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PREFACE

This volume contains progress reports presented by the Aquatic Species Program subcontractors at the SERI Biomass Program Review held in Washington, D.C., June 23 - 25, 1982. These reports present and discuss research advances achieved by the subcontractor during the preceding year. The SERI Biomass Program receives its funding through the Biomass Energy Technology Divison of the Department of Energy.

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INTRODUCTION

The Aquatic Regular Species Program (ASP) is concerned with how plant biomass that naturally occurs in wetland or submerged areas is utilized. Processes are being developed in this program to make use of those aquatic species, capitalizing on their inherent capacity for rapid growth as well as on their extraordinary chemical compositions. The cultivation of salt-tolerant species on poorly utilized, low-value lands is emphasized, particularly where conventional agriculture is not economical. Candidate species include microalgae—unicellular plants that are natural factories for converting sunlight into high-quality oils; macroalgae—large, chemically unique plants that can be easily fermented to methane gas or alcohols; and emergents—plants that grow rooted in waterways and bogs, but are partially exposed above water.

Techniques for intensive cultivation of aquatic species in managed systems need to be developed. Within the next five years, the conditions and resources necessary for sustained systems operations should be defined, design parameters examined, and experimental facilities developed. In succeeding years, we must focus on resolving major technical hurdles in systems operations, integration, and component performance.

Research and development activities in the Aquatic Species Program are conducted in four mutually dependent areas: species selection, cultivation requirements, processing technology, and systems integration. Species selection involves the collection, identification, and characterization of plants classified in each category under the program's purview. Selections are made according to data comparing growth rates, light requirements (both intensity and wavelength responses), temperature tolerance, nutrient demands, salinity and pH limitations, and chemical compositions. Cultivation requirements are often established empirically through effects-of-variables studies. Processing technology involves the harvesting, fractionation, and conversion of aquatic plants into fuels or other energy products. Systems integration provides the definition of components required for fully functional, practical systems.

Present activities focus principally on the first two of these research and development opportunity areas but rely heavily on guidance provided by systems integration analyses.

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MICROALGAE AS A SOURCE OF LIQUID FUELS

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INTRODUCTION

The production of planktonic microalgae has a number of distinct differences, compared with conventional agriculture (Table 1). Two key disadvantages are the microscopic size of most planktonic microalgae and the low standing biomass for optimal productivity. A major constraint is the need for a supply of pure or enriched CO_2. In addition, the hydraulic nature of the production system imposes unique constraints on the engineering design of a production system. For the production of fuels, only low-cost designs can be considered. A key problem in such a design is the harvesting of the microalgae, which involves the separation and concentration of dilute microscopic particles.

Two different approaches to microalgae production have been developed over the past three decades: (1) very shallow (<10 cm) and highly mixed systems with or without transparent covers, and (2) somewhat deeper systems (>15 cm) with gentler mixing. An example of the former is the Czechoslovak system, which consists of a sheet flow of algal culture traveling over an inclined surface provided with ridges [1]. Systems up to 1000 m^2 have been built in Bulgaria. The advantage of such a system is that it can produce a very concentrated culture of microalgae, up to several grams per liter. Thus, harvesting is less of a problem, and centrifugation may be feasible for more highly priced products.

The other approach, first introduced by Oswald and colleagues, involves a much simpler system: large ponds, up to 100 acres each, divided by 200-ft-wide channels and mechanically mixed at relatively low velocities. They developed such "high-rate ponds" for wastewater treatment and proposed such a system for energy production. Harvesting was to be by "autoflocculation," a high pH induced coprecipitation of microalgae with Mg^{++} and other ions [2]. The first step in the practical development of such systems was

TABLE 1. COMPARISON OF ALGAE CULTURE AND AGRICULTURE

Parameter	Algaeculture	Agriculture
Biomass yield (mt/ha/yr)	25-90	2-25
Protein yield (mt/ha/yr)	10-40	0.2-2.5
Water use (mt/mt biomass)	500-1,000	1,000-10,000
Water quality	Poor to bad	Good to fair
Annual production cycles	100-200	1-3
Nutrient use efficiency	High	Low
Capital and labor use	High	Low
Weather influence	Minor	Major
Pest effects	Minor	Major
Carbon source	CO_2 (10%-100%)	CO_2 (0.03% CO_2)
Product moisture	Wet (>75%)	Dry (<25%)

the development of a low-cost integrated wastewater treatment-fuel production process. A number of supported R&D projects with this objective were carried out by the authors and colleagues at the University of California, Berkeley, from 1975 to 1980 [3-7].

Early in 1979, EnBio, Inc. (formerly Ecoenergetics, Inc.), based on an analysis of microalgae production technology, submitted to SERI a proposal to develop a concept for the production of high lipid (oil) microalgae biomass through controlled nitrogen limitation. In September of 1980, EnBio, Inc., received a contract to design and build an experimental facility with which this concept of microalgae production could be tested and developed. The facility was based on the concept of a high-rate pond (HRP) production system [8]. A parallel project was initiated at the University of Hawaii to design, build, and test a shallow, covered pond system based on an invention first tested there [9, 10].

In August of 1981, EnBio, Inc., was awarded a contract by the DOE Office of Energy Research, cofunded by the Biomass Systems Technology Division, to carry out a more detailed analysis of the liquid fuels from microalgae option. The following were the major objectives of this contract:

- Review the state of the art of microalgae biomass production
- Conduct a comparative engineering and cost analysis of the two systems investigated by SERI, emphasizing cost differences
- Develop capital and operating cost figures and provide fuel costs

- Assess potentials for by-products and total resources
- Recommend needed research and development.

Here, we present the key results of this project.

COMPARISON OF THE SCS AND HPR SYSTEMS

The first task was to compare the two systems that are or have been the subject of DOE-SERI research and development. The first system is referred to herein as the "shallow covered system," or SCS system (Figure 1) [9, 10]. The key components are a cover for IR radiation blockage, to screen out supposedly inhibitory light quality; a very shallow depth (5.0-7.5 cm); air lift pumps, to carbonate, mix, and harvest by foam fractionation; and sufficiently rapid mixing to maximize productivity through a "flashing light" effect. The HRP system was described in greatest detail in a 1978 report to DOE [11]. The system (Figure 2) consisted of twenty 40-hectare high-rate ponds with large settling ponds arranged between them, to harvest the algae. The two systems are compared in Table 2. Important differences in operating and design parameters are apparent and are reviewed next.

One reason for a $CuSO_4$ cover in the SCS system is the supposed inhibitory effect of IR radiation. A detailed literature review, including key references cited by the inventor [9], did not support the contention that long wavelength light is inhibitory to microalgae

TABLE 2. COMPARISON OF THE SHALLOW COVERED (SCS) AND HIGH-RATE POND (HRP) SYSTEMS

Parameter	HRP	SCS
Microalgae species	Not specified	*P. tricornutum*
Depth	25-30 cm	5-7.5 cm
Cover	None	$CuSO_4$ filter
Mixing velocity	30 cm/sec	30 cm/sec
Mixing system	Paddlewheel	Air lift pump
Liner	Compacted soil	Required
Water source	Brackish, fresh	Seawater
Harvesting	Settling	Foam flotation

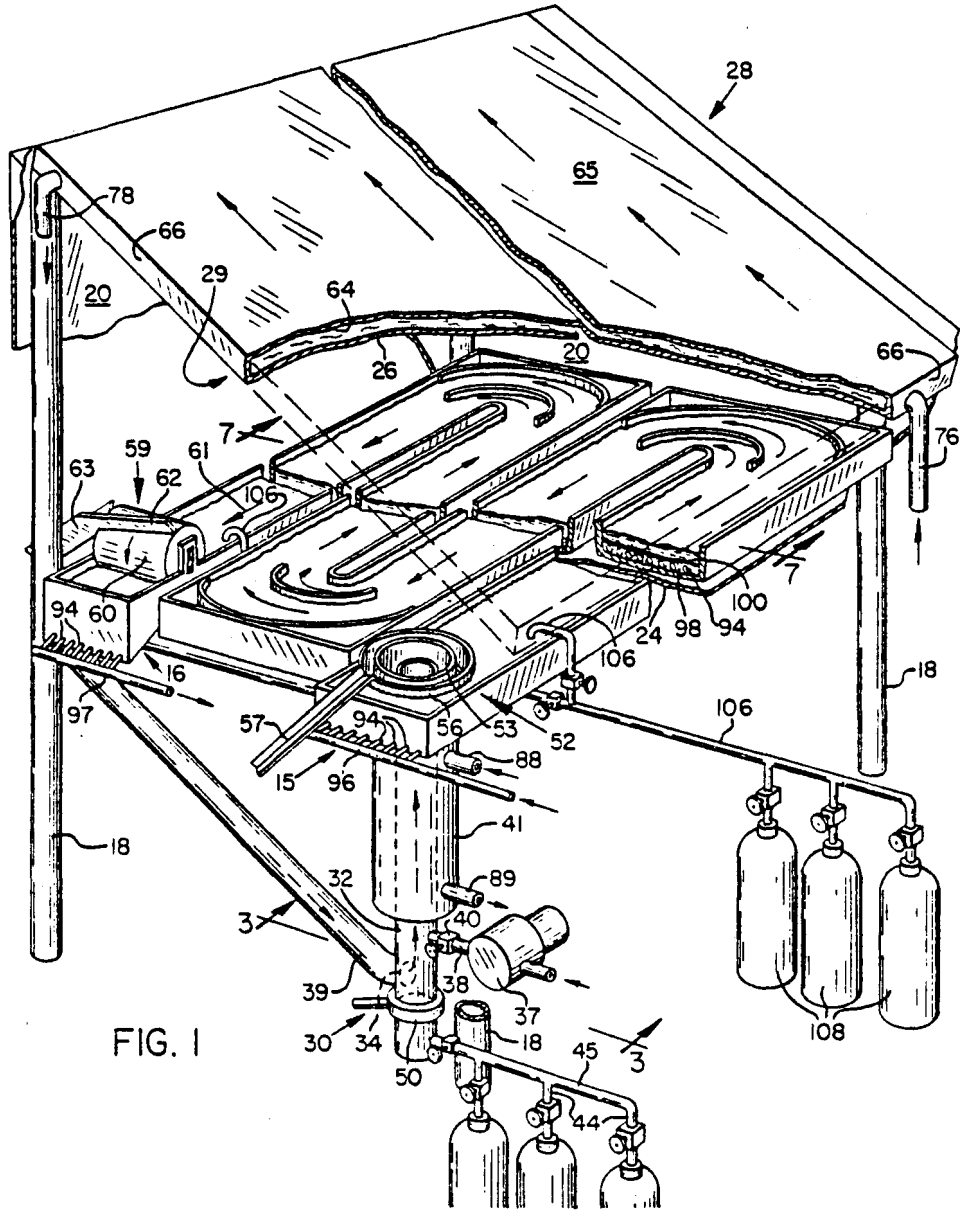

FIG. 1

KEY PATENT CLAIMS

- GAS LIFT PUMP MECHANISM FOR CIRCULATION, CARBONATION AND HARVESTING
- HEAT EXCHANGE FOR TEMPERATURE CONTROL
- FOAM FRACTIONATION / SKIMMING HARVESTING
- $CaSO_4$ SOLUTION IN COVER TO REMOVE INHIBITING IR.
- MIXING TO ACHIEVE FLASHING LIGHT EFFECT

Figure 1. Patented Shallow Covered System [10]

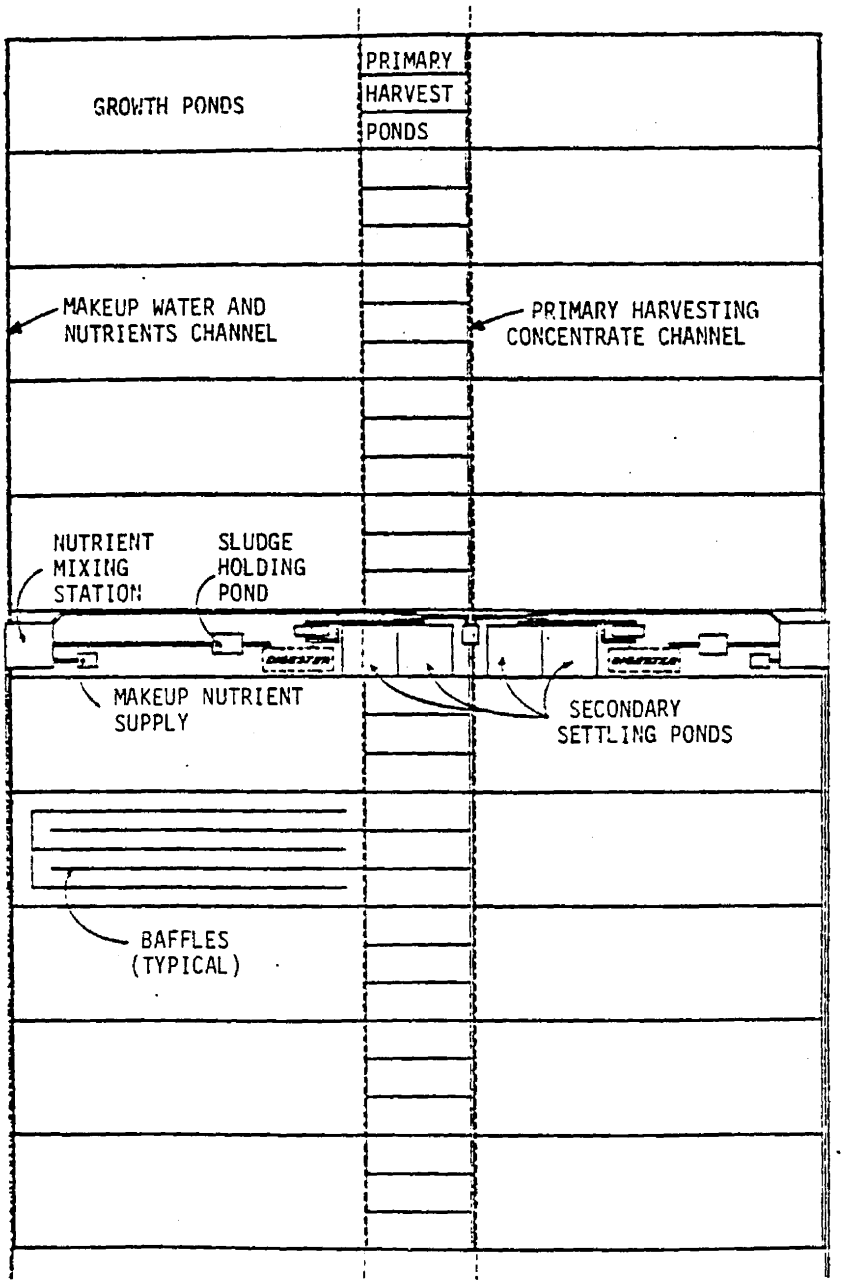

Figure 2. Plan View of 2000 Acre High Rate Pond Module [11]

production. Recent data by William Thomas (see his paper in these proceedings) also fail to support such a contention. Another reason for the $CuSO_4$ filter was to allow temperature control in these ponds. Table 3 summarizes our heat balance calculations. A $CuSO_4$ filter is effective at maintaining a lower temperature in shallow ponds; however, this assumes that the $CuSO_4$ filter is kept at ambient temperature, which requires very large and expensive heat exchangers. Coupling this waste heat production to a Rankine cycle engine is not considered practical, because of low temperature differentials and high capital costs (over $20,000/average kW). Even with a $CuSO_4$ filter at ambient temperatures, temperatures in the pond would still rise to 50°C, which, unless further in-pond heat exchangers are used, is well above the optimal (10°-22°C) reported for Phaeodactylum tricornutum [12], the organism of choice in the SCS. Thus, we conclude that the use of a $CuSO_4$ filter is not warranted for either scientific or engineering reasons. Of course, $CuSO_4$ is a potent algicide, and this imposes some other constraints on its use.

TABLE 3. HEAT BALANCE CALCULATIONS FOR A COMPLETELY COVERED SHALLOW MICROALGAE POND SYSTEM

Time of Day	Ambient Air Temperature (°C)	Solar Insolation ($cal/km^2/min$)	Water Temperature (°C)	
			With $CuSO_4$[a]	Without $CuSO_4$
7 a.m.	7.2	806	35.1	37.5
8	10	1270	36.7	40.8
9	15.5	1704	39.5	46.0
10	18.3	2040	47.9	52.2
11	21.1	2246	46.6	58.6
12	23.9	2325	56.3	64.8
1 p.m.	25	2246	53.0	69.7
2	26.1	2040	54.8	73.1
3	26.7	1704	53.4	74.4
4	26.7	1270	54.1	73.5
5	25.5	806	52.3	69.5
6	23.9	342	49.5	65.0
7	21.1	0	46.6	59.8
8	18.3	0	43.9	55.8
9	15.5	0	42.2	52
10	13.3	0	41.2	50.9
11	16.7	0	40.5	49.6
11	10	0	40.0	48.7
1	8.9	0	39.7	47.3
2	7.8	0	39.2	46.5
3	6.6	0	38.3	42.3
4	5.5	0	37.0	43.5
5	9	0	34.6	41.0
6	4.4	342	31.7	37.9

[a] $CuSO_4$ assumed to be maintained at ambient temperature.

Covered systems (without an IR filter or heat exchangers) could possibly operate with extreme thermophilic microalgae. Open ponds will exhibit lower nighttime temperatures, and, because of water evaporation, would reach a maximum temperature of about 40° C (70° C for covered ponds). Water loss is considerable for open ponds, up to 1 cm per day. The choice of a covered or open system must be based on the relative cost of water versus cover costs. Other factors must also be considered, such as CO_2 transfer and the potential for improved control of the microalgae culture.

Our theoretical analysis of CO_2 transfer suggests that both sumps and covers are viable alternatives for CO_2 transfer. Covers could be preferred for systems operating at low pH and pure CO_2, while sumps are preferred at high pH and with flue gas (10%), CO_2. The amount of area that must be covered for CO_2 transfer depends on many factors, and is complicated by the possibility of a "chemical enhancement" effect. It appears that both SCS and HRP would need about the same extent of coverage, 5%-10% for a pure CO_2 supply. A complete cover over the ponds would prevent loss of CO_2 to the atmosphere, so that overall it is useful in CO_2 transfer.

Costs of covering materials are, however, high (Table 4). Our capital cost estimates for installed covers are about $\$12.6/m^2$, or \$126,000/ha for a "long-life" (10-yr) system. Short-life plastic would be cheaper but would have much higher maintenance costs and operating uncertainty. Assuming optimistic factors for depreciation (10 yr), maintenance and other fixed costs, and return on investment (ROI, before taxes) of 10%/yr, total capital charges for the long-life cover would be \$30,000/ha/yr or \$150/bbl of oil, assuming 200 bbl of oil/ha/yr. Thus, it is apparent that a cover by itself would make any fuel output uneconomical by a large factor, even using these extremely favorable assumptions. So, covers, except for partial ones in connection with CO_2 transfer, can be excluded from consideration in fuel production. Water savings would not compensate for such a cost, until they reach almost 100 times what farmers pay today.

TABLE 4. COST OF POND COVERS

	$/m^2 (Installed)
A. Long-Life System (fiberglass panels)	
Materials (4 oz. Filon^R)	\$ 8.20
Supports	1.60
Anchoring, miscellaneous	2.20
Total	12.00
B. Short-Life System (inflated cover)	
Material (6 mil UV polyethylene)	1.60
Supports	2.70
Blowers, miscellaneous	1.10
Total	\$ 5.40

TABLE 8. COST OF MICROALGAE FUEL PRODUCTION BY AN HRP SYSTEM

[System Description: Twenty 40-ha growth ponds, harvesting by settling (bioflocculation) followed by centrifugation, carbon and nutrient recycle after oil extraction, productivity of 62.5 mt/ha/yr with 40% oil (lipids) content ≅ 160 bbl of oil equivalent per acre per year. Optimistic costs cases only, pure CO_2 at $22/mt or flue gas CO_2 from coal at $33/mt. Return on investment before taxes: 10%.]

	Flue Gas	Pure CO_2
Capital costs ($/ha)		
Growth ponds	$ 4,340	$ 4,340
Harvesting system	7,280	7,280
Processing (oil extraction)	3,710	3,710
CO_2 supply	3,200	670
Indirect capital costs	6,785	5,860
Land costs	1,190	1,190
Working capital (3 months)	1,335	1,485
Total capital costs	$27,850	$24,520
Operating costs($/ha/yr)		
Labor and overhead	$ 1,410	$ 1,410
CO_2 and nutrients	1,365	2,100
Water	495	495
Utilities	1,450	865
Maintenance, taxes, insurance	1,210	1,040
Depreciation	1,685	1,460
Total operating costs	$ 7,615	$ 7,370
$/bbl of oil	65	61

potential in reducing output fuel costs. The concept of integrating microalgae fuel production and wastewater treatment [3-7] has a significant near-term potential, but the ultimate total energy contribution impact is only about 0.05 quads [20].

Thus, microalgae fuel production systems must be economically viable in their own right, without needing to rely on other products or outputs for cost support. This would restrict this technology to only the simplest and lowest-cost production processes, embodied in the concept of the HRP system, as well as to favorable sites where land, water, and, most important, CO_2, are present at low cost. The ability of microalgae to use land and water resources that are not used in conventional agriculture suggests CO_2 as the limiting resource in microalgae production. The potential resource base is uncertain at the present time. A large number of very diverse CO_2 resources exist (power plant flue

gases, coal, geological CO_2 industrial gases, seawater, etc.); their availability and aggregate potential must be established.

Before microalgae fuel systems can be seriously considered for practical development and private investment, a number of fundamental research projects must validate the basic assumptions made in this analyses:

- Establish parameters controlling microalgae species dominance and stability in outdoor systems
- Study mechanisms and control of bioflocculation and other low-cost harvesting systems
- Document productivities of microalgae cultures in such systems
- Determine optimal strategies for maximizing oils or fermentable carbohydrate production
- Investigate effects of fluctuating parameters (pH, light, temperature) on algal productivity
- Improve microalgae strains that are competitive in pond systems.

Until this research is successfully carried out, economical microalgae fuel production will remain an unproven concept.

ACKNOWLEDGMENT

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COST BUDGETING FOR MICROALGAE SYSTEMS

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INTRODUCTION

Most important business decisions are made under conditions of uncertainty. The decision maker must choose a specific course of action from among those available, even though the consequences of these alternative actions depend on events that cannot be predicted with certainty. SERI program managers experience similar problems in allocating a finite budget among various high-risk research projects for which results are not known at the time of the decisions. Not unlike corporate managers, SERI program managers must establish the research priorities and initiatives that have a significant effect on the progress of technology or that significantly reduce the uncertainty of possible outcomes. This paper presents a methodology for decision evaluation that may be applied to assess the impact of large-scale microalgae production systems on existing U.S. fuels and chemicals markets.

The large-scale production of microalgae offers considerable promise for obtaining high-value fuels and chemicals currently derived from petroleum and natural gas feedstocks. Microalgae-derived products from existing commercial production facilities typically include specialty chemicals (e.g., vitamins); health food for people; and protein-supplement feeds for animals. These products are marketed through either fixed, long-term contracts or as a by-product credit from sewage oxidation ponds. These marketing schemes allow current producers to recover their production costs with even low yields of microalgae.

Despite these specialized commercial activities, the full potential of microalgae has yet to be realized in the fuel and bulk chemical markets because of technical and market constraints. Recent laboratory studies sponsored by SERI indicate sustainable yields of 50 to 70 dry ash-free (DAF) tons per acre-year as compared to existing commercial yields of approximately 20 DAF tons per acre-year [1]. Other SERI research activities indicate that, with proper species selection and environmental stress of the culture, the total lipid content of algae cells may increase to 50%-80% of cell dry weight, yielding valuable unsaturated fatty acids, triglycerides, phospholipids, pigments sterols, and carotenoids [1].

OBJECTIVES

The objectives of the SERI Microalgae Cost Budgeting Task are (1) to assess the potential of systems development for large-scale microalgae production of fuels and chemicals; and (2) to assist in the programmatic evaluation of research objectives to determine

commercial viability. An overview of the methodology proposed in this task is presented in Figure 1 and discussed in the following section.

METHODOLOGY

Introduction

Despite the promise microalgae products offer in the fuel and chemical markets, there are numerous research paths to prepare the technology for eventual commercialization. On the scientific and technical side, system design and performance need to be examined to ascertain whether, in fact, commercially produced microalgae can achieve sustainable yields of high-value fuels and chemicals. In addition to these activities, economic or commercial considerations need to be addressed. These latter activities are as important as technical concerns, because applied research cannot be conducted independent of its potential uses. Applied research is directed toward the development of products that will fulfill needs. Thus, the dynamic interactions of a competitive marketplace must feed back to and assist in directing research [3].

Microalgae production provides an excellent example of the need to conduct systematic research that integrates many scientific, engineering, and economic disciplines. The ability of microalgae species to produce different products having specific applications in hundreds of end-use markets compounds the complexity of establishing research priorities.

Some of the applicable scientific, engineering, and economic concerns associated with large-scale microalgae production are presented in the schematic overview shown in Figure 2. The scientific objectives include determining yield potential, algae composition, and culture growth parameters. Specific knowledge acquired in species screening leads to engineering research requirements that quantify production costs for various design options. Estimates of economics of scale, design optimization, and processing equipment requirements are important parameters in this area. The final research area is the product(s) definition. The ability of microalgae-derived products to compete in existing and future fuels and chemicals markets depends on profitability, which in turn is related to the product supply and demand relationships.

The complex interrelationships that exist within and among research areas necessitate a systematic appraisal of all these factors so that research budgets are allocated toward the most cost-effective projects and so that realistic goals are established. To conduct this systematic appraisal of research activities, a mathematical treatment of the decision-making environment is proposed. By modeling the physical and economic interrelationships involved in microalgae products, a decision to choose one research alternative over other possible research opportunities involves consideration of the likely consequences and specific objectives appropriate to the decision problem faced by the R&D sponsor.

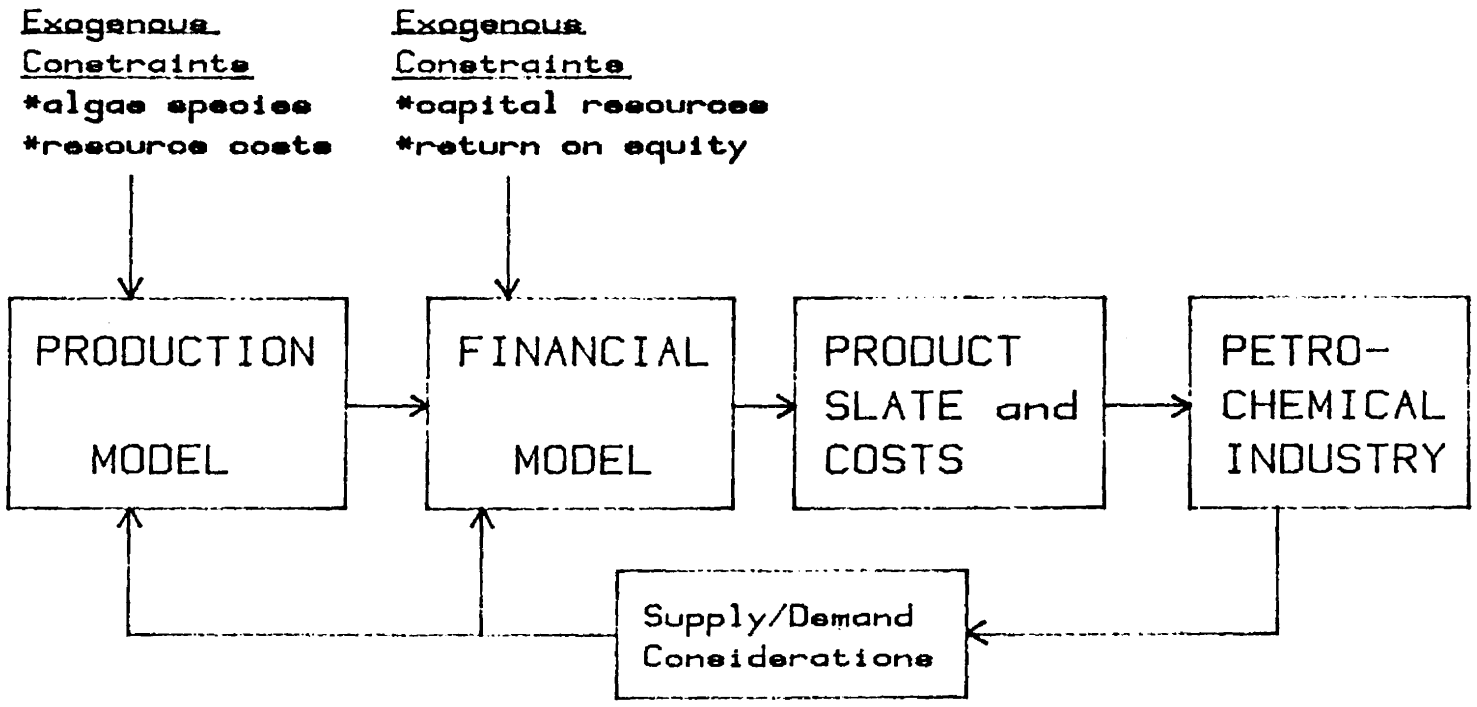

Figure 1. Overview of Microalgae Production Assessments

TABLE 3. OUTPUT FROM CO-PRODUCT PRICING MODEL BY PRODUCT WEIGHT AND VALUE

	Products Price by Weight ($/lb of Chemical Product)			
	Glycerine	Fatty Acids	Protein	Beta-Carotene
Algae plant				
Fixed costs	0.131	0.131	0.131	0.131
Tot. var. costs	0.116	0.116	0.116	0.116
Subtotal	0.247	0.247	0.247	0.247
Refining cost				
Fixed costs	0.06	0.02	0	0
Tot. var. costs	0.07	0.03	0	0
Subtotal	0.13	0.05	0	0
Total mfg. cost	0.377	0.297	0.247	0.247
Margin	2.12	0.841	1.01	84.56
Contribution	0.61	0.10	0.13	20.80
CSR	0.77	0.41	0.53	0.99
Total manufacturing cost/ton algae =		574.00		
		Revenues exceed mfg. costs		

	Products Price by Value ($/lb of Chemical Product)			
	Glycerine	Fatty Acids	Protein	Beta-Carotene
Algae plant				
Fixed costs	0.033	0.003	0.003	24.64
Tot. var. costs	0.029	0.003	0.003	21.87
Subtotal	0.062	0.006	0.006	46.52
Refining cost				
Fixed costs	0.06	0.02	0	0
Tot. var. costs	0.07	0.03	0	0
Subtotal	0.13	0.05	0	0
Total mfg. cost	0.192	0.056	0.006	46.51
Margin	4.17	4.45	43.45	0.45
Contribution	0.70	0.22	0.25	0.98
CSR	0.88	0.87	0.99	0.05
Total manufacturing cost/ton algae =		576.48		
		Revenues exceed mfg. costs		

Notes:
- Assumed glycerine yield and price = 15% at $0.80/lb.
- Assumed protein yield and price = 40% at $0.25/lb.
- Assumed lipid yield and price = 37% at $0.25/lb.
- Assumed beta-carotene yield and price = 0.5% at $20.90/lb.
- Refining process not optimized for algae plant [Ref. 2].

specific product yield from selected algal species, and market pricing schemes. To maximize gross revenues, a facility operator would track the individual product profitability and, within the limits of production flexibility, emphasize those products having the highest measures. Production changes to maximize yields of these products may be accomplished by changing physical parameters such as nutrient levels, light, or temperature, or by changing algae species.

The margin for each product represents the ratio of current market prices to total manufactured costs. This measure provides a gross indication for the per-unit manufacturing profit for each product. The contribution, or the difference between sales price and total variable cost per unit of production, represents the product's contribution to total fixed expense and to net annual profit. This estimate, along with the contribution to sales ratio (CSR), indicates which products should be emphasized to achieve maximum profits at that production volume and product price [5].

Linear Programming Model

The production of microalgae-derived products for potential end-use markets can be represented as a network of physical and/or chemical transformations defined by specific material flows. These material flows and production equipment requirements for each design option can be modeled by a set of linear equalities and inequalities representing a production process. Similarly, the supply and demand interactions of the end-use markets for microalgae products can be modeled by a set of linear equations. The linear equations may then be used as a tool to provide first-order approximations on the complex interactions that occur between different parts or the production process, and its subsequent impact on profitability.

An objective function that may, for example, maximize the market share for microalgae in the fatty-acids market or, alternatively, that seeks to minimize production costs, is used to achieve an optimal solution, given the physical and/or financial constraints on the entire system. The solution yields a combination of values and conditions for the biological and physical parameters of the model that may be compared with the current state of the art. Comparison of these values provides insight into research gains required to achieve optimal values. At this point, a programmatic evaluation of the likelihood of occurrence can be made. In areas where unrealistic research goals have been set, alternative methods of design or combinations of lesser research objectives can be assessed by the linear programming model to achieve similar results. This allows various technologies to be assessed in a competitive environment that will indicate which technologies are feasible or, if appropriate, the specific conditions under which some technologies dominate others. If one technology does not predominate under all possible production systems, research on several candidates would be justified.

The linear programming model is formulated under the assumption that all the input data are known with certainty. This is not currently the case for many microalgae production schemes. However, these technologies are presently the object of extensive R&D on process improvements and costs definition. The degree of success this research will realize, though, is unknown. Thus, the basic linear programming model is not adequate

for comparing alternative processes, especially in light of the need to establish research priorities that will make the most of future opportunities.

An approach to circumvent the problem of allowing for uncertainty in research opportunities would be to construct a linear programming model that represents production and economic environments using available data from cost-budgeting activities, and then constructing "decision trees" that characterize the dimensions and nature of the uncertainty. The decision tree provides a representation of the decision problem and its environment from the perspective of a research sponsor and potential investor [6]. Decision-tree analysis provides for the incorporation of uncertainty in the context of a programming model.

The decision tree is constructed to permit comparative evaluations of each technology design and the research initiatives specific to that design. For each design option, an approach to solve the decision tree and establish research priorities might be to use net present value of savings over a base case. Determining which research offers the highest return may be derived by using expected value of the net present savings if probabilities of success were assigned to each branch. Alternatively, the determination could be made by linguistic comparison of the potential savings. In either case, a mathematical treatment of the various research outcomes is performed that enables the research sponsor to assess potential gains objectively.

The principle advantages of the methodology described include

- A clearer understanding of how scientific knowledge and R&D contribute to the development of alternative new technologies.
- A more precise representation of the uncertain decision environment within which sponsored research operates.
- A mathematical formulation of the uncertain research environment that is independent of the way a method of evaluating R&D decisions is chosen. Attention then may be focused on developing the types of data required for further analysis and establishment of consistent assumptions for the evaluation methods subsequently employed.
- Introduction of the notion of competition among alternative processes for R&D funding and provision of information about the relative returns each research opportunity provides.

Preliminary Results and Discussion

Specific cost analyses for microalgae production systems require more detail in costing biological and harvest engineering. Despite the need for cost refinements, preliminary analyses of microalgae production can be conducted by using ranges of values for the critical parameters.

One technique used in preliminary evaluations of microalgae technology is the "spider diagram". This diagram for microalgae production is shown in Figure 3. A base case (not representative of the state of the art) is plotted with a corresponding return on equity.

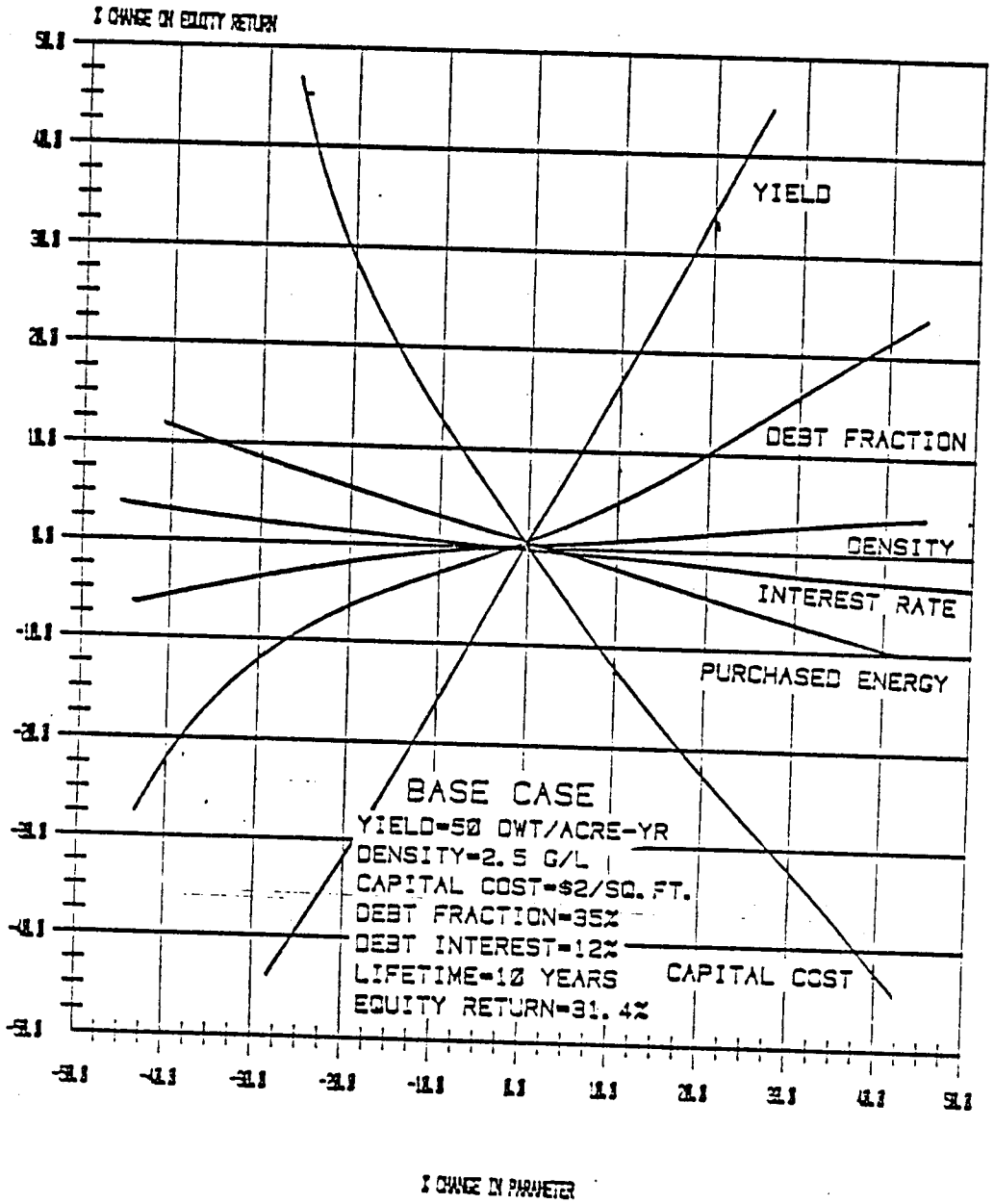

Figure 3. Spider Diagram for Microalgae Systems

Percentage changes in individual parameters are plotted to illustrate the impact each parameter may have on the dependent variable, profitability. Visually, the parameter lines having the highest effect on profitability will have steeper slopes. As shown in Figure 3, yield (tons per year) and capital cost ($/ft^2) had steeper slopes and, therefore, greater effect on profitability. Other parameters had less impact than yield or capital cost. However, because maximum variation in all parameters was ±50%, several of the parameters may have a more significant effect than indicated if other realistic values outside the range of the spider diagram had been used. Despite this somewhat focused analysis, it is apparent that small changes in yield and capital cost have a significant effect on a project's total return on equity.

Figure 4 presents the relationship between yield and capital cost for three revenue (or market) scenarios. A minimum 15% return on equity (ROE) was assumed to be the profit criterion needed to calculate the relationships shown in the figure. Other assumptions include 35% debt fraction at 12% interest, a lifetime of 10 years, and a culture density of 500 mg/L. The first entry market for microalgae products is shown to be $2500/ton of algae. This market assumes a specialty chemical (e.g., vitamins) product slate of relatively high value. To meet the criterion of a 15% ROE, the feasible options must lie above the specific revenue line. For the $2500/ton or the $1000/ton market, the figure shows that low yields can achieve 15% profitability provided relatively low capital costs are also achieved. This scenario demonstrates the current marketing environment for many commercial microalgae facilities where high value specialty chemicals such as vitamins and other health foods are produced in open ponds, where yields of up to 30 tons/acre-year are achieved. The emphasis has been to reduce capital cost as low as possible to be economically feasible. Thus, without new construction techniques, additional capital cost reductions would be difficult. However, the bulk chemicals and fuels markets (assumed to be $500/ton) require low capital cost and relatively high yields. The ability to reach these "price stable" bulk commodity markets is important because the large volume of consumption in these markets allows more stability over the long term than the specialty chemical markets which lose their dominance within seven years [7]. Extrapolation of the $500/ton revenue line indicates that minimum yield of approximately 38 tons/yr is required even if no capital investment is associated with the microalgae facility.

To summarize, an attempt to obtain preliminary assessments of microalgae production indicates that to penetrate the bulk fuels and chemicals markets may require low capital costs and high yields of algae. Other process improvements, separately or combined, may help reduce the need for the low capital cost and high yield, although more detailed cost and process data are required. This task will assist in refining these unknowns and identify other information needs so that more realistic cost estimates can be made.

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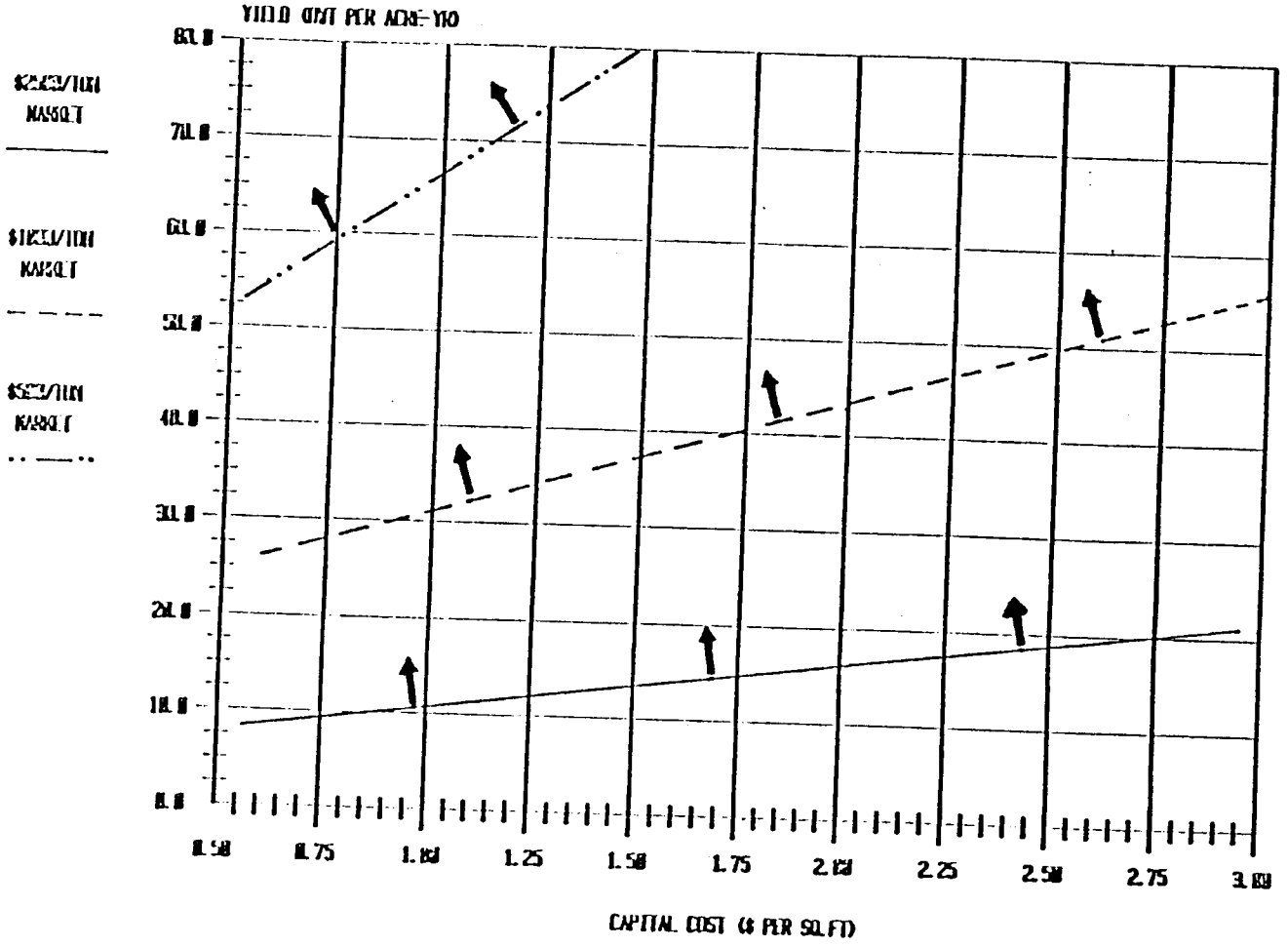

Figure 4. Microalgae Yield and Capital Cost Goals (15% minimum return on equity)

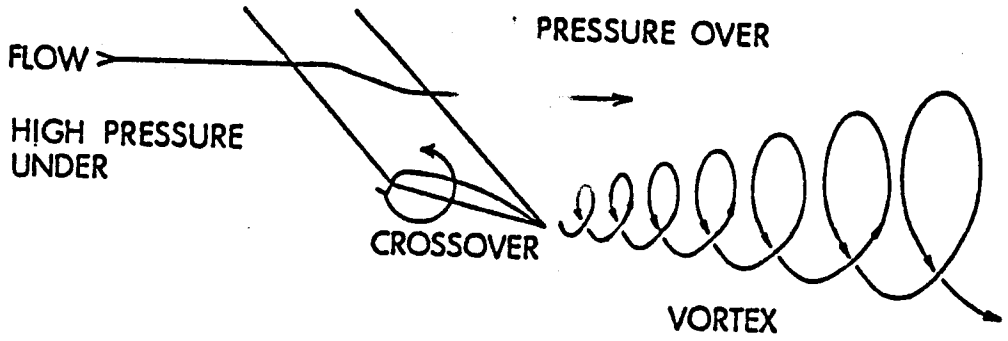

Figure 2. Schematic of a Single Foil Showing Mechanism of Vortex Production

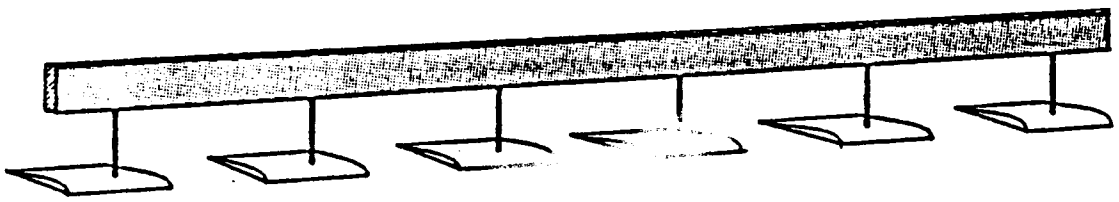

Figure 3. Foil Array Used to Generate Parallel Vortices Across Width of Flume

produces the sort of systematic mixing which is necessary to produce the flashing light effect. Since the edges of the foils offer little resistance to the flow in the flume and since water moves within the vortices at right angles to the direction of flow in the flume, the foils require very little additional head to keep flow at the desired rate of ~ 30 cm·s^{-1}. In our 40-m-long experimental flume, placement of foil racks at intervals of about one meter produced a change in head of less than 0.5 cm. Foils such as these are effective in producing systematic vertical mixing throughout the culture depth only in a shallow flume. An additional advantage of the use of such foils is that so-called "ground effects" cause scouring of the bottom and preclude sedimentation of cells. Thus, have been able to identify a mechanism to mix the culture systematically that requires relatively little energy input.

SUMMARY OF RESEARCH PROGRESS

1. Preliminary Work and Choice of Test Species

The organism designated for the initial experiments was the marine diatom Phaeodactylum tricornutum. This species was chosen because of its known tendency to dominate outdoor mass cultures; its wide range of tolerance to pH, salinity, and temperature [7,11,16,20]; its ability to withstand buffeting [23]; and the fact that excretory products produced by P. tricornutum appear to be inhibitory to the growth of bacteria as well as other algae [7,8,23]. Thus, the possiblity of establishing and maintaining a monoculture of P. tricornutum for long periods of time is promising. In our present outdoor flume, we have been able to maintain a culture of P. tricornutum continuously for seven months. Most important is the fact that the lipid content of P. tricornutum is anomalously high among algae. The lipid content may reach 80% of the dry weight under appropriate growth conditions [2,5,10,15]. The great majority of the carbon in P. tricornutum is allocated to either lipid or protein, and the latter is an economically valuable by-product. Preliminary work by Raymond [23] in a 5-m^2 prototype flume indicated that solar energy conversion efficiencies as high as 13% might be achieved in a shallow, densely populated flume. Projected production figures from Raymond's early work were about 50 g·m^{-2}·d^{-1} of ash-free dry weight. This figure may be compared to annual production figures of at most 15 g·m^{-2}·d^{-1} from conventional algal mass culture systems [6]. Raymond projected lipid and protein production of 15.3 g·m^{-2}·d^{-1} lipid and 24.5 g·m^{-2}·d^{-1} protein based on the performance of his prototype. The former figure translates into about 150 bbl of oil per acre-year based on the energy content of crude oil and lipid.

2. Research Accomplishments of the First Year

The first two years of our contract called for the construction of a 50-m^2 prototype flume to determine what rates of production could be achieved on a sustained basis in a system designed to take advantage of the flashing light effect. Construction of the flume itself and associated support facilities took approximately one year. Schematics of the finished flume are shown in Figures 4 and 5. The flume is constructed of modular 4 ft by 8 ft sections made of fiberglass. Circulation is achieved by means of an air lift pump. While construction of the flume was in progress, a number of laboratory-scale continuous culture experiments were conducted to develop analytical techniques and to explore potential problems. These studies showed that (1) continuous exposure of the

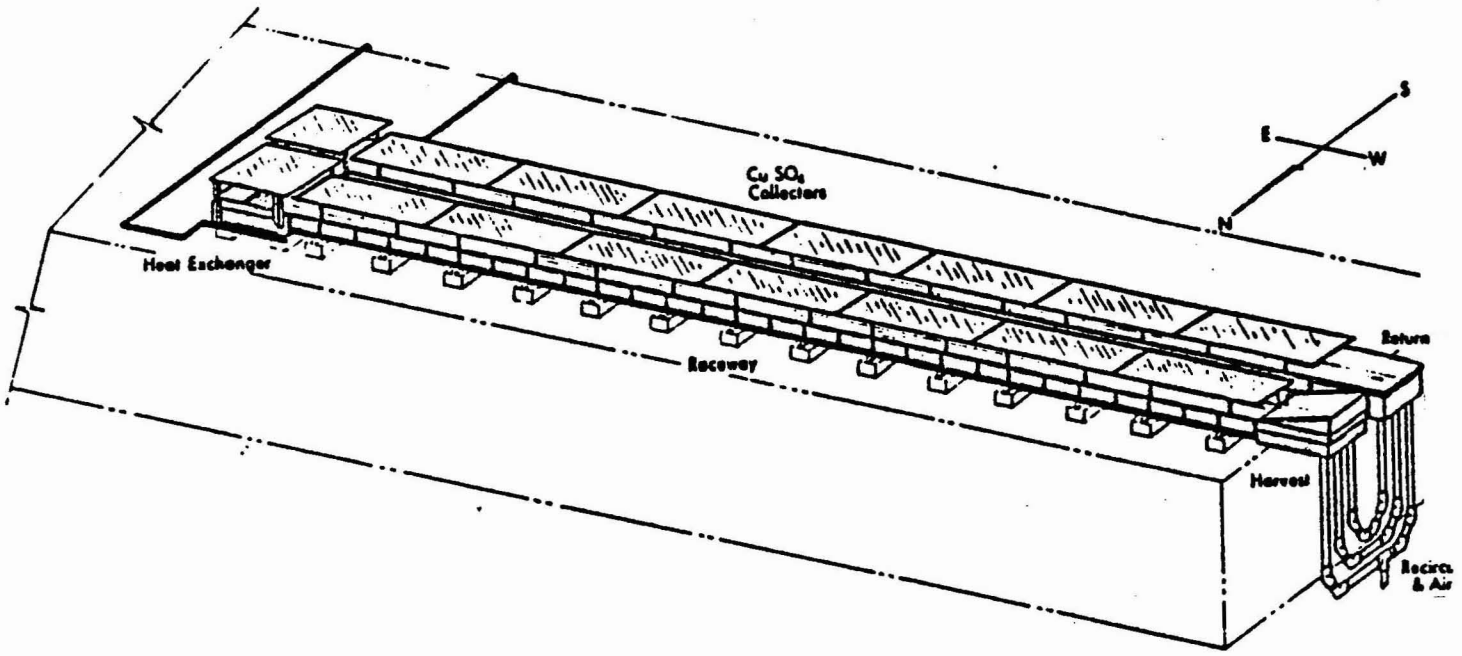

Figure 4. Perspective of 50-m^2 Flume Located on Roof of Experimental Facility

cells to temperatures in excess of approximately 25° C markedly reduced production. Growth is slowed to almost zero at a temprature of 28° C. (2) The culture is not only tolerant of wide ranges in pH, but can also withstand rapid changes in pH. (3) Raising the pH to greater than 10 caused rapid flocculation and settling of the cells. (4) Regular dilution of the culture stimulates production, perhaps because of the removal of an inhibitory waste product. (5) Maximization of both protein and lipid production occurs at essentially the same set of growth conditions. Maximum production occurs when the culture is growing under nutrient-saturated, light-limited conditions at a rate equal to or slightly less than half the maximum growth rate μ_m. (6) Growth is a linear rather than exponential function of time at cell densities greater than about 10^7 per mL.

3. Research Accomplishments of the Second Year

Almost all research conducted during the second year was performed in the 50-m^2 flume. Initially, we were unable to achieve cell densities in excess of 10^7 per mL. However, we learned from the laboratory-scale, continuous culture work that CO_2 addition to the culture was necessary to produce cell densities in excess of 10^7 per mL. By bubbling additional CO_2 into the medium, we were able to achieve cell densities of $>2 \times 10^8$ per mL in the laboratory-scale experiments. CO_2 addition to the flume produced cell densities in excess of 2×10^7 per mL, but additional problems began to develop at that point. A Monad predator initially described in P. tricornutum mass cultures by Raymont and Adams [24] began to appear in our flume culture, and rapidly grazed down the population. In response to this problem, we began to explore several mechanisms for predator control. Those that worked well included the following.

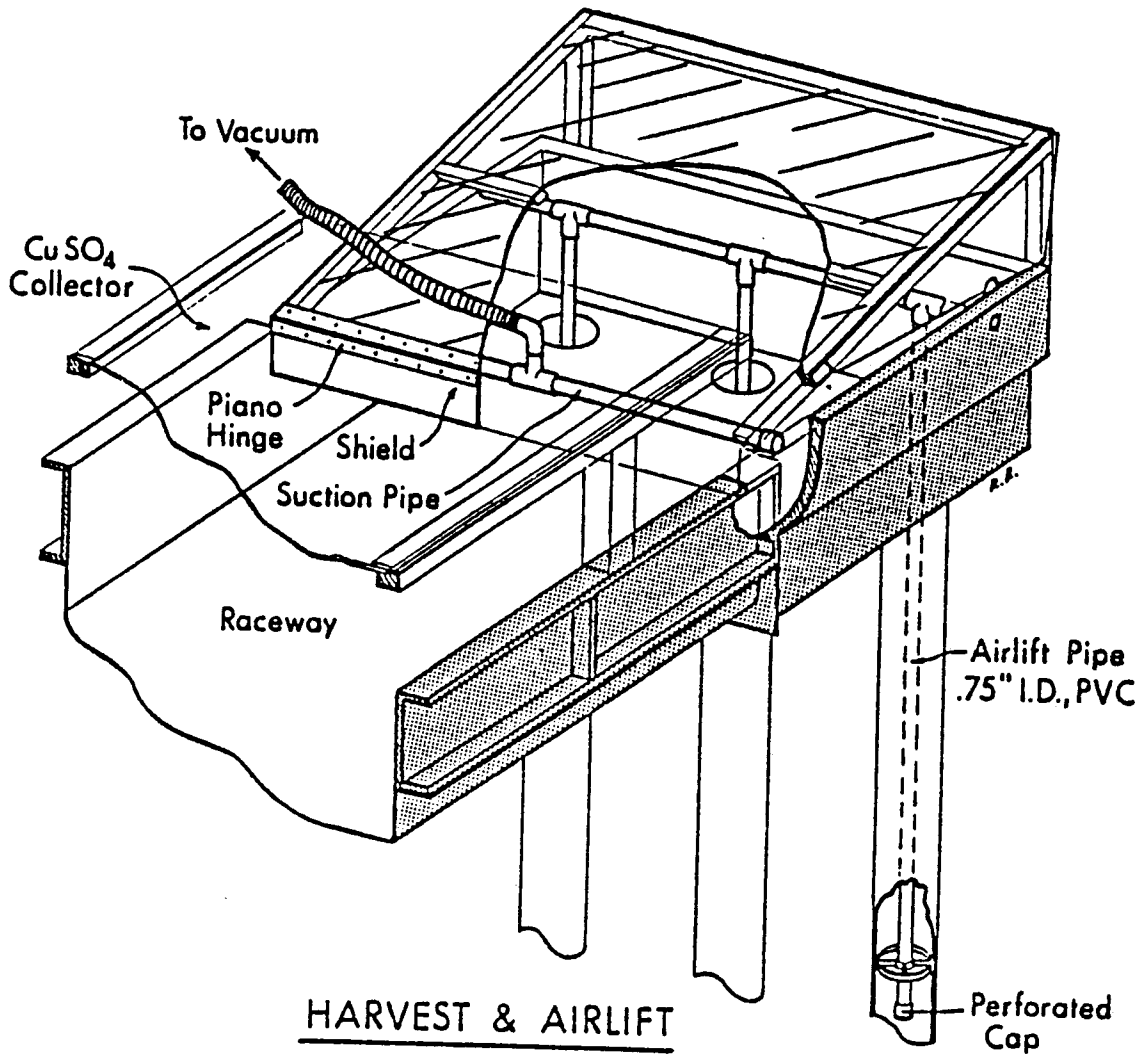

Figure 5. Detail of Airlift System and Foam Suction Harvester Used on 50-m^2 Flume

- Sonicating. Sonicating a sample of culture for a matter of seconds killed the Monad but did not affect P. tricornutum.
- Sedimentation. Sedimentation effectively removes the ameboid form of the Monad, but does not remove the flagellate form. (See Raymont and Adams [24] for a discussion of these two forms).
- Chemicals. Certain chemicals such as quinine and potassium antimonyl tartrate were effective in killing the Monad, and at appropriate concentrations were not harmful to P. tricornutum.
- pH. Raising the pH of the culture to greater than 9 greatly slows the growth of the Monad or kills it. The effect of high pH on the Monad may well be due to NH_3 toxicity, since NH_4 is converted in significant amounts to NH_3 as pH rises from 8 to 9.5. As we noted before, a pH of 9-9.5 does not appear to affect the growth of P. tricornutum adversely.

Through a combination of pH control and periodic sedimentation, we were able to produce cell densities in the flume consistently over 2×10^7 per mL and have maintained the flume culture continuously for seven months. The highest cell density achieved to date in the flume has been 4.6×10^7 per mL. The highest monthly production rate has been 11.6 $g \cdot m^{-2} \cdot d^{-1}$ (Table 1), a figure which may be compared to the best previous long-term production of 10 $g \cdot m^{-2} \cdot d^{-1}$ in a mass culture of P. tricornutum [6]. Thus, we have already surpassed the best previous production achieved in a P. tricornutum culture. However, we feel that large improvements may still be made in system production by optimizing design characteristics and operating procedures. This optimization procedure has been hampered to date by the fact that we had only one experimental flume.

During the second year of research, we developed the idea of using foils to effect systematic vertical mixing in the flume. Observation of the effect of the foils in the flume indicate that the foils mix the culture at a frequency of about one hertz, and that the bottom is effectively scoured by the ground effects of the foils. Figure 6 gives a rough idea of how the vortex rotation rate varies as a function of the angle of the foils and the distance downcurrent from the foil array. Studies such as these are needed to determine the optimum foil angle as a function of culture depth and foil shape and size.

TABLE 1. RECORD OF P. TRICORNUTUM PRODUCTION IN 50-m^2 FLUME DURING PERIOD 11/81 to 4/82

Month	Production (g dw $\cdot m^{-2} \cdot d^{-1}$)	Light Intensity (E $\cdot m^{-2} \cdot d^{-1}$)	Efficiency (%)
Nov.	3.0	23.6	1.5
Dec.	2.4	22.5	1.3
Jan.	4.6	30.7	1.8
Feb.	10.5	37.0	3.4
Mar.	11.6	33.6	4.2
Apr.	11.3	40.2	3.4

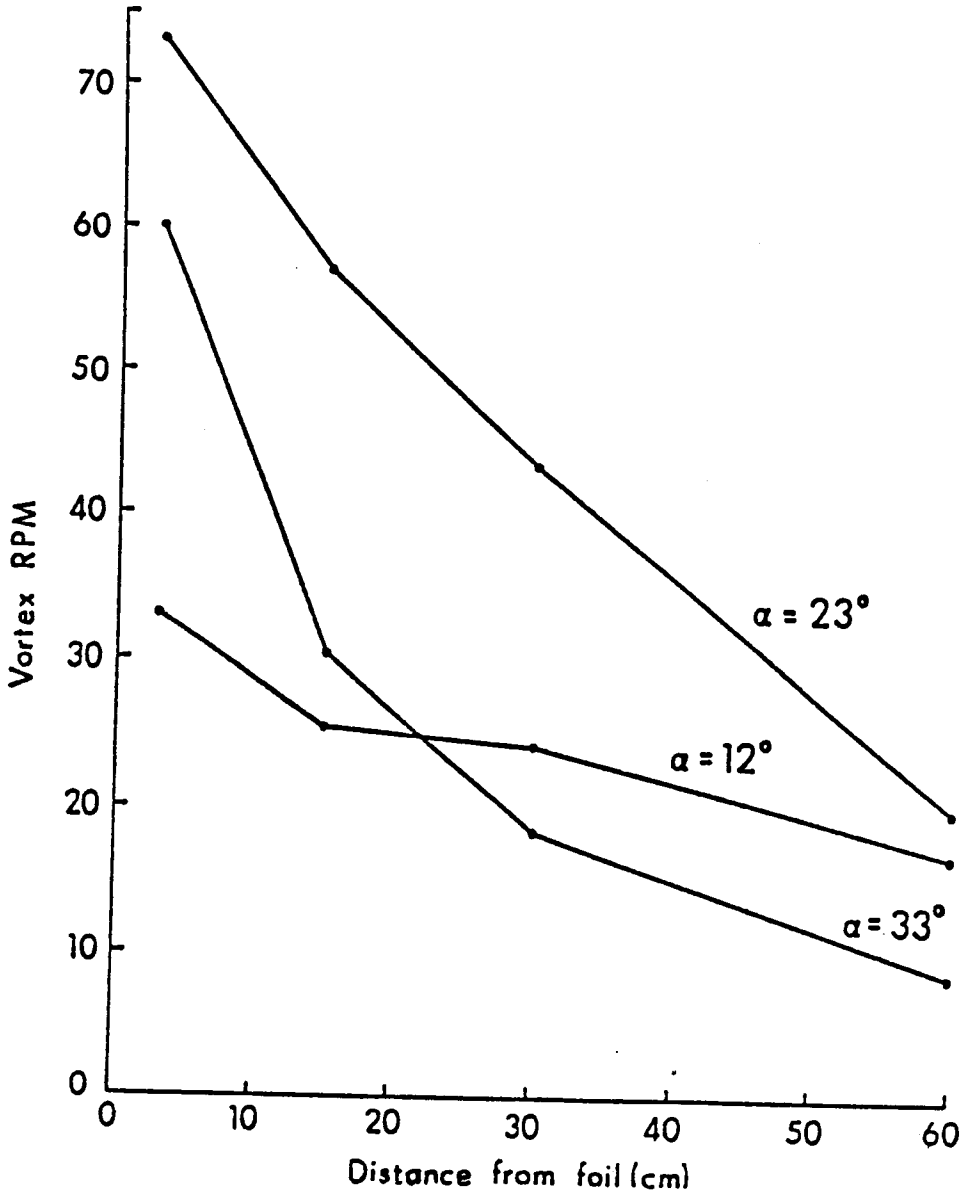

Figure 6. **Approximate Dependence of Vortex Rotation Rate on Angle of Attack and Distance Downcurrent from Foil** (Flume is 7.5 cm deep)

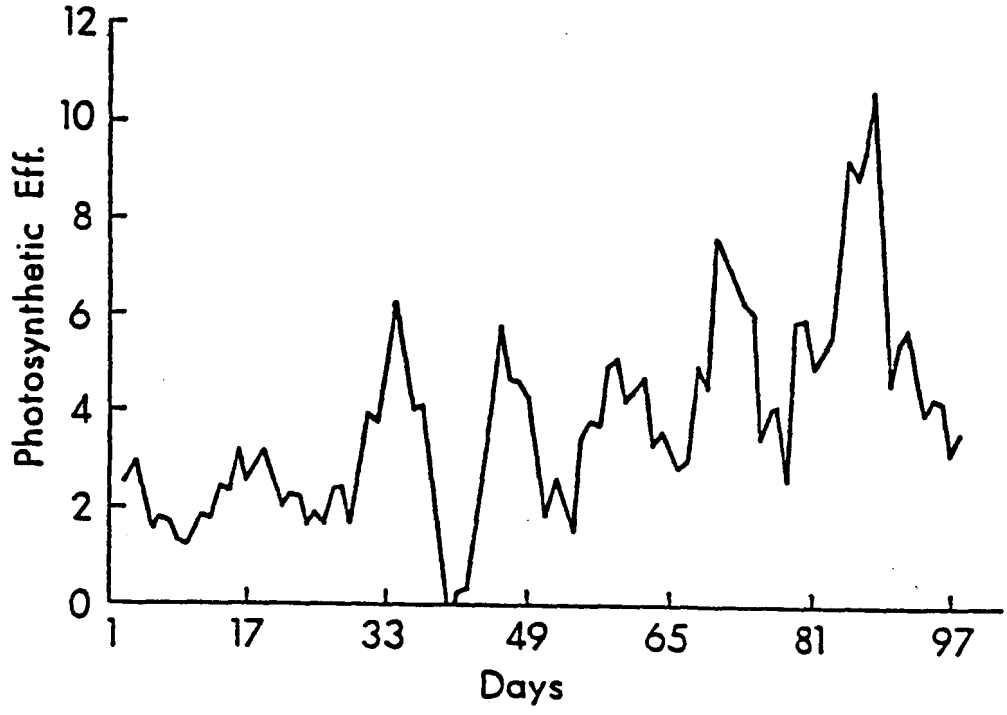

Figure 7. Five-Day Running Mean of Photosynthetic Efficiencies During First 100 Days of 1982 in 50-m^2 Flume

Table 1 summarizes production results that were obtained during the period November 1981 through April 1982 in the 50-m^2 flume. During the first three months of that period, production efficiencies averaged only 1.5%. In late December, the first set of foils was installed in the flume; and in the latter half of January, successful control of the Monad predator was finally achieved through the use of high pH. Production efficiencies during the last three months averaged 3.7%, more than a twofold increase over the previous three-month period. Furthermore, five-day running means of photosynthetic efficiencies peaked at over 10% in April (Figure 7) and showed a generally increasing trend during the six-month study period. Our most recent studies indicate that the troughs in the production efficiency cycle (Figure 7) can be largely eliminated by running the culture in a semicontinuous rather than batch mode. If these troughs can be largely eliminated, production from the system can be expected to more than double.

CURRENT RESEARCH

Work completed during the last six months has made it obvious that efficient optimization of the production system cannot be achieved unless multiple raceways are available to allow for direct comparison of treatment effects. This fact is particularly apparent when one considers that a large number of variables may affect production in important ways. As a result, we are presently constructing five 8-m^2 flumes similar in design to the 50-m^2 flume with which we have worked during the past year. Four of these smaller flumes will initially be used in a fractional factorial design experiment to determine the parameters and parameter interactions that are most important in determining algal production. The particular parameters to be studied will include culture depth, cell concentration, flow rate, temperature, light quality (blue light versus ordinary sunlight), pH, salinity, and nitrogen source (NH_4^+ or urea). Once the most important

variables and interactions have been determined, production will be maximized by using all five small flumes in a first-order factorial design to determine the local slope of the production surface, the method of steepest ascent to approach the maximum region, and a second-order factorial design to obtain a local representation of the maximum region.

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BIOLOGICAL AND ENGINEERING PARAMETERS OF ALGAL MASS CULTURE

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INTRODUCTION

Biomass is recognized as the most important of the renewable energy technologies in terms of its potential impact on the nation's future energy needs, both in the next two decades and in the long term. The long-term potential of biomass for energy stems from the ability of carefully focused research and development (R&D) to make major qualitative improvements in the production and conversion of biomass feedstocks into useful fuels and petrochemical substitutes. Among long-term biomass options, aquatic biomass, particularly microalgae systems, deserves particular attention. Microalgae systems produce extremely high yields of high-quality feedstocks for liquid fuels and chemicals. Moreover, they appear to be able to do this using low-quality land and water resources—an absolutely essential prerequisite for widespread use in the United States and elsewhere.

The SERI Aquatic Species Program has developed and coordinated an active microalgae R&D program over the past several years to develop a firm technical data base on algal systems from which business people can make commercialization decisions. Microalgal systems have great potential, and this program constitutes precisely the sort of long-term, high-risk, high-payoff research and development that many in government feel the nation should continue to invest in. On the other hand, increasingly serious budget constraints mandate that the microalgae program have as clearly articulated a strategy as possible.

The objective of the study is to support the development of such a strategy by evaluating relevant continuing microalgal studies, identifying key technical parameters and uncertainties, and eventually articulating a set of research and development recommendations to strengthen specific opportunities for applications within the overall Aquatic Species Program.

PLAN OF ACTIVITIES

The successful mass cultivation of microalgae depends on the complex interactions of a variety of physical, biological, and engineering parameters. The relative and absolute importance of pH, salinity, temperature, genetic manipulation, systems depth, flow rate, turnover, and nutrient composition on the growth rates and yields of microalgal species is not entirely clear for any particular species, much less among species. On the other hand, a broad objective of the Aquatic Species Program is to establish the cost and benefit values related to design and operating parameters of microalgal systems so that

informed decisions can be made in systems development. This study is designed to evaluate and compare available experimental and theoretical data from current or completed experiments or experimental systems to codify our understanding of the state of the art in mass algal culture systems. Based on this "data base," important technical uncertainties can be identified and "high-leverage" experiments or system modifications can be proposed that can be used to help plan a continuing microalgae R&D program.

The first step in this process will be to identify and evaluate technically as many microalgal studies as possible that relate to systems development. This involves literature searches, discussions with U.S. government agencies supporting work in the field (e.g., DOE, NSF, EPA, NOAA), and communications with private groups having similar interests. Related activities in other countries will also be tracked as far as possible. The purpose of this phase of the study will not be to compare or evaluate the various systems or species, but to develop a consistent data base of understanding of algal systems and subsystems that is as broad as possible. This base will then be used for further evaluations and analyses.

That analysis will be the foundation of the second phase of the study. Key technical parameters and uncertainties associated with microalgal culture will be identified. The emphasis will be on looking at issues from a systems perspective, so that uncertainties or issues that have the greatest potential effect on the economics of an overall system can be identified. This focus will allow the R&D program to emphasize reducing the uncertainties associated with the development of this technique.

Microalgal systems development could be studied from two extreme points of view: (1) emphasizing capital-intensive systems, involving relatively high technology and maximum algal yield; or (2) investigating lower technology, less capital-intensive systems that focus on minimizing material costs. This study need not choose between these two extremes. Practical, cost-effective systems will probably evolve that include elements of both approaches. It may be more appropriate to try to determine what combination of these approaches is likely to produce long-term benefits, and how these choices depend on a particular system application.

The final phase of the study will be to integrate the results of the analysis, after extensive discussion within the R&D community, into a proposed agenda of R&D tasks important to the development of cost-effective microalgal systems. SERI could then use the results to develop more long-term plans for the Program.

CONCLUSION

By the next annual review of the Aquatic Systems Program, the study just described should be complete. If the study is successful, it will probably raise more questions than it answers. To develop the broad scientific and engineering data base on which system development decisions can be analyzed, however, requires the cooperation of the R&D community. We anticipate working directly with scientists and engineers to develop our information and hone our analyses. The entire community should benefit.

STUDIES ON THE PRODUCTION AND ACCUMULATION OF OIL AND LIPIDS BY MICROALGAE

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Microalgae of aquatic and soil origins contain an oxygenic photosynthetic system that is tightly coupled to an active, versatile biosynthetic metabolism. Under optimal culture conditions with net intake of CO_2 and H_2O, microalgae act as efficient solar energy-to-biomass transducers. Peak productivity in excess of 125 metric tons ha^{-1} yr^{-1} and mean productivity of about 51 metric tons ha^{-1} yr^{-1} have been reported for high-rate algal ponds [1]. Certain microalgae are known to produce oils as storage materials that function as food and energy reserve for the cells [2]. These storage oils and lipids are, for the most part, not covalently linked to cellular, structural constituents such as membranous proteins or cell-wall polysaccharides. Thus, they are readily extractable and easily separated from the rest of the algal mass. Algal oil and lipids are characterized by a high reduced carbon content (such as $-CH_3$, $=CH_2$, etc.) relative to that of the oxygenated carbon (such as -COOH, =CO, etc.). Therefore, they have high caloric values and can be converted into fuels with minimal additional energy. The storage oil and lipids from most species of algae consist mainly of neutral lipids such as esters of fatty acids. They contain fatty acids of varying lengths that may also include hydroxyl, keto, epoxy, and other functional groups. Both conjugated and unconjugated unsaturates, small-ring systems, and highly aromatic structures are also present in varying quantities. As a result, algal oils and lipids are a rich source of chemicals and may serve as renewable sources of feedstock material for the petrochemical industry. Finally, the nonlipoidal fraction of the algal mass is a rich source of single-cell protein and also has high economic value.

The great potential of algae for lipid and chemical production is based on the two features of the organisms just outlined: (1) high photosynthetic efficiency and a resulting high rate of net biomass production, and (2) a large fraction of the total algal mass stored as oils and lipids [3]. Unfortunately, based on currently available information from a few oleaginous species, these two important attributes of microalgae appear to be mutually exclusive. Frequently, culture conditions that are most conducive to high rates of vegetative reproduction tend to suppress the active production and accumulation of intracellular storage oils and lipids. Thus, massive amounts of oil and lipids are present only among old, resting cells that do not exhibit the high level of photosynthetic activity and efficiency associated with young, actively dividing cells. In order to overcome this problem, we have embarked upon an algae-screening project to broaden the biological foundation of this new technology. Using these high lipid producers as test organisms, we hope to develop a better understanding of the biosynthetic pathways, the photophysiological responses, and the regulatory aspects of lipid metabolism and oil accumulation in algae.

SCREENING OF ALGAL STRAINS WITH HIGH OLEAGINOUS CAPACITY

The original cytochemical staining technique developed in FY 1980 for the microscopic visualization of intracellular storage lipids in algae [4] was improved by the addition of a brief heating and drying cycle during the preparation of the algal samples. This improved procedure consistently produced uniformly stained alga specimens with most species of unicellular and colonial microalgae. (The only difficulty is encountered with a few species of highly gelatinous algae that produce massive amounts of viscous, extracellular slime.) Using this method, we have identified eight species of algae that exhibit an unusually high capacity to produce and accumulate oil and lipids (see Table 1). The most exciting prospect resulting from our survey of oleaginous algae in FY1981 is the isolation of a green alga (temporarily designated "CHLS01") from a local site. This organism is a fast-growing (mass doubling time = 10 to 15 h for exponential phase cultures), oil-producing strain of Chlorella. Our preliminary results indicate that, when grown in nitrogen-limited liquid media, the alga readily attained a cell density in excess of 2 g (dry weight)/L and at the same time accumulated approximately 30%-45% of its total mass as intracellular storage oils and lipids. Thus, in terms of lipid productivity under laboratory cultivation, CHLS01 is as good as Neochloris oleoabundans (see Table 2). In addition to the identification of individual, high oil producers, the most significant contribution of our screening activities is the emerging generalization that massive accumulation of intracellular storage lipid is not restricted to a few species of atypical microalgae species. Rather, it appears to be a phenomenon widespread among the green algae.

THE PHYSIOLOGICAL RESPONSES OF OLEAGINOUS ALGAE TO NITROGEN LIMITATION

In analyzing the parameters that affect oil accumulation in algae, we focused our attention on the effect of nitrogen supply because of the previously reported stimulation of lipid production in algae by nitrogen deficiency [5]. In order to study the effect of nitrogen limitation quantitatively, we developed a sensitive, reproducible analytical procedure for nitrate using ion chromatographic assay. This procedure can monitor the level of nitrate which is the sole source of nitrogen in the medium. The course of nitrate utilization by N. oleoabundans is depicted in Figure 1 (Curve A). In this batch culture experiment with nitrate-limited medium (0.5N-BBM-8), free nitrate became totally depleted 55 h after inoculation of the alga. Active algal growth was observed as long as the level of nitrate in the medium was in excess of a few nmoles/mL. During this phase of rapid growth, chlorophyll a accumulated at a rate proportional to the rate of increase in total cell mass. However, the chlorophyll a content of the culture decreased sharply immediately after the complete depletion of nitrate (see Curves A and B). This suggests that chlorophyll a might be metabolized by the cells during severe nitrogen deficiency. The effect of a drastic decrease in the level of chlorophyll a on the photosynthetic activity of the alga is currently being investigated. Figure 1 also illustrates another important feature of nitrogen depletion. Although cell division stops shortly after the onset of complete nitrate depletion, accumulation of biomass (i.e., the total dry mass of the culture) continues long afterwards (Curve C). In fact, more than 50% of the total biomass is synthesized after total nitrate depletion. Using the Nile Blue A cytochemical staining technique, we observed that a major fraction of the biomass synthesized during this latter period appeared as storage oils and lipids. A very similar response to nitrogen

TABLE 1. THE OLEAGINOUS MICROALGAE IDENTIFIED DURING FY 1980-81

Organisms	Source	Oil Accumulation		Comments
		Solid Media (4 weeks)	Liquid Media (7-12 days)	
Botryococcus braunii	UTAC[a]	massive [b]	N.T.[c]	Extremely slow grower
Chlorella pyrenoidosa	UTAC	moderate[b]	slight[b]	16 h doubling time
Chlorococcum olefacien	UTAC	massive	slight	variable growth in liquid
Chlorococcum pinguideum	UTAC	massive	N.T.	
Neochloris oleoabundans	UTAC	massive	35%-54%[d] of dry wt.	10-15 h doubling time
Neochloris texensis	UTAC	massive cultures)	slightly massive (in old liquid	
Neochloris pseudostigmata	UTAC	massive	N.T.	slow grower in liquid
CHLS01[e]	SERI	massive	30%-45%[f] of dry wt.	10-15 h doubling time

Notes:

[a]UTAC stands for University of Texas Algal Collection, Austin, TX.
[b]Massive accumulation of oil means that the storage oil (as visualized by the Nile Blue A staining) occupies more than 50% of the intracellular space of the algal cells. Moderate indicates that the storage oil occupies approx. 20% to 50% of the intracellular space. Slight accumulation denotes that storage oil is detectable by the Nile Blue A staining method, but it occupies less than approx. 20% of the total cellular volume.
[c]N.T. stands for not tested.
[d]Estimated by gravimetric determination of total algal mass and extracted lipids.
[e]CHLS01 is a very fast-growing species of fresh-water, unicellular, green alga (genus and species identification is yet to be completed) isolated from a local site next to SERI's temporary building.
[f]Estimated from the specific heat of combustion of the total dry mass of the alga.

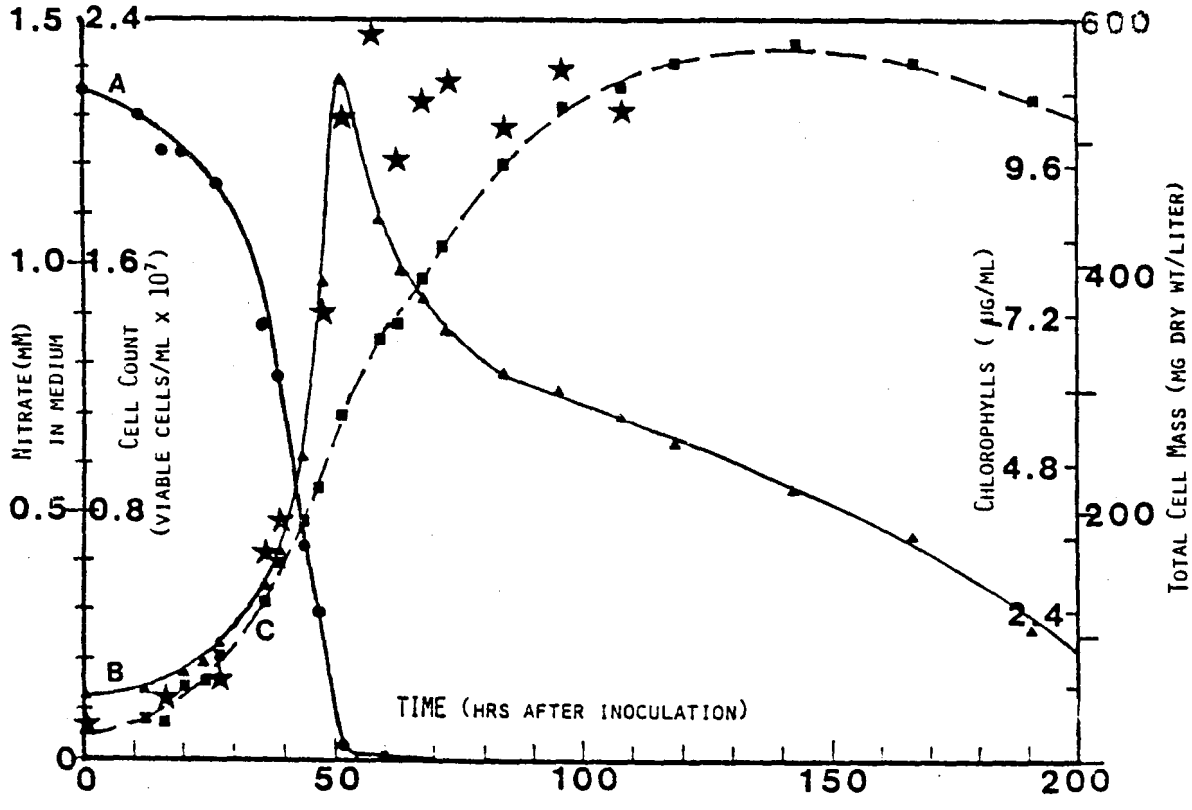

Figure 1. Effect of Nitrate Supply on Growth and Biomass Accumulation

A dense, green suspension of N. oleoabundans (250 mL) in the late exponential phase of growth was inoculated at time = 0 into 12 L of 0.5 N-BBM-8 medium. The culture was continuously bubbled with air enriched with 0.5% CO_2 (V/V) and grown at 28°-31°C under continuous illumination of 2500-3000 lux provided by cool white fluorescence lamps. Samples of 200-250 mL were withdrawn from the culture at the indicated time for analysis. Keys: Nitrate concentration (Curve A, •); chlorophylls per unit volume of culture (Curve B, ▲); total algal mass dry weight per unit volume (Curve C, ■); and viable cells per unit volume (★).

TABLE 2. ALGAL MASS AND OIL PRODUCTION BY *N. OLEOABUNDANS*.
(Batch cultures in 0.5N-BBM-8 medium)

1.	Culture duration	7-8 days per batch
2.	Inoculum	1%-2% v/v (of active, green cells in the late exponential phase) or 3%-5% v/v (of oil-rich resting cells in the late stationary phase)
3.	Final cell density	0.9 ± 0.1 g dry wt. L^{-1} 3.6 ± 0.4 g wet wt. L^{-1}
4.	Average daily productivity	
	(a) Total algal mass	110-130 mg dry wt. L^{-1} day^{-1}
	(b) Total lipids	48-54 mg dry wt. L^{-1} day^{-1}
	(c) Neutral lipids (triglycerides & carotenoids)	37-42 mg L^{-1} day^{-1} 37-42 mg L^{-1} day^{-1}

starvation was also observed in the newly isolated strain of oleaginous alga, CHLS01. Other readily observable physiological changes associated with the accumulation of intracellular oil and lipid are (1) an increased ratio of carotenoid-accessory pigments to chlorphylls (Figure 2), and (2) a great reduction in the complexity of the intracellular membranous systems of the algae (Figure 3). These are important photophysiological responses that directly or indirectly affect the photosynthetic apparatus of the algae and which, therefore, will strongly modify the photosynthetic productivity and lipid metabolism of these organisms. A detailed analysis of these structural-functional aspects of algal oil production and accumulation is under way.

ANALYSIS OF ALGAL OIL AND LIPID PRODUCTION

Using one of the fast-growing species of oleaginous algae, N. oleoabundans, we obtained a rough estimate of potential oil productivity under the routine culture conditions used in our laboratory. Table 2 summarizes the results of those studies. If the value of lipid production rates in Table 2 was used to calculate the potential oil production rate (by linear extrapolation, assuming an average depth of 25 cm for the algal pond), an unrealistically high value of approximately 1200 bbl of algal oil per acre per year would have been obtained. This is because, under laboratory conditions, the growth of the organisms was neither limited by the availability of CO_2 nor by the input flux of the radiant energy.

To obtain a more realistic and accurate estimate of algal oil and lipid production, we analyze the effect of (1) the net energy conversion efficiency of photosynthesis (P.S.E.); (2) the energy density of the algal oil relative to that of the nonlipoidal part of the algal mass; and (3) the oil content (as a percentage of the total dry mass) of the algae. The result of this calculation is presented in Figure 4. It is evident that using fast-growing (a P.S.E. of 2%-3% averaged over the entire batch culture duration) and highly oleaginous (oil content of 40%-70%) species, the annual production of algal oil will be in the range of 50-100 bbl/acre. The absolute maximum production rate for algal oil is limited to approximately 220 bbl $acre^{-1}$ yr^{-1}, which assumes an oil content of 70% and P.S.E. value of 6% obtained year-round under average U.S. insolation (4 kWh/m^2), and the algae are assumed to grow almost year-round (i.e., 360 days/yr). However, for large-scale cultivation of algae for lipid production, the rate of annual oil yield is most likely to be limited to a range of 50-120 bbl $acre^{-1}$ yr^{-1}.

ANALYSIS OF LIPID COMPOSITION OF NITROGEN-STARVED ALGAE

In collaboration with Dr. T. Tornabene, we have examined the lipid composition of the oily lipids (which comprised 35%-54% of the total dry algal mass) from N. oleoabundans. Triglycerides are the predominant components, and they account for 80% of the total lipids. Aliphatic hydrocarbons, sterols, pigments, glycolipids, and phospholipids make up the remaining lipid fraction. Saturated, monounsaturated, and diunsaturated octadecanoic acid represented approximately 50% of the total fatty acids. Similarly, triglycerides are found to be the predominant component in the storage lipids of oleaginous species Chlorella, Scenedesmus, and Chlorococcum. Detailed lipid composition will be

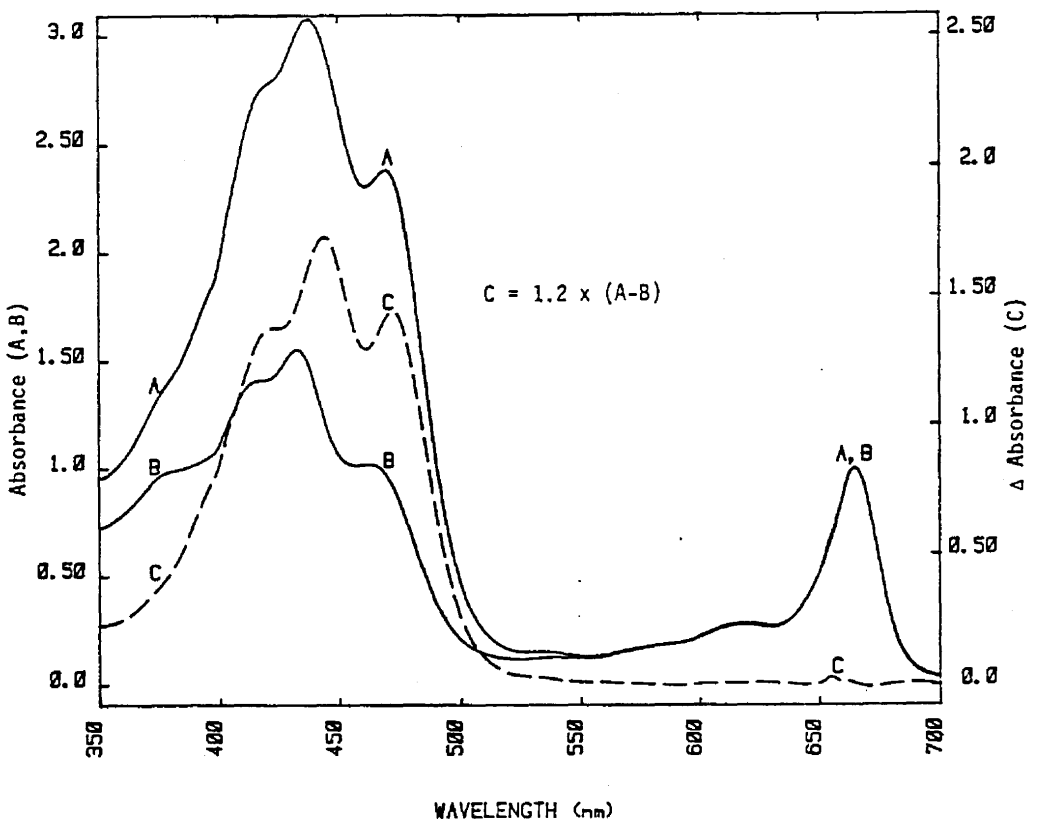

Figure 2. Changes in the Photosynthetic Pigments of N. oleoabundans Under Nitrogen-Limited Growth

Samples for spectral analysis were obtained from experiments described in Figure 1. Spectrum A: oil-rich, yellow-green cells taken at time = 190 h. Spectrum B: green exponential cells taken at time = 46 h. Both spectra were obtained from a 90% ethanol extract of the total pigments and were normalized at the red absorption peak (λ = 666 nm) of chlorophyll a. Spectrum C was the difference spectrum (i.e., A - B, expanded by a scale factor of 1.2 for clarity) which shows the characteristic absorption peaks of carotenoids in the 350-500 nm region.

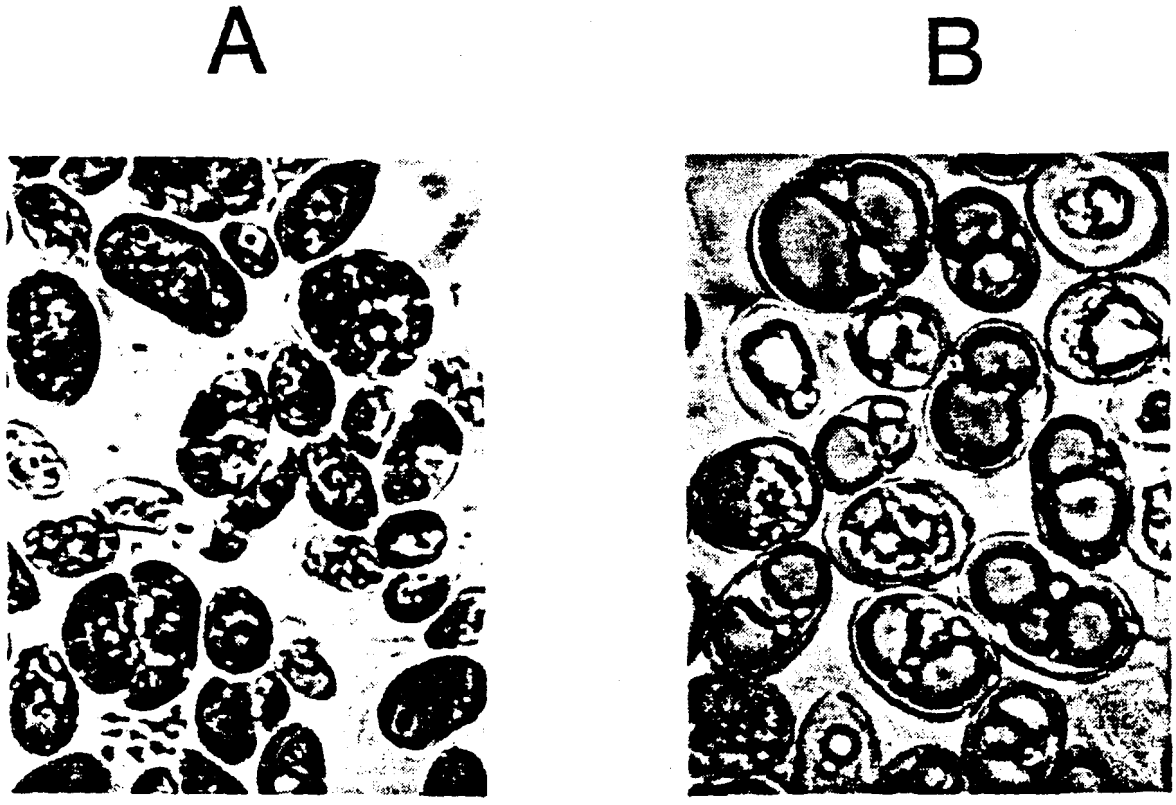

Figure 3. Structural Changes Associated with Oil Accumulation in Cells of "CHLS01"

Photomicrographs (2,800 X) of non-oily young cells and old, nitrogen-deficient, oil rich cells of "CHLS01." The extensive intracellular structures in the young, green cells (A) were greatly reduced and replaced by structureless oil globules in the old cells (B).

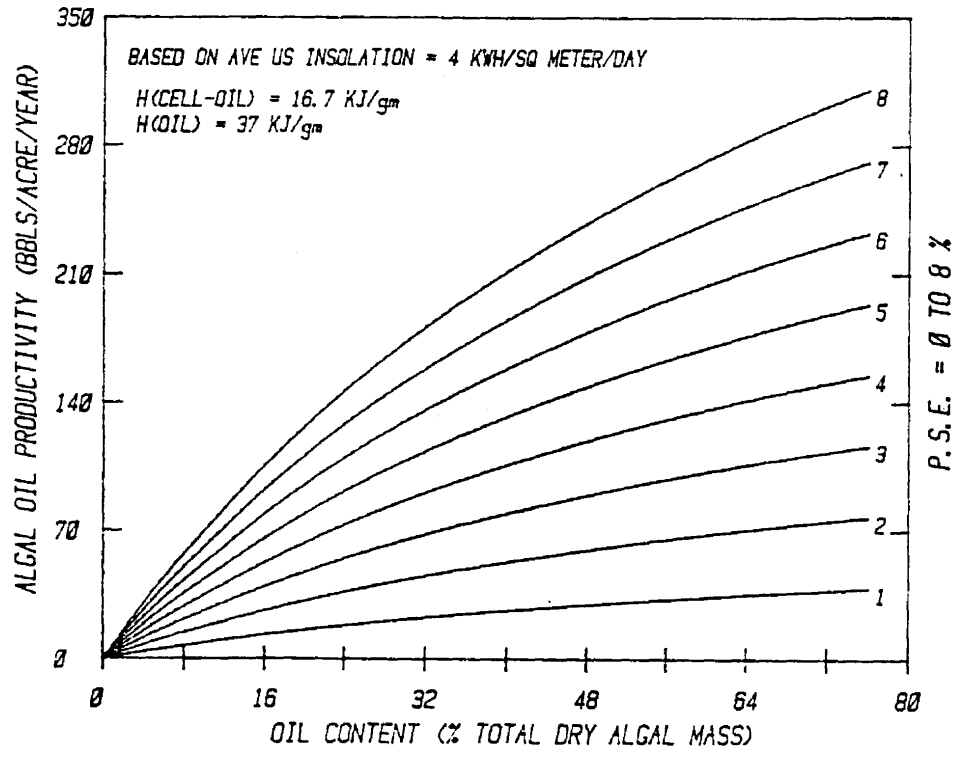

Figure 4. Projected Algal Oil Production Rate Using Oleaginous Microalgae

The following assumptions are used in the calculation of algal oil productivity: (a) annual averaged U.S. isolation equals 4 kWh m^{-2} day^{-1}; (b) oil content of the algae is allowed to vary from 0% to 80% (X-axis) of the total dry algal mass; (c) a value of 37 kJ g^{-1} (typical for triglycerides) is used as the specific heat of combustion for algal oil while that of the non-oily part of the algal mass is assumed to be 16.7 kJ g^{-1} (a typical value for carbohydrates and proteins); and (d) an average photosynthetic energy conversion efficiency of 1% to 8% (as indicated to the right of each curve) of the total incident solar radiation was maintained for a growing season of 360 days per year.

continued in FY 1982-1983. The identification of unusual fatty acids with unique chemical properties or high market value will be emphasized.

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RESOURCE ASSESSMENT FOR SALINE AQUATIC BIOMASS PRODUCTION SYSTEMS

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ABSTRACT

The siting, design, and operation of aquatic biomass production systems are integrally related to available resources and the environment within which such systems must function. The need for resource assessment as an ongoing, key component of the Aquatic Species Program results from the intrinsic and inseparable influences of natural resource and environmental conditions upon the total research program, leading to the ultimate development and establishment of viable systems. The climate, water, and land resources (and related environmental factors) of greatest interest at present include insolation, water supply/demand, land use/cover, growing degree-days, topography, severe storm incidence, water chemistry, land ownership, soil characteristics, and geology.

Resource assessment research objectives for FY 1982 are threefold: (1) to provide natural resource/environmental inputs to the ASP research plan; (2) to provide an initial stratification of the southwestern United States into zones of varying potential for siting saline microalgae and emergent plant production systems; and (3) to develop a resource assessment plan to provide refined and continuing inputs to research plans, system design, and regional stratification based on advanced geographic information system (GIS) techniques. This presentation provides an interim report on progress to date. The importance of each of the resource and environmental factors is briefly considered. The effort required to assess and characterize such factors for the southwestern United States is described.

INTRODUCTION

The feasibility of using microalgae or other aquatic biomass production systems as a significant source of renewable energy for the United States depends on the availability of adequate land, water, and climate resources. Therefore, assessing and characterizing the natural resources and environmental constraints affecting such systems is an integral, vital part of the overall aquatic species program at the Solar Energy Research Institute (SERI). The objectives of this task, being undertaken by the Renewable Resource Assessment and Instrumentation Branch (RRAIB), are given below.

Resource Assessment Objectives

The following objectives relate specifically to the effort undertaken during FY 1982. They imply but do not explicitly state the Resource Assessment objectives that will be required in future years.

I. To develop a resource assessment plan to provide continuing inputs to research plans, system design, and regional stratification.

II. To provide an initial stratification of the southwestern United States into zones of varying potential for siting saline microalgae and emergent plant production systems.

The work performed in FY 1982 can be applied to aquatic biomass production systems in general. The emphasis, however, is on microalgae production systems.

Resource Assessment Status

At the time this interim paper was prepared, most of the resource needs and environmental constraints associated with microalgae production systems had been identified. These will be described later.

Procedures for the initial stratification of the southwestern United States have been selected. The use of transparent gray-tone overlays to designate varying classifications of land, water, and climate suitability fits within the time and money constraints of this effort. This procedure is expected to yield a good preliminary evaluation of the resource and environmental constraints that may be imposed on aquatic biomass production systems in the Southwest. However, the complex nature of the resources and environmental constraints for these systems will probably necessitate a computerized geographical information system to further refine the efforts undertaken in FY 1982.

Data and information have been provided to the ASP office and to Battelle's Columbus Laboratory, which is working on a parallel task. The collection, evaluation, and presentation of data and information affecting the other ASP research tasks will be a continuing and important part of the resource task.

Major data sources for land, water, and climate information have been identified at this time. Many of these sources are located at the Denver Federal Center, which greatly facilitates the characterization and collection of data by RRAIB personnel. Initial contacts have been made with many of the federal agencies that have data of interest to this task, and some limited contacts with state agencies have also been made. Follow-on contacts will involve evaluation and characterization of the data and the data sources.

The data collection and reduction procedures required for performing the initial stratification of the southwestern United States are well under way at this time. Most of the climate data are in hand, land ownership and topographic maps (for computation of slope) have been obtained, and about half of the Landsat images required for evaluating land use/cover for the southwestern United States are on order. Although this initial stratification will be cursory, with mostly national- scale maps, it will provide the first estimate of the availability and suitability of land, water, and climate resources in the Southwest. The resource assessment plan, which is the primary product of the FY 1982 effort, has been outlined, and all subsequent efforts undertaken at present are directed toward its completion.

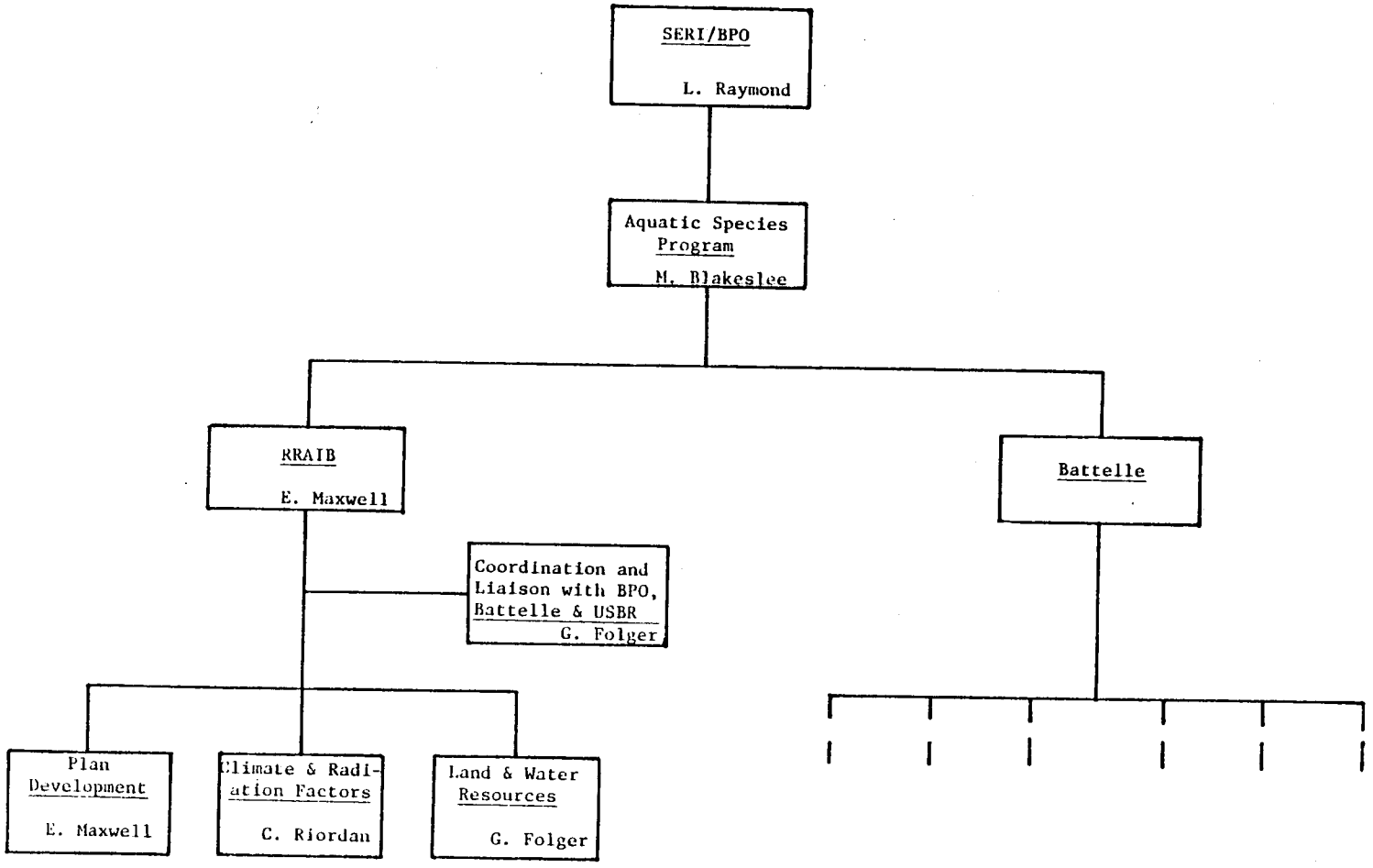

Figure 1. Chart of Organizational Relationships

TASK ORGANIZATION AND APPROACH

The RRAIB task for resource assessment is closely paralleled by a task undertaken at Battelle's Columbus Laboratory. The organization chart in Figure 1 shows the relationship between the Biomass Program Office, the ASP Office, and Battelle. Note that a liaison officer, A. Gray Folger, has been designated to maintain communications between the program offices, RRAIB, and Battelle. Because of the parallel development of system design, criteria, resource requirements, and environmental and other constraints, there will be some unavoidable overlap of activity. Limiting overlapping to an acceptable level requires frequent communications, which are the primary responsibility of the liaison officer.

SYSTEM DESCRIPTION

Only microalgae biomass production systems are described in this interim paper. These descriptions are based on information provided by the ASP office and are essentially limited to system parameters and characteristics that relate to natural resource requirements, environmental constraints, and environmental impacts.

System Diagram

An artist's conception of a microalgae biomass production system is shown in Figure 2. This conceptual diagram is of value primarily in discussing the natural resource requirements and environmental constraints of such systems.

Based on current system production estimates, a fuel oil system must be sized at 100 square miles or larger in order to supply a 50,000-barrel-per-day refinery, which is about the smallest refinery presently constructed by the petroleum industry. The individual, connected circulatory channels within which microalgae will be grown would range in size from 30 to 60 feet wide and 150 to 400 feet long. The system is shown installed on essentially flat or gently rolling terrain over or very close to a subsurface saline water supply. The majority of the land surface within the 10-by-10-mile region will be covered by shallow ponds or channels which will probably be covered in turn with a transparent plastic material. Covering the ponds or channels may not be absolutely necessary, but in the southwestern climate, covering will reduce evapotranspiration and the introduction of contaminants from wind-blown materials. The resource/environmental significance of this system concept is considered in the following section.

Resource Requirements and Environmental Constraints

The primary resource and environmental parameters of significance to a microalgae biomass production system such as the one shown in Figure 2 are listed in Table 1. Under the general category of climate, insolation and growing degree-days are considered to be resources of interest, whereas severe storms and precipitation represent environmental constraints. Solar radiation is a direct, essentially controlling resource required for the production of biomass by terrestrial or aquatic plants of any type. Although microalgae grow everywhere from the tropics to the arctic, it is expected that a certain minimum level of insolation will be required for cost-effective production of biomass as an energy resource.

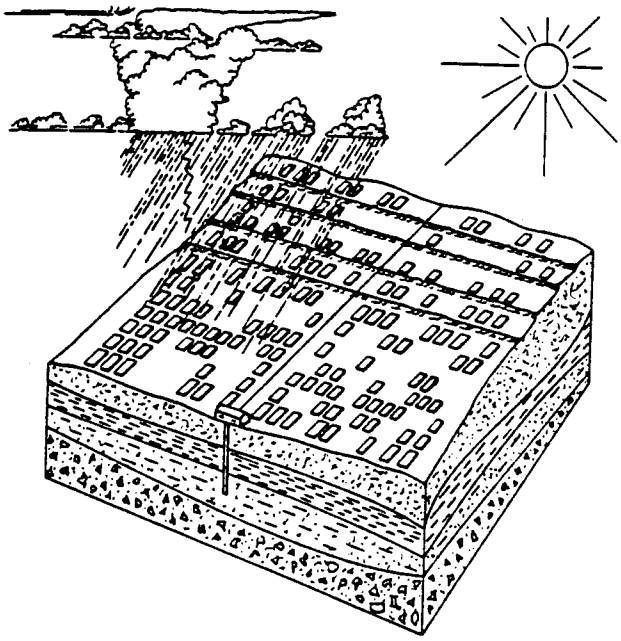

Figure 2. **Microalgae Biomass Production System** (Artist's Concept)

TABLE 1. RESOURCE AND ENVIRONMENTAL PARAMETERS

Climate	Water	Land
Insolation	Supply/demand	Use/cover
Growing degree-days	Location	Topography
Severe storms	Salinity	Soils*
Precipitation	Chemistry*	Ownership
		Geology

*Battelle has primary responsibility for these.

Growing degree-days constitute a parameter employed by the agricultural community to estimate the growth rate of agricultural crops. Growing degree-days are defined as

$$GDD = 1/2(T_{max} + T_{min}) - T_{base} \quad , \tag{1}$$

where T_{base} = the minimum effective temperature for plant growth
T_{max} = the maximum daily temperature (an upper limit is usually set)
T_{min} = the minimum daily temperature, which if less than T_{base} is set equal to T_{base} .

The upper limit to T_{max} represents the maximum temperature that will affect the growth rate of the plants. The upper limit for T_{max} and T_{base} are, of course, plant-dependent. Therefore, values for the microalgae species of interest will have to be determined before growing degree-days can be computed.

Severe storms represent an environmental constraint for covered systems. Plastic covered ponds would be especially susceptible to damage or total destruction from hailstorms, tornadoes or the high winds associated with such storms. Even for an uncovered system, contaminants from high winds and the damage that could be incurred from hailstorms and tornadoes represents a serious constraint. A concern associated with precipitation, a constraint primarily for covered systems, is for damage that could be inflicted by a snowfall of about two inches or more. Average annual precipation could be a limiting factor relative to the recharge of aquifers or the annual feeding of streams or reservoirs.

The four parameters found under the general category of water resources include (1) supply/demand; (2) location (these represent resources of interest); (3) salinity; and (4) chemistry (these generally represent constraining factors that could limit the availability or the usefulness of the water resources).

The complexities of water law and water distribution systems in the southwestern United States will almost surely present the most difficult and limiting problems of importance to these systems. It is no exaggeration nor does it represent an overstatement of the seriousness of this problem to note that many small wars and thousands of legal battles have been fought over water rights in the western United States. Microalgae's tolerance for saline water may alleviate some of these problems, but since the use of saline water resources could affect fresh-water supplies in one way or another, we must expect that water rights will remain a prominent factor in the future development of these systems.

The location of both surface and subsurface water resources will be important regardless of supply and demand problems, simply from the standpoint of locating sites near suitable land and climate resources. This is a relatively straightforward problem, compared with supply and demand. Eventually, some new exploration for water resources should be required as sites are selected in a particular region.

The salinity of water resources takes on special importance for aquatic production systems for the reasons stated in this paper. Some salinity information is available for many of the resources of interest, but again, we expect that new data will be required. The chemistry of the water is also important, primarily to identify chemicals that might be toxic to a particular microalgae species. Since the species are still being selected and studied, the present chemical evaluations of water resources must be general.

The primary land resource of interest can simply be described as suitable surface locations for the construction of aquatic production systems. With the exception of geology, therefore, the parameters found in Table 1 under the land category represent constraints that will limit the total acreage available for this application. And land use and land cover will constrain the installation of aquatic biomass production systems, for a number of reasons. Certain land uses may give the land such a high value that converting it to biomass production would be economically unfeasible. Other land uses entail sensitive environmental or political constraints that eliminate such lands from consideration, including national parks, wilderness, and certain military reservations. Furthermore, because of the scarcity of national forests in the southwestern United States, any region covered by forests might also be eliminated from serious consideration.

Topography will be a limiting factor for these systems, because the installation of large, shallow ponds requires relatively flat terrain. As a first approximation, lands having a slope greater than 10% will be designated as unsuitable or limiting for these systems. These slope limitations will probably be altered at a later date when more design information is available. Soils also represent a constraint on aquatic production systems, for several reasons. A certain minimum soil depth will be required in order to construct a pond at a reasonable cost. The porosity, permeability, and compactibility of the soils will be important relative to the effectiveness of efforts to seal the pond and reduce water losses to an acceptable level. The microalgae provide some natural sealing, but that will also depend on soil characteristics.

Current land ownership can be both a political and an economic constraint. Certain private lands could be prohibitively expensive, and land ownerships such as Indian Reservations could represent a political limitation. The significance of ownership as a constraint can be assessed accurately only from detailed discussions with the landowners involved.

Geological resource information is required primarily to determine the characteristics of subsurface aquifers and the geologic strata through which they are recharged. This will be important both from the standpoint of the long-term characteristics of the water supply and the potential for affecting fresh-water supplies above or below the saline subsurface reservoirs of interest.

RESOURCE AND ENVIRONMENTAL INFORMATION NEEDS

The first step in any resource assessment task to determine the specific information and data needed to characterize the resource in terms of its importance to a particular use. The results of the evaluation of resource/environmental information needs for microalgae production systems are given below.

Climate Information Needs

The most important climate variable is insolation. Since shallow ponds are the collectors of solar radiation for these systems, monthly means of total global horizontal insolation are the primary data of interest. However, since the photosynthetic activity of most plants reaches an asymptotic value at some finite level of solar radiation, the statistical distribution of the insolation is also of interest.

Calculation of growing degree-days requires the collection of maximum/minimum temperatures as indicated by Eq. (1). Since the limiting temperatures for Eq. (1) would be different for different species of microalgae, alternative temperature-related statistics may be of value. These include mean temperatures, the length of freeze-free periods for a region, and the probability of temperatures occurring below set thresholds.

As noted, severe storms are of interest in relation to potential damage to systems and to the potential for introducing contaminants to open systems. Therefore, occurrences of thunderstorms, hail, and high winds are important in siting and operating aquatic production systems. Such information is routinely collected at weather stations and should be readily available for this task.

Water Information Needs

Water supply and demand must be addressed from the standpoint of both surface and subsurface water resources. Information associated with surface water resources includes reservior storage, stream flow, and water rights. Subsurface water resource information needs include identification of known aquifers, general ground water distribution, draw-down versus recharge rates, and water rights. The water flow available from existing wells constitutes an important source of information. The location of all of the water resources of interest to this task relative to the location of suitable land resources represents one of the more critical information needs.

The chemistry of the water resources, including salinity, pH, and toxic chemicals, represents another critical information need. Establishing the criteria for the constraints that water chemistry may impose on microalgae production systems is one of the key responsibilities of Battelle's Columbus Laboratories. RRAIB will make use of information provided by Battelle to assist in the development of the plan for future resource assessments.

Land Information Needs

The availability of land that can meet the criteria for biomass production systems represents another critical constraint. Factors that impact on land availability and suitability include current land use/land cover. Land that is currently being used for irrigated agriculture and land that has been developed for housing, commercial, or industrial uses will obviously be too expensive to be considered as a biomass production site. Equally constraining will be land uses such as national parks, wildlife refuges, wilderness areas, and certain types of military reservations. Land cover constraints will be encountered primarily in relation to forested lands which, for economic or environmental reasons, or both, will be unavailable for this application. Other land uses and covers will be more or less constraining depending on political, environmental, and economic considerations.

The slope and general shape of the land represent another limiting constraint. Obviously, systems that consist of shallow ponds on the order of 60 by 400 feet, clustered together in areas covering up to 100 square miles, cannot be installed on terrain having even moderate relief. Flat to gently sloping terrain will be required for the installation of even moderate-sized systems. At present, a criterion of 10% or less slope has been established. In the future, the impact of various slopes from 0 to as much as 20% will be evaluated.

RESOURCE AND ENVIRONMENTAL INFORMATION SOURCES

Sources of climate information are relatively few and also represent few data collection problems. Sources of land information present a more complex picture; a number of federal and state agencies hold a portion of the data and information of interest to this task. Water resources, however, present the major problem relative to the location and characterization of data and data sources. Data sources that at this time have been identified, located, and in a few instances, examined are described briefly in the following subsections.

Climate Information Sources

The primary climate information sources are the National Climatic Center, the Solar Energy Research Institute, and state climatology offices. The National Climatic Center archives data collected by the National Weather Service, military weather services, and other national and international sources including the World Meteorological Organization. SERI has one of the most complete collections of solar radiation data and accompanying sunshine and cloud-cover information. This information has recently been compiled into a solar atlas, which represents the most concise and comprehensive collection of solar information available on a national scale. In some instances, however, individual states have more detailed climatological information for part or all of the state. These data will be collected and used to improve the national maps whenever possible.

Water Information Sources

The primary source of water resource information is the Geological Survey of the U.S. Department of the Interior, commonly known as the USGS. The USGS is the principal federal agency responsible for collecting and reporting data on water resources. Although other federal, state, and local government agencies collect water data, as do private entities such as engineering firms, primary responsibility for coordinating water data acquisition, storage, and dissemination resides with the USGS.

The USGS has surveyed and monitored geophysical resources for many decades. Water resource investigations of the agency are reported in numerous publications and maps. Geological Survey publications of special interest include series of hydrologic unit maps, hydrologic investigations atlases, water-supply papers, and selected circulars, professional papers, and bulletins. A recent group of reports in the professional paper series provides summary appraisals of groundwater resources by water resource region. However, these publications are primarily interpretive—that is, they present analyses and interpretations of field data rather than listing the complete actual basic data.

Much of the basic water resources data can be located and accessed via a system of comprehensive data bases that have been developed and improved by the USGS since the mid-1960s. The Office of Water Data Coordination of the USGS has published several editions of the Catalog of Information on Water Data, for each of 21 water-resources regions, which provide a hard-copy version of a computerized file of water data acquisition activities. Rather than provide the data, the Catalog provides information on where and by whom data are being collected, the type of data collected, and how the data can be obtained. The Catalog is updated with information obtained by the NAWDEX (National Water Data Exchange) program of the USGS. The function of NAWDEX is to

provide a central source of water data information by indexing the data collection activities of various water-oriented organizations throughout the nation. Access to NAWDEX information is obtained through two subordinate data bases—the Master Water Data Index and the Water Data Sources Directory—and a network of local assistance centers, of which two are located in Colorado (one is at the Denver Federal Center).

The USGS also maintains an accessible, comprehensive data base containing water resources data called WATSTORE (Water Data Storage and Retrieval System). The WATSTORE system includes (1) continuous or daily data on surface water, groundwater, and water quality; (2) annual peak stream-flow values; (3) chemical analyses of surface and groundwater; (4) geologic and inventory data for groundwater sites; and (5) summary data on water use. WATSTORE access and assistance are also available locally.

While its purpose is not to provide water data per se, several other large water data bases are accessible through NAWDEX by virtue of reciprocal agreements. Included in these are (1) the STORET (Storage and Retrieval) system of the U.S. Environmental Protection Agency; (2) the Environmental Data and Information Service (EDIS) of the National Oceanic and Atmospheric Administration (NOAA); (3) an extensive bibliographic data base of the Water Resources Scientific Information Center (WRSIC) of the U.S. Department of the Interior's Office of Water Research and Technology; and (4) several state data bases, such as the Texas Natural Resources Informaton System (TNRIS).

The challenge in utilizing these sources of water data for the purposes of evaluating groundwater availability for microalgae production systems is one of selecting and obtaining needed data efficiently. Water data have been collected and studied for a variety of purposes, but most notably for determining water supply, monitoring water quality, and avoiding water-related disasters. Only in recent years has groundwater been studied for quality and quantity determinations and, even so, the primary purposes for these investigations have been related to conventional uses of water. The water requirements and tolerances of microalgae production systems may dictate special data needs for which additional data should be collected.

Land Information Sources

Landsat satellite images will be primary sources of land-use and land-cover information. A great deal of level-one and level-two land-use/land-cover information can be obtained from photointerpretation of Landsat False Color Composite transparencies or prints. Table 2 shows the level-one and level-two classifications established by Anderson (1972). Landsat resolution does not permit the extraction of all of these land-use classes, but most of the information that is particularly important for the selection of aquatic production sites can be obtained from Landsat. Figure 3 is a black and white Landsat print of part of the southwestern United States which illustrates the kind of information that can be extracted and is pertinent to this task.

Topographic information could be extracted from Landsat, as is apparent from Figure 3, but the primary source of slope information for this task will be the topographic maps supplied by the USGS. During FY 1982, slope information for the initial stratification of the Southwest was obtained from 1:500,000-scale state topographic maps. It has been determined, however, that the use of this scale will overestimate areas of land having a slope of 10% or less by as much as 50%. Future regional stratifications should be conducted initially with 1:250,000-scale quadrangle maps that will reduce the overestimation of flat terrain to 20%-30%. Finally, when specific locations are being

Figure 3. Landsat Print of Southern Part of the San Luis Valley, Colorado

TABLE 2. U.S. GEOLOGICAL SURVEY LAND-USE AND LAND-COVER CLASSIFICATION SYSTEM FOR USE WITH REMOTE SENSOR DATA

Level I		Level II	
1	Urban or Built-up Land	11	Residential
		12	Commercial and Services
		13	Industrial
		14	Transportation, Communications, and Utilities
		15	Industrial and Commercial Complexes
		16	Mixed Urban or Built-up Land
		17	Other Urban or Built-up Land
2	Agricultural Land	21	Cropland and Pasture
		22	Orchards, Groves, Vineyards, Nurseries, and Ornamental Horticultural Areas
		23	Confined Feeding Operations
		24	Other Agricultural Land
3	Rangeland	31	Herbaceous Rangeland
		32	Shrub and Brush Rangeland
		33	Mixed Rangeland
4	Forest Land	41	Deciduous Forest Land
		42	Evergreen Forest Land
		43	Mixed Forest Land
5	Water	51	Streams and Canals
		52	Lakes
		53	Reservoirs
		54	Bays and Estuaries
6	Wetland	61	Forested Wetland
		62	Nonforested Wetland
7	Barren Land	71	Dry Salt Flats
		72	Beaches
		73	Sandy Areas Other Than Beaches
		74	Bare Exposed Rock
		75	Strip Mines, Quarries, and Gravel Pits
		76	Transitional Areas
		77	Mixed Barren Land
8	Tundra	81	Shrub and Brush Tundra
		82	Herbaceous Tundra
		83	Bare Ground Tundra
		84	Wet Tundra
		85	Mixed Tundra
9	Perennial Snow or Ice	91	Perennial Snowfields
		92	Glaciers

selected for the installation of a biomass production system, the use of 7.5-ft. quadrangle maps at a scale of 1:24,000 will be required to assess surface characteristics accurately.

Land ownership information is available from many sources, including state and county files, but a general assessment of land ownership in the southwestern United States will be obtained from the Bureau of Land Management (BLM) land status maps. These maps show such categories as Indian Reservations, national and state forest lands, BLM and state lands, wildlife refuges, national grasslands, national parks, military reservations, developed lands, and private lands, including some special designations such as the Spanish Land Grants in New Mexico. These BLM state maps are considered adequate for the initial stratification and may be adequate until the selection of a specific site is required.

Information on soil characteristics can be obtained from the Soil Conservation Service (SCS) and similar state agencies. State-level soil maps are generally available, and larger scale quadrangle maps are being prepared for much of the southwestern United States. The detailed quadrangle maps will not be available for all regions, however, and in fact, specific information needed to site and design a microalgae biomass production system will not be available for many regions of interest. Special soil surveys will also be required when specific sites are being evaluated.

Geology maps available from the USGS and state survey offices should be completely adequate for the assessment of geological controls over aquifers and aquifer recharge areas. Special studies to evaluate the significance of the geology are likely to be required in most instances, however.

In addition to the sources noted in this paper, the land use and land cover and associated map series being prepared by USGS under the Land Use and Data Analysis Program (LUDA) will be used for areas where maps are available. This series provides land use/land cover, hydrologic units, census data, and federal land ownership on a common base map using a 10-acre minimum mapping unit for urban and water-covered areas and a 40-acre minimum mapping unit for other areas. Unfortunately, this series is far from complete for the areas of interest.

SUMMARY

Numerous sources of land, climate and water resource information will be characterized. Key data will be collected and transferred to a series of overlay maps to stratify the Southwest into zones of suitability for microalgae biomass production systems. The results of the stratification will be used to select potential sites for future experimental or prototype systems, or both. The overall study will be concluded with the development of a resource assessment plan to provide guidance to future resource evaluation and site selection projects.

EMERGENT AQUATICS: STAND ESTABLISHMENT, MANAGEMENT AND SPECIES SCREENING

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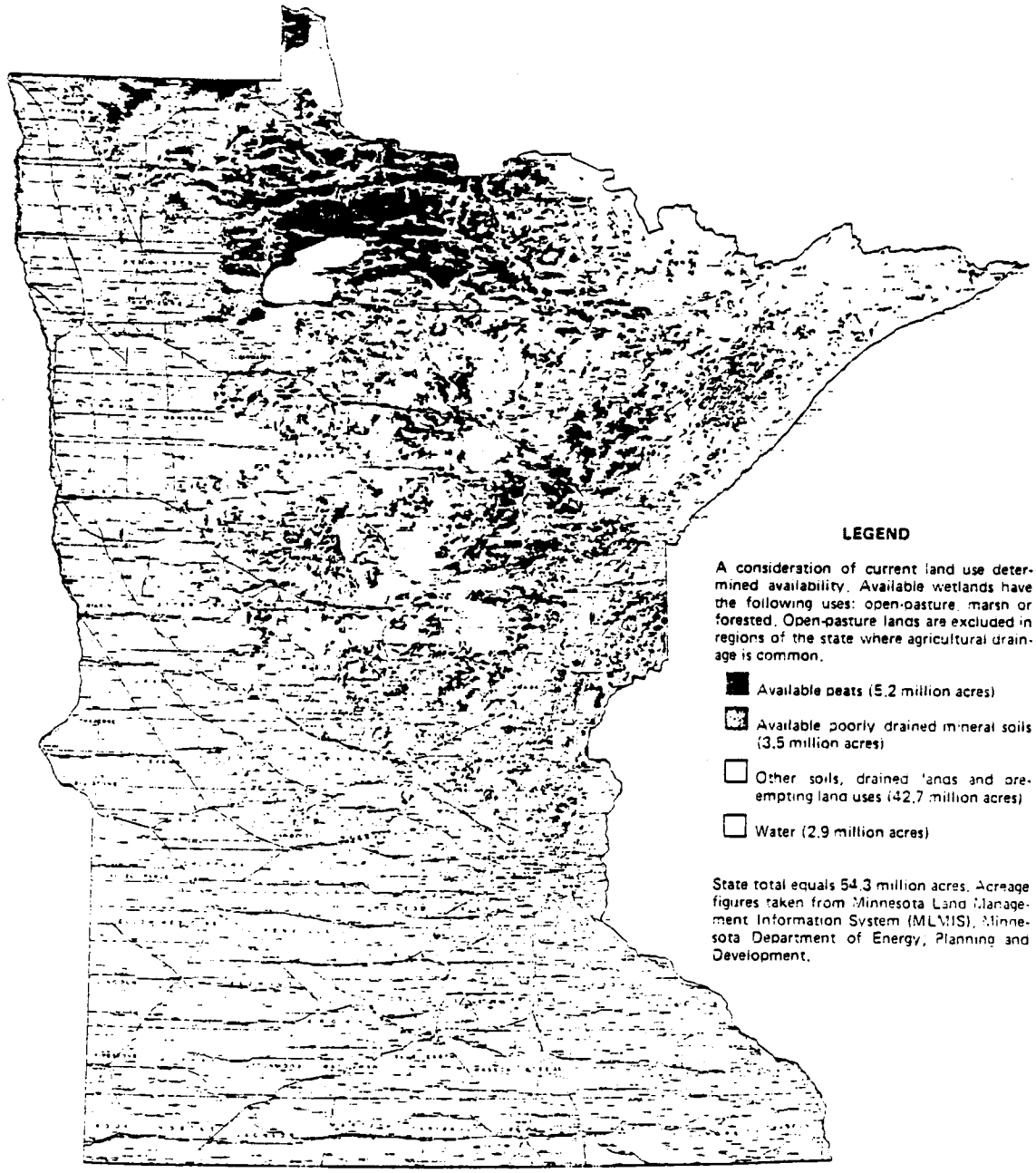
INTRODUCTION

The possible use of wetlands to produce biomass energy crops is the focus of a research effort at the University of Minnesota. Wetlands dominated by Typha spp. (cattails) and other emergent vegetation, such as Phragmites (reeds) and Scirpus (rushes), are one of the most productive natural systems in the temperate zone (1). Minnesota with over two million hectares of peatland and 1.4 million additional hectares of wet mineral soils appears to have considerable potential for wetland crop production (Figure 1). Among the attractive features of this system is the fact that wetland crops would not compete with traditional crops for prime agricultural land. The use of peatlands for the production of a renewable resource also offers an attractive alternative to peat mining.

Its high yield potential and attractive chemical composition make Typha a particularly viable energy crop. The Minnesota research effort has demonstrated that total annual biomass yields between 25 and 30 dry tons/hectare are possible in planted stands (2). This compares with yields of total plant material between 9 and 16 dry tons/hectare in a typical Minnesota corn field. At least 50% of the Typha plant is comprised of a belowground rhizome system containing 40% starch and sugar (3). This high level of easily fermentable carbohydrate makes rhizomes an attractive feedstock for alcohol production. The aboveground portion of the plant is largely cellulose and although it is not as easily fermentable, it can be gasified or burned.

The high productivity of Typha can be rationalized in a number of ways. Of primary importance is the fact that cattails are not limited by the availability of water. Also, the canopy architecture appears to increase the efficiency with which directly incident and reflected sunlight can be utilized in photosynthesis. Because of the upright leaf angle in the foliage canopy, a greater proportion of the leaf area is exposed to direct sunlight. In contrast with most crop plants, Typha begins growth early in the spring from shoots developed the previous fall, and remains active until the leaves are killed by frost in the fall. Because of their adaptability to a wide range of temperatures, they are able to remain active through a greater proportion of the growing season. Each of these factors undoubtedly contributes to Typha's success as solar energy collector, but the relative importance of each has not as yet been carefully assessed.



Produced by the Center for Urban and Regional Affairs, University of Minnesota, under contract with the Minnesota Energy Agency, November, 1981.

Figure 1. AVAILABLE WETLANDS FOR BIO-ENERGY PURPOSES: LAND USE AND DRAINAGE CONSTRAINTS

The rate of Typha biomass accumulation is greatest, and almost constant, between June 15 and September 15. Thus, the decreasing day length through July and August appears to be compensated for by increased photosynthetic capacity as the foliage canopy develops. Growth rates during the period of maximum production average about 40 g/m^2/day in agricultural soils (4), and about 30 g/m^2/day in peat soils (5). Growth rates for agricultural crops of nearly 50 g/m^2/day have been reported, but normally for only relatively short periods of two or three weeks. A growth rate of 30-40 g/m^2/day sustained for a two month period is unusual. Thus, the high seasonal yields of cattails are due more to a prolonged moderate level of growth than to an unusually high spurt of photosynthetic activity.

Studies of Typha stand establishment and management, harvesting methods, equipment needs and design, environmental constraints and the overall economics of wetland energy crop production are currently under way administered by the University's Bio-Energy Coordinating Office (BECO). The major long-term objective of the multidisciplinary research program is to examine the possibility of developing an efficient and renewable energy system in an environmentally sound fashion, using available indigenous resources.

This report summarizes the first year results of a series of studies funded by the Solar Energy Research Institute (SERI). It is divided into the following sections:

- Typha Stand Establishment and Management
- Typha spp. Nutrient Experiments
- Wetland Species Comparisons and Micropropagation

TYPHA STAND ESTABLISHMENT AND MANAGEMENT

In order to establish large stands of Typha, it is necessary to investigate appropriate planting material and land preparation options. Because the plants being considered are perennials, it is hoped that stand establishment need occur only once.

Planting material can be in the form of seed, seedlings, or rhizome pieces. Seed is by far the least costly method of establishment, but has problems of competition from weeds, slow first-season growth, and low first-season yields. Transplanting seedlings or rhizomes into fields results in rapid stand establishment and good first season yields, but has the disadvantage of high cost.

The effects of different land preparation schemes are being studied to aid in the selection of the best system for water and weed control, and to enhance information on the feasibility of using Typha to reclaim mined peatlands. Excavation and rotovation are the two major land preparations being investigated. The possibilities of chemical weed control are also being examined.

Weed control and water control are linked in that appropriate water levels during stand establishment limit the types of weeds which will grow. Different methods of land preparation will affect both the water regime and the weed concentration in a certain area. Potential advantages of excavation are

- reduction in the amount of water used for irrigation because of closer proximity to ground water;
- elimination of dike maintenance;
- reduction in competitors resulting from removal of the surface seed bed;
- and possible utilization of the excavated peat.

Considerable interest has developed in recent years in the mining of peatlands for energy production. This activity, if developed, will occur in a small but significant portion of the total wetland resource base if Department of Energy standards for fuel grade peat are followed (6). If mining does occur, biomass production appears to be an attractive method of reclamation. Since excavated peatlands would be too wet for most terrestrial plants unless extensive drainage systems were developed, emergent aquatic species appear to be particularly appropriate for biomass production.

To determine the relative effectiveness of different planting material for Typha stand establishment, trial plots started with seed, seedlings and rhizomes were compared. Stands established with rhizomes appear to be the most consistent and productive under a wide variety of conditions. Preliminary results indicate that stand establishment with seed results in relatively low first-season yields; mature stands could probably be established within 2-3 years. Transplanting greenhouse-grown seedlings appears promising and may be particularly appropriate for establishing cutting beds of superior genotypes.

To test the feasibility of growing wetland crops on mined peatlands, two areas were excavated by removing 0.6m and 1.5m of peat (Figure 2). For comparative purposes, a third area was simply rotovated. Typha stands were successfully established on both excavated areas. After one growing season the most significant difference was in competitor control (Table 1). Removing the seed bank by excavating is an effective, though costly, weed control method. The intermediate excavation (0.6m) required the least amount of water control. Preliminary data indicate that when peat is removed nutrients may be less available under flooded conditions. Land preparation can have an effect on first season yields by altering the competitor seed bank, the availability of water, and nutrient availability.

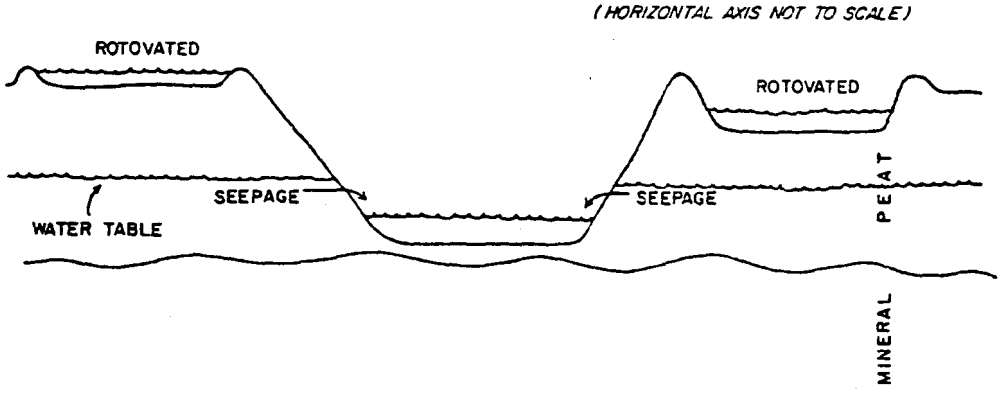


Figure 2. EXCAVATION SCHEME - ZIM, MINNESOTA

Table 1. TYPHA EXCAVATION STUDY AT ZIM, MN
ANALYSIS OF VARIANCE SUMMARY
COMPARISON OF PLANTING MATERIAL

Excavation Depth (m)	Planting Material	Dry Weight (MT/HA) AG	BG	TTL	Competitor	Shoot Density (Per m^2)	CLD** Percent
0.0	Rhizome	2.7 *	3.4 *	6.2 *	3.1	25	35
0.0	Seedling	0.4	0.5	0.9	4.0	19	62
0.6	Rhizome	4.0 *	5.2 *	9.2 *	0.0	33	40
0.6	Seedling	2.1	3.2	5.3	0.0	42	35
1.5	Rhizome	3.8	4.9	8.6	0.1	27 *	18
1.5	Seedling	3.3	4.8	8.1	0.0	52	18

AG = aboveground BG = belowground TTL = total
*Significant difference at $\alpha = 0.05$.
**CLD = insect-caused central leaf damage.
Note: Values represent means of four plots. Planting dates were May 29,30 (0.0,0.6m) and June 3 (1.5m).

TYPHA SPP. NUTRIENT EXPERIMENTS

The selection of cattail (Typha spp.) as a potential bio-energy crop was based on a number of factors described in the introduction. The high productivity of these plants in natural stands coupled with the low opportunity costs of land which could be used to grow them has led to research into methods of production in newly established, managed stands. The ultimate goal is to determine a management system which maximizes yield and minimizes inputs resulting in an energy resource that is economically competitive with other renewable and non-renewable energy resources.

One component of production costs which could significantly affect the final cost of this resource is that of nutrients required to attain high yields. In a natural system, nutrient recycling greatly reduces the need for additional nutrient inputs. In a bio-energy production system, nutrients are removed from the system when the biomass is harvested. High, sustained yields require that these nutrients be replaced. This can be accomplished to varying degrees by natural biological and physical processes and by application of fertilizers. The actual amounts and types of nutrients which need to be replaced through fertilizer application will depend on how much is replaced by natural processes and the overall nutrient requirements of Typha.

In agronomic crops, nitrogen, phosphorus, and potassium are the macronutrients of major concern because of their cost and effect on yield. This will, no doubt, also be the case for Typha production. In addition to these macronutrients, other macro- and micronutrients could prove to be limiting, especially in the anaerobic organic soils where cattails will be grown. Copper is an example of a micronutrient often found limiting in organic soil (7).

The need, then, exists to determine the following information for Typha in a field situation:

- The nutrient requirements needed to achieve high yields
- How nutrients affect yield and the partitioning of yield into above and belowground components
- How to minimize the nutrient input by avoiding unnecessary losses of fertilizer through luxury consumption or denitrification.
- What amount of nutrients are removed from the system during harvest.

It is important to note that nutrients are only one component affecting yield. Planting stock, density, competition from weeds, and other factors including temperature, soil conditions, and insects all influence yield. It is also important to note that results presented here are first-season results from which conclusions must be interpreted cautiously.

Methods

A 0.5 ha paddy with a well-decomposed organic soil was used for the study. Prior to use by this project, the land was uncultivated.

The experimental design was a blocked complete factorial experiment with three levels of nitrogen (0, 75 and 150 kg/ha), two levels of phosphorus (0 and 150 kg/ha), and two levels of potassium (0 and 300 kg/ha). The nitrogen was applied as urea to slow down the denitrification process, and all fertilizers were incorporated to a depth of 10-15cm.

Commercially obtained planting stock consisting of rhizome pieces and shoot bases was used to plant each of 48 3m x 5m plots at a density of $9/m^2$. Planting occurred in mid-May and replanting portions of ea[illegible]ot occurred in mid-June. Replanting was necessary because of the low surviva[illegible]te of initial planting stock.

The field was flooded to an average depth of [illegible]m during the growing season.

Results and Discussion

Results showing average first-season dry weights and shoot densities for each of the twelve fertilizer treatments are shown in Table 2. The range of dry weights and shoot densities is also shown. Shoot densities ranged from 11 to $48/m^2$; dry weights ranged from 1.5 to 14.5 mt/ha. An analysis of variance which was run on this data indicates that some of the differences in yield and density can be attributed to fertilizer treatment. A summary of this analysis is shown in Table 3. Although there is a trend toward increased yields and densities with increasing

Table 2. TYPHA FERTILIZATION STUDY

Fertilizer Rate (KG/HA)			Shoot Density (Per m^2)		Dry Weight (MT/HA)					
					Aboveground		Belowground		Total	
N	P	K	Mean	Range	Mean	Range	Mean	Range	Mean	Range
0	0	0	22	14-28	2.71	1.38-4.79	3.19	1.59-5.40	5.90	2.97-10.19
75	0	0	21	17-25	2.97	1.89-4.02	3.33	2.48-4.72	6.30	4.37- 7.56
150	0	0	24	15-29	3.12	0.90-5.14	3.07	0.64-5.52	6.19	1.54-10.66
0	150	0	25	11-44	2.87	0.99-6.65	3.44	1.29-7.36	6.31	2.28-14.01
75	150	0	34	28-40	4.83	3.17-5.95	5.29	2.52-7.91	10.12	5.69-13.86
150	150	0	28	20-34	3.87	1.76-5.55	4.06	2.03-6.58	7.93	3.79-12.13
0	0	300	30	24-37	4.52	2.62-6.14	4.32	3.11-6.52	8.84	5.73-12.58
75	0	300	25	21-32	2.56	1.27-3.67	3.31	1.92-4.78	5.87	2.19- 8.35
150	0	300	32	26-39	5.35	4.52-6.52	5.49	3.85-7.29	10.83	8.52-13.81
0	150	300	26	15-36	3.05	1.45-4.55	3.59	2.00-4.94	6.64	3.45- 9.49
75	150	300	34	20-48	4.40	1.64-6.70	5.04	2.34-7.76	9.44	3.98-14.46
150	150	300	32	25-41	3.99	3.00-5.34	4.64	3.30-6.10	8.62	6.30-10.37

Table 3. TYPHA FERTILIZATION STUDY
ANALYSIS OF VARIANCE SUMMARY

Fertilizer Main Effect	Rate (KG/HA)	Mean Shoot Density (Per m^2)	Mean Dry Weight (MT/HA) AG	BG	TTL
Nitrogen	0	26	3.28	3.64	6.92
Nitrogen	75	28	3.69	4.24	7.93
Nitrogen	150	29	4.08	4.31	8.39
Phosphorus	0	25 *	3.54	3.78	7.32
Phosphorus	150	30 *	3.83	4.34	8.18
Potassium	0	26 *	3.39	3.73 *	7.12
Potassium	300	30 *	3.98	4.40 *	8.37
Combined	150-150-300	32	3.99	4.64	8.62

AG = aboveground BG = belowground TTL = total

fertilizer rates, this trend is statistically significant only in the cases of phosphorus and density, potassium and density, and potassium and belowground dry weight.

Partitioning of biomass into above and belowground portions of the plant was unaffected by fertilizer treatment. The percent of total dry weight found in the aboveground portion averaged 47% overall.

In order to explain the large variability in yield which, for the most part, was unexplained by fertilizer treatment, several other factors affecting yield were analyzed. An analysis of variance was run examining the effect of fertilizer treatment on the dependent variables affecting yield. The significance of the treatment and overall means of the difference variables are shown in Table 4. Relationships between these variables and yield and other factors affecting yield were examined using correlations.

Survival rate as measured by the number of plants replanted in June or the densities in July was on average quite low and highly variable. A strong correlation exists between the July density and both final density and final yield indicating that the factor or factors affecting survival rate also strongly influenced final yield. From Table 4, it can be seen that an average of half the plants were replanted in each plot.

It can also be seen that fertilizer treatment had no effect on the number replanted or the density in July. Correlations between water levels in each plot,

Table 4. SIGNIFICANCE OF FERTILIZER TREATMENT ON VARIABLES AFFECTING FINAL YIELD

Dependent Variable	Fertilizer Treatment			Overall Mean
	Nitrogen	Phosphorus	Potassium	
Survival Rate and Density				
Number Replanted	-	-	-	4.5/m^2
July Density	-	-	-	10.7/m^2
Final Density	-	*	*	27.7/m^2
Density Increase	-	*	-	17.0/m^2
Competitors				
Competitor Dry Weight	-	-	-	132 g/m^2
Tissue Nutrient Concentrations				
Aboveground Nitrogen	-	-	-	15,500 ppm
Belowground Nitrogen	-	-	*	17,300 ppm
Aboveground Phosphorus	-	*	-	2,040 ppm
Belowground Phosphorus	-	**	-	3,750 ppm
Aboveground Potassium	-	-	**	12,000 ppm
Belowground Potassium	-	-	**	15,600 ppm

*Significant difference at $\alpha = 0.05$
**Significant difference at $\alpha = 0.01$
-No significant difference

the number replanted and the density in July showed no relationship. There is, however, reason to suspect that the planting stock used resulted in a low survival rate. The quality of the commercially obtained stock was highly variable in terms of size and number of viable buds and shoots. Also, yields from plots planted with this material in other experiments were significantly lower than those from plots planted with other stock (see section on Wetland Species Comparisons and Micropropagation).

The effect of fertilizer treatment on density was previously mentioned. Both phosphorus and potassium applications resulted in a statistically significant 20% increase in shoot density (Table 3). Although final density is strongly correlated with yield in this experiment, it does not appear that this density increase resulted in statistically significant higher yields except, possibly, in the case of belowground dry weight which was significantly higher in plots where potassium was applied.

Fertilizer treatment had no effect on competitor dry weight. Competitor dry weights were relatively high compared with Typha yields which may be the result of the slow early development of the cattail plants. After the cattail plants began growing vigorously in mid-season, the spread of competitors appeared to be controlled.

Application of phosphorus and potassium significantly and, in some cases, dramatically increased tissue concentrations of these nutrients. In the case of potassium, aboveground concentrations increased from 7,020 ppm to 16,900 ppm and belowground concentrations increased from 12,500 ppm to 18,800 ppm with the application of 300 kg/ha of potassium. Application of 150 kg/ha of phosphorus resulted in increases of above and belowground concentration from 1,910 ppm to 2,160 ppm and 3,630 ppm to 3,880 ppm, respectively. Nitrogen application did not result in increased tissue nitrogen concentrations which were statistically significant.

Although tissue phosphorus and potassium concentrations were increased by the application of fertilizer, there is either no correlation between concentration and yield or a negative correlation indicating that tissue nutrient concentration of phosphorus and potassium was not the limiting growth factor. Tissue nitrogen concentration also shows a negative correlation with yield. It appears based on tissue nutrient comparisons and soil results that nutrient availability was quite high to begin with in all plots, and applied fertilizer resulted in luxury consumption by the plants. This negated the effect of the treatment on yield in the first season, but may be extremely important in the second season since Typha stores nutrients in its rhizome system.

To ensure that micronutrients were not limiting in this experiment, they were applied to the field at the beginning of the season. Results of tissue micronutrient analysis indicate that these nutrients were not limiting, and results of correlations indicate no relationship between productivity and micronutrient concentration.

After examining these factors which may have influenced yield, it is difficult to explain the large variability in yield. Problems related to establishing the stand appear to have had the greatest impact on first season productivity.

In addition to the factors affecting first season productivity discussed above, other factors will affect second season yields. At the start of the second growing season, an established root system plus reserves of carbohydrates and nutrients in the rhizome system should result in rapid early season growth. Differences in nutrient reserves and nutrient availability attributed to first season fertilizer treatment could result in significantly higher yields in the second season.

Based on analysis of nutrient standing crop (defined as grams of nutrient per m^2) and fall soil nutrient levels, it appears that the fertilizer treatment will carry over into the second season. Figure 3 shows the nutrient standing crop for nitrogen, phosphorus, and potassium at the end of the first growing season. With the exception of aboveground nitrogen amounts, all increases resulting from fertilizer treatment are statistically significant. Figure 4 shows the available phosphorus and potassium levels in the soil at the end of the first season. Fertilizer application resulted in statistically significant increases in soil fertility. Based on these results, it is reasonable to expect that second season yields and densities will be affected by the nutrient treatment.

Conclusions

While conclusions are difficult to arrive at for field experiments after only one year of research, the following tentative conclusions can be reached:

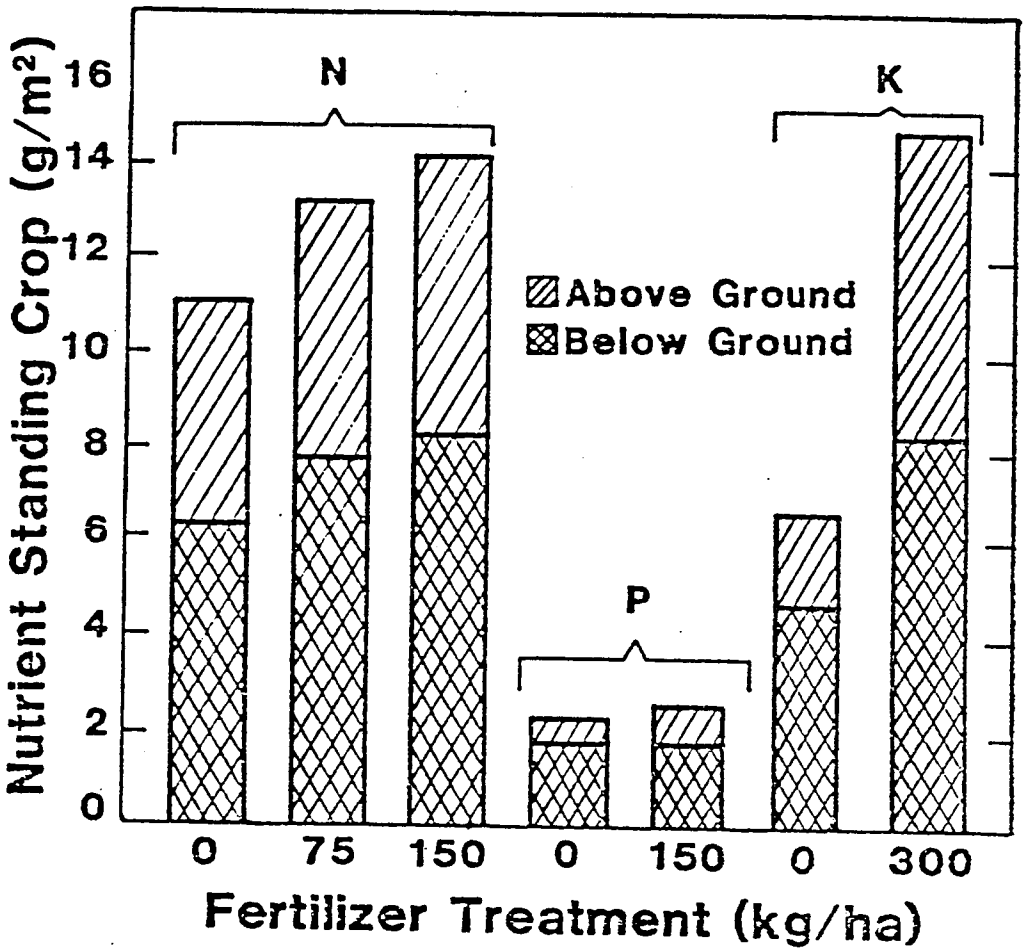


Figure 3. NUTRIENT STANDING CROP AT THE END OF THE FIRST GROWING SEASON

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CULTIVATION OF MACROSCOPIC MARINE ALGAE

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ANNUAL YIELD OF GRACILARIA IN MASS SUSPENDED CULTURE

Gracilaria tikvahiae was grown outdoors in a 24,000-L (28.7-m^2) aluminum tank for one complete year beginning 16 March 1981. The culture was continuously aerated, maintaining the seaweed in suspension, and was provided with 3.8 exchanges of seawater per day. The seaweed was completely removed from the tank every one to three weeks--depending upon season and growth rate--weighed, and incremental growth removed. The starting biomass of 86.2 kg (3 kg wet wt./m^2) was then soaked for 24 h in a 3800-l aerated nutrient solution containing 5000 μm NH_4^+-N, 500 μm $PO_4^{\equiv}$-P, and a chelated iron/trace metal mixture before being returned to the culture tank. That tank was cleaned while the Gracilaria was enriched in a separate tank.

Mean daily yields for the growth periods between each harvest are shown in Table 1. Mean yield for the complete year was 25.4 g dry wt./m^2 day, equivalent to 82 dry tons/ha yr (48 ash-free dry tons/ha yr). Note that the annual total includes the 40 days the culture was out of production during enrichment (i.e., one day every one to three weeks).

This is the first time that sustained yields have been monitored throughout the year for any seaweed in a large, mass culture system. They are essentially the same as those reported earlier for Gracilaria grown in smaller, 2000-L (2.4-m^2) aluminum tanks under similar conditions of aeration and water exchange, but with nutrients added continuously. The larger-scale study demonstrates that there is no adverse scaling factor in that culture system and that the concept of intermittent or pulse feeding of nutrients is a viable method for sustained culture.

Table 1. YIELDS OF *GRACILARIA* IN 24,000-L ALUMINUM TANK WITH CONTINUOUS AERATION, 3.8 EXCHANGES OF SEAWATER PER DAY, AND PULSE-ENRICHMENT FOR 24 H AT THE END OF EACH GROWTH INTERVAL

Starting Date	Days[a]	Yield (g dw/m^2 day)
3/16/81	—	--
3/26	9	23.4
4/6	10	35.1
4/20	13	32.4
5/4	13	30.2
5/18	13	22.7
6/1	13	22.2
6/8	6	32.1
6/15	6	32.3
6/22	6	42.2
6/29	6	39.6
7/6	6	37.7
7/13	6	30.6
7/20	6	27.8
7/27	6	34.2
8/3	6	34.2
8/10	6	37.2
8/24	13	27.8
8/31	6	21.9
9/8	7	15.7
9/14	5	18.8
9/21	6	16.0
9/28	6	16.8
10/5	6	21.5
10/12	6	29.6
10/19	6	35.7
10/26	6	32.7
11/2	6	28.1
11/9	6	20.4
11/16	6	22.6
11/23	6	15.8
11/30	6	19.3
12/7	6	19.4
12/14	6	6.6
1/5/82	21	19.9
1/18	12	8.7
2/1	13	21.9
2/16	14	25.3
3/2	13	26.5
3/15	12	25.6
Total days:	325	Mean yield: 25.4

[a]Does not include days seaweed was soaked in enrichment solution in the dark.

These data will be used in the final economic and energy evaluation of different seaweed culture systems.

RECYCLING OF DIGESTER RESIDUE AS A NUTRIENT SOURCE FOR GRACILARIA MASS CULTURE

A second 24,000-L aluminum tank culture of Gracilaria was grown from 2 November 1981 to 17 March 1981 under the same conditions as described previously. The harvest from this culture was digested anaerobically and the starting culture was enriched, after removing incremental growth, in the liquid digester residue rather than in an inorganic nutrient solution. The nitrogen concentration in the digester residue was adjusted to 5000 µm by dilution with well water or by addition of NH_4Cl. Yields of Gracilaria grown in this way were compared with those obtained for the same period in the inorganic nutrient-enriched culture described above (Table 2). Yields for the two were very nearly the same; the slightly lower mean yield in the residue-enriched culture was attributable to toxic levels of some unidentified ingredient of the residue that occasionally burned the growing tips of the Gracilaria, significantly reducing yields during the ensuing growth period (e.g., those starting 5 November and 6 January). The nature and sporadic occurrence of that toxicity is not known but could perhaps be avoided by greater dilution of the residue.

SPRAY CULTURE

Ascophyllum nodosum was collected from intertidal rocks in Falmouth, Mass., on 1 May 1981 and was grown outdoors in perforated, polypropylene Nestier® trays that were originally designed for oyster culture. The plants were sprayed with unenriched seawater with commercial garden hose nozzles at rates that ranged from 0.5 to 0.35 and averaged 0.20 mL/cm^2 min. The seawater, taken from Vineyard Sound, Mass., was pumped to the Woods Hole Oceanographic Institutions Environmental Systems Laboratory seawater system. Before 1 June and after 15 September, it was heated by means of an impervious carbon heat exchanger system to 20° C. At other times, ambient seawater temperature was used.

Once a week, the seaweed was placed in cloth mesh bags and immersed for 24 h in an aerated, 500-L nutrient solution containing 1000 µm NO_3-N, 100 µm $PO_4^{\equiv}$-P, and 1000 µ g/L Fe-EDTA with other trace elements. The iron/trace metal mixture was the commercial preparation Orthogreenol®.

Initial stocking density was approximately 3.0 kg wet wt./m^2. Each week before enrichment, the Ascophyllum was drained and weighed. A sample of the alga was oven-dried at 80° C for 24 h to obtain the relationship between wet and dry weight and thereby to permit yields to be expressed in units of dry weight. On 25 August, the algae in each tray was harvested back to a density of approximately 3.0 kg wet wt./m^2, and a new series of experiments was started.

Table 2. YIELDS OF GRACILARIA IN 24,000-L CULTURES ENRICHED WITH INORGANIC MEDIUM AND DIGESTER RESIDUE

Starting Date	Days	Mean Yield (g dry wt./m^2 day)[a]	
		Medium	Residue
10/26	6	32.7	
10/29	6		22.6
11/2	6	28.1	
11/5	7		5.4
11/9	6	20.4	
11/11	7		23.2
11/16	6	22.6	
11/18	6		12.5
11/23	6	15.8	
11/24	8		22.3
11/30	6	19.3	
12/2	7		21.1
12/7	6	19.4	
12/9	7		8.9
12/14	6	6.6	
12/15	21		29.1
1/5	21	19.9	
1/6	14		1.4
1/18	12	8.7	
1/23	15		25.8
2/1	13	21.9	
2/5	12		20.8
2/16	14	25.3	
2/18	12		12.8
3/2	13	26.5	
3/3	14		26.1
x		20.5	17.8

[a]Does not include days seaweed was soaked in enrichment solution in the dark.

Growth of Ascophyllum was tested with several different spray schedules: (A) continuous spray, (B) spray off from 2100 to 0500 h, (C) spray off for one hour at 0400, 0800, 1200, 1600, 2000, and 2400 h, (D) spray off for 4 h at 1000 and 2200 h. Three Nestier trays with a total area of 0.94 m^2 were used for each treatment and the weight of seaweed in each group of three trays was lumped to calculate growth (% increase/day) and yield (g dry wt./m^2/day), respectively.

Experiments were started on 9 May, but because of dehiscence and subsequent loss of reproductive receptacles, weight losses were initially recorded in all trays, obscuring growth of the algae. This weight loss normally occurs as the plants pass the peak of their reproductive cycle in spring. A net increase in

weight was first noted near the end of May and growth data were recorded then until mid-October, when the experiments were concluded.

After the reproductive cycle ended in mid-June, the plants all sprouted numerous adventitious vegetative branches. Presumably because of thallus detachment from the substrate and loss of orientation, the branches were mostly lateral with only a few apical and irregularly pinnate, this being characteristic of the morphological variants ecad mackaii and ecad scorpioides. In September, the plants began to form reproductive receptacles again. Unlike natural attached populations of Ascophyllum, receptacles of the experimental plants occurred less frequently, and were ovate, obovate, or irregular in shape. These irregularities are also characteristic of unattached Ascophyllum ecads.

Ascophyllum remained healthy and grew in all treatments except the series in which the seawater spray was stopped for four hours during both day and night. The algae in those trays lost weight from the outset and died by mid-June, apparently unable to withstand four hours of desiccation at mid-day. In natural environments Ascophyllum plants are protected from excessive desiccation at low tide by either a canopy of Spartina or by settling into tide pools, under rocks, or in mud. Our plants were grown in suspended perforated Nestier trays, which allowed drying air to circulate around them, thereby desiccating the Ascophyllum beyond natural levels.

Weight increases resulting from the other three treatments are shown separately for the May-August and August-October experiments in Figure 1, and calculated specific growth rates and yields are given in Table 3. Yields in

Table 3. EFFECTS OF DIFFERENT SPRAY REGIMES ON THE GROWTH AND YIELD OF ASCOPHYLLUM

Regime[a]	Dates	Biomass (kg.w.w.) Start	Biomass (kg.w.w.) End	Growth[b] (% day^{-1})	Yield[b] (gdwm^{-2} d^{-1})
A	5/29-8/18	3.05	5.98	1.2	14.3
	8/25-10/13	3.53	5.00	0.7	10.5
B	6/8-8/13	3.30	5.23	1.0	8.8
	8/25-9/1	3.43	5.44	0.9	12.6
C	5/29-8/18	2.24	3.58	0.7	6.0
	8/25-9/1	3.45	3.74	1.2	13.2
D	6/8-6/19	weight loss followed by mortality			

[a] A = continuous spray.
B = spray off at night (2100-0500 h).
C = spray off one hour at 0400, 0800, 1200, 1600, 2000, and 2400 h.
D = spray off four hours at 1000 and 2200 h.

[b] Means of observations every 1-2 weeks corresponding to points in Figure 1.

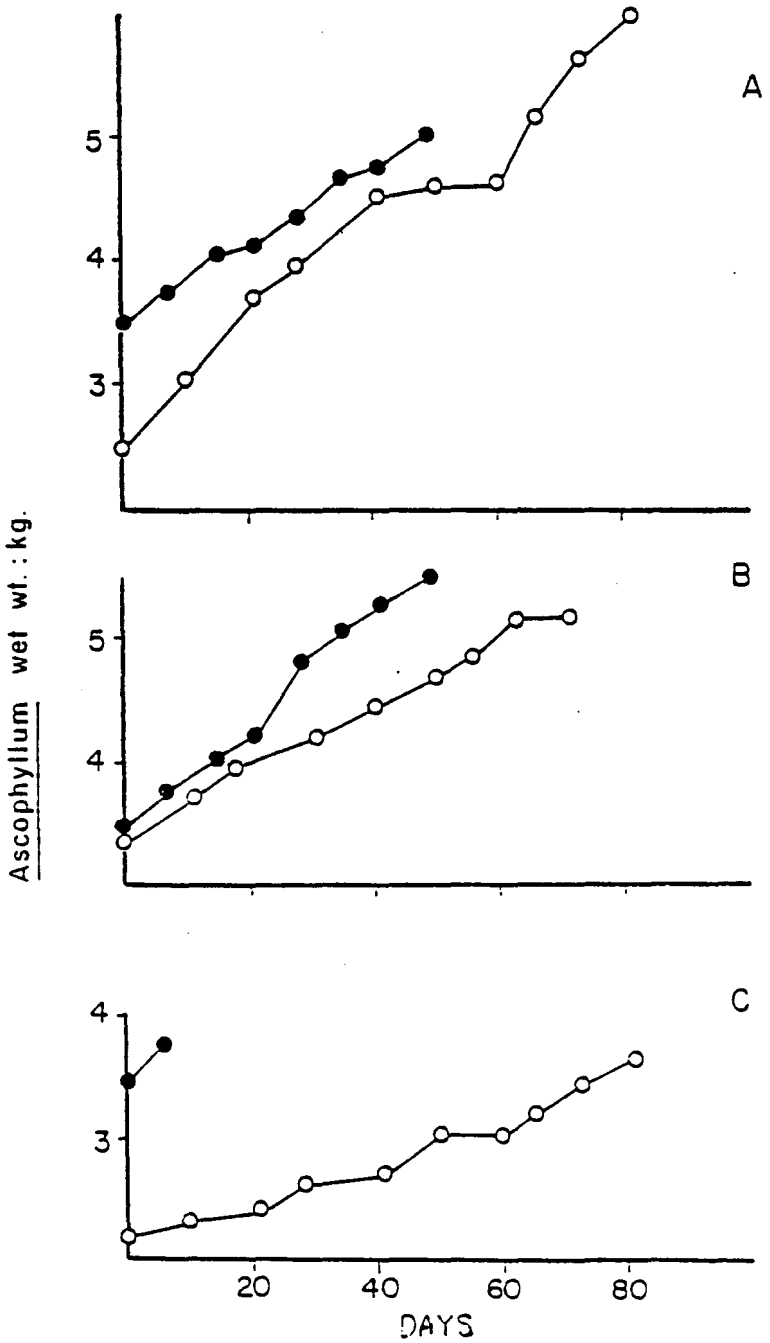

Figure 1. Ascophyllum Nodosum Weight Increase with Time (When Grown in Spray Culture with (A) Continuous Spray, (B) With Spray Off from 2100 to 0500H, (C) With Spray Off for One Hour at 0400, 0800, 1200, 1600, 2000, and 2400 H. Open Circles, Experiments Started 5/29 or 6/8. Closed Circles, Experiments Started 8/25.)

Treatment C (spray off one hour six times a day) were lower than the others, primarily because the experiment started with a lower biomass (2.24 kg versus 3.0 kg in the others), yield being a function of both biomass and growth rate. The other half of that experiment was unfortunately terminated after one week because of technical problems. Otherwise, growth and yield were as variable within as between treatments, indicating, as might be expected, that this intertidal alga does not need continuous spraying to survive and grow, an important economic consideration if the technique were to be used in commercial cultivation.

The mean yield of about 12 g dry wt./m^2/day (excluding the low-density yields of Treatment C, discussed earlier), over a six-month growing season in New England would be equivalent to an annual production of about 22 dry metric tons/ha.

Spray cultures of Gracilaria and Ulva were initiated at Harbor Branch Foundation in January 1982, growing both species of algae in the same Nestier trays that were successfully used in the studies with Ascophyllum. Several different commercial spray heads were employed, producing spray comparable to that obtained in the earlier Ascophyllum studies with garden hose nozzles ranging to a fine mist and a still finer fog of seawater. The algae were enriched by weekly soaking in a concentrated nutrient solution, thereby avoiding the growth of epiphytes on the target species. Although the results for the first few weeks were encouraging, both species began to sunburn and bleach in February and all cultures were dead by March.

COMPARATIVE METHANOGENESIS BY THREE SPECIES OF SEAWEEDS

Three species of seaweeds were examined for their digestion properties. These included the rhodophyte, Gracilaria tikvahiae; the chlorophyte, Ulva sp.; and the floating pelagic phaeophyte, Sargassum fluitans. Gracilaria and Ulva were grown at the Harbor Branch Foundation's aquaculture facility, while Sargassum was collected from the Ft. Pierce, Fla., beaches. The Gracilaria digester consisted of 1 kg wet wt. of chopped (2-3 cm lengths) seaweed, 0.8 L of seawater, and 0.2 L of inoculum. For the Sargassum digesters, 1 kg wet wt. of shredded Sargassum (about 0.5 cm pieces) was used with 0.5 L of seawater, and 0.2 L of inoculum. The Ulva digesters initially contained 1 kg wet wt. of chopped seaweed (pieces 2-3 cm on a side), 0.7 L of seawater, and 0.2 L of inoculum. However, because of acid difficulties during startup, all but one Ulva digester was restarted, with 0.5 kg of seaweed, 1.2 L of seawater, and the same dosage of inoculum. A previously described 120-L digester, restarted with 20 kg of anaerobic sediments and 10 kg of Gracilaria, provided the inoculum source in each case.

The digesters consisted of sealed 2-L Nalgene® bottles, placed in boxes filled with poured-foam insulation. A gas line made from Nalgene tubing ran from each digester to an inverted 1-L graduated cylinder. The cylinders were filled with a 0.05N sulfuric acid solution, and were placed in a tub containing approximately 4 cm of the acid solution. Gas production was monitored by displacement of the acid solution. The digesters were housed in an insulated building; its temperature was maintained at 28° C ±3° by means of heat lamps.

Gracilaria and Sargassum digesters were loaded at rates corresponding to 20-, 30-, 40-, or 50-day retention times, while the Ulva digesters were operated at 30-, 40-, or 50-day retention times. Before the experiment, the seaweed of each species was prepackaged in 100-g wet wt. packets and frozen to minimize possible effects of using different batches of seaweed. Unfortunately, not enough Sargassum could be collected at once, so two batches of this seaweed were necessary. The digesters were loaded according to one of two schedules by adding either 10% or 20% of the wet weight of seaweed in the digester at each loading. The equal amount of digester residue removed during loading contained both solid and liquid residue phases. The digesters were agitated only during loading. At this time, a digester's contents received a 10-25-sec stirring. Since the 10% exchange digesters were loaded twice as often as their 20% counterparts, the latter set of digesters was swirled for the same amount of time when the 10% digesters were loaded. The data reported here represent a ten week period commencing four to six weeks after initiating differential loading.

Gas samples and digester residue samples were taken either at each loading, or on alternate loadings, depending upon a digester's retention time and loading schedule. The digester residue samples were separated into solid and liquid phases by filtering through a 0.5-mm mesh. Determination of the volatile solids content of plants or solid digester residues consisted of drying samples for four days at 70° C, followed by measurement of weight loss after combustion at 550° C in a muffle furnace for four hours. Biogas production was measured daily; gas samples were analyzed for methane content using an MSA total hydrocarbon analyzer standardized against known amounts of methane. All gas production numbers have been corrected to standard conditions of temperature and pressure.

Pertinent information about each species of seaweed tested is presented in Table 4. Most importantly, Sargassum contains the greatest amount of volatile solids per unit of wet weight, followed by Ulva, then Gracilaria. Energy content per unit wet weight is also highest in Sargassum, lower by 15% in both Gracilaria and Ulva.

Initially, within two days following inoculation, all of the digesters displayed a significant drop in pH. Ulva reacted the most drastically, dropping from 7.2-7.3 at inoculation to 5.8-5.9. Gracilaria and Sargassum reacted

Table 4. COMPOSITION AND ENERGY CONTENT OF SEAWEEDS

Organism	Dry Wt.	Volatile Solids		Nitrogen	Energy
	(% wet wt.)	(% wet wt.)	(% dry wt.)	(% dry wt.)	(C/g VS)
Sargassum	13.4	8.4	62.8	1.04	4.2
Gracilaria	10.8	6.6	60.9	2.07	4.5
Ulva	11.6	6.9	59.7	2.35	4.3

similarly, with the pH of Gracilaria digesters falling to 6.1-6.2, while Sargassum digesters dropped to 6.2-6.3. These pH changes were countered by titrating the digesters back to the neutral range with sodium hydroxide. The digesters required daily titration for a period of 8 to 10 days before the pH stabilized at 6.7-6.8. Titration with alkali proved to be a more effective method of pH control than the addition of carbonate buffers in these saltwater digesters.

Initially, there was concern that exchanging as much as 20% of the digester volume would shock or disrupt digester performance, either by disturbing the organisms or causing harmful pH fluctuations. However, those digesters showed similar characteristics to the ones in which 10% of the biomass was exchanged at each loading. There were, however, two noticeable trends. The 20%-loaded digesters characteristically combined a slightly higher methane content with a somewhat lower biogas production per gram of volatile solids added. However, T-tests between matched digesters indicated no difference at the α-0.05 level. Therefore, the data presented in Table 3 represent the combined 10% and 20% exchange results for each retention time. These results permit seaweed digesters to be run with only half the maintenance necessary if loading exchanges of 10% or less were required.

All three species of seaweed showed increased methanogenesis with increased retention time, but the methane output patterns were somewhat different for each species (Table 5). Sargassum maximized its methane production at the 30-to-40-day retention times. Gracilaria, on the other hand, did not attain high methane production until the 40-to-50-day retention times. Ulva displayed good methanogenesis for all three retention times tested. The greatly increased gas production of Ulva per gram of volatile solids added is particularly noticeable, especially at higher loading rates. The gas output of Sargassum slightly exceeded that of Gracilaria at higher loading rates, while Gracilaria's production eventually surpassed Sargassum's at longer retention times. Note also that yields of 60% or more of methane could be obtained from each species. With high gas production coupled to high methane content, Ulva displayed the best bioconversion efficiency.

Figure 2 portrays the trends in daily biogas and methane production at different retention times. For each species, the maximum daily biogas output occurs at the lowest retention time. However, under those conditions, methane content is quite low for Gracilaria, but is near 50% for both Sargassum and Ulva. These changes in methane content with retention time result in shifting optimization for daily methane production to longer retention times for Gracilaria. The daily methane output of Sargassum, on the other hand, is similar for 20-to-40-day retention times, because of fairly equal compensation between biogas production and methane content, but it drops off significantly at the 50-day retention time.

Of the three marine macrophytes tested, Ulva appears to be more readily digestible than Gracilaria or Sargassum. This is indicated by (a) the large initial pH drop of Ulva digesters, suggesting a rapid release of volatile organic acids; and (b) the gas produced per unit of volatile solids reduced. The latter reflects a higher amount of volatile solids destruction per unit of gas produced, most notably at shorter retention times. The relatively poor performance of Gracilaria at low retention times most probably results from

Table 5. CHARACTERISTICS AND EFFICIENCY OF SEAWEED METHANOGENESIS

Organism	Retention (days)	pH	Biogas Produced (l/g VS added)	Methane Produced			VS Reduced (%)	Energy Recovered (% VS as methane)
				(% Biogas)	(l/g VS added)	(l/g VS reduced)		
Sargassum	20	6.9	0.16	49.5	0.08	0.39	20.2	17.0
	30	7.0	0.19	58.5	0.11	0.42	27.4	24.5
	40	7.1	0.22	67.5	0.15	0.44	33.3	31.5
	50	7.2	0.25	57.4	0.14	0.34	40.4	30.6
Gracilaria	20	6.8	0.14	36.8	0.05	0.29	18.2	10.7
	30	6.9	0.16	39.5	0.06	0.30	21.2	12.8
	40	7.1	0.25	58.4	0.14	0.45	31.8	28.9
	50	7.2	0.30	62.7	0.19	0.48	39.4	38.3
Ulva	30	6.9	0.29	50.3	0.14	0.34	41.3	30.4
	40	7.0	0.34	59.0	0.20	0.39	50.4	41.7
	50	7.2	0.38	60.6	0.23	0.41	56.1	48.1

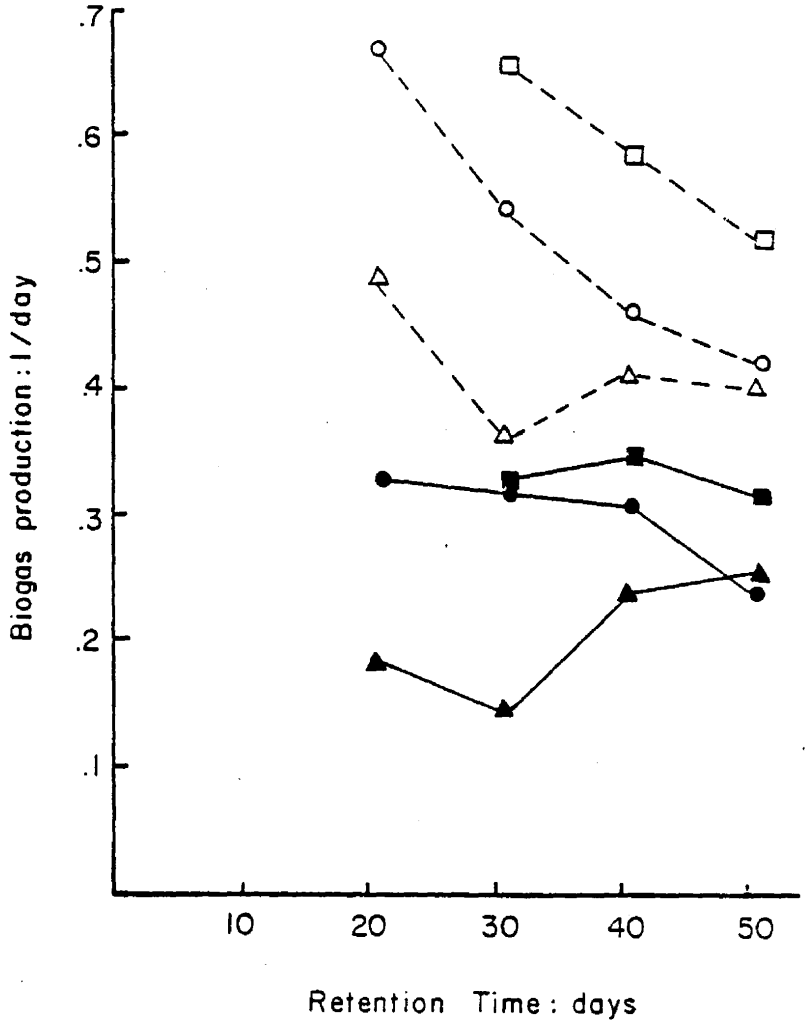

Figure 2. Daily Gas Production vs. Retention Time [From Anaerobic Digestion of the Seaweeds Sargassum (Circles), Gracilaria (Triangles) and Ulva (Squares). Open Symbols and Dashed Lines: Total Biogas. Closed Symbols and Solid Lines: Methane.]

initial resistance of agar to microbial attack, as we have previously found. However, the earlier studies also showed that, with time, agar becomes a suitable substrate for methanogenic fermentation. Those observations correlate well with the present performance of Gracilaria at longer retention times. The present results suggest that once initially resistant polysaccharides, such as agar, are partially degraded, they provide a good methanogenic substrate. The agar content of Gracilaria may be very important in determining optimum retention time.

Sargassum presents a different picture. This brown seaweed appears to contain some biochemical constituents that provide a relatively digestible methanogenic substrate, which accounts for the good performance at low retention times. However, at the longest retention time (50 days), Sargassum's performance falls off considerably, suggesting that a fairly resistant, poor substrate component, possibly a fiber fraction, must also be utilized as a methanogenic substrate. Sargassum does contain a larger fiber fraction and a smaller soluble carbohydrate fraction than either Gracilaria or Ulva (according to unpublished data). There is also the possibility that the Sargassum digesters became nutrient-limited, since the C/N ratio of the feed exceeded 20.

METHANOGENESIS AS A FUNCTION OF THE CELLULAR NITROGEN CONTENT OF SEAWEEDS

Gracilaria and Ulva were soaked for 24 h in a concentrated nutrient solution and then grown for different periods of time in unenriched seawater, producing a supply of each species that had been nutrient-starved to different degrees and with different chemical composition. The criterion for the latter was tissue nitrogen content.

The algae were then loaded into batch digesters at 500 g (wet wt.) to 1 L of seawater and 0.2 L of inoculum from an old digester. The digesters were operated at 32° C ±3° C.

The nitrogen content of the high-, medium-, and low-N plants of each species are shown below, in % dry wt.

	High N	Medium N	Low N
Gracilaria	2.8	2.8	1.4
Ulva	2.4	2.0	0.8

Methane production per gram of volatile solids added is shown in Figure 3.

The medium- and high-nitrogen Gracilaria, which were not very different in their nitrogen content, produced about the same amount of methane and were somewhat higher in this respect that the low-N plants, possibly reflecting the somewhat lower digestibility of agar, which is higher in the N-starved plants.

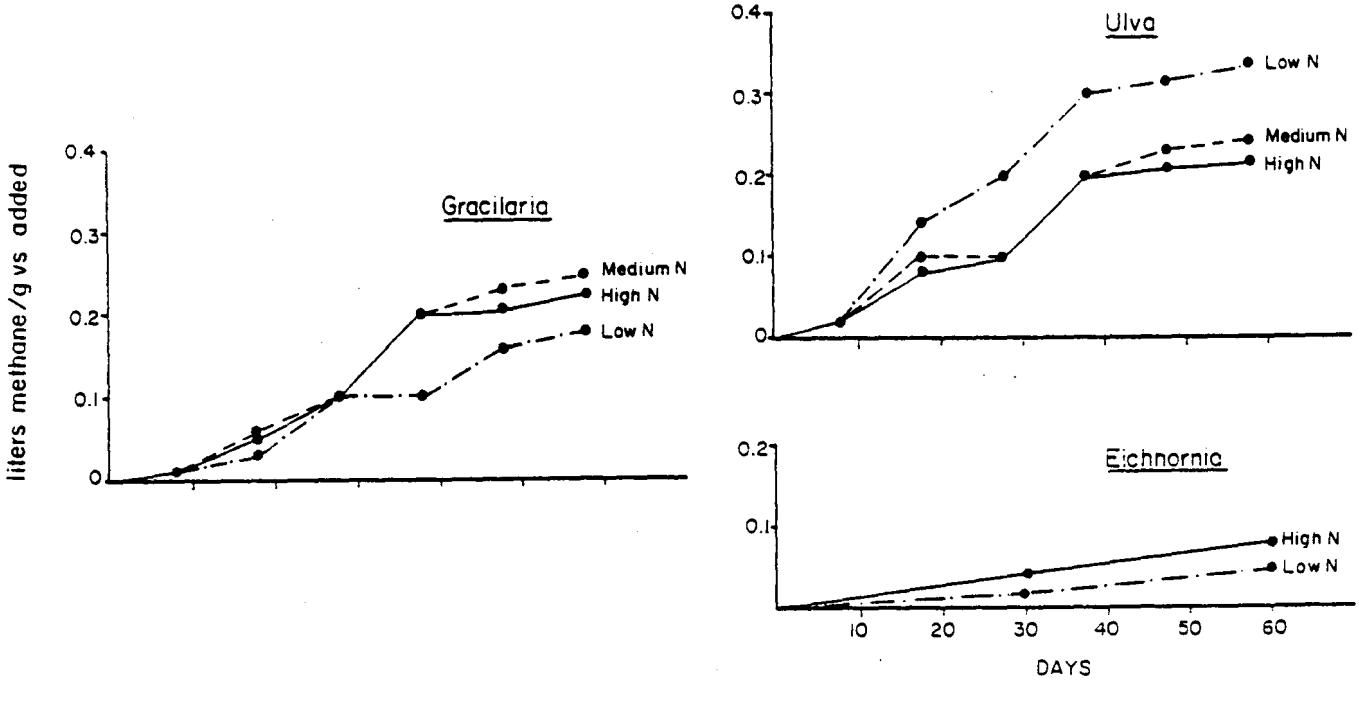

Figure 3. Methanogenesis of Aquatic Plants with Different Tissue Nitrogen Contents

The opposite effect was seen in Ulva, in which the low-N plants produced significantly more methane per unit of volatile solids added than did the medium- and high-N plants, which were, again, very similar. Presumably, this reflects the fact that the green algae produce starch and other relatively labile carbohydrate storage products when they become nitrogen-limited.

The opposite effect is seen in some old, unpublished data for the water hyacinth (Eichhornia crassipes), in which low-nitrogen (0.99%) plants produced significantly less methane than did the high-nitrogen (2.08%) plants. In that plant, as in many higher plant species, nitrogen deficiency leads to the elaboration and storage of structural, ligno-cellulosic carbohydrates that are resistant to bacterial digestion. Note that water hyacinths are, in general, a less successful substrate for methane production than are either of the seaweeds, though the experiments were not done simultaneously or with the same equipment, so the comparison may be invalid.

NITROGEN UPTAKE AND STORAGE BY ULVA LACTUCA

In our earlier studies, we found that when the red alga Gracilaria tikvahiae became nitrogen-starved and assumed a pale yellow, straw coloration, it was capable of rapidly assimilating ammonium-nitrogen from a concentrated solution (e.g., ~25-30 mg/L N), doubling its tissue nitrogen concentration in as little as six hours. Uptake of ammonia-N was more rapid than that of nitrate-N, and was initially the same in either light or dark, but persisted longer in the light, apparently requiring a newly produced photosynthetic product for continued conversion of the inorganic nitrogen to amino acids or some other form of organic nitrogen. The rapid uptake was clearly not associated with growth per se and could only be interpreted as nitrogen storage.

On the latter assumption, it was subsequently shown that Gracilaria that had been soaked in a concentrated nutrient solution and increased its tissue nitrogen content to 3.5%-4.0% of its dry weight could then be grown in unenriched seawater for periods of as much as two weeks without any further enrichment of any kind and at essentially the maximum growth rate that could be achieved under the given conditions of illumination, temperature, and other environmental variables. This discovery was significant because it meant that the seaweed could be grown in unenriched seawater, normally extremely impoverished of nutrients during most of the year in tropical to semitropical surface ocean water. Continuous or even pulsed enrichment of flowing culture systems is not only uneconomical and wasteful of nutrients, but also encourages the growth of epiphytes, usually filamentous green, brown, or red algae that have hitherto represented the most difficult and persistent problem in large-scale, commercial seaweed culture efforts.

With shifting emphasis in our program to the cultivation of the green alga Ulva lactuca, additional experiments were carried out to determine whether nutrient-deficient Ulva, too, was capable of the rapid assimilation and storage of ammonium-nitrogen. Five kilogram samples of N-starved Ulva (also a pale, straw-yellow color in contrast to the bright, apple green of well-fed plants and with a tissue-N content of about 1.0% dry weight), were placed in a 500-L cylindrical, clear fiberglass tank which was aerated sufficiently to keep the seaweed in suspension. The tank was located outdoors in full sunlight and the nitrogen content of the water and the plants was monitored for 30 h beginning at 0900. A replicate but somewhat smaller experiment (2.8 kg

in 2800 L) was carried out in a completely darkened container over the same period of time.

The results (Figure 4) show that Ulva behaves essentially the same as Gracilaria. Uptake of ammonium-N was initially rapid in both the light and dark, but slowed down in the dark after three hours, while it continued in the light until natural darkness occurred, at which time some of the stored ammonium was released back into the water. The following day, however, that and the remaining initial ammonium-N were again rapidly assimilated. Table 6 shows that the nitrogen removed from the water was, in fact, taken up by the Ulva, the plants doubling their tissue nitrogen content in six hours.

The experiment described here was repeated several times during the summer of 1981, each time with the same results as shown in Figure 4 and Table 6, but with uptake rates varying slightly as a function of the initial N-content of the seaweed.

YIELDS OF SEAWEEDS IN STAGNANT SEAWATER WITH THE ADDITION OF CO_2 AND BICARBONATE

A chronic problem in Gracilaria culture to date has been the need to exchange the seawater at least several times per day to obtain high yields. Experiments reported last year revealed that Gracilaria does not photosynthesize at pH above 9.0, presumably because there is very little free CO_2 available at that pH, and Gracilaria is believed to be unable to utilize bicarbonate directly as a carbon source. Since the pH of Gracilaria cultures receiving little or no exchange of seawater often exceeds 9.0 and frequently 10.0, poor growth under those conditions was believed to be the result of carbon limitation.

Ulva, on the other hand, was found to continue photosynthesis at higher pH levels than did Gracilaria and, therefore, was believed to be able to use bicarbonate as a carbon source, at least more successfully so than could Gracilaria. Ulva could presumably grow under more stagnant, high-pH environments.

Short-term photosynthesis measurements may not necessarily be directly translated in terms of growth, however, so additional growth studies have been carried out to investigate the phenomenon further. These were still in progress at the time of this writing, but preliminary results, described below, appear convincing.

Gracilaria was grown in 700 L (1.6-m^2) concrete tanks in aerated culture at a density of 3.0 kg wet wt./m^2. Once a week the complete culture was removed and weighed, and incremental growth was removed. The starting density was then soaked for 24 h in a concentrated, aerated nutrient solution (1000 µm NH_4-N, 100 µm $PO_4^{\equiv}$-P, Fe-EDTA, and trace metals) before being returned to the culture tank. Six replicate cultures were established. One received fine bubbles of CO_2 gas, one received concentrated HCl, and one received both HCl

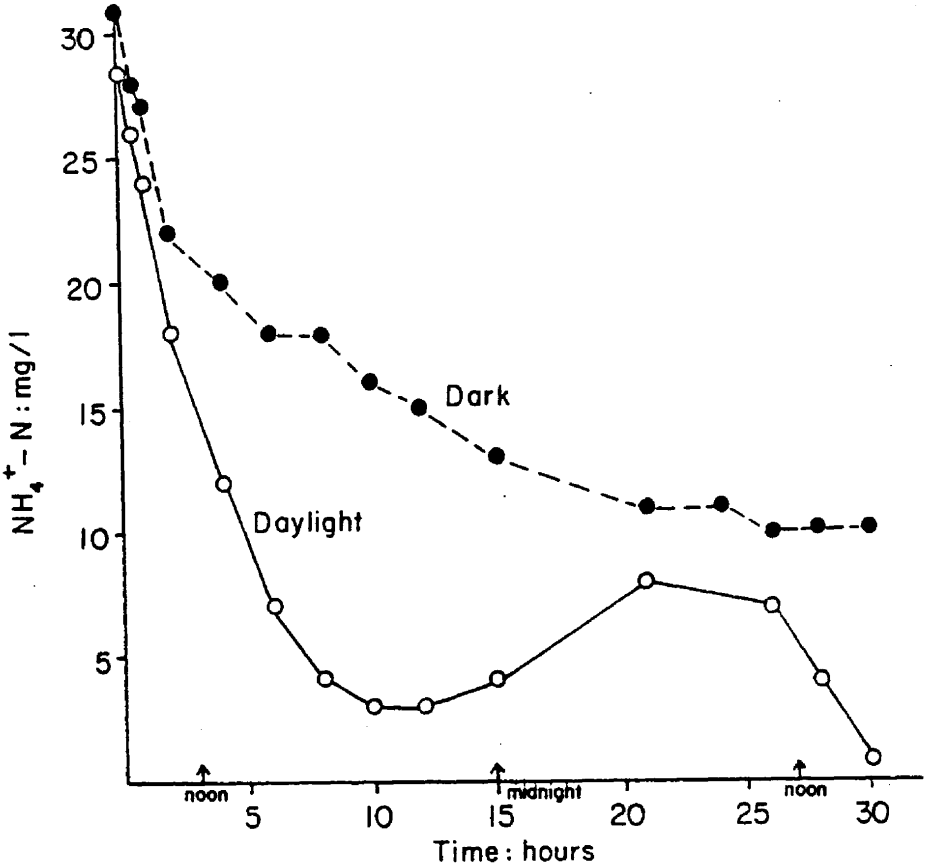

Figure 4. Uptake of Ammonium-Nitrogen by Nutrient-Deficient ULVA

and a $NaHCO_3$ solution. The respective additions of these ingredients was controlled by a Fisher Accumet Model 650 pH controller with a solenoid valve so as to hold pH in each tank at 8.0. One tank received only bicarbonate with an acid or pH control. One control received no additions. All five tanks were stagnant, with no exchange of seawater. A second control, with no additions, received eight exchanges of seawater per day. Yields were monitored weekly for two weeks and the results are shown in Figure 5.

The addition of CO_2 and of the acid-bicarbonate mixture, with pH held at 8.0, resulted in the same high yields as did the culture with eight exchanges of seawater per day, the first time that such high yields have been obtained in stagnant seawater. The addition of acid alone, controlling pH at 8.0, simply drove all the natural carbon in the seawater to CO_2 and bubbled it off to the air. Addition of bicarbonate at uncontrolled, high pH provided more carbon in an unusable form, although growth was slightly enhanced in that culture.

Table 6. NITROGEN REMOVED FROM WATER AND ASSIMILATED BY N-DEFICIENT ULVA

Hours	N Removed from Water (g)	N Assimilated by Ulva (g)	N-Content of Ulva (% dry wt.)
0	0	0	1.75
2	7.9	8.9	3.08
6	11.0	12.2	3.60
26	12.1	13.9	3.93

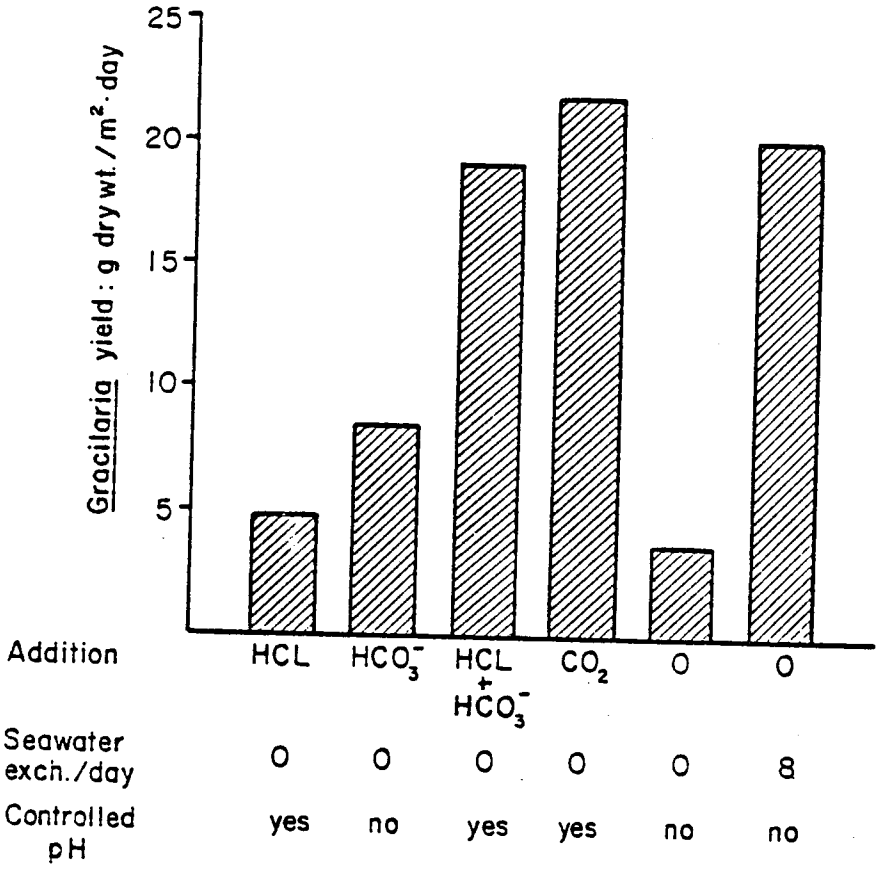

Figure 5. Growth of Gracilaria in Stagnant Seawater with Added CO_2 and Bicarbonate and Controlled pH

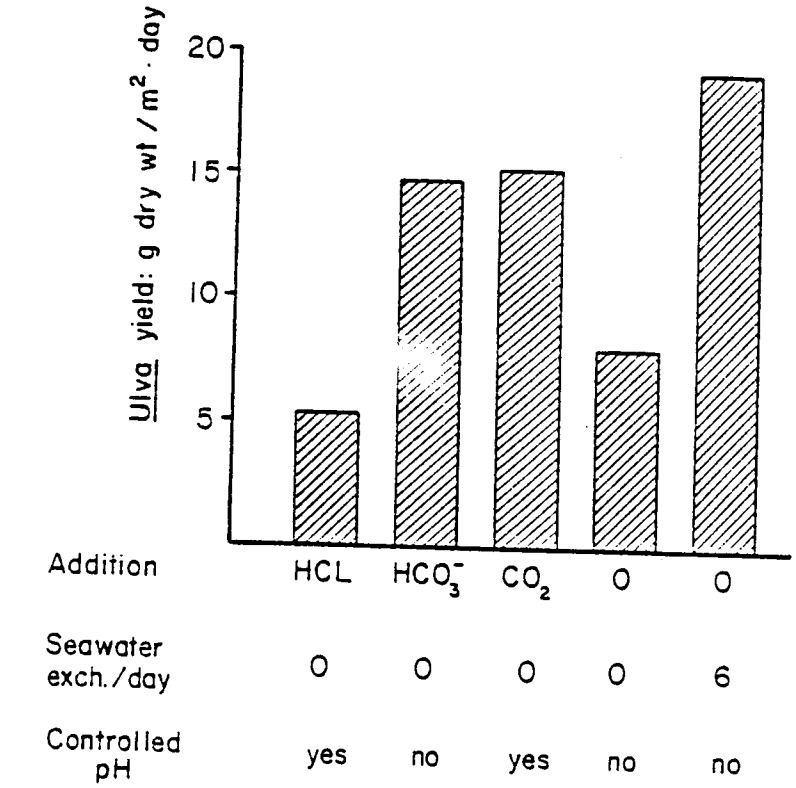

Figure 6. Growth of Ulva in Stagnant Seawater with Added CO_2 and Controlled pH

A similar experiment with Ulva was in progress at the time this paper was written. Results for the first growth period 13-19 May 1982 are shown in Figure 6. The major difference from Gracilaria is that addition of HCO_3^- alone, with no pH control and with pH levels consistently above 9.0 during the day, resulted in growth that was roughly the same as that obtained from CO_2 addition with pH controlled at 8.0 and nearly as much as obtained from six seawater exchanges per day. This confirms the conclusion from the short-term photosynthesis experiments that Ulva, unlike Gracilaria, can continue to grow at high pH if a supply of carbon is available, even if it is present predominantly as bicarbonate.

WATER HYACINTH WASTEWATER TREATMENT SYSTEM

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ABSTRACT

A prototype water hyacinth wastewater treatment system has been in operation for two years at Walt Disney World, near Orlando, Florida. Typically, the hyacinth system removes 80%-90% total suspended solids and BOD from the influent stream. Major impacts on the quality of water exiting the system are seasonal variations in solar radiation; air and water temperature; operational problems, particularly harvesting equipment breakdown; and retention time in the ponds. Phosphorus and nitrogen removal have a strong seasonal dependence, with removal rates varying from 10% in the winter to 70% in the summer. Nitrogen removal rates are quite dependent on retention times, with a retention time of 5 days appearing to be a critical limit for the establishment of an active population of denitrifying bacteria. The hyacinth biomass productivity of the system was approximately 66.7 dry metric tons/ha/yr (30 dry tons/acre/yr) during the second year of operation. An Experimental Test Unit (ETU) for anaerobic digestion of hyacinths to methane should be installed by late 1982.

INTRODUCTION

The Water Hyacinth Wastewater Treatment System at Walt Disney World has been developed with the cooperation of industry, government, and academia. The participants in this project include Aquamarine Corp., Boyle Engineering, the Environmental Protection Agency (EPA), the National Aeronautics and Space Administration (NASA), United Gas Pipeline Company, the University of Arizona, the Department of Energy through interagency agreement with EPA, the Gas Research Institute (GRI), the Reedy Creek Improvement District (RCID), and several subsidiary companies of Walt Disney Productions. Although a prototype demonstration for unconventional wastewater treatment may seem an unusual project for Walt Disney Productions, it is consistent with Disney's concept for EPCOT, the Experimental Prototype Community of Tomorrow, one goal of which is to provide a proving ground for innovative emerging technology. The Water Hyacinth Wastewater Treatment System is the first of what is hoped to be many special demonstration projects for EPCOT.

System Design—Project Components

The water hyacinth project has recently completed the expansion of its facilities. With the addition of two new channels sponsored by the Solar Energy Research Institute, the evaluation of operating parameters will proceed at a much more rapid and flexible pace. The data presented in this paper, however, are from the three-channel system described in previous reports [1]. In late 1982, the Gas Research Institute will begin construction of an Experimental Test Unit which will include a 1200-gal anaerobic digester for the bioconversion of water hyacinth and primary sludge to methane gas. Normal operating flow rates will be 2000 lb/day of a combined water hyacinth/primary sludge blend.

Operations

The hyacinth system described previously was operated in the same mode from January 1981 to October 1981. Primary effluent from the Reedy Creek Wastewater Reclamation Plant continued as the influent to the three channels. The flow to channel 1 was 25,000 gpd with a retention time of 3.6 days; the flow to channel 2 was 21,000 gpd, with a 4.3-day retention time; and channel 3 continued with 17,000 gpd and a 5.3-day retention time. All channels were operated in parallel at a depth of 14 in.

Table 1 shows the average daily flow by month for all three channels, as well as average retention time and average percentage of water lost from evapotranspiration.

Monitoring

The treatment performance was monitored by the testing schedule shown in Table 2. In addition this schedule, the following trace metals are being tested once per quarter in the influent and effluent of each channel: boron, calcium, magnesium, potassium, cadmium, chromium, copper, iron, lead, manganese, zinc, and arsenic. The tissue studies performed by the University of Arizona have been synchronized with the sampling dates for trace metals in the effluent.

RESULTS

Water Quality

Seasonal differences, retention times, and operating problems continue to be the major impacts on system performance. Typically, the best performance occurs in channel 3 (5.3 days retention) during the summer when the harvesting schedule continues without interruption. Conversely, the worst system performance occurred in channel 1 (3.6 days retention) during January and February 1981, when mechanical breakdowns prevented scheduled harvests.

Secondary treatment goals of 80%-90% reduction in suspended solids and BOD_5 were met in channels 2 and 3 in April, May, and June 1981. Channel 1, with the shortest retention time, averaged 7.1% lower suspended solids removal than channel 2 and 9.6% lower BOD_5 removal than channel 2 during the 6 months from January to June. Even with the 3.6-day retention time, channel 1 exceeded 80% BOD_5 removal in January, May, and June and more than 80% total suspended solids removal in January, April, and May (Figures 1 and 2).

TABLE 1. WATER BUDGET (gpd)

Month	Channel	Influent	+	Rainfall	-	Evapotranspiration	=	Effluent	% Loss
Jan.		24,613	+	97	-	3,420	=	21,290	13.8
		20,673	+	97	-	3,420	=	17,350	16.5
		16,736	+	97	-	3,420	=	14,455	20.3
Feb.	1	25,176	+	1,274	-	1,939	=	22,511	14.9
	2	21,148	+	1,274	-	1,939	=	18,483	17.6
	3	17,120	+	1,274	-	3,939	=	14,455	21.4
Mar.	1	25,194	+	493	-	3,843	=	21,845	13.3
	2	21,163	+	493	-	3,843	=	17,814	15.8
	3	17,132	+	493	-	3,843	=	13,783	19.0
Apr.	1	24,210	+	130	-	4,364	=	19,976	17.5
	2	20,337	+	130	-	4,364	=	16,103	20.8
	3	16,463	+	130	-	4,364	=	12,228	25.7
May	1	24,448	+	663	-	4,220	=	20,891	17.3
	2	20,536	+	663	-	4,220	=	16,979	20.5
	3	16,625	+	663	-	4,220	=	13,068	25.4
June	1	21,737	+	2,688	-	8,518	=	15,907	26.8
	2	18,259	+	2,688	-	8,518	=	12,429	31.9
	3	14,781	+	2,688	-	8,518	=	8,951	39.4

Nitrogen and phosphorus removal by the hyacinth treatment system is not as straightforward as BOD_5 and TSS removal. Denitrification, ammonification, nitrosification, and nitrification are well documented effects known to occur in sewage treatment facilities. Likewise, the effect of redox potential on solubility of phosphate ions is also well documented. The mass balance of nitrogen in the hyacinth channels (Table 3) shows that

TABLE 2. MONITORING

(A) Water Quality: Influent and Effluent, Each Channel

(1) Daily Tests
- pH
- D. O.
- Water temperature
- Total insolation
- Total rainfall
- Air temperature and relative humidity
- Total influent and effluent flow

(2) Twice per week tests
- TSS
- TS
- NH_4^+-N
- O-PO_4
- T-PO_4
- NO_3-N
- BOD_5
- TKN

(3) Twice per month tests

- Total and fecal coliforms
- Chlorides
- Alkalinity

(B) Biomass Production—each 60' cell

Density (lb/ft^2): 1 per week

6% to 40% of the nitrogen entering the channels may be lost to the atmosphere through denitrification. The data for nitrate, nitrite, and ammonia removal provide corroborative, if circumstantial, evidence for active microbial effects on the nitrogen flux in this system. The reaction $2NH_3 + 3O_2 \quad 2HNO_2 + 2H_2O + 79$ kcal does not proceed under even mildly acidic conditions. Thus, for the first three months of the year, when the pH is below 7.0 (Table 4), ammonification is the dominant process, resulting in a slight increase in ammonia values exiting the system. As ambient temperatures rise, plant growth increases and begins to buffer the system, and the pH rises to neutral or mildly basic conditions. Under these conditions, the activity of nitrifying bacteria increases and causes a rise in the effluent concentrations and mass of nitrites and nitrates and a decrease in the effluent concentration and mass of ammonia. (Figures 3-5). The reduction of nitrate to N_2 gas by the reaction $2NO_3^- + 10e^- + 12H \; N_2 + 6H_2O$ is still

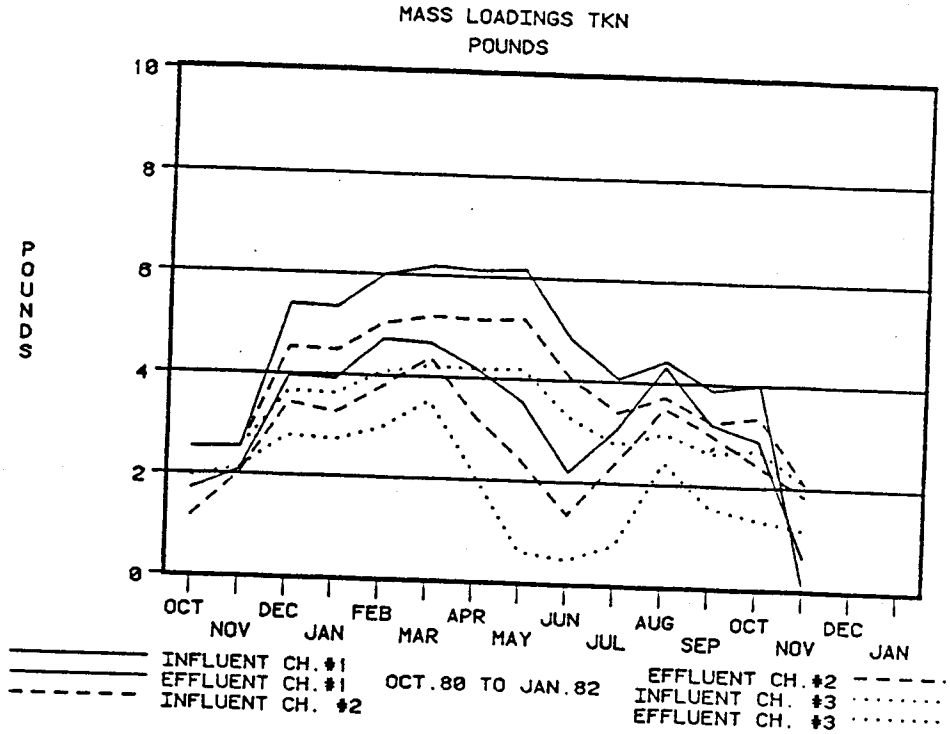

Figure 1

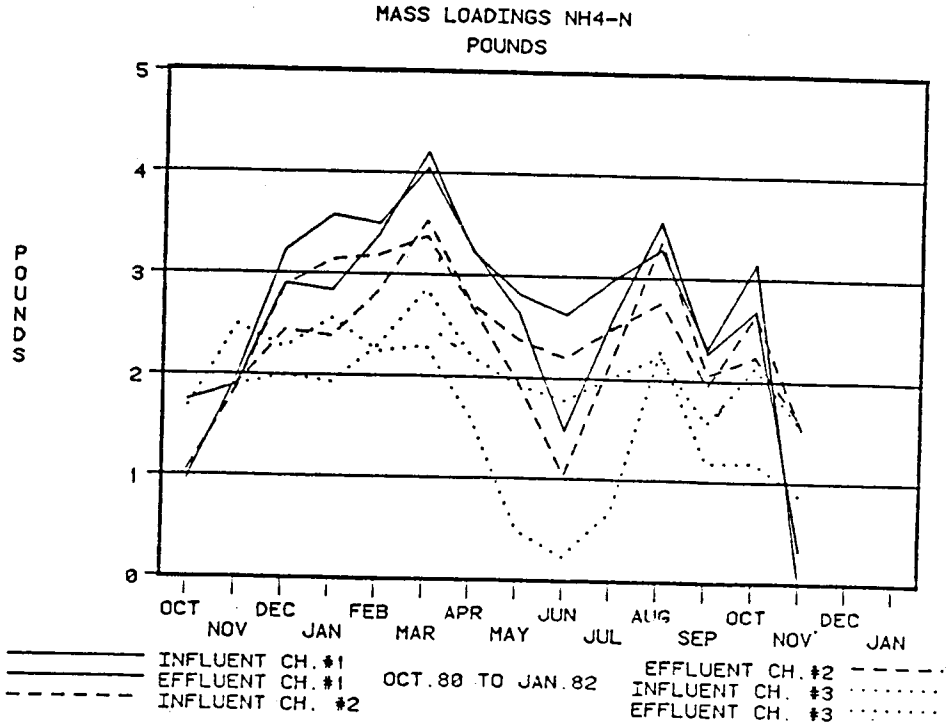

Figure 2

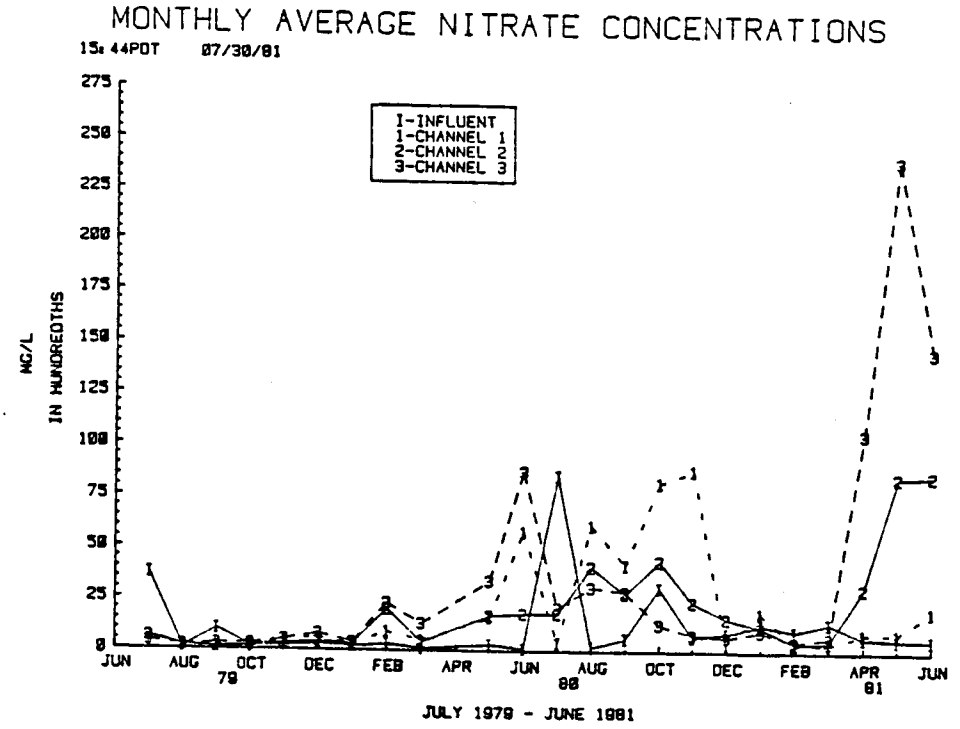

Figure 3

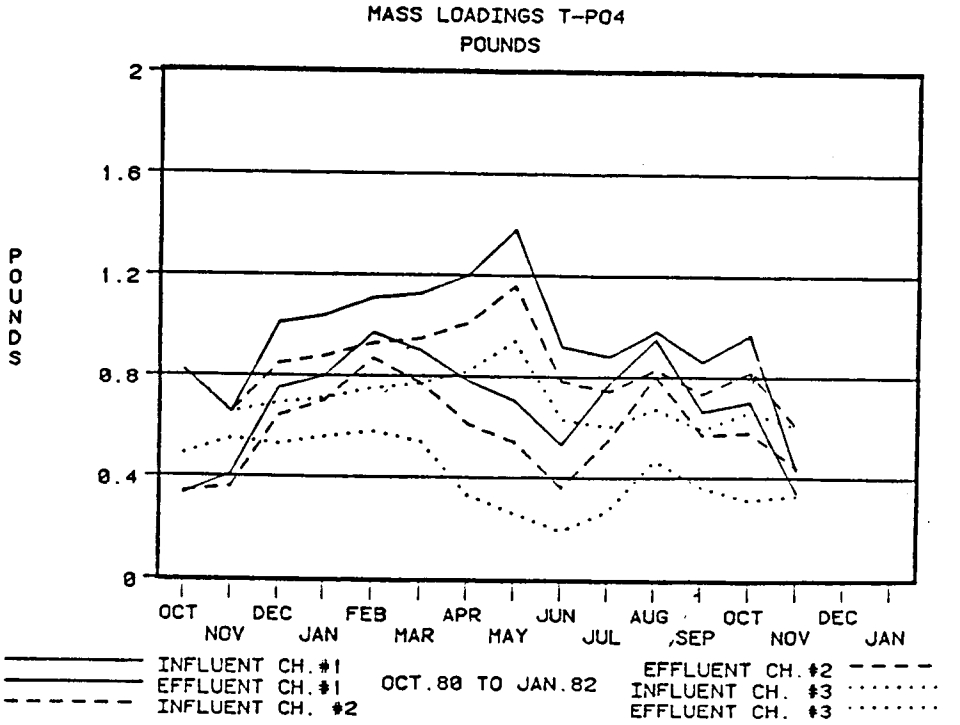

Figure 4

TABLE 3. NITROGEN MASS BALANCE

Channel	Period (days)	N Influent (TKN)	= N Effluent (TKN)	+ N Plants (estimated)	+ N_2 Gas (assumed)
1	104	620.13	396.4	188.0	(36.0)
2	104	493.75	256.91	183.5	(53.3)
3	65	232.44	36.33	102.2	(93.9)

not well understood, but the rise in BOD removal in April, May, and June may reflect the availability of NO_3 as a terminal electron acceptor in the effluent water. Although the pH and dissolved oxygen data are incomplete, they still provide supporting evidence for nitrogen transformation within the system.

In contrast to the transformations of nitrogen, no organisms are known that reduce phosphates or oxidize them as a source of energy. Almost all of the phosphorus in this system exists as soluble phosphates, the solubility of which is controlled in part by the redox potential of the surrounding water. During periods of low redox potential and low dissolved oxygen concentrations, phosphates—particularly orthophosphates—are released into the surrounding water; whereas, during periods of high redox potential (higher levels of dissolved oxygen), phosphates are both precipitated as insoluble salts and taken up by

TABLE 4. AVERAGE DAILY VALUES BY MONTH FOR DISSOLVED OXYGEN (mg/L) AND pH AT EFFLUENT WEIR OF EACH CHANNEL

	1981					
	Jan.	Feb.	Mar.	Apr.	May	June
	D. O.					
Channel 1	0.7	0.7	0.6	0.6	0.6	1.0
Channel 2	0.7	0.6	0.5	0.5	0.7	1.0
Channel 3	0.6	0.6	0.6	0.6	0.7	1.0
	pH					
Channel 1	6.4	6.6	6.9	—	—	—
Channel 2	6.4	6.6	7.0	—	—	—
Channel 3	6.6	6.6	7.1	6.9	7.1	7.0

bacteria and higher plants as long chain polyphosphates. Again, the increase in phosphate removal in the months of May, June, and April (Figure 6) in the case of channel 3 follow the general trend of increasing levels of dissolved oxygen and, presumably, higher redox potential. Although the widely varying concentrations of phosphorus in the hyacinths and microflora preclude the construction of an accurate mass balance for phosphorus without month-by-month data for P concentration in the plants, the observed seasonal variations in removal correlate with available D. O. data.

Cover Studies

1981-82 was the first year that the inflatable cover was in place throughout the winter, allowing comparisons to be made between covered and uncovered channels treating primary effluent. The effects of the cover on water quality are not clear. There is no significant difference in suspended solids and BOD_5 reduction. However, the removal percentages for SS and BOD_5 in both channel 2 and channel 3 are the highest yet recorded for the months of January and February.

During December, channel 3 (covered) showed a greater reduction in $T\text{-}PO_4$, TKN, and $NH_4\text{-}N$ than channel 2 (uncovered), but during January these comparisons were difficult to make, since effluent concentrations of these parameters were higher than influent concentrations. These observations suggest that internal cycling of nutrients play an important role in overall system dynamics.

Trace Metals

Data on growth limitations by trace elements is inconclusive at present. Early reports indicated that calcium and magnesium might be limiting at some time during the year, but further detailed studies are necessary to confirm this observation. Data from the trace element analysis of influent and effluents of the hyacinth channels are given in Table 5.

Iron values in the effluent are higher than normal for secondary effluent; however, they appear to exhibit a seasonally dependent reduction in concentration as well as a dependence on retention time. Iron has been implicated in the phosphorus cycle and may provide insights into the fluxes of this element in the hyacinth system.

Productivity

Harvesting data for the period is given in Table 6. As previously noted, there was no harvest until March 17, because of mechanical problems with the front-end loader. Harvest data are quite variable, with values ranging from 21 to 68 dry tons per acre year. The source of this variation is not clear at present, but seems to be sampling error rather than short-term variation in the productivity of the channels. At present, there does not appear to be any significant difference in productivity from the three channels, nor does there appear to be any significant difference in productivity caused by the harvesting schedule. In the future, the $1\text{-}m^2$ PVC-vexar mesh basket productivity sampling technique will be changed by increasing the sample size (i.e., the number of baskets) and standardizing the weighing procedure. The larger sample size should help to eliminate some of the variability that makes comparisons of culture techniques difficult.

The density-yield study reported in the U.S. EPA quarterly reports 9 and 10 [2] was repeated during April and May 1981. The restocking density needed to maximize productivity appears to have a wider range during spring and summer than during fall and

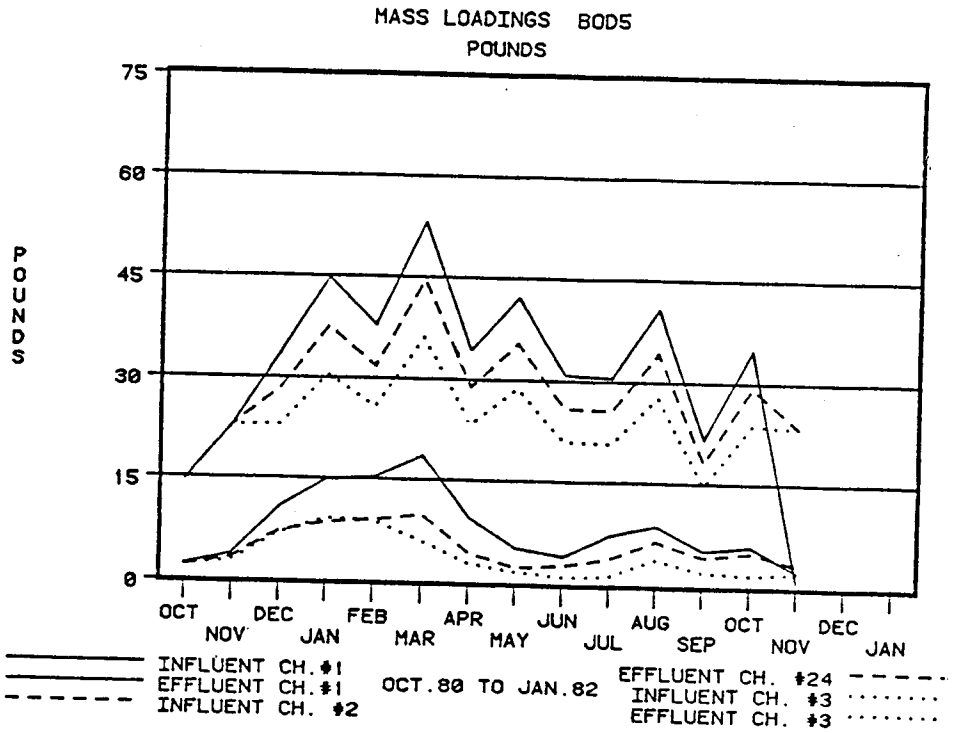

Figure 5

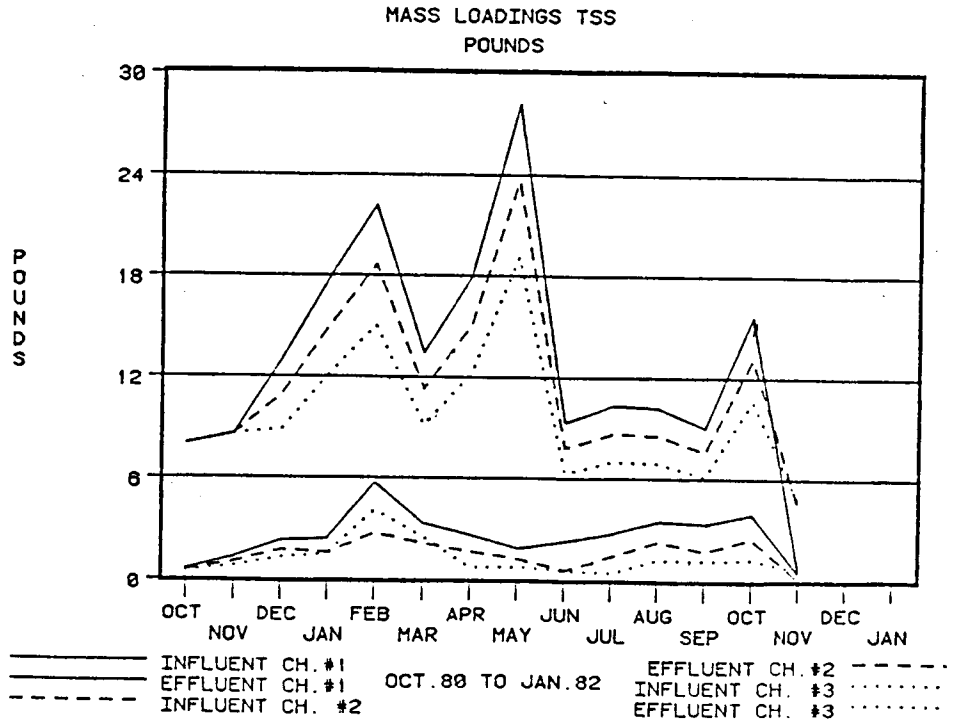

Figure 6

TABLE 5. TRACE METALS

Metal	Influent	26 June 1981 #1	#2	#3
Calcium (ppm)	26.6	23.6	26.2	18.2
Magnesium (ppm)	5.9	5.7	5.1	4.4
Potassium (ppm)	9.2	7.6	3.6	0.6
Cadmium (ppb)	0.2	0.15	0.15	0.2
Chromium (ppb)	2.02	4.13	2.02	3.26
Copper (ppb)	33.36	18.92	13.42	6.45
Iron (ppb)	491.1	337.5	126.1	113.8
Lead (ppb)	4.61	2.89	1.73	2.55
Manganese (ppb)	13.86	14.09	9.54	5.09
Zinc (ppb)	24.14	11.91	14.76	22.36
Arsenic (ppb)	5.49	4.75	5.75	6.81
		30 Mar. 1981		
Boron (ppm)	0.34	0.31	0.44	
Calcium (ppm)	27.20	28.50	30.40	31.3
Magnesium (ppm)	9.7	5.6	6.1	5.8
Potassium (ppm)	11.4	8.0	10.0	9.6
Cadmium (ppb)	0.27	0.21	0.23	0.21
Chromium (ppb)	1.57	1.57	1.75	1.48
Copper (ppb)	54.88	18.95	21.38	18.43
Iron (ppb)	617.9	494.5	377.4	358.9
Lead (ppb)	5.55	8.15	10.98	11.82
Manganese (ppb)	14.45	14.32	16.12	13.41
Zinc (ppb)	102.1	59.84	60.17	145.5
Arsenic (ppb)	3.46	2.45	2.3	2.56

winter, when the previous study was made. As a result of this study, restocking density will be maintained at 2 lb/ft^2 during spring and summer.

The effects of the cover on water hyacinth productivity are clear cut. During the period in which the cover was inflated (from 4 December 1981 to mid-March 1982), channel 3 (covered) averaged 31.3 dry tons per acre year. During this same time period, channel 2 (uncovered) produced 14.6 dry tons per acre year. It is important to note that during this time period, channel 1 produced approximately 26.9 dry tons per acre year but still suffered a frost dieback, as did channel 2. Channel 3, on the other hand, did not suffer from any frost dieback as long as the cover was inflated. Although we have no empirical data as yet, it is worthwhile to note that the root/shoot ratio of the hyacinths in channel 1 appears higher than channel 2. This may account for the relatively more rapid recovery from frost damage shown in channel 1.

TABLE 6. WATER HYACINTH PROJECT HARVESTING DATA

Period	Total Wet Pounds	Mean Harvest Frequency (days)	Mean Yield ± 1 Std. Dev. (dry tons/acre/yr)
Channel 1			
3/17/81 - 5/5/81	91,038	16.3	36.3 ± 7.2
5/19/81 - 7/1/81	53,157	14.0	33.2 ± 8.8
7/14/81 - 9/28/81	21,141	12.0	14.6 ± 5.9
10/8/81 - 11/24/81	22,540	19.5	11.2 ± 2.9
12/8/81 - 2/18/82	48,807	25.4	26.9 ± 24.6
Channel 2			
3/24/81 - 5/13/81	78,888	25.0	30.5 ± 9.8
5/21/81 - 6/23/71	42,100	10.2	39.2 ± 20.3
7/6/81 - 9/4/81	36,453	12.0	39.9 ± 25.5
9/23/81 - 11/12/81	7,569	23.0	4.2 ± 3.9
12/14/81 - 2/22/82	46,371	51.3	9.0 ± 10.9
Channel 3			
5/5/81 - 6/29/81	49,457	7.8	30.1 ± 16.9
7/9/81 - 8/27/81	37,758	7.0	52.3 ± 22.9
9/1/81 - 10/15/81	70,295	8.1	57.6 ± 36.1
11/9/81 - 2/2/82	91,440	22.0	30.4 ± 21.5

CONCLUSION

The water hyacinth system at Walt Disney World has demonstrated that it can achieve secondary wastewater treatment standards. The second year's operation has also increased hyacinth biomass productivity, compared with the first year's results [1]. However, the major lessons from the second year's operation concern the importance and value of overcoming operational problems. Equipment failure, insect attack, and frost damage have had significant effects on system performance. Some of these problems may be solved by improving the design of second-generation facilities, while some, such as insect attacks, have been solved by anticipation and preventive treatment.

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LABORATORY SELECTION OF MICROALGAE FOR MAXIMUM LIPID AND PROTEIN PRODUCTION

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ABSTRACT

Compared with higher plants, microalgae are attractive for biomass energy because of their higher yields and photosynthetic efficiencies. Using dense, thin cultures, we have screened various algal species for the above parameters and for their lipid, protein, and carbohydrate content. Nitrogen deficiency and light intensity effects have been assessed. The highest yield (21.5 g dry weight m^{-2} day^{-1}) and efficiency (12.2%) were obtained with Phaeodactylum at a light intensity equaling 39% of maximum sunlight at La Jolla and N sufficiency. Lipid and protein yields were 5.62 and 13.0 g m^{-2} day^{-1}, respectively. Dry weight, lipid, and protein yields translate to 30.4, 7.87 and 18.2 tons $acre^{-1}$ $year^{-1}$. Comparative higher plant yields average 7.3 g dry weight m^{-2} day^{-1}. Increases in light intensity or cell density did not increase yields or efficiencies. Nitrogen limitation increases Phaeodactylum lipid content from 20% to 30% of the dry weight, but lipid yield is reduced (to 2.39 g m^{-2} day^{-1}) because of low overall dry weight yield. No other species (Dunaliella, Monallantus, Tetraselmis, Isochrysis, or Botryococcus) produced yields as high (dry weight, lipid, or protein) or were as efficient as Phaeodactylum. Nitrogen deficiency did not increase lipid content in any of these other species and, in Dunaliella and Tetraselmis, carbohydrate, rather than lipid, levels were greatly increased by deficiency.

Because coastal land areas for growing marine algae are limited, we will study algae that might be utilized in desert areas having saline water resources. A literature review on desert algae is in progress. We plan to isolate such algae in the field and culture them in the laboratory as we have with the marine algae outlined above. Thus, in the field and laboratory, we will select candidate microalgae for outdoor desert application to oil and protein production.

INTRODUCTION

Pictures of planet Earth taken from outer space have dramatically increased our awareness that we live in a finite world. The only reasonably infinite input into this system is energy from the sun. Superimposed on this input is the high energy input from the combustion of fossil fuels, supplies of which are also used for petrochemically derived plastics and other specialized items. Fossil fuels, however, will eventually be depleted and, in the long term, except for the energy developed from fusion, we will be dependent on solar energy. Therefore, we need to develop technologies to utilize energy from the sun.

Producing biomass by plant photosynthesis can capture some solar energy, but much basic work needs to be done, both on the photosynthetic process itself and on its applications. This research is high-risk and socially useful in the long term, but the results will not be immediately applied.

As biomass producers, algae are attractive in that they often have higher yields and are more efficient in utilizing light energy than higher plants. This comparison was originally made experimentally by Wassinck et al. [1], and Shifrin [2] has recently compiled additional comparative data. Taking the 31 values given by Shifrin (his Table 6.5), the mean yield of higher land plants is 7.3 g m^{-2} day^{-1}, while microalgae values range from 15-25 g m^{-2} day^{-1}. Thus, the concept of having outdoor ponds to produce microalgae biomass is attractive, and such installations are being studied and developed at a number of locations throughout the world. In fact, outdoor mass culturing was the subject of a recent symposium held in Israel [3].

Much work has been done on the application of freshwater green algae in outdoor culture, but the marine forms have been less well investigated. Therefore, since seawater is an obvious resource of water and, at least, of some elements that algae need, we have been investigating the potential of marine microalgae in the laboratory where conditions can be reasonably controlled for each species. We have measured yields and light utilization efficiencies and have studied the effects of nitrogen deficiency, since lack of this element can shift algal metabolism from protein production to components such as lipids or hydrocarbons that have a higher energy content.

To a large extent, our efforts have been "horizontal," in that several species have been compared under similar conditions of high nutrient supply and at light intensities approaching maximum sunlight intensities. However, some of our work has been "vertical," in that we have varied conditions extensively for one species, Phaeodactylum. This work was conducted in support of the algal raceway research of Dr. Edward Laws.

The eventual utilization of marine microalgae depends on the ability to install ponds along sunny coastlines, but land is generally in short supply along the U.S. coast. Therefore, we are turning our attention to desert algae, and a literature review and future plans will be discussed at the end of this paper.

MATERIALS AND METHODS

Microalgal cultures were generally obtained from our extensive culture collection of marine algae. Monallantus (GSB sticho) was obtained from Dr. R. R. L. Guillard of Woods Hole; Botryococcus was obtained from Dr. S. Lien of SERI. Marine cultures were grown in filtered Scripps Pier seawater enriched with 15 mg at liter^{-1} N, 1.25 mg at P liter^{-1}, and the trace metal mixture in medium "f" [4]. Ammonium was generally used as an N source, but nitrate was used for Isochrysis. Botryococcus was grown in an artificial medium (modified Chu No. 10 enriched as for marine cultures). Before each main experiment, the algae were precultured to a high density at 21°C and a light intensity of 0.056 cal cm^{-2} min^{-1}. Dense cultures were then poured aseptically into the main culture apparatus. This is diagrammed in Figure 1. The light source was a 2000-W tungsten-halide stage lamp operated on a 12:12 L/D cycle. This light was filtered through a 7-cm thickness of flowing tapwater and generally through a 2-cm thickness of 3% $CuSO_4$ to remove infrared radiation. Light intensity was not uniform over the culture surface and the measurements are those integrated from 143 separate measurements taken at the culture surface.

The 3.3-L culture could be operated in batch mode (without pumping) or in the continuous mode by pumping in filter-sterilized media. The culture container was made of plexiglass plastic, and media, aeration, overflow, and sampling tubes were of glass and silicone rubber. Temperature was controlled by a water jacket which was not very effective; the main temperature control was to place the whole apparatus in a cold room.

Sampling was carried out daily by removing 100 mL of cell suspension. Harvests for proximate cell analyses were 240 mL and cells were filtered on glass fiber filters (dry weight and proximate analyses samples) and centrifuged for caloric measurements. Growth was measured by dry weight, optical density (at 750 nm), cell numbers (Coulter or hemocytometer counts), and in vivo chlorophyll fluorescence.

Total cellular carbon, nitrogen, and hydrogen analyses were performed with a Hewlett Packard CHN analyzer [5]. Protein was calculated as 6.2 x %N. Cellular carbohydrate was carried out with the phenol-H_2SO_4 method [6]. Lipid was measured by extracting filtered cells with methanol-chloroform, followed by washing the extracts with water. Aliquots of the water-insoluble extract were then dried under N_2 and the residue weighed on a microbalance [7]. Ash was measured by combusting dry weight filters at 450°C overnight and reweighing. Caloric content of the cells was determined by Philipson microbomb calorimetry on centrifuged cell samples.

Growth was linear, rather than exponential, in these dense cultures, and yields were determined from linear regression of dry weight versus time (batch measurements) or by multiplying the overflow volume in liters day^{-1} by the dry weight liter^{-1} in the culture (continuous culture).

Efficiency was determined from the ratio of calories of cell material produced day^{-1} to calories of light supplied day^{-1} in the photosynthetically active range (400-700 nm). Calories of cell material produced were taken from measured caloric values or by multiplying the cell carbon in milligrams by 11 [8].

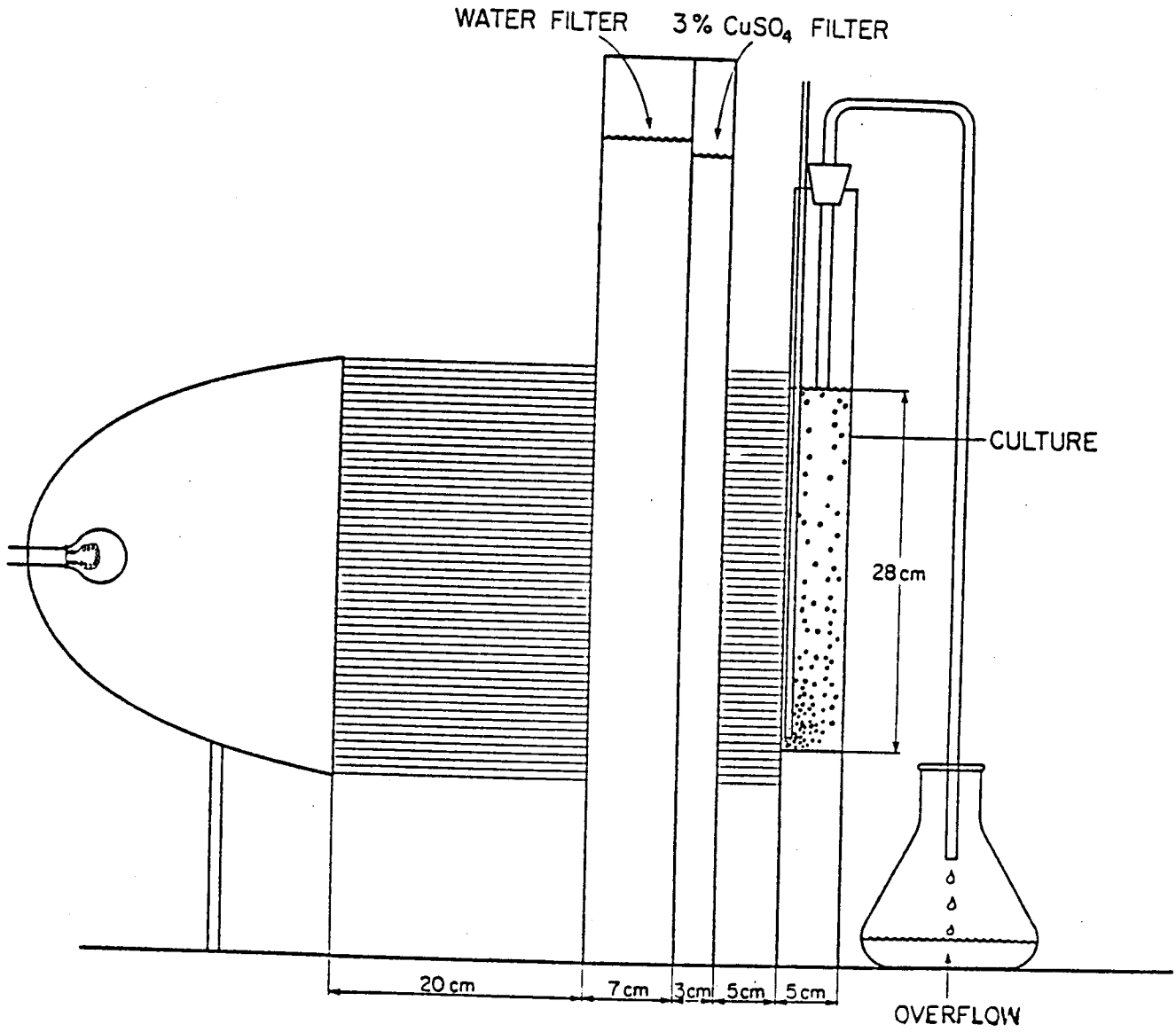

Figure 1. Diagram of Continuous Culture Apparatus

RESULTS AND DISCUSSION—PRESENT STUDIES

Comparison of Several Species

Cellular yields and efficiencies and lipid and protein yields for six algal cultures are shown in Table 1. It will be seen that Phaeodactylum in batch culture gave the highest yield and efficiency. Nevertheless, this yield is slightly less than those in deeper outdoor cultures of freshwater algae where maximum yields as high as 25 g m^{-2} day^{-1} have been reported [3]. However, this efficiency (12.2%) is generally higher than those found in outdoor cultures, but is similar to that reported by Raymond [9] for Phaeodactylum in his initial small raceway experiment in Hawaii. From our results, Phaeodactylum remains the best candidate marine microalgal species for outdoor application. The freshwater species, Botryococcus, has a high lipid and hydrocarbon content [10] but is a very slow-growing species. We have experimented with various media for this species but have not been able to increase growth rates with any medium other than modified Chu 10.

These yields can be translated to tons $acre^{-1}$ $year^{-1}$ by multiplying by 1.4 [2] or to Btu $acre^{-1}$ $year^{-1}$ (assuming 5,000 cal g^{-1}) by multiplying by 29.6×10^6 [2]. Thus, our value for Phaeodactylum converts to 30.4 tons $acre^{-1}$ $year^{-1}$ or 6.42×10^8 Btu $acre^{-1}$ $year^{-1}$. One quad of energy (10^{15} Btu)/year could be produced on 1.56×10^6 acres of

Table 1. YIELDS AND PHOTOSYNTHETIC EFFICIENCIES OF SEVERAL MICROALGAE AT MODERATE LIGHT INTENSITIES AND NUTRIENT SUFFICIENCY

Alga	Yield (g dry wt. m^{-2} day^{-1})	Efficiency (%)	Lipid Yield (g m^{-2} day^{-1})	Protein Yield (g m^{-2} day^{-1})
Phaeodactylum (batch culture)	21.7	12.2	5.62	13.0
Phaeodactylum (continuous culture)	16.2	6.2	3.22	9.5
Dunaliella (batch culture)	8.7	3.5	—	—
Dunaliella (continuous culture)	12.0	3.8	2.59	8.4
Monallatus (batch culture)	7.1	4.0	1.42	2.8
Tetraselmis (batch & cont. cultures)	1.3	7.6	4.48	—
Isochrysis (batch culture)	6.7	2.8	1.77	—
Botryococcus (batch culture)	4.0	1.7	1.20	—

Phaeodactylum outdoor cultures. However, these estimates are rough and it is doubtful that our highest yield could be sustained in a continuous or semicontinuous pond culture. Further experimentation—particularly with culture thickness, turbulence, and sun screening, such as that being done by Dr. Laws in Hawaii—will refine such estimates more realistically.

Effects of Nitrogen Deficiency on Cellular and Lipid Yields and on Efficiency

Effects of nitrogen deficiency were tested by pumping in an N-free medium. Such experiments were carried out with four species, and deficiency in Isochrysis is presently being studied.

The results are shown in Table 2. Deficiency generally reduced cellular yields and efficiencies, sometimes greatly. In Phaeodactylum, the lipid content of the cells increased from 20% to 30% of the dry weight, but the lipid yield was reduced because the overall cell yield was greatly reduced. Nitrogen deficiency in Dunaliella and Tetraselmis resulted in carbohydrate (up to 60% of the dry weight) rather than lipid storage. Monallantus has been reported to increase its lipid content greatly under N-deficient conditions [2]. We did not confirm this result. In our experiments, lipid content decreased from 20% to 12% of the dry weight.

We felt that, at this stage of our research efforts and knowledge, future efforts to increase lipid yield in microalgal cultures should not include N deficiency as in the experimental regimes. It seems more desirable to concentrate on a means of maximizing overall cellular yields and efficiencies.

Table 2. EFFECTS OF NITROGEN DEFICIENCY ON YIELDS AND PHOTOSYNTHETIC EFFICIENCIES OF SEVERAL MICROALGAE AT MODERATE LIGHT INTENSITY

Alga	N Status	Yield (g dry wt. m^{-2} day^{-1})	Efficiency (%)	Lipid Yield (g m^{-2} day^{-1})	Protein Yield (g m^{-2} day^{-1})
Phaeodactylum	+N	16.2	6.2	3.22	9.5
	-N	7.8	4.1	2.39	1.7
Dunaliella	+N	12.0	4.2	2.59	8.4
	-N	10.2	3.8	1.40	2.7
Monallantus	+N	[illegible]	4.0	1.42	2.8
	-N	6.6	3.7	0.79	1.3
Tetraselmis	+N	18.3	7.6	4.48	—
	-N	15.6	6.5	1.87	—

Effects of Light Intensity on Phaeodactylum Yields and Efficiencies

Several batch culture runs were carried out with this alga at different light intensities, ranging from 32% to 69% of the maximum daily photosynthetically active sunlight intensity at La Jolla. The results are shown in Table 3. Generally, the lower light intensities resulted in the highest yields and efficiencies. High intensities resulted in reduced growth as did the lowest intensity tested. Removal of the $CuSO_4$ filter increased the irradiance in the red portion of the spectrum. At about the same overall light intensity, the yield and efficiency was increased when the filter was removed. Thus, in outdoor cultures, it may not be necessary or desirable to include this filter. The lower culture density (Experiment 1) provided higher yields than a higher density did (Experiment 2).

These experiments were performed to support, if possible, Dr. Laws' raceway studies. For instance, he may be able to increase yields by putting sunscreening material over his culture and not using a $CuSO_4$ filter.

FUTURE WORK ON DESERT ALGAE

Annotated Literature Review

Before starting research work on desert algae, we decided to prepare an annotated bibliography of papers on algae in the southwestern United States and on their habitats. This review has been done by computer searches, library work, correspondence, and personal contacts. So far, over 150 papers have been found, photocopied, read, and abstracted. Key words for each include author, state, water type, habitat, algal taxa, etc. The bibliography will be searchable by microcomputer. Most papers just list the

Table 3. EFFECTS OF LIGHT INTENSITY ON YIELDS AND PHOTOSYNTHETIC EFFICIENCIES OF PHAEODACTYLUM WITH NUTRIENT SUFFICIENCY

	Mean Light Intensity (g cal min^{-1})	Percent Max. Daily Sunlight at La Jolla	Yield (g dry wt. m^{-2} day^{-1})	Efficiency (%)
Experiment 1				
(Culture density	0.124	39.5	21.7	12.2
= 416 to 1586	0.186	59.6	21.2	8.1
mg dry wt. L^{-1}	0.216	68.9	17.1	5.4
Experiment 2				
(Culture density	0.100	31.9	8.0	6.0
= 1500 to 3000	0.129	41.1	13.3	7.9
mg dry wt. L^{-1})	0.163	51.9	12.4	5.8
	0.207	65.9	11.4	4.2
	0.152[a]	48.4	19.9	10.0

[a] $CuSO_4$ solution filter removed to give more red light.

species in a given area, but some include data on habitat chemistry and algal ecology. In California, most papers concern the Salton Sea, Mono Lake, Death Valley, and various springs. In Arizona, there have been several studies on freshwater algae in the northern part of the area and on soil algae. Pyramid, Walker, Big Soda Lakes, and some springs have been investigated in Nevada, and there is a pertinent book, Algae of the Western Great Basin, by La Rivers. In Utah, algae have been studied in Great Salt Lake and Utah Lake, and a few papers on freshwater algae were found. Few papers were found on desert algae in Oregon, Colorado, New Mexico, and Texas. Hot springs generally contain blue-green algae and diatoms. Moderately saline lakes contain these taxa plus green algae; extremely saline lakes contain mainly green algae; i.e., Dunaliella and Coccomyxa. The preparation of this review is still in progress.

Future Field Studies of Desert Algae

Using information from our review, we will be in a good position to start field work on the selection and isolation of species from the desert that might be suitable for large-scale culture there. We plan to design and have built a mobile field laboratory on a motor home chassis which we can drive to desert towns and in which we can select and isolate suitable species and bring them back to La Jolla for laboratory mass culture studies (see below). This field work will go on for the next year and a half, and two of us will spend about six months in the field during several short trips.

We plan to set up several small chemostats in the mobile laboratory and pump enriched natural water into them at relatively high dilution rates. The candidate faster growing species should outcompete slower growing species. Natural illumination will be provided through windows in the vehicle, and temperature control will be provided by its air conditioning. Thus, we will let the growth system itself select suitable species for further maximization of yields and efficiencies in laboratory continuous cultures.

When fast-growing species come up, we will isolate them into unialgal culture by the usual procedures—micropipetting, serial dilution, agar plating, etc. We plan to do isolation in the mobile laboratory, but some may be done at La Jolla. There will be lighted incubators aboard the mobile facility so that we can bring the algae back without losing them.

Cultures from the chemostats will also be tested in the field for temperature tolerance with a lighted temperature gradient block [11]. Salinity tolerance experiments on chemostat-cultured algae will also be done using filtered natural water concentrated by reverse osmosis to avoid changes in ionic ratios or diluted with demineralized water. We will also stain cells with "C12 Basic Blue 12" [10] for the field selection of those that might have a high lipid or oil content.

Some chemical analyses for inorganic constituents will be done in the field, but this work will be minimal so that we can devote more time (and space in the field laboratory) to biological studies. However, we will bring back water samples for complete inorganic analyses by a commercial laboratory. We have located a reliable commercial firm that has been doing such analyses for over 50 years.

Future Laboratory Studies on Desert Algae

Once we have brought desert algae back to La Jolla, we plan to determine which species can be grown in really dense culture and have potentially high yields. These preliminary studies will be done in serum bottles incubated in a fluorescent-lighted water bath. Various further tests on temperature and salinity tolerances of the isolated algae will also be done. We plan to use natural water that we will bring back as a base for media rather than artificial media. This will simplify medium formulation and reduce the necessity for analytical chemical data on the water.

These preliminary studies will involve tests on about 10 species. From them we will choose no more than three species for further efforts using continuous cultures. These will be set up in four chemostats similar to that shown in Figure 1. Our main goal is to determine the conditions under which yields and efficiencies are maximized. Light intensity, culture thickness, temperature, and culture density are obvious parameters to investigate; others may become apparent as the work proceeds.

With support from the Bio-Energy Council, we are presently developing a system to automate growth and temperature measurements. We have found that light transmitted through the culture is correlated with dry weight. Transmitted light measurements will be made every half hour and the data stored on microcomputer disk for later analysis. Thus, we will be able to plot and print the growth measurements for each day and make decisions on a more "real time" basis than daily measurements provide. We also plan to interface this data-gathering computer with an Apple home computer via a telephone circuit so that culture decisions and data analysis can be made.

We will do fewer cellular chemical analyses than we have previously, but a few CHN, protein, carbohydrate, lipid, and caloric measurements will be done for calculations of constituent yields and efficiencies. Cell samples will also be shipped to Dr. Tornabene at Georgia Tech for detailed lipid analyses.

Other SERI Research Needs

We feel a need for a raceway installation in the desert so that our results on desert algae can be applied in a timely fashion. Thus, we could interface with more applied studies, as we have done with Dr. Laws' investigations. We think our field desirable if such an installation were presently being built.

CONCLUSIONS

Of the marine species that we have studied, Phaeodactylum remains the most suitable candidate for outdoor application. Nitrogen deficiency does not seem to be a suitable means of increasing lipid yield.

Future work will be directed toward finding a desert alga that can, for example, give a sustained yield of 30 g m^{-2} day^{-1} with a lipid content of 35% of the dry weight, and be easily cultured at a location having an ample saline water supply. Such a species would yield 10.5 g lipid m^{-2} day^{-1} or 14.7 tons lipid $acre^{-1}$ $year^{-1}$. At a caloric content for lipid of 9500 cal g^{-1}, this works out to 1.11×10^9 Btu $acre^{-1}$ $year^{-1}$. Thus, to produce 1 quad of energy would require 900,000 acres. Admittedly, these estimates are optimistic. Such yields and lipid contents may not be easy to achieve and sustain in an outdoor system.

ACKNOWLEDGMENTS

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CHEMICAL PROFILES OF MICROALGAE WITH EMPHASIS ON LIPIDS

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ABSTRACT

Neochloris oleoabundans was cultivated in a mineral medium deficient in nitrogen. The yield of oily lipids was 35%-45% of cell dry weight. Triglycerides comprised 80% of the total lipids. Aliphatic hydrocarbons, sterols, pigments, glycolipids, and phospholipids comprised the remaining lipid fraction. Saturated, monounsaturated, and diunsaturated octadecanoic acid represented approximately one-half of the total fatty acids.

An assessment was made on the suitability of Neochloris as a substrate for bacterial growth and as a source of fatty acids for hydrocarbon synthesis by bacteria. Pseudomonas spp. grew well on Neochloris; however, the algal fatty acids were not assimilated directly into bacterial hydrocarbons through its normal condensation route. The algal fatty acids were oxidized to the acetate level and then reassembled into bacterial fatty acids which were condensed into hydrocarbons. The total lipid content of the Pseudomonads remained at 7%-8% of the dried cell weight and the relative intensities of the individual lipid classes were relatively unchanged.

INTRODUCTION

The purposes of this project are (1) to advance the analytical data for species selection and characterization, and (2) to provide a data base from which potential chemical and energy potential from microalgae can be identified. These will be accomplished through the specific objectives of (1) examining the cultivation conditions on the chemical composition of microalgal species with emphasis on lipid production, (2) studying control points and regulatory mechanisms for production of desirable chemicals, (3) investigating harvesting and processing techniques for lipid extraction and recovery, and (4) providing standardization of materials and methods required to quantitatively identify the chemical constituents of microalgal species.

This report reviews the results obtained from a study conducted this past year on the lipid productions of a green alga. The test organism was chosen primarily on the basis of the report by Archibald [1] who described Neochloris cells that appeared to have oil droplets intracellularly or extracellularly, respective to different species. The oil-producing potential of Neochloris was substantiated by the studies of Lien [2], who employed a staining procedure specifically for neutral lipids and oils of algal cells. Since the growth parameters of this alga have been reported [1-4], and there were no reports on the lipid composition of Neochloris, the chemical nature of the total lipid production by Neochloris was studied. Cells were removed from the growth medium when they visually appeared to contain the greatest amount of oil. This condition generally occurred when the cells were old or when the nutrient growth medium became deficient in nitrogen.

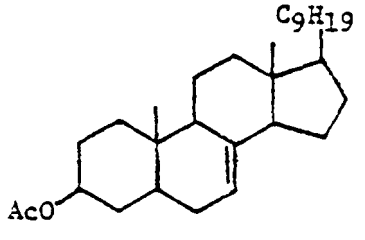
RESULTS

Total Lipids

Batch cultures of N. oleoabundans, grown in the N-limited BBM medium, accumulated a lipid content that accounted for 35%-54% of the cell dry weight. Complete extraction of the lipid components was judged by the absence of nonsaponifiable lipids and trace amounts of fatty acids in alkaline hydrolysates of extracted cells. The proportions of lipids fractionated on a silicic acid column and separated by TLC in neutral and polar organic solvents are given in Tables 1 and 2, respectively. Multiple pigmented complexes existed in all eluates except the hexane eluate, and were not characterized further. More than 80% of the total lipids were separated by TLC developed with neutral solvents (Table 1). The analytical data are presented according to the sequence of elution from the silicic acid column.

Hexane eluate. One predominant component was detected in the hexane eluate that comprised approximately 0.7% of the total lipids. This component, comprising 96.6% of the fraction, was identified as n-heptadecane ($C_{17}H_{36}$). The remaining 3.4% of the hexane fraction consisted of a mixture of relatively small amounts of n-pentadecane ($C_{15}H_{32}$), n-hexadecane ($C_{16}H_{34}$), heptadecane ($C_{17}H_{34}$), and a possibly branched $C_{17}H_{34}$ hydrocarbon, the sum of which accounted for less than 0.4% iso-octadecane ($C_{18}H_{38}$, 1%); n-octadecane ($C_{18}H_{38}$, 0.4%); unidentified (0.6%); branched $C_{19}H_{38}$ (0.5%); and n-nonadecane ($C_{19}H_{40}$, 0.5%).

Benzene eluate. The components present in the benzene eluate represented 3.9% of the total lipids (Table 1). The fraction consisted principally of unidentified pigments and specific components that cochromatographed with steryl esters, methyl esters, and free sterols. The composition of the sterol fraction is summarized in Table 3. Seven individual sterols were detected; however, only four of them were in sufficient quantity for a tentative determination of their structures. The principal component was a C-28 sterol; its mass spectrum is summarized under sterol 4 in Table 4. The compound has a fairly large molecular ion at m/e = 442, which is usually observed for most Δ^7-sterols [4]. The ions at m/e = 427, 382, and 367 arise from the loss of a methyl group, acetic acid, and a combination of both, respectively. The peak at m/e = 315 is formed by elimination of the alkyl side chain. A mass difference of 127 AMU indicates a saturated side chain with nine carbon atoms. The ion at m/e = 228 involves the elimination of the side chain and 27 additional AMU, most likely C16 and C17. Further loss of the acetate residue produces a peak at m/e = 229. This group of ions is characteristic for Δ^7-sterols [4]. The loss of the side chain and ring D produces an ion at m/e = 273 [5]. The most abundant ion of the spectrum at m/e = 225 results from the elimination of the side chain and acetic acid. Additional loss of ring D generates the ion at m/e = 213. The mass spectral pattern is consistent with a Δ^7 sterol of the following structure.



The most prominent ions of the other sterols are listed in Table 4. Compound 2 displays a pattern similar to sterol 4. The ring system has two sites of unsaturation as indicated by the ion at m/e = 313 (M-side chain). The second double bond appears to be located in

ring D, because ions involving the loss of ring D or parts of it (m/e 288, 229, 213) appear at the same mass numbers as in sterol 4. The second double bond is presumably located in ring B; however, the exact position cannot be determined with certainty. The mass spectrum of sterol 1 with a molecular weight peak of m/e = 438 and an ion at m/e = 311 (M-side chain) suggests a ring system with three double bonds. Unfortunately, only a small quantity of material was present, resulting in a poor mass spectrum and preventing further speculation on its structure. Sterol 3 (Table 4) had a molecular weight ion at m/e

TABLE 1. CHARACTERISTICS OF NEUTRAL LIPID COMPONENTS OF N. OLEOABUNDANS

Av. R_f Values	Eluates from Silicic Column					Probable Identification of Compounds
	Hexane	Benzene	$CHCl_3$	Acetone	MeOH	
0.88	0.7	—	—	—	—	Hydrocarbons
0.79	—	1.2	—	—	—	Steryl esters
0.69	—	0.2	0.1	—	—	Methyl esters
0.59	—	—	73.5	7.1	—	Triglycerides
0.50	—	0.2	0.1	0.3	0.1	Pigment
0.48	—	0.3	0.1	0.4	0.1	Pigment
0.47	—	0.2	0.1	0.4	0.1	Pigment
0.46	—	0.6	—	—	—	Pigment
0.46	—	—	0.7	—	—	1,3 diglyceride
0.42	—	0.5	—	—	—	Pigment
0.42	—	—	0.1	—	—	1,2 diglyceride
0.39	—	0.7	0.5	—	—	Free fatty acids and free sterols
0.39	—	—	—	1.1	—	Pigment
0.30	—	—	—	1.1	—	Pigment
0.25	—	—	—	0.1	—	Pigment
0.18	—	—	0.1	—	—	Monoglyceride
0.00	—	—	—	1.3	8.0	Polar lipids

Note: Data obtained from TLC developed with neutral lipid solvent A. R_f values for authentic lipids were found to be eicosane, 0.88; cholesteryl oleate, 0.79; methyl stearate, 0.69; tripalmitin, 0.50; 1,3-dipalmitin, 0.45; 1,2 dipalmitin, 0.41; myristic acid 0.39; monopalmitin, 0.17. Values expressed as percentage of the total lipids were determined with a recording Zeineh soft laser scanning densitometer.

TABLE 2. CHARACTERISTICS OF POLAR LIPID COMPONENTS OF N. OLEOABUNDANS

Av. R_f Values	Hexane + Benzene + $CHCl_3$	Acetone	MeOH	Probable Identities of Components
0.91	79.9	10.5	0.4	Neutral lipids
0.83	—	0.8	0.2	Monogalactosyl diglyceride
0.43	—	—	1.1	Diphosphatidyl glycerol
0.38	—	—	2.4	Phosphatidyl ethanolamine
0.33	—	0.2	0.8	Phosphatidyl serine
0.30	—	0.3		?
0.28	—	—	0.8	Digalactosyl diglyceride
0.23	—	—	1.5	Phosphatidyl glycerol
0.18	—	—	0.7	Phosphatidyl choline
0.08	—	—	0.3	Phosphatidyl inositol

Note: Data obtained from TLC developed with polar lipid solvent B. R_f values for authentic lipids were: tripalmitin, 0.91; monogalactosyl diglyceride, 0.83; phosphatidic acid, 0.62; steryl glycoside, 0.52; diphosphatidyl glycerol, 0.43; phosphatidyl ethanolamine, 0.38; phosphatidyl serine, 0.33; digalactosyl diglyceride, 0.28; phosphatidyl glycerol, 0.23; phosphatidyl choline, 0.18; phosphatidyl inositol, 0.08. Values, expressed as a percentage of the total lipids were determined with a recording Zeineh soft laser scanning densitometer.

TABLE 3. COMPOSITION OF THE STEROL FRACTION OF N. OLEOABUNDANS

Component Identity	Retention Time (min)	Area (%)
2-methyl-heptadecanoic acid	21.7	7.0
2-methyl-octadecanoic acid	24.7	19.6
Unid. sterol	31.4	17.0
Unid. sterol	34.3	4.2
Unid. sterol	35.2	1.4
Sterol 1	49.5	6.9
Sterol 2	51.4	7.0
Sterol 3	59.7	6.0
Sterol 4	58.9	30.4

Note: Retention times were determined on a 10 m x 0.25 mm SP2100 fused quartz capillary operated from 60 to 240 C at 6 C min^{-1} with He at 8 psi.

TABLE 4. SUMMARY OF THE MASS SPECTRAL FRAGMENTATIONS OF THE ISOLATED STEROLS

Fragmentation	Sterols			
	1	2	3	4
M	438	440	428	442
M - CH_3	423	425	413	427
M - AcOH	378	380	368	382
M - (AcOH + CH_3]	—	365	—	367
M - side chain	311	313	315	315
M - (side chain + C16, C17)	—	288	—	288
M - (side chain + 42)	—	—	—	273
M - (side chain + AcOH)	251	253	255	255
M - (side chain + C16, C17, + OAc)	—	229	—	229
M - (side chain + AcOH + D-ring)	—	213	—	213

= 428 and was tentatively identified as cholest-14-en-3 β-ol on the basis of its mass spectrum and retention time.

Two fatty acids were identified in the sterol fraction exclusively (Table 3). The fatty acids were identified as 2 methyl-heptadecanoic acid and 2 methyloctadecanoic acid. The α-methyl branch was identified on the basis of the predominant ion at m/e = 88 resulting from a McLafferty rearrangement.

$CHCl_3$ eluate. The chloroform eluate contained about 75% of the total lipids (Table 1). The principal lipid cochromatographed with a triglyceride standard. An additional 7% of the lipids that cochromatographed with triglyceride standard also appeared in the acetone eluate. Deacylation products of these components from each eluate isolated by preparatory TLC yielded only fatty acids and glycerol in a ratio of 3 fatty acids to 1 glycerol. Less than 1% each of 1,3 and 1,2 diglycerides and monoglyceride were detected in the $CHCl_3$ eluate. The triglycerides represent about 80% of the total lipids. Refractionation of the acetone fraction on a silicic acid column with a greater lipid load capacity only partitioned 20% of the triglycerides into the $CHCl_3$ eluate, the remainder again eluted into the acetone eluate. The fatty acid profiles of the triglycerides from the $CHCl_3$ and acetone eluates (purified by elution from TLC plates) were not significantly different. Additional components (0.5%) in the $CHCl_3$ eluate cochromatographed with free fatty acids standards.

Acetone eluate. In addition to the triglycerides found in the acetone eluate and previously described together with the triglycerides occuring in the $CHCl_3$ eluates, the acetone eluate contained most of the pigments extracted from the cells. The pigments were not analyzed. A polar fraction that did not migrate from the origin in the neutral lipid solvent represented about 1.3% of the total lipids (Table 1). The acetone eluate run on TLC in a polar lipid solvent revealed three polar components (Table 2). Two of the components cochromatographed with monogalactosyl diglyceride and phosphatidyl serine, respectively, while the third did not cochromatograph with authentic standards and was not identified. The unidentified compound with an R_f value of 0.3 (Table 2) gave a positive reaction to alkaline $AgNO_3$, indicating the presence of vicinal hydroxal groups.

MeOH eluate. The polar lipids isolated in the MeOH fraction were those commonly found in microbial systems. The principal component cochromatographed with phosphatidyl ethanolamine (Table 2). The remaining components were tentatively identified as phosphatidyl choline, phosphatidyl serine, phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl inositol, monogalactosyl diglyceride, and digalactosyl diglyceride (Table 2).

The tentative identification was supported by ninhydrin stain for primary amines, a phosphate stain and an alkaline $AgNO_3$ stain for vicinal hydroxides.

Alkaline and Acid Hydrolyses of Polar Lipid

Two-dimensional chromatographic separation of the water-soluble components from mild alkaline deacylation of the combined acetone and methanol eluates demonstrated that most of the components cochromatographed with those of established standards. These data supported the tentative identification assigned to the intact lipids given in Table 2. Two of the ninhydrin positive spots had R_f values identical to glycerolphosphorylserine and glycerolphosphorylethanolamine standards. The third compound was not identified. Four compounds that were periodate-Shiff positive had R_f values that corresponded identically to authentic standards of glycerolphosphorylinositol, glycerolgalactose,

glycerol (galactose)$_2$, and glycerol. The total composition of the deacylated fraction was visualized by staining first with an o-tolidine stain followed with an acid molybdate solution. The remaining compounds identified by comigration to standards were glycerolphosphorylcholine, glycerolphosphorylglycerol, and diglycerolphosphorylglycerol. Three additional components were indicated in the mild alkaline hydrolysate that were not detected in the int t lipids studies by TLC (Table 2). One of the compounds cochromatographed with glycerolphosphatidyldimethylethanolamine. The other two compounds were not identified.

Analyses of the hydrolysates obtained from acid hydrolyses of deacylated lipids supported the identifications (Table 2) by the detection of ethanolamine, monomethylethanolamine, choline, serine, and an unidentified amino compound (Table 5). Acetylation of the water-soluble components of the acid hydrolysate and analyses by GLC/GC-MS further supported the identification assigned to the lipids (Table 6). The large quantity of glycerol detected in the polar lipids was attributed to the presence of triglycerides in the acetone eluate. The identification of inositol and galactose is consistent with the assigned identification of phosphatidyl inositol, monogalactosyl diglyceride, and digalactosyl diglyceride based on chromatographic properties. Additional components in the acetone and M_eOH eluate are listed in Table 6. In addition to glycerol, inositol, and galactose, some heptose and trace amounts of mannose and glucose were identified. The electron impact (e.i.) fragmentation pattern of the heptose showed peaks at m/e 433, 361, 289, 217, and 145 which are expected from the cleavage of the alditol chain. Ions formed by the elimination of Ac_2O, HOAc, and ketene were also detected. The amount of material available did not allow isolation of the sugar in sufficient quantity to determine its exact structure. The chemical ionization spectrum (CH_4- and NH_3-C.I.) of the two components eluting shortly after the heptose showed a molecular weight M = 506, similar to that of a heptose. The e.i. mass spectra of the two unknown compounds are essentially identical to each other, but significantly different from the expected pattern of a heptose. The mass spectrum consisted of a base peak at m/e = 159, which is a characteristic fragment of 2-deoxy sugars. Minor peaks at m/e = 115, 145, 200, 243, and 331 were detected. This information along with the C.I. spectrum was not sufficient to propose a structure for the unknown sugars.

Fatty Acid Composition

The fatty acid composition of the $CHCl_3$ eluate collected from a silicic acid column and which consisted predominantly of triglycerides (Table 1) is given in Table 7. The range of acids was from C_{14} to C_{20} with predominantly evennumbered carbon fatty acid chains. The even-numbered carbon chains existed as saturated, mono-, and diunsaturated ones. The fatty acid fraction also contained a relatively substantial quantity of odd-numbered carboxylic acids existing as saturated, normal, and branched chains. MeOH-HCl hydrolysis of the polar lipid fraction provided fatty acid methyl esters like those described in Table 7. Two additional fatty acids were isolated from the sterol fraction and identified as 2-methyl-heptadecanoic acid and 2-methyl-octadecanoic acid (Table 3). These two α-methyl branched acids were not detected in the triglyceride preparations nor the polar lipid fraction.

Neochloris Cells as a Culture Support Medium

The purpose of this pilot study was to determine if Neochloris cultures could support growth of Pseudomonads, and if the high concentration of neutral lipids in the form of

TABLE 5. CHARACTERISTICS OF H_2O SOLUBLE COMPONENTS IN ACID HYDROLYSATES OF TOTAL POLAR LIPIDS

R_f Value	Probable Identification
0.47	Ethanolamine
0.44	Monomethylethanolamine
0.42	Dimethylethanolamine
0.39	Choline
0.28	Amino compound
0.25	Serine
0.21	Amino compound

Note: Components were chromatographed on 1 mm Whatman paper in pyridine-ethyl acetate-H_2O (x2) in one dimension. The chromatograph was visualized with ninhydrin or CoSCN. The components were identified by comparing R_f values to those of known and established standards.

TABLE 6. NEUTRAL SUGARS RECOVERED FROM THE ACID HYDROLYSED DEACYLATED POLAR LIPIDS

	Rel. Composition (%)	Rel. Retention Times (min)	M+18 (NH_3-P.C.I)
Glyercol	82.8	6.3	236
Mannose	trace	27.4	452
Galactose	2.6	28.1	452
Glucose	trace	28.3	452
Inositol	4.9	28.5	450
Heptose	4.8	37.7	524
Unidentified	3.6	38.1	524
Unidentified	1.3	38.4	524

Note: The data were obtained by GC and GC-MS of the alditol acetate derivatives. The retention times were recorded on a glass column (2.5 m x 31 mm) packed with 10% SP2330 on Chromasorb W AW. The column was operated at a He-flow rate of 30 mL/min from 120°-235° at a rate of 4°/min. Relative amounts were determined from the FID signal, calibrated with standard compounds if available. The molecular weights of the alditol acetate were obtained by chemical ionization mass spectrometry (P.C.I.) using NH_3 or CH_4 as a reactant gas.

TABLE 7. FATTY ACID METHYL ESTERS OF TRIGLYCERIDES OF N. OLEOABUNDANS

Peak	Fatty Acid Identity	Molec. Wt.	Area %	Position of Double Bond
1	14:1	240	0.4	
2	14:0	242	1.6	
3	iso-15:0	256	1.0	
4	15:0	256	0.4	
5	16:2	266	2.5	
6	16:1	268	3.5	Δ^7
7	16:0	270	15.0	
8	iso-17:0	284	8.4	
9	C17:1	282	1.0	
10	17:0	284	3.3	
11	18:2	294	7.4	
12	18:1	296	36.0	Δ^9
13	18:0	298	11.0	
14	iso-19:0	312	0.5	
15	19:1	310	0.1	
16	19:0	312	0.3	
17	20:1	324	2.5	Δ^{11}
18	20:0	326	2.1	

Note: Components were separated on SP2100 fused quartz capillary as in Table 3. The components were identified by relative retention times and their mass spectral pattern taken as each component eluted from the column. The double-bond positions of the major monounsaturated acids were accomplished by mass spectrometry of the acid when in the pyrrolidine ester form. See the text for descriptions of two additional fatty acids found in hydrolysates of the sterol fractions.

triglycerides would stimulate hydrocarbon production. Over the last 10 years 12 species/mutants of a gram negative Pseudomonad were isolated that synthesized 3%-5% of its dry cell weight in branch, unsaturated, acyclic hydrocarbons. The hydrocarbons are synthesized by condensation-reduction reactions of two long chain fatty acids. These organisms are bacteria that are normally cultivated on a trypticase soy medium. One Pseudomonad strain was tested on viable broth cultures of Neochloris, autoclaved broth cultures, and saponified broth cultures. The test culture grew well in each preparation providing total cell production between 0.4 and 0.8 g of total cells per liter. The saponified broth culture preparation was the best support medium for the cultivation of the bacterium. Since the nature of the hydrocarbons and fatty acids of the bacterium were significantly different from the alga, these two lipid factors were evaluated. The results of the study clearly showed that the algal fatty acids were utilized by the bacterium; however, the algal fatty acids were not assimilated directly into bacterial hydrocarbons through the bacterial's normal condensation route. The algal fatty acids were oxidized to the acetate level and then reassembled into bacterial fatty acids which were condensed into hydrocarbons. Cell free preparations of the bacterial cells, however, contained enzymes in a particulate fraction that incorporated algal fatty acids into bacterial hydrocarbons.

DISCUSSION

Nitrogen-starved N. oleoabundans cells were harvested from media deficient in nitrogen. The cells exhibited the same appearance of cellular oil droplets previously described [1] and attributed to a high oil-producing capacity [2]. Repeated preparations of the cells consistently provided liquid hydrophobic compounds that constituted 36%-54% of the cell dry weight; more than 80% of the oil was triglycerides. The remaining constituents were predominantly pigments, sterols, glycolipids, and phospholipids. The phospholipids are those commonly found in microorganisms. The broad range of phospholipid types found were those expected in "old" cells or those cultivated in nutritionally deficient media. The occurrence of the saturated form of the hydrocarbon was not typical of green algae, which commonly contain the monounsaturated form [6]. The saturated n-C17:0 that constituted 97% of the hydrocarbon fraction is more typically found in red algae. The glycolipid fraction consisting of galactose, glycerol, and inositol derivations is usually expected. The occurrence of heptose and two unidentified sugars, however, are not typical sugars found as constituents of glycolipids in algae. The heptoses, together with small quantities of mannose and glucose, are sugars typically found in lipopolysaccharides of bacteria. The harvested algae were not axenic cultures; however, the bacterial content was in relatively small concentrations. In addition, the relatively large amount of heptose and unidentified sugars (Table 6) was inconsistent with that expected in a lipid extract, assuming the source of the sugars is from the lipopolysaccharides of bacteria. Similar considerations can be made on the existence of odd-numbered carbon fatty acids (Table 7). The source of these fatty acids, however, was triglycerides; typical contaminating bacteria are not abundant producers of triglycerides. Whether or not some of the fatty acids and sugars are contributed by bacteria is secondary to the importance of the amount of oily lipids produced by photosynthetic microorganisms in mineral media.

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RESOURCE ASSESSMENT FOR MICROALGAL/EMERGENT AQUATIC BIOMASS SYSTEMS IN THE SOUTHWESTERN UNITED STATES

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INTRODUCTION

Aquatic biomass systems in the southwestern United States must utilize the available resources of the area as efficiently as possible. This research project is designed to facilitate the selection of production systems using aquatic species [microalgal and emergent aquatic plant species (MEAP)] that effectively exploit potential resources of the Southwest.* In cooperation with SERI's Renewable Resource and Instrumentation Branch, four specific subobjectives of the project are as follows:

- To identify available land resources
- To identify available water resources
- To select aquatic plant species (microalgal/emergent aquatics)
- To identify and rank research needs.

The first activity of the project was to develop specific criteria for identifying resources and species.

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*The area under consideration in this study is restricted to U.S. land, S of 40° latitude and W of 100° longitude.

TASK 1. IDENTIFICATION OF LAND AND CLIMATIC RESOURCES

To develop a data base from which to assess the land and climatic resources of the southwestern United States for the eventual development of microalgal and emergent aquatic biomass production systems, it is necessary to (1) identify the pertinent land and climatic factors that are important in siting biomass production systems, and (2) develop qualitative and quantitative screening criteria for each factor. These criteria are necessary to provide guidance and organization to SERI's data collection. The important land- and climate-related factors identified so far are as follows:

Land-Related Factors

- Soil characteristics
- Land ownership/availability
- Land use/cover.

Climate-Related Factors

- Solar radiation
- Temperature
- Severe weather.

Land-Related Factors

Soil Characteristics

Biomass production systems currently include raceway systems, open-pond systems, and enclosed systems. Construction materials for these systems are likely to include concrete, steel, earth, and synthetic liners (in cases where soils are highly permeable). The relevant characteristics of soils at the biomass production system site are found to be (1) slope or topography, (2) depth to bedrock, (3) textural classification, (4) permeability classification, (5) salinity, (6) hydrologic grouping, (7) risk of corrosion, and (8) taxonomic unit (association).

The soil slope criterion, for example, has been broken down as follows: level or nearly level land (0%-2% slope), gently sloping land (2%-5% slope), and gently sloping land or greater (more than 5% slope). And adequate soil depth is required to permit the shallow excavations (1.5-1.8 m) necessary for the construction of the physical facilities, to provide stable support for foundations of concrete and steel structures without excavating rock. Rock excavation over much of the project area could significantly affect the economics of site construction. Because of the scale of the project, and because the most economical type of construction would be one in which the culture media level would be at or slightly above the existing ground elevation, screening criteria for soil depth have been categorized as follows: very deep soils (greater than 60 in.), deep soils (40 in. to 60 in.), and moderately deep soils (20 in. to 40 in.). This criterion limits the ideal site to locations where soil depth exceeds 60 in. In sloping terrain, because the entire project site ideally would have to be smoothed and leveled to a single elevation, greater minimum soil depths would be required to permit necessary cut-and-fill operations.

Land Ownership/Availability

Land ownership is a critical parameter in the siting of a project of this scope and must be taken into consideration. Not only must land areas in the Southwest having adequate soil characteristics be identified, the land must be available for developing biomass production systems. The chief categories of land ownership depicted on various land ownership state maps are (1) federal lands controlled by various federal agencies including the U.S. Department of Interior, U.S. Department of Defense, U.S. Department of Agriculture, and U.S. Department of Energy; (2) lands controlled by the various states; (3) land controlled by various Indian organizations; and (4) land controlled by private entities. It is likely that adequate land exists in each of these ownership categories.

U.S. Department of Interior. Land with adequate characteristics for aquatic biomass production may be available from lands controlled by various agencies under the Department of Interior; the primary agency is the Bureau of Land Management (BLM). Land ownership maps of various dates and categories could be available for each state from an appropriate BLM office. Each contiguous available land unit over about 800 ha (2000 acres) should be identified, along with various acquisition alternatives.

U.S. Department of Defense. Suitable land may also be available from lands controlled by various agencies under the Department of Defense. The primary agencies controlling land in the eight-state study area are the Departments of the Army and the Air Force.

U.S. Department of Agriculture. Various agencies under the U.S. Department of Agriculture may control land suitable for aquatic biomass systems. The primary USDA agency controlling land in the eight-state study area is the U.S. Forest Service. Unlike the BLM, the Forest Service does not maintain state-level offices, so the regional offices would be the most appropriate starting points.

U.S. Department of Energy. Land with adequate characteristics could be available from that controlled by the Department of Energy or agencies under its control. Information on Department of Energy landholdings in the study area should be available from the real estate section of DOE or a real estate information source at a specific DOE agency.

State Lands. Land suitable for aquatic biomass systems may be available from the holdings of the various states in the study area. State holdings are particularly significant in the states of Utah, Arizona, and New Mexico. The land administration system is probably quite different in each state and the various classifications or categories of state land are probably different in degree of availability in each state. In Arizona, for example, the coordinating agency is the Arizona Land Department, and in Texas it is the General Land Office. Similar coordinating state agencies may exist in other states of the study area. State-owned parcels of over 800 ha should be identified during initial screening.

Climate-Related Factors

Solar Radiation

Intensive outdoor production of algal biomass could be limited by several major factors, including water availability, minerals, carbon source, temperature, and light. In cultures kept under optimal conditions with respect to the first four factors, growth generally is limited by the amount of sunlight energy available to the cells in the culture [1]. The

The most promising source of water for MEAP systems appears to be saline groundwaters. Drilling to sufficient depths, it is likely that saline groundwaters will be encountered throughout the study area. Provided that adverse effects on freshwater aquifers can be prevented, these deeper groundwaters are potentially attractive sources for several reasons:

- Given the water rights situation in the Western United States, it would be difficult if not impossible to obtain adequate surface water (as, for example, from the Central Arizona Project) or shallow groundwater for the anticipated project scale.
- These resources are presently considered to be a nuisance when compared with less saline waters and often exhibit disposal problems during exploratory well drilling.
- Depending on the geologic formation, nutrients required for plant growth may be already present in significant quantities.

Concerns regarding the use of this source of water include the following:

- Identification of data indicating where saline groundwaters may be found, their depth, and quantities available
- Obtaining a consistent set of data using the same definition of salinity
- Potential for downward movement of freshwater due to withdrawal of more dense saline waters
- Environmental protection of potable water aquifers from contamination due to water seeping through the bottom of the culture system or due to the need to discharge a waste stream (known as blowdown) to maintain the salt levels within an acceptable range.

Gross Water Yield. This criterion refers to the amount of water available, without regard to depth, quality, or institutional barriers. Ideally, this information should be presented on an areal basis (for example, cubic meters per minute per hectare) to facilitate an analysis that recognizes the scale of MEAP systems. The information is displayed in this manner to make sure that a sufficient number of wells can be drilled to supply the system without having to pump water over long distances. Yield per unit area is a critical parameter which recognizes that multiple wells will be needed and that the overall MEAP system size will be dictated by the area needed to obtain the water.

Unfortunately, specific information will not be available except in isolated areas. Typically, three types of information are reported: (1) subjective impressions of flows obtained without a pumping test, (2) capacity (liters/minute) data for a single isolated well, and (3) capacity (liters/minute) and drawdown (reduction in surface elevation) in meters from which specific capacity (liters/minute-meter) can be computed.

Depth to Water. Suitable quantities of water may be found only at considerable depth. Much of the operating cost associated with delivering water to the site will be attributable to bringing the water from the aquifer depth up to the surface.

Depending on the geologic configuration of the aquifer, the physical distance between the ground surface and the level of the aquifer may not be the controlling factor. Aquifers are broadly characterized as unconfined or confined. An unconfined aquifer is one in which the water in the pores of the medium is exposed to atmospheric pressure and which is not overlain by strata that prevent a downward movement of precipitation. Depth to water in these situations refers to the physical depth in the aquifer where water

is encountered. A confined aquifer, on the other hand, is one overlain and underlain by impermeable strata such that the water at a particular location may be at a pressure other than atmospheric. In these situations, a well drilled to tap the waters can exhibit a water level considerably higher than the physical upper limit of the water-bearing strata. In extreme cases, this level may actually be at or above the ground surface, creating an artesian condition. Various degrees of confinement between the two extremes are typically encountered.

In many cases, determining the depth to water is difficult because the water levels are a function of the pumping rate and the rate of recharge. The recharge rate is an important concept in the long-term use of wells for MEAP systems. Aquifers are subjected to a state of dynamic equilibrium when water is withdrawn. In arid regions, this water may have been in groundwater storage for long periods of time; withdrawal may require very close control to avoid continually declining levels over time. Where the pumping rate consistently exceeds the recharge rate, a condition known as groundwater "mining" exists. Often, this practice leads to a complete depletion of the resource and subsidence of the land surface above the aquifer.

As in the case of groundwater quantity, the manner in which the data are recorded varies greatly. When water is found incidentally to other purposes (for example, oil exploration), the supervising geologist may note only the drilling depth at which the water was first observed. On the other hand, exploration for geothermal sources may provide a very detailed evaluation of water levels as a function of pumping rate.

Data should be mapped for the most productive aquifer(s). A data resolution of 30 m (100 ft) may be acceptable, but the number and spacing of the isopleths should be determined by taking available information into consideration.

Current and Potentially Competing Uses. This criterion addresses the significant question of whether institutional constraints exist to the use of physically available water. Most of the western states have permitting programs that appropriate to potential users the right to a certain amount of water obtained from a particular source. When a potential user acquires a claim, his or her priority to use the water is based on the date of the claim, and subsequent claims are lower in priority. Thus, a distinction should be made between current users who obviously hold permits and potential future uses that are projected but not currently permitted. If a claim for a MEAP system water permit is filed beforehand, no conflict exists. Data on the status of water rights to saline aquifers should be mapped to indicate the degree of restriction that exists. Three or four levels should be considered:

Level 1: No current usage, no reasonable potential usage (unrestricted)
Level 2: Potential future usage based on projected activities in the near term (5 years) but no current usage
Level 3: Inchoate permit holders or projected water-using activity in the near term, or both
Level 4: Current significant usage (most restricted).

Data should be available for most of the study area on the current status of allocation permits. Less reliable or detailed information may exist for projected activities such as mineral resource extraction or geothermal systems.

Major Concerns/Capable of Being Ameliorated by a Moderate Degree of System Engineering

Water Quality. Water of adequate quality must be provided to MEAP systems to allow maximum growth rates and environmentally acceptable disposal of residuals. From the standpoint of salt tolerance, microalgal species can be found that span the salinity range from freshwater to highly saline water. Therefore, if the salinity is maintained in a given range, it becomes a matter of matching the species to the system. For a given system, the salt concentration in the channels (or ponds) is a function of the evaporation rate, the salt concentration in the make-up water, and the salt losses from the system. The two major salt-removal mechanisms are seepage through the channel (or pond) bottom and moisture removal in the harvested crop.

If no other means of removing salt is provided, the concentration of dissolved solids is then fixed. Assuming that the seepage is reduced to negligible amounts to prevent contamination of surface water supplies, the salt concentration at the tail end of the system will increase by about a factor of 20. This means that a species must be selected that tolerates the resulting concentrations. Alternatively, the system could be set up to provide varying growth conditions (increasing in salinity) in successive channels (or ponds), allowing the growth of different species. From an engineering standpoint, it is most practical to withdraw a continuous blowdown stream to maintain salt content at the desired level.

However, the latter approach creates a continuous waste stream that must be disposed of in an environmentally sound manner. The most economical method of disposal would be to simply pump the brine to evaporation ponds, but solar-assisted evaporators (for example) may be cost-effective if the need for make-up water can be reduced significantly by recycling.

Along with ions such as sodium and chloride present in brines, hardness cations such as calcium and magnesium will be present. In some geologic formations, these ions may be in equilibrium with solid phase minerals. Withdrawal of groundwaters from the geologic setting, addition of CO_2 via aeration or sparging, and algal growth (use of bicarbonate ion and carbonic acid) may alter the pH of the system and may cause the formation of calcium carbonate. The most pronounced effect of precipitation of significant amounts of calcium carbonate would probably be the reduced effectiveness of the incident solar radiation because of the increased shading of the culture.

Finally, water obtained from deeper aquifers may contain phytotoxic concentrations of some trace elements. These waters would either require treatment to reduce the concentrations or would have to be avoided altogether.

Salinity or Total Dissolved Solids Concentration. High concentrations of salinity will limit flexibility in operating the system and in extreme cases will require a blowdown stream to make the system feasible at all. Salinity, while having a precise chemical meaning, is used rather loosely in practice. Typically, a distinction is made between fresh and saline groundwaters at a dissolved solids concentration of 1000 mg/L. This is often the only distinction possible, given the available information.

Ideally, chemical analysis data will allow a further delineation of the range above 1000 mg/L into the following subranges as defined by the U.S. Geological Survey:

Slightly saline	1,000-3,000 mg/L
Moderately saline	3,000-10,000 mg/L
Very saline	10,000-35,000 mg/L*
Briny	More than 35,000 mg/L*.

Further resolution of the isopleths would have to be justified by the data. Mapping of dissolved solids data should be limited to the most productive aquifers as defined in the water quantity criteria description.

Soil Textures (Permeability). The ability of the native soils to prevent the transmission of water is an important economic consideration in determining the feasibility of a MEAP system. If water losses due to leakage are significant, not only will the overall water usage increase, but the potential for environmental degradation will also exist.

Some types of soils, particularly those containing large percentages of clay and silt, tend to minimize seepage. At the other extreme, soils comprised predominantly of sand or gravel-sized materials tend to be quite permeable.

The data used for mapping should be uniformly available. Given the study area size, a generalized soils map of each state should provide adequate resolution for purposes of this analysis. Areas should be categorized based on soil associations as follows:

- Level 1: Associations consisting predominantly of impervious soils or subsoils (e.g., homogeneous clays and silty clays)
- Level 2: Associations consisting predominantly of poorly drained soils or subsoils (e.g., very fine sands, organic and inorganic silts, and loams)
- Level 3: Associations consisting predominantly of well drained soils or subsoils (e.g., clean sands, clean sand and gravel mixtures, and clean gravels).

Chemical Characteristics—Toxic Substances. Maximum growth rates for MEAP systems can be obtained only if the concentrations of algal toxins are below a threshold level. Considering the nature of groundwater, most of these substances are likely to be trace metals or nonmetals (for example, arsenic or boron). In coastal areas, other types of toxic substances such as industrial pollutants could be important. Phenolic compounds and free cyanides are two additional constituents relevant to toxicity evaluation. Although there is evidence in the literature that synergisms or antagonisms may exist in the presence of multiple toxins, it will not be possible to account for these effects.

Available data on trace chemical analysis should be acquired for the most productive aquifers identified in the water quantity subtask. It may be possible to limit the number of factors mapped if a tentative list of potentially toxic substances can be compiled.

Also, two options for mapping the data have been identified. If all of the concentrations are combined, the factor could be mapped as "total toxin concentration." The limited availability of data will probably preclude combination criteria mapping. Therefore, the recommended mapping approach is simply to map the available chemical species individually in appropriate ranges.

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*Waters in this range will require a blowdown stream to control salt concentrations.

It is not possible at this time to specify the ranges since the range boundaries should be sensitive to the toxic thresholds of individual species. As work progresses, more refined estimates of ranges can be provided.

Minor Concerns, of Economic Significance Only

Water Quality and Nutrients. Additional fertilizer elements will be needed for maximum growth potential. Of the macronutrients (other than carbon) nitrogen, phosphorus, and perhaps potassium will require augmentation. Concentrations of these elements may vary significantly, and waters having higher concentrations (other factors being equal) should be more favorable. If cyanophytes or other nitrogen-fixing species are cultured, the nitrogen concentration may be irrelevant. Trace nutrients, such as iron, copper, and zinc, may also be supplied in different quantities by various water sources. However, it may not be possible to define crop requirements as precisely as for macronutrients.

Chemical Characteristics—Nutrients. This criterion is similar in approach to that described for toxins, requiring the acquisition and mapping of data on concentrations of individual chemical constituents. Additional information is needed on nutritional requirements before the criterion range specifications can be developed, for these reasons:

- The range boundaries should be sensitive to differences between species needs.
- The probable significance of this source of nutrients should be evaluated by trial computation before any significant level of data manipulation effort.

TASK 3. SELECTION OF SPECIES

Several species of microalgae and emergent aquatic plants may be amenable to large-scale aquaculture. Species selection for a given system should be based on specific criteria that allow efficient utilization of available resources. The goal of the present task is to identify the specific criteria for selecting species and to characterize the various species with respect to these criteria.

This task was scheduled for completion by the end of July 1982, and meanwhile the important criteria for species selection were gleaned from the work of others. These criteria are provided in Table 1.

It is difficult to order the criteria. Previous efforts at growing microalgae, mostly for single-cell protein production, generally have attempted to provide nutrients, temperature, water, etc., under nonlimiting conditions in order to force radiant energy as the limiting condition [4]. Since light energy may not be the limiting condition to large-scale aquaculture, and since single-cell protein may not be the desired end-product, criteria other than radiant energy may be of equal importance. Rather than attempting to rank the criteria now, we present examples of the rationale behind criteria selection.

Growth Rate and Yield

Growth rates for different species vary markedly and depend on conditions such as nutrient supply, culture dilution rate, culture mixing, etc. When light energy is limiting,

TABLE 1. CRITERIA FOR SPECIES SELECTION FOR LARGE-SCALE AQUACULTURE

- Growth rate
  - Optimum
  - Minimum
  - Short-term
  - Long-term
- Yield
  - Short-term
  - Long-term
- Photosynthetic efficiency
- Response to environmental changes
  - Light
  - Salinity
  - Nutrients
  - Toxic conditions
    - metals
    - organics
  - Temperature
  - Scale-up
  - Dilution rates
- Growth requirements
  - Radiant energy
  - Water
    - use rate
    - efficiency
    - fresh
    - brackish
    - saline
    - hypersaline
    - pH
    - temperature
  - Nutrients
    - nitrogen
    - phosphorus
    - carbon
    - organic
    - inorganic
    - others
- Biochemical composition
  - Protein
  - Carbohydrates
    - monosaccharides
    - polysaccharides
  - Lipids
    - triglycerides
    - terpenoids
  - Others (e.g., phenolics)

it has been found that maximal growth rates (dry wt. m^{-2} day^{-1}) generally are obtainable only in the short term (less than one month). In addition, scaling up from small "bench top" culture units to larger units may result in a decreased growth rate. Goldman [6] has indicated that 30 to 40 g dry wt. m^{-2} day^{-1} represents the maximum yield obtained in the short term with microalgae when natural sunlight is the forced limiting condition, regardless of the other cultural conditions such as species or culture system. This rate is related directly to the photosynthetic conversion efficiency, which is approximately 5%

under the best conditions. Whether the photosynthetic conversion efficiency of algae in mass cultures can be improved is not clear. It may be more important to determine the optimum growth rate of the various species as opposed to a maximum growth rate [7]. This optimum growth rate is based on several factors such as dilution rate, light penetration with the depth of the culture, cell senescence due to photooxidation or photorespiration, etc. Clearly, criteria such as growth rate, maximum yield, and photosynthetic efficiency are related. Species of microalgae and emergent aquatic plants that may be useful in large-scale aquaculture systems need to be delineated in terms of these criteria. Where information is lacking, further research can be done.

Biochemical Composition and Response to Environmental Changes

The exact biochemical composition of some microalgae species can be altered by adjusting cultural conditions, such as altering the glycerol content of Dunaliella by changing the salinity level of the aqueous medium [8]. Thus, the biochemical compositions of some microalgae reportedly may be manipulated to consist of over 65% protein, up to 75% lipids, or up to 58% carbohydrates [9]. Furthermore, the responses to altered cultural conditions appear to be specific to the species.

Apparently, then, many of the biochemical characteristics in Table 1 can be environmentally manipulated, depending on the species. It is critical to delineate what is currently known concerning the species-specific end-product composition in order to plan future research and to be able to assess the current and future potentials for producing chemicals from aquatic biomass.

Requirements for Growth and Product Biosynthesis

As discussed earlier, most previous work with microalgae has employed cultural conditions that are light-limiting. In fact, in large-scale aquaculture, for chemicals production the limiting factor may be carbon dioxide and/or water availability. Thus, it will be important to determine which particular species of microalgae and emergent aquatic plants are the most efficient users of limited resources: inorganic carbon and water of various salinity levels, pH, and temperature.

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