NASA CONTRACTOR REPORT 166615

Problems Associated with the Utilization of Algae in Bioregenerative Life Support Systems

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CELSS

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Problems Associated with the Utilization of Algae in Bioregenerative Life Support Systems

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CELSS

INTRODUCTION

The systems presently used for human life support in space require that food, water, and oxygen be stored, that excess atmospheric carbon dioxide be removed, and that other human wastes be collected and stored. Lengthy missions or large crews dictate that large masses of consumable supplies be taken along or resupplied. Ultimately, a point will be reached where the regeneration of consumables will be economically competitive with the cost of their initial transport or resupply.

It is possible to envision a number of systems that in theory at least would be capable of recycling human waste gases, liquids, and solids, and regenerating food, oxygen, and potable water. Among such theoretical systems, those based on the use of biological processes are very attractive and have been the subject of extensive study in the Controlled Environment Life Support System (CELSS) program. This NASA-sponsored program is directed at studying the feasibility of constructing a bioregenerative life-support system for use in extraterrestrial environments. A bioregenerative life-support system can be described as one that uses rigorously controlled and integrated biological and physicochemical processes to regenerate the supplies consumed in life-support systems.

A biological process that is likely to be central to the successful development of such a system is photosynthesis. Photosynthesis has the potential for simultaneously carrying out most of the major functions of a life-support system: production of food, of oxygen, and of potable water, and the removal of carbon dioxide. Among those organisms capable of photosynthesis, there are two kinds of microorganisms — the green algae (Chlorophyta), and the blue-green algae (Cyanophyta) — that have a number of characteristics that make them attractive candidates for inclusion in a bioregenerative system. These algae grow rapidly, their metabolism can be controlled, they produce a high ratio of edible to nonedible biomass, and their gas-exchange characteristics are compatible with human requirements. In addition, many strains of blue-green algae can convert gaseous nitrogen into ammonium, a biologically useful form of nitrogen.

Unfortunately, there are a number of problems associated with the use of microalgae in life-support systems. For example, there are questions about the adequacy and acceptability of algal-derived food, about harvesting and processing of the algae, and about the long-term stability of the algal cultures. And although ground-based experimentation will be essential for identifying and resolving some of these problems, others can only be approached by appropriate spaceflight experimentation. With this in mind, a workshop was held at the Ames Research Center on November 28-29, 1983, for the purpose of bringing together a group of experts to discuss two major issues: (1) the identification of the major problems associated with the use of microalgae in a bioregenerative life-support system; and (2) the identification of algae-related research issues that must be addressed through spaceflight experimentation.

The contributors to this Workshop were scientists with expertise in a variety of pertinent areas, including algal growth and physiology, the production of food from nonconventional food sources, and human nutrition.

WORKSHOP AGENDA

Problems Associated With the Utilization of Algae in Regenerative Life-Support Systems for Space Habitation

NASA Ames Research Center
Moffett Field, California 94035

Room 361, Building 239 (Life Sciences)

Monday, November 28, 1983

Session I (9:00 to 11:30)

Presentation of the Problem
Chair: Dr. M. M. Averner

9:00 - 9:05 Dr. H. P. Klein
Opening Remarks

9:05 - 9:20 Dr. R. D. MacElroy
The Status and Role of Regenerative Life-Support Research in NASA

9:20 - 9:50 Dr. M. M. Averner
The Potential Functions of Algae in Regenerative Life-Support Systems

9:50 - 10:30 Dr. Richard Radmer
The State of the Art in Sustained Culture of Algae under Space-Related Conditions

10:30 - 10:45 Coffee Break

10:45 - 11:30 Dr. Marcus Karel
The Problem of the Conversion of Nonconventional Substrates to Human Food

11:30 - 1:00 LUNCH

Session II (1:00 to 3:45)

Round-Table Discussion of Problems of Algal Utilization
Moderators: Drs. Radmer and Karel

3:45 - 4:00 Coffee

Session III (4:00 to 5:00)

Space-Flight Experiments Utilizing Algae
Chair: Dr. Lester Packer

4:00 - 4:20 Introduction
Dr. M. M. Averner

4:20 - 4:50 Opportunities for and Constraints on Space Flight Experimentation
Dr. Edward Merek

Tuesday, November 29, 1983

Session IV (9:00 to 12:00)

Identification of Research Issues to be Addressed by Space-Flight Experiments
Moderators: Drs. MacElroy and Averner

12:00 - 1:30 Lunch

Session V (1:30 to 3:30)

Summary and Wrapup
Chair: Dr. Marcus Karel

UTILIZATION OF ALGAE

Algal Growth

For the past 50 years or so, algal-culture studies have been closely intertwined with studies of photosynthesis. Green algae, for example, Scenedesmus and Chlorella, have proved to be good models for higher plants. Several programs in applied algal culture have also been undertaken in the last 30 years, notably at the Carnegie Institute, and in programs sponsored by the United States Air Force and NASA on bioregenerative life support during the period of the mid-1950's to mid-1960's. Obviously, applied algal culture is not a new undertaking.

Species of algae for space-related culture- Most of the work on algal physiology related to sustained culture under space-related conditions has been done on a relatively few species of green (eukaryotic) and blue-green (prokaryotic) algae. By far the most popular have been several species of Chlorella, which are consequently the best characterized organisms. The primary species currently considered for space-related applications are: (1) Chlorella (green), several species; (2) Scenedesmus (green); (3) Anacystis (blue-green) = Synechococcus; and (4) Spirulina (blue-green).

We should emphasize that there is no ideal alga. For example, Spirulina is not necessarily superior with respect to food value; it is simply the best studied in that respect. In some cases, there may be a definite trade-off between ease of culturing and harvesting; for example, Chlorella versus Spirulina.

L. Packer and his colleagues suggest that the prokaryotic N_2-fixing cyanobacteria (blue-green) species deserve primary consideration, based on their following attributes:

1. For N_2-fixing species, nutrient requirements are minimal, and no class of organisms exists in nature with simpler nutrient requirements. Except for inorganic nutrients, an aqueous environment, N_2 or air, aerobic species will generally grow without auxillary substances.

2. Species can be selected that exhibit short generation times.

3. Species can be selected that exhibit a wide tolerance for environmental stresses, such as extreme drafts, high temperature, high salinity, and sudden exposure to such conditions.

4. These organisms utilize phycobiliproteins and phycobilisomes as light-harvesting systems, enabling these organisms to grow well in a broad range of light intensities between 600 and 650 nm, a range in which most other photosynthetic organisms will not grow well.

5. Strains are available that grow as unicellular organisms in suspension culture, and filamentous suspension culture with or without sheath material. Strains can be selected that grow in aggregates, and in many configurations. It is possible to obtain isolates from nature that fulfill desired growth associations.

6. Many species produce gas vacuoles and will, therefore, be of interest for culturing in zero-gravity conditions.

7. Properties (5) and (6) can be exploited for purposes of harvesting or collecting cultures.

8. Under nitrogen limitation, the organisms will fix atmospheric N_2; thus, they can participate in a CELSS in which the nitrogen recycling and regeneration of biologically useful nitrogen and biomass is essential.

9. Most species produce a high content of proteins, in some cases as much as 70% of the total dry weight.

10. Under appropriate growth conditions, the relative proportion of carbohydrates that can be accumulated in the form of glycogen, lipid, or protein or any combination thereof can also be modulated, particularly by manipulating nitrogen limitations of growth.

11. If needed, cultures can be synchronized. Growth under steady-state conditions has been achieved with many species.

Two other points made by R. Lewin:

1. Contamination could be reduced (if not eliminated) by growing algae capable of tolerating extreme conditions, say high pH and high salinity (e.g., Spirulina), low pH (e.g., Cyanidium and Spermatozopsis can tolerate pH less than 2 or 3), and high temperatures (e.g., Cyanidium and Mastigocladus can tolerate temperatures as high as 60°C).

2. Algal products could be adjusted to have about 50% protein (in high-nitrogen media) or almost no organic nitrogen: glycerol (10%-30% from Dunaliella); mucilage (80% from Porphyridium grown on sponge in a flowing system); starch (perhaps 50% from green unicells); glucose, mannitol, or maltose from cells "permeabilized" (cf. zoochlorella in symbiotic systems, algae in lichens, etc.).

Light- The effect of light is probably the most important consideration in the design of alga culture systems. An alga culture system basically follows Beer's Law. Examples of well-characterized rate-versus-intensity curves for algae are available. As pointed out by J. Myers, such characteristics are not fixed, even for a particular species; since algae are capable of adapting to different light conditions, changes in light intensity can elicit corresponding changes in the alga's physiology.

The productivity of an alga culture for a given culture system can be derived. Productivity follows some rather general rules: maximum productivity occurs at relatively high population densities, and at a specific growth rate equal to about one-half the maximum growth rate.

San Pietro suggested the use of "stacked cultures" to maximize the use of light quality and intensity (assuming the light source to be broad-spectrum white light). Under natural conditions, essentially every part of the spectrum from 300-950 nm is utilized by one or another organism. In green algae, maximal absorption is due to chlorophyll-a and chlorophyll-b, with absorption maxima at 675 and 650 nm, respectively. In general, most of the energy in the 500-650 nm range is not used by green algae. However, the blue-green algae contain accessory pigments (phycobiliproteins) that absorb in the range of 500-650 nm, and this energy can be used photosynthetically by transfer to chlorophyll. Depending on the geometry of the culture arrangement, one could have stacked or concentric cultures of green and blue-green algae,

depending on placement of the light source; above or below for stacked cultures and central for concentric cultures. This might allow for (1) maximal use of available light energy and (2) major production of two different products (protein, carbohydrate, or lipid), one each by the two cultures. One could even go further and possibly include a culture of photosynthetic bacteria (light absorption beyond 700 nm) which take up carbon dioxide in the absence of oxygen evolution but require an oxidizable substrate (organic or inorganic).

CO_2 and O_2- The effects of CO_2 and O_2 have not been worked out in as quantitative a manner as those of light. However, one can generally run alga cultures so that these factors are not limiting. One relevant recent finding is that some algae are able to pump HCO_3^- (bicarbonate), and thus concentrate inorganic carbon in a manner similar to that found in C_4 plants.

The main features of carbon dioxide utilization in the present context are

1. CO_2 is a substrate, and air levels (0.03%) are not saturating. Algal cultures are generally run on 1%-5% CO_2 in air.

2. The enzymology of CO_2 uptake (i.e., Calvin cycle, etc.) is well understood.

3. HCO_3^- transport by algae is recognized but not well understood. It does appear to function as a "CO_2 pump."

4. Problems relating to mass transfer and interfaces need to be addressed (e.g., bubble size and aeration rate).

Oxygen is both a product and an inhibitor of photosynthesis (the inhibitory effect is generally discussed in terms of the Warburg effect, photorespiration, or photoreduction). In general, deleterious O_2 effects at atmospheric levels can be circumvented by using high CO_2 tensions. The concentration relations between O_2 and CO_2 are quite complex, and depend on such factors as specific physiology, HCO_3^- pumping, and mass transfer.

In summary, the problem of CO_2 and O_2 is not as well characterized as the light problem. In general, O_2 effects are competitive with CO_2. Both problems (i.e., CO_2 availability and O_2 inhibition) can be solved by using CO_2-supplemented air. Under these circumstances, the algae are substrate-saturated and the competitive O_2 inhibitory effects are minimized.

Temperature- The responses of algae to temperature are not unusual, compared with those of other organisms. In general, algae have a wide range for growth, with given species having an optimal growth range of 2°C-3°C. Thermotolerant Chlorella have an optimal growth range of 39°C, and some thermophilic blue-greens have been reported with optimal growth ranges greater than 50°C. Although there was some early interest in the applicability of high-temperature strains for mass culture, there has in general been little success in increasing productivity by the use of these strains. One side benefit is that minimal cooling of such cultures would be required.

Mineral nutrition- The main mineral requirement for algal growth is fixed nitrogen in a proportion of about one nitrogen atom per six carbon atoms. Urea, ammonia, and nitrate have all been used as nitrogen sources, and there have been some experiments using human wastes. Other required macroelements are K, Mg, S, P. In addition,

about 10 microelements seem to be required for optimal growth. There have been a few reports of mineral recycling systems being used for continuous algal production.

Depending on the method used to dispose of organic waste (particularly human feces and nonutilizable algal products from the CELSS) the level of bacterial denitrifying activity will vary. A continuous loss of biologically usable fixed nitrogen, at whatever rate, will be a serious problem in a CELSS program during prolonged spaceflight. As emphasized by Packer, the use of nitrogen-fixing cyanobacterial species would circumvent this process, reclaiming nitrogen lost by bacterial action and reintroducing it in a usable form into the biosystem.

Engineering algal cultures- Given what is known, how do we engineer algal cultures for space-related applications? Algal growth can be considered as a balanced chemical equation:

$$6.14\ CO_2 + 4.7\ H_2O + HNO_3 + \text{LIGHT} \rightarrow C_{6.14}H_{10.3}O_{2.24}N + 8.9\ O_2$$

Assimilatory quotient = 0.69

or

$$6.14\ CO_2 + 3.7\ H_2O + NH_3 + \text{LIGHT} \rightarrow C_{6.14}H_{10.3}O_{2.24}N + 6.9\ O_2$$

Assimilatory quotient = 0.89

The system can be considered in the following terms:

1. Known: the yield as a function of intensity and wavelength

2. Can determine: the yield as a function of CO_2 and O_2 for the specific configuration (one generally uses CO_2-amended air)

3. Choose: nitrogen source, its concentration being scaled to the desired cell density.

Work to date suggests that there are no insurmountable problems with respect to algal cultures (on Earth); in general, they can be run and considered as biochemical (chemical) reactors.

Modifications via molecular genetics- Several new and intriguing possibilities have recently arisen as a result of developments in molecular biology and genetics. It now appears that in the very near future one will be able to specifically alter the nutritional (or other quality) of an alga; for example, one could clone polylysine synthesis into lysine deficient Spirulina. Although these studies are in their infancy, there are ample precedents in the more highly developed work on E. coli and yeast. The current status of molecular genetics with respect to some algae of immediate interest to CELSS is

1. Anacystis (blue-green): has known transformation system

2. Chlamydomonas (green): has known transformation system

3. Spirulina (blue-green): no plasmids?

4. Chlorella and Scenedesmus (greens): no data

The unanswered question in all of these studies to date has been how will these modified organisms behave in culture? It should be possible to answer this question within the next 5 yr or so, if the necessary research programs are undertaken.

Processing Algae

General considerations- In an algal regenerative system, a given amount of regenerated oxygen will be associated with a given total biomass of algae (X) and a fraction of this biomass (Y) will be utilizable as food. The basic problems of the research and development to be undertaken, if the system is to fly, arise from the following relationships. The biomass X is a function of the species of alga selected, of growing conditions, of harvesting methods, and of other factors. The biomass fraction Y is obviously a function of X, but is also a function of species, of growing conditions, and of harvesting methods (i.e., for any given total X the resulting Y will depend on the above, because, for example, different species have different protein-to-cell-wall ratios). The fraction Y is also a function of a number of food processing variables, such as method of purification, method of preparation, and palatability; it is a function of a number of external variables, such as availability of other food components to blend with the algae to improve taste or nutrition, motivation of the crew to eat marginally edible materials, and the presence of other biomass conversion systems (yeast, fish).

Some of the important issues related to the use of algae for human food in space are

1. Determining the maximum amount of algal material that can be used as a result of limitations imposed by acceptability, nutrient content, and freedom of contamination.

2. Establishing the availability of other nutrients (foods or components) to blend with the algae-derived components, and determining their compatibility with respect to taste, nutrient content, and chemical reactivity.

3. Determining how to formulate and process acceptable foods using algal and non-algal components.

Optimization of the algal system will require the consideration of all of the above plus other variables. In order to obtain the necessary information to begin work toward this optimization, it appears that there is an urgent need for research in food processing and nutrition, and in algal physiology as it affects food processing and nutrition.

Feasibility of converting algal biomass to edible components- Direct consumption of single-cell biomass without purification is not feasible in amounts that would be of any significance to biomass recycling. The purification (removal of undesirable components) or isolation (recovery of edible components in relatively pure form) may be necessitated by physiological concerns (nutrition and toxicology) and palatability concerns. The following concerns have been identified at the workshop as being well established by past work with several species of algae.

1. Physiological (undesirable components): Nucleic acid excess, recyling of potentially toxic metals, photosensitizing and toxic pigments, steroids, excess protein-to-calorie ratio in the potentially edible biomass, nondigestible components of cell wall, potentially nondigestible, and physiologically disturbing carbohydrates (diarrhea, flatulence), toxins, and allergens of unknown origin.

2. Organoleptic: Previous studies reveal offensive flavors, tastes, and perhaps textures and colors in minimally processed algae.

It is important to (1) establish the feasibility of extracting purified edible components from algal biomass, (2) establish the feasibility of converting these components to food, (3) scale down relevant processes to space conditions, (4) analyze the relationships between the degree of utilization of the biomass (yield) and the weight of required equipment.

It appears that recovery of part of the protein may be the least difficult task, followed by recovery of part of the lipid. However, recovery of carbohydrates, which in a large measure are in the cell walls, will require substantial chemical processing, and the carbohydrates may not be of nutritional value (wrong carbohydrates). This conclusion is premised on composition of algae under the combinations of growing conditions currently considered "optimal" for trouble-free recycling of oxygen.

Following are some of the specific, although broadly defined, tasks of this research into the processing of algae for human consumption in space:

1. Establishing methods of cell rupture (chemical, physical, enzymatic)

2. How to concurrently reduce nucleic acids and extract crude components (chemical, physical, enzymatic)

3. How to isolate and purify desirable components; for example, by ionic strength, centrifugation, physical separation (filtration, dialysis)

4. How to manage by-products

5. Determine properties of purified ingredients

Some alternatives in processing are shown in figure 1. As shown in figure 1, nucleic acid reduction is a major processing objective and could be accomplished by heat-shocking the cells to activate endogenous nucleases and incubating them at reduced temperatures to hydrolyze the nucleic acids and excrete the digested nucleotides. Extracellular nucleotides in the liquid phase would then be recycled as nutrients for algal culture following cell harvest. Alternative methods might involve alkaline extraction of whole cells or exogenous nucleases under similar thermal processing conditions. Harvesting of cells might involve centrifugation, filtration, or chemical flocculents. Flocculents are not favored because they are generally unsuitable for human consumption at required dosages. For large organisms, for example, Spirulina, cells could be recovered by a rotary filter of large mesh size. Smaller organisms, for example, Scenedesmus, would be more difficult to filter and would require pressure gradients at filtration-cake resistances that could not be reduced by conventional filter aids that would make cell rupture more difficult. Following cell rupture, it might be possible to use a formulated edible filter aid as a nutritional supplement to solids consisting primarily of carbohydrate and protein. Algal walls are reported to contain 10%-20% of cell nitrogen and

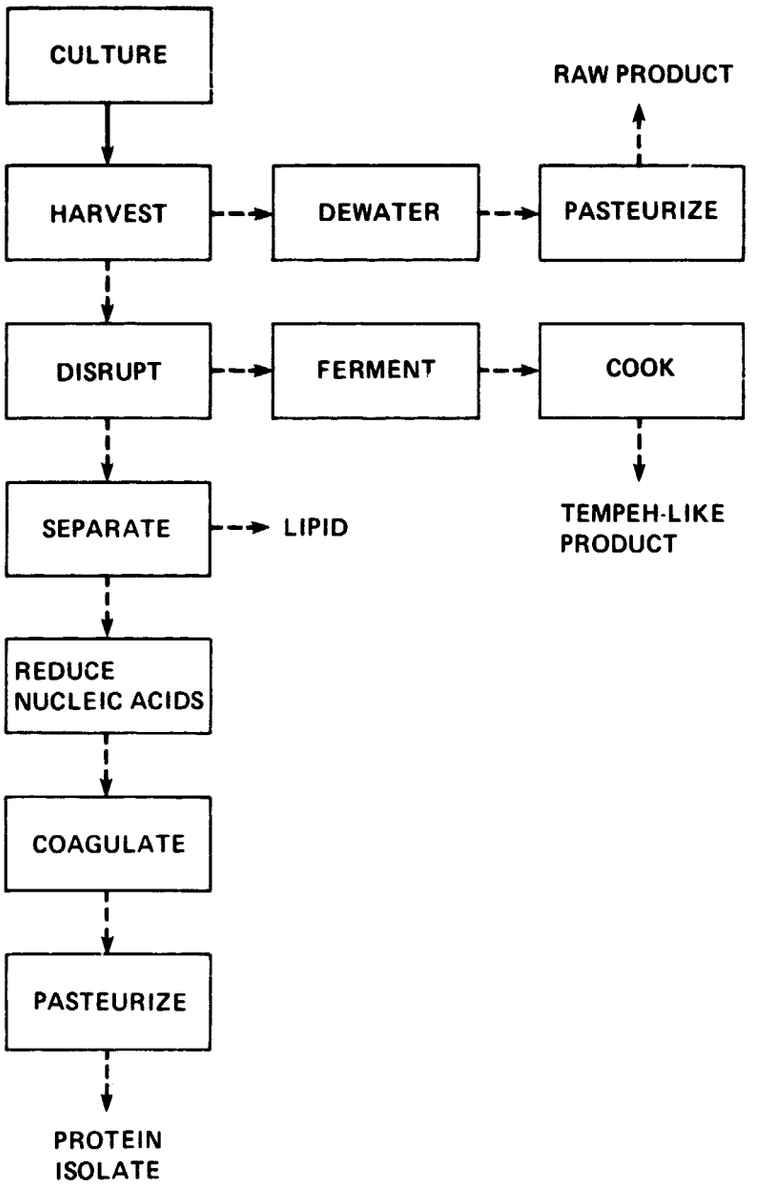

Figure 1. Alternatives for Production of Food from Algae.

varying levels of cellulose, rhamnose, and pectin, which are not digested in normal mammalian metabolism.

Protein and lipid recoveries could be enhanced by in vitro treatment of whole cells with cellulase mixtures or by in vivo fermentation of harvested cells in the solid state before cell rupture. Cell disruption might involve sonication, ball-milling, homogenization, or pressing, followed by separation of the solids phase from a two-component liquid phase containing the major soluble proteins and insoluble lipids as cytoplasmic droplets. The lipids could then be recovered by density differences permitting phase separation with protein recovery by thermal coagulation of the aqueous phase. The solids might then be digested in vitro by enzymes to give a mixture of amino acids and sugars which could be separated by chromatography or filtration, or recovered by drying the solids for use as emergency rations or as conventional food supplements. Alternatively, to increase the digestibility of solids, the disrupted solids could be fermented in the solid state by a food-grade organism producing extracellular enzymes that hydrolyze cell-wall constituents. Such methods are widely used to produce traditional oriental foods and could probably be developed for algal processing to yield a tempeh-like product of improved flavor, texture, and digestibility, which could be cooked and eaten alone or with conventional food supplements. A preliminary estimate of available calories per pound of dry algae based on 50% recovery of major nutrients for the unit operations described is shown in table 1. The estimate is speculative, of course, but it does provide a basis for preliminary consideration of nutritional requirements. The estimate suggests that additional carbohydrate would be required for nutritionally balanced diets.

TABLE 1.- ESTIMATED CALORIC VALUE OF ALGAE BASED ON 50% RECOVERY OF MAJOR FOOD CONSTITUENTS (Based on 1 lb of solids)

Constituent, %		Approximate weight distribution, g				Calories	
		Total	Wall[a]	Cytoplasm	Recovery	Unit	Total
Protein	45	204	30	174	102	4.5	459
Nucleic acids	5	23	-	23	-	-	-
Lipid	15	68	18	50	34	9.5	323
Carbohydrate	25	114	100	14	57	3.7	211
Fiber	5	23	23	-	-	-	-
Ash	5	22	-	22	-	-	-
Total	100	454	171	283	193	5.1	993

[a]Including cell membrane.

Modifying the algae regenerative system for food components- It will also be important to analyze the potential for modifying the algal oxygen regenerative system in order to facilitate the utilization of biomass as food. In particular, the possibility for growing algae with a high carbohydrate (preferably starch) content in a form easily recovered by physical means, such as large granules, should be studied.

Other food processing considerations that may affect the selection of algal species were pointed up by Packer. In particular, Packer called attention to some of the advantages of cyanobacteria. Most of the major proteins of cyanobacteria should be readily accessible for fractionation. Being prokaryotic, it is in principle relatively easier (than with eukaryotic algae) to design methods combining enzyme treatment with mild mechanical treatment or osmotic shock to break the organisms open and free their internal contents.

Major protein fractions in the cyanobacteria, as in other photosynthetic organisms, are the ribulose bisphosphate carboxylase protein fraction (fraction I protein), which represents the major single protein in most eukaryotic and prokaryotic photosynthetic organisms. This protein is perhaps a third of the total in cyanobacteria. Some species produce this protein in the form of "carboxysomes." In the electron microscope, these are seen as huge macromolecular aggregates.

The cyanobacteria are also special in that the light-harvesting pigments are phycobiliproteins and phycobilisomes (phycobiliproteins organized into granules). Phycobilisomes are relatively loosely attached by electrostatic means to thylakoid membranes. These granules can be readily released by mild ionic strength washing of membranes in ruptured cell homogenates. Rapid and simple isolation procedures are state-of-the-art. Also, experiments have shown that temporary functional uncoupling of such phycobilisomes will occur by subjecting cyanobacteria to a transient salt shock.

These two fractions, total phycobiliproteins and fraction I protein, would be about 50% of all the cellular protein proteins that should, in principle, be readily available for processing for nutrient use for human consumption. These protein fractions would not, a priori, be expected to contain either as cofactors or as unwanted trace elements, metallic or undesirable lipid substances.

Cyanobacteria have already been widely used as a source of food for animals and for humans in various parts of the world. Indeed, in China, it has been used for centuries.

Combining algal systems and other synthesis methods- The potential for combining the algal oxygen regeneration system with a synthetic or a higher plant component to reduce the dependence on the algal biomass for the nutritional and edibility requirement will also have to be determined. Some possibilities include high-carbohydrate-content plant (sugar beet or sugar cane) and chemical synthesis of glycerol.

Analysis of the waste system requirement- It is necessary to reanalyze the waste system required for the various regenerative systems under consideration. This point was also stressed by Myers and Mudgett.

SPACEFLIGHT EXPERIMENTS

General Considerations

Because the extensive discussions devoted to other areas of interest, only brief attention was given to spaceflight experimentation at the Workshop. Because of its importance to the proper planning of future CELSS-related spaceflight experimentation, however, it is strongly recommended that an additional Workshop be held in the near future to address this topic. The following is a summary of both the Workshop discussion and additional comments submitted later by the participants.

It is the consensus of the participants that there are no insuperable problems related to the growth of microalgae in space and that in general algal growth characteristics in space would be expected to be similar to ground-based cultures. The major research issues lie in the areas of long-term culture stability, the optimal design of algal growth reactors, and postgrowth harvesting and processing.

Research in these areas will start in ground-based laboratories, but predicted behaviors must be verified under actual flight conditions. Data so obtained will be critically important to total CELSS system design, to mathematical model development and validation, and to testing of regulation and control.

Algal Growth Characteristics

Although short-term growth of algae would seem to present no problems, long-term exposure to the space environment may affect culture stability or viability. For example, the Shuttle cabin atmosphere has been reported to contain many volatile organic compounds outgassed from Shuttle structural materials. Continual exposure of the algal cultures to such compounds may allow their accumulation until they reach toxic levels. Other problems that may only be manifested after long-term exposure of the algae to the spacecraft environment include mutagenic effects of ionizing radiation, microbial contamination of the algal cultures, and application of techniques of heat and mass transfer on fluid and particle behavior. Biological parameters that might be affected include rates of growth, photosynthesis and respiration, algal composition, and the excretion of organic and inorganic compounds. These factors could have negative effects on the algae as a reliable and stable component of a bioregenerative system. To assess properly the long-term behavior of algal cultures, spaceflight opportunities lasting for several hundred algal generations, corresponding to several months of continuous growth, are required.

Algal Growth Reactor

Of central importance in the successful utilization of microalgae in a bioregenerative system is the algal growth reactor. Although certain design problems and potential approaches to their solution can be defined, we anticipate that several technical advances will be necessary to ensure that the appropriate tests, both on the ground and in space, can be made. Some of the problems that must be addressed are gas-liquid separation; the behavior and transport of culture medium; cell adherence to surfaces; optimal lighting techniques and configuration, considering efficiency of utilization; and heat-transfer minimization.

All present ground-based algal growth reactors use processes that are dependent on gravity, such as gas-bubble sparging and mixing, and overflow harvesting. These processes will have to be replaced with functionally analogous processes that can operate in the space environment. Thus, the effects of microgravity on particle, fluid, and gas-bubble behavior, as they affect culture aeration and mixing, must be determined. Other considerations include reactor size and weight, culture illumination, reactor monitoring, and control. It is possible that a single reactor design might serve the requirements of many experimenters, but it is also likely that various experimental goals might require experiment-specific designs or extensive modifications to a single selected design.

Harvesting and Processing

Utilizing algae for food will require that cells be separated from the culture medium, and that the harvested material be processed to produce food. Physical methods, instead of chemical ones, should be used to the maximum extent possible. Such methods will involve heating and moving fluids, or the use of forced convection, for most of the methods under consideration. For example, filtration, chromatography, and dialysis, are all methods that depend on mass transfer coefficients. These coefficients may be affected by fluid behavior and particle properties that themselves may be altered in microgravity. Similarly, conventional heating and cooling methods depend on heat-transfer coefficients that may also be affected by fluid behavior. Thus, the effects of microgravity on both particle and fluid properties and flow behavior, are of interest with respect to the design and optimization of such processes. There is an additional question of whether phase separations obtained by centrifugation can be maintained when the centrifugal acceleration is removed.

FUTURE WORK

Areas that are recommended for future CELSS-related algal culture studies are summarized below.

Establish criteria- Establishing a conceptual CELSS framework goes hand-in-hand with the suggestions of several of the Workshop participants (e.g., Lewin). These criteria need not be definitive; a series of possible scenarios would suffice (e.g., artificial light versus sunlight; x men; y liters total volume, z years duration, w kilowatts, s gallons per day sewage capacity, and v percent sewage recycle), so that discussions can have a starting point. Most of these could be derived from early NASA and USAF data.

Myers suggested a plus-and-minus point system that could be applied to any life-support system: positive points for items such as reliability, minimum components for complete support, and use of known technology, and negative points for production of any component not utilizable by the crew, for example, time for management, and power, volume, and mass requirements, to establish criteria for scenario selection.

Myers' concern is that historically there has been a repeated pattern of avoiding difficult parts of a proposed system on the premise that workers on other parts of the system would somehow resolve the problems associated with them. For example, it was earlier supposed that someone would solve the problem of feeding algae to humans, and no one was concerned about the recycling of the algal medium, since it was assumed that someone else would handle waste management.

At the Workshop, there was little discussion of power requirements. For artificial light the minimum input electrical power requirement per man will be 4 kW, 96% of which is a heat load that has to be dissipated. From this starting point, trade-offs between large systems with low-intensity lamps (e.g., fluorescents) and more compact systems with higher-intensity lamps, lower efficiency, and higher power requirements can be made. Even in crudest form a point system would force a disciplined approach; and even if no two people could agree, the exercise would tell where the uncertainties are.

A similar considerations was addressed by Mudgett, who attempted to estimate the effects of algal culture on O_2-CO_2 balance in terms of assimilation and respiration. A comparison of reciprocal yields in mass balance suggests three operating modes in continuous culture:

1. Maintain constant cell density with the objective of optimizing biomass productivity

2. Vary cell density to consume CO_2 at its rate of evolution in respiration

3. Vary cell density to produce O_2 at its rate of consumption in respiration

According to his computations, each alternative leads to imbalance. His considerations indicate the need for an integrated control system to maintain balanced gas conditions for algal culture and human respiration, and suggest that turbidimetric control may not be the optimal approach to this problem.

At present, because of our lack of defined rules and goals, we have no means by which to implement or evaluate the above considerations.

Expand CELSS-related algal culture studies- There are several aspects to the problem of algal-related studies, all of which must be coordinated with the related problem of food production.

During the past two decades there has been little emphasis on the culture of algae for space use, and as a result its development has not kept pace with some of the related areas. It should be relatively straightforward to obtain species-specific (or strain-specific) culture and production data on the algae of interest. Even some of the more arcane data (e.g., possible effluent volatiles) should be obtainable, and any related problems solvable, without undue effort or expense.

Other topics to be addressed in the near future might include the following.

1. The suitability of nitrogen-fixing cyanobacterial species. Studies of growth rate, ability to withstand environmental shocks, presence or absence of gas vacuoles, and effects of nitrogen limitation would have to be undertaken to determine the conditions under which optimum accumulation of protein (fraction I and particularly phycobilisomes) and starch or fat accumulate.

2. Different algae or culture conditions, for example, growth on surfaces in moist air (e.g., Protosiphon, Hormidium) or enmeshed and immobilized in artificial polyurethane sponge (e.g., Porphyridium).

3. Problems related to heat and mass transfer. Mudgett made some preliminary calculations for gas transfer in algal culture based on generalized equations for mass balance in an agitated culture vessel.

Similar preliminary considerations of heat transfer in algal culture were based on generalized equations for energy balance in a countercurrent heat exchanger.

Spaceflight experiments- At present there are few reliable data concerning the effect of microgravity on algae. It may be that there is no effect on the algal physiology per se, in which case the problem reduces to one of handling gases, liquids, and small particles in space. (The effects of ionizing radiation can probably be addressed adequately on Earth.)

Several participants stressed the value of developing and maintaining algal culture systems in microgravity in the near future. Immediate questions to be addressed would include the following.

1. The stability and viability of algal organisms during long-term exposure to microgravity is unknown (San Pietro). The "leasecraft" vehicle may provide the best experimental approach to answering this question. The initial information necessary is a complete ground-level photosynthetic characterization of a variety of algae (green and blue-green). Multiple cultures of these algae could then be placed aboard the leasecraft vehicle so that cultures of the various algae could be returned to Earth at 6-month intervals. The returned cultures would then again be subjected to a complete ground-level photosynthetic characterization to determine any changes resulting from exposure (6 months, 1-yr, 1.5 yr, etc.) to zero gravity. For long-term flights (up to 5 yr), it will be necessary to have on board some backup cultures of algae since it is unlikely that a single continuous culture will operate optimally and without accident for the duration of a long spaceflight.

2. It will also be necessary to determine for how long a continuous algal culture will function optimally and without significant change (in assimilatory quotient, in product formation, etc.) under zero gravity. Initial experiments might well be with short-term flights and emphasize simple measurements (e.g., CO_2 uptake, O_2 evolution, change in pH). Depending on the results of these experiments, one could vary culture conditions such that one of a variety of possible products (protein, carbohydrate, lipid) is accumulated.

3. It is clear that an algal CELSS will require many mechanical and experimental manipulations in progressing from an algal culture to a useful product. It should be possible to test many of these manipulations in a complementary set of experiments so that the results are applicable to an algal CELSS. The following are examples of the types of manipulations that should be considered. (a) Stirring and bubbling of cultures: What methods are available and have those methods been tested previously in other experiments? (b) Liquid flow in continuous culture: How does one collect culture fluid continuously at zero gravity—by overflow or circumferentially? (c) Monitoring devices; (d) Illumination: power availability and spectral quality: Can one grow more than one algal culture with the illumination available as described above? (3) Harvest of algae: What methods are applicable in zero gravity, and have any of those methods been tested previously?

This is an opportune time to evaluate processed algae as a food source. There are no significant unknowns with respect to algal culture (on Earth), and the problems of algal culture in space may be readily (but not necessarily cheaply) solved.

NASA should undertake a program to study and develop methods for processing algae for human food. These methods should be based on either separation of undesirable or unacceptable components from the edible fraction. These studies should begin with Spirulina (an edible alga) and an easily grown, well-characterized alga such as Scenedesmus or Chlorella. Once the question of feasibility has been answered, more detailed questions related to, for example, the energy costs and yields, of the food-processing system can be addressed. The choice of algal species may be an important aspect in the long-term success of this project (most terrestrial land plants do not provide acceptable food). However, the aforementioned species may be adequate, if not ideal. In any event, we should not attempt to find the ideal alga: there will be too many trade-offs with respect to nutrition, processing, culturing, and harvesting, and a CELSS should probably have more than one algal component.

Finally, as noted above, we should not overlook the attractive opportunities available through the use of genetic engineering techniques. This area is in its infancy, particularly with respect to algae. However, it will probably mature rapidly enough that it could make a substantial contribution to the CELSS program.

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