No attempt was made at this stage 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.

RESULTS AND DISCUSSION

Before we could ascertain whether organic compounds had any effect on autotrophically grown S.obliquus, it was necessary to determine culture conditions in which light was limiting growth. Therefore, algal cultures were grown autotrophically at different incident light intensities in order to determine a truly growth-limiting light intensity. We observed that above an incident light intensity of about 70µE m^{-2} s^{-1} the growth rate of S.obliquus was independent of light intensity in our system, i.e., the light was saturating (data not shown). Figure 1 shows the autotrophic growth kinetics observed at three light intensities, namely 46, 80 and 144 µE m^{-2} s^{-1}. Note that an incident light intensity of approximately 46 µE m^{-2} s^{-1} resulted in a growth rate equivalent to half the maximum observed rate. Accordingly, subsequent experiments requiring light-limited growth of S.obliquus were performed at an incident light intensity of approximately 50µE m^{-2} s^{-1}.

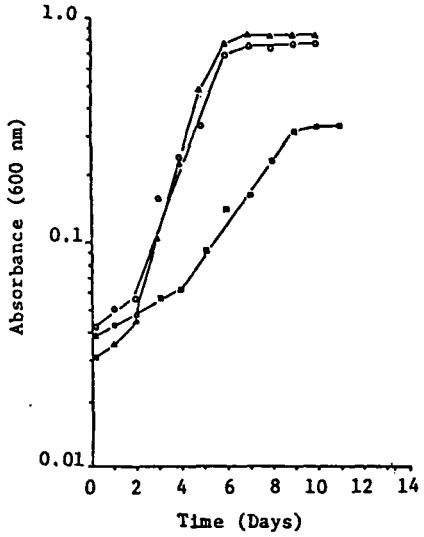

Fig. 1. Culture dry weight as a function of time for growth of S.obliquus at different light intensities. Cultures were grown in Roux bottles containing basal medium, bubbled with 2% CO_2. Cell growth was assayed by performing dry weight measurements on duplicate 20 ml samples taken at daily intervals. Light intensities in µE m^{-2} s^{-2}: O---O,80 ; □---□,46 ; Δ---Δ,144

Table 3 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 S.obliquus. In these experiments the organisms were grown either in the dark or under limiting light, and the growth medium supplemented with various carbon sources; i.e., carbon dioxide (either 0.03%, the amount in air, 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 (µ) was then calculated from the doubling times. Each value in Table 3 represents the mean of two separate experiments.

TABLE 3. Growth rate and doubling time of S.obliquus as a function of available energy source.

GROWTH CONDITION	CARBON SOURCE	DOUBLING TIME (h)	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
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 3 (items 1a & 1b) show the growth rates and doubling times observed when S. obliquus was cultured under limiting light (ca. 25 $\mu E\ m^{-2}\ s^{-1}$) with unamended air (containing 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 S. obliquus 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 (air) 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 under the same light intensity, 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 3a and 3b above, there was no appreciable difference between the rates observed with or without added CO_2, and indeed, little significant effect of light at all. The observed lack of additivity of the dark and light rates may reflect the existence of a "Kok effect" i.e., the suppression of respiration by photosynthesis /6/. Whatever the ultimate cause, these results do indicate that an algal extract prepared from S. obliquus can support the growth, either heterotrophically or mixotrophically, of this same organism.

CONCLUSIONS

Our results show that hydrolyzed S. obliquus 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.*

We wish to emphasize that a scheme of this type may provide the means to avoid, or at least minimize, futile energy cycles associated with carbon flow in a CELSS. The only significant energy input into the biological carbon cycle is through photosysthesis-related processes, which use this energy to produce the reduced carbon compounds that comprise higher plant and algae. This energy is subsequently either harvested (via biological oxidation processing by the crew) or lost via spurious oxidation or waste processing. As shown in Figure 2, the most straightforward, but energy inefficient pathway of carbon flow would be a scheme in which plant and algal waste materials would be recycled via the waste processing system. A more complex, but more energy-efficient pathway would involve a scheme in which this route were short-circuited, and the reduced, and energy-rich waste biomass reused before oxidation. Such a recycling system could result in increases in the energy efficiency of carbon flow (and air regeneration) approaching 50%, compared to a system in which this non-edible material is oxidatively decomposed by standard waste handling techniques.

*An important point, not addressed by the rather preliminary experiments discussed above, is the relative efficacy of the different algal fractions for the support of algal growth. Ideally, the various algal fractions that cannot be converted to a useful component of the human diet would, after proper processing, prove to be efficient carbon and energy sources for Scenedesmus. However, at present we cannot rule out the possibility that the fractions supporting algal growth in the above experiments are the very same fractions that are best suited for the production of human food. A definitive answer to this question would require a more detailed study of both the food processing technologies applicable to Scenedesmus and detailed heterotrophic and mixotrophic growth studies of this alga.

REFERENCES

1. Radmer, R. and B. Kok, Light Conversion Efficiency in Photosynthesis, Encyclopedia of Plant Physiology, New Series, Vol. 5, (A. Trebst and M. Avron, eds.), Springer-Verlag, Berlin, pp 125-135 (1977)

2. Zelitch, I., Improving the Efficiency of Photosynthesis, Science 188, 626-633 (1975)

3. Karel, M. and A. Kamarei, Feasibility of Producing a Range of Food Products from a Limited Range of Undifferentiated Major Food Components, NASA Contractor Report 177329 (1984)

4. Neilson, A. and R. Lewin, The Uptake and Utilization of Organic Carbon by Algae: an Essay in Comparative Biochemistry, Phycologia 13, 227-264 (1974)

5. Hutner, S.H., L. Provasoli, A. Schatz and C.P. Haskins, Some Approaches to the Study of the Role of Metals in the Metabolism of Microorganisms. Proc. Am. Philos. Soc. 94, 152-170 (1950)

6. Hoch, G., O. Owens and B. Kok, Photosynthesis and Respiration, Arch. Biochem. Biophys. 101, 171-180 (1963)