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PREFACE

This volume contains progress reports presented by the Aquatic Species Program subcontractors and SERI researchers at the SERI Biomass Program Review held in San Diego, California, March 9-10, 1983. These reports present and discuss research advances achieved by the program participants during the preceding year. The SERI Biomass Program receives its funding through the Biomass Energy Technology Division of the Department of Energy.

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Approved for

SOLAR ENERGY RESEARCH INSTITUTE

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INTRODUCTION

The Aquatic Species Program (ASP) addresses the utilization of plant biomass that naturally occurs in wetland or submerged areas. Processes are being developed through this program to make use of such aquatic species, capitalizing on their inherent capacity for rapid growth as well as their extraordinary chemical compositions. Emphasis is placed on salt-tolerant species for cultivation on poorly utilized, low-value lands, where conventional agriculture is not economic. 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.

Research supported by the ASP is broken down into biological research, engineering research, and technology assessment. Biological research includes the "manipulation" of microalgae in the laboratory. This manipulation can take many forms, all of which are attempts to force the microalgae to produce the desired product as a larger-than-natural fraction of their cell weight. One method involves alteration of the microalgae's aquatic environment such that they naturally respond to these stimuli by producing more lipids. Subjecting certain microalgae to a deficiency in the nutrient nitrogen stimulates lipid production at the expense of proteins (which require nitrogen for their manufacture). Alternatively, scientists can attempt to change the way the microalgae naturally respond to their environment. This can be done by tampering with the cell's genetic machinery, which is responsible for reproduction and metabolic regulation. This can be done by mutagenesis or genetic engineering.

All of the above-mentioned techniques for microalgae manipulation can be performed in the laboratory as part of the ASP biological research. One activity assumed to be done prior to and along with the manipulatory research is species collection and screening. Through this process, scientists attempt to find species of microalgae that naturally produce significant quantities of the desired product, are amenable to manipulation for increasing the quantities of this product, and can thrive in the environment foreseen for eventual mass production. It is also necessary to define each species' nutritional needs and tolerances to variations in such environmental parameters as temperature, pH, and salinity. These tolerance-levels are important because it would be too expensive or even impossible to maintain rigid control over these parameters in the field.

Engineering research is concerned with the productivity of the algae under the actual field conditions to be encountered in the mass production system. The engineer is concerned with not only the algal growth unit, but also the harvesting technique and the processing technology necessary to turn out the final product or products. The bulk of the engineering research to date has dealt with the algae growth unit.

This research progresses from analysis of theoretical and laboratory data to conceptualization of system designs, followed by construction and operation of the various designs and, finally, performance and cost comparisons of each concept to decide which are worthy of scale-up for final debugging. The initial theoretical analysis step provides a standard against which prototype performance can be measured. Using only theoretical considerations allows definition of the maximum possible performance or yield in such terms as metric tons of lipid product per hectare per year. No limitations connected to the operation of a real system are included. Instead, only limitations imposed by nature are

considered—mainly, availability of sunlight and the efficiency of its use by the organism. The introduction of laboratory data into the analysis defines a more realistic performance goal by defining the limits of manipulation and control of the algae and their environment. In the laboratory the scientist can use very sophisticated equipment on a very small scale to exert the maximum possible control over the microalgal environment. This degree of control is very expensive. When dealing with economic realities, the engineer is faced with trade-offs between cost and productivity.

In response to this dilemma, the ASP is sponsoring engineering research using a high-cost, high-productivity prototype system and a lower-cost, lower-productivity prototype system. The first is operating at the University of Hawaii and is termed a "shallow covered raceway" (SCR) system. The second is run by EnBio, Inc., of California and is called a "deep open raceway" (DOR) system.

To determine which type of design—the high-cost, high-yield or low-cost, low-yield—is more cost-effective, it is necessary to know or to be able to project the costs of building and operating each system at the time the technology is scheduled for commercialization. To do this, the supply and demand of the required inputs (water, carbon, and the sundry nutrients) must be calculated, as must the market potential for the intended products. This task is the objective of the third area of research in the ASP—technology analysis. The resource assessment subtask is concerned with the availability of the required resources, and the economic evaluation subtask with cost/benefit and ultimate marketability analyses.

ALGAL OIL PRODUCTION AND LIPID METABOLISM RESEARCH

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1.0 Objective

The long-range goal of this task is to establish an adequate biological resource pool of oil-producing microalgal species and to expand the relevant technical informational foundation needed to support and to sustain the development of a new, energy-efficient, photosynthetic energy technology for the production of fuels and chemicals from inorganic nutrients using microalgae. In FY81 under Task 1036.20, the Photoconversion Research Branch at SERI formulated the following three areas of research and scientific task research objectives to implement the above-mentioned, long-range goal.

1.1 Establishment and maintenance of an oil-producing microalgal culture collection at SERI to serve as a national algal resource center with the explicit purpose of promoting and supporting research and technical development activities in algal oil/lipid production technology.

Under this research objective, species of microalgae are acquired through:

(i) Direct purchasing from currently established general purpose algal collection centers (such as American-Type Culture Collection, University of Texas, Cambridge Collection, Gottingen Collection, etc.);

(ii) New isolation of algal strains by staff from local sites and other special field locations having unusual environmental parameters;

(iii) Deposition of algal samples from domestic and foreign universities' algal researchers and from other SERI aquatic species program subcontractors.

These algal samples are screened for their oleagious capacity and the ones that demonstrated the capacity to produce and accumulates storage oil/lipids in bulk are retained and maintained as SERI's oleaginous algae stock collection for:

(i) Species identification by our in-house phycologist (Dr. K. Spencer) in consultation with external experts (Drs. Richard Starr, John A. West, Paul C. Silva and Ralph A. Lewis).

(ii) Initial species characterization with respect to their growth properties and oil-lipid productivity.

Upon proper documentation, a catalog will be published (September-December 1983) and updated as warranted. These algal species will be made available, upon request, to all subcontractors of SERI's aquatic species program and other DOE's investigators. (Distribution of the SERI's newly isolated oleaginous species to private sectors and non-DOE agency will be conducted according to SERI and DOE's legal guidelines.)

This area of research is an extension of our highly productive species selection program carried out during the last two-and-a-half years. Technical listing and cataloging of our current collection of the oleaginous algal species is needed for our internal use. The updating and revision of such records are needed periodically. Thus, publication of the catalog of SERI's oleaginous algal collection on an annual basis will not require a significant amount of additional resources but will provide valuable information for SERI's aquatic species program. The establishment of SERI's oleaginous Algal Collection not only will serve as a resource center for all subcontractors of the SERI's aquatic species program but also will ensure that the program can build up a diverse gene pool for the algal lipid research such that future genetic manipulation programs would have the necessary genetic foundation to draw upon.

1.2 Analysis on the Environmental Adaptability of the Oleaginous Microalgae

The specific research objectives are:

(1) To conduct a systematic characterization on the effect of key environmental parameters on: (a) the photosynthesis, respiration and related energy conversion processes; (b) the rate of overall algal mass production; and (c) the composition and rate of lipid biosynthesis.

(2) To compile data on the upper and lower ranges of the key environmental parameters for the oleaginous microalgae with respect to (a) the survival of the organisms, and (b) the maintenance of an adequate cell mass and lipid productivity.

(3) To extend the ranges of environmental adaptability by a positive species selection and isolation procedures using a large population of both wild-type and mutagenized algal cells.

The environmental control of algal cell metabolism and lipid biosynthesis will be investigated by two different but complementary approaches. The first approach is the ecological method, which analyzes the metaboliic activity and cellular composition of the naturally-occurring algal samples and correlates the observed physiological and biochemical data to the prevailing environmental parameters of the natural habitat of the organism. In the second approach, carefully-designed laboratory experimental procedures are applied to provide a systematic examination of the effects of key

environmental parameter(s) on the cellular metabolism and lipid biosynthesis using selected strains of high-oil producers.

Data gathered by both approaches will be integrated with the results obtained via the basic studies on the biochemistry and physiology of the microalgae to develop a mechanistic understanding of the "triggering" process and to identify the key regulatory bichemical agent(s) involved in the promotion of lipid biosynthesis (see Section 1.3).

To extend the environmental tolerance and adaptability of the oleaginous species, both UV and chemical mutagenesis procedures will be employed to isolate genetically-altered strains adapted to a given set of specific environmental conditions.

1.3 Biochemical and Physiological Analysis of the Oleaginous Microalgae.

The primary research objective here is to develop a full understanding on the biochemistry and physiology of the oil-producing microalgae with special emphasis on:

(1) The detailed biochemical pathway and enzymatic constituents involved in the biosynthesis of storage lipids in algae;

(2) The regulatory process involved in the transition of algal cells from their normal low-lipid-producing metabolic state into the stress-induced, high-oil-producing metabolic state.

(3) The roles of photosynthetic reductants and light-driven ATP production in the biosynthesis of the storage lipids in algae; and

(4) The photophysiological adaptation of the structural organization and functional activities of the photosynthetic apparatus accompanying the transition of cells from their low-lipid to high-lipid metabolic states.

Production of lipids and oil from CO_2 and H_2O represents a net storage of chemical free energy generated by the primary energy conversion process of oxygenic photosynthesis. The energy efficiency of the overall lipid biosynthesis depends critically on the coordination of the light-driven, reductant-generating reactions and those of the complex light-independent carbon metabolism of the algal cells. The basic studies on the biochemistry and physiology of the oleaginous microalgae will generate the information basis needed to devise any biochemical, physiological or genetic manipulation technique to enhance lipid production in algal cells. The following two technical approaches will be employed to implement the above-mentioned objectives:

(1) Basic biochemical and physiological studies will be conducted with the fast-growing, high-lipid-producing

organisms identified and selected under work described in Section 2. These organisms (such as Chlorella SOl, Scenedesmus 308, and Neochloris oleoabundans, etc). are ideal experimental species for biochemical analysis because a dramatic change in the capacity of lipid biosynthesis can be readily controlled by simple manipulation of their nutrient supply (see Fig. 1, Section 2). Initially, nitrogen limitation will be used exclusively to activate the biosynthesis of storage lipids. Later experiments will employ other nutritional and environmental agents to induce lipid accumulation.

(2) Well-proven photobiological, biochemical, anad biophysical techniques will be used to characterize the photosynthetic activity and related cellular energy conversion processes. Both in vivo and in vitro experiments will be performed to study the bichemical pathway and enzymatic constituents in lipid biosynthesis. ^{14}C- and ^{3}H-labelling techniques will be utilized in these studies. Kinetic results on the metabolic intermediates of fatty acid and lipid metabolism will be correlated with the data obtained on the profile of the enzymatic activity to define the synthetic pathway.

2.0 Accomplishments

The major technical accomplishments since the last program review meeting (June, 1983) are listed below:

2.1 Established SERI's oleaginous microalgal culture collection and completed the preliminary taxonomical listing of SERI's current collection (see Table I) of 31 algal isolates from 11 genera of eucaryotic microalgae. Documentation and cataloging of their oleaginous capacity and growth characteristic under laboratory conditions are currently in progress. In addition, we also isolated 7 additional strains of oil-rich strains of microalgae from local and remote field samples. Two of these new isolates are local strains (S03 and S09) which are fast-growing, salt-tolerant, high-lipid producers.

Table I. SERI Algal Taxonomic List of Oleaginous Microalgae

- Chlorophyta
  - Chlorophyceae
    - Volvocales
      - Chlamydomonaceae
        - *Haematococcus* sp
      - Chlorococcales
        - Chlorococcaceae
          - *Chlorococcum aureum* 1768
          - *C. citriforme* 1769
          - *C. croceum* 1770
          - *C. oleofaciens* 107
          - *C. pinguideum* 774
          - *C. refringens* 1783
          - *C. scabellum* 1233
          - *Neochloris oleoabundans* 1185
          - *N. pseudostigmata* 1249
          - *N. pyrenoidosa* 777
          - *N. texensis* 1980
          - *Spongiochloris spongiosa* 1
        - Protosiphonacese
          - *Protosiphon botryoides* 92
        - Dictyosphaeriaceae
          - *Botryococcus braunii* (g)
            (t)
      - Chlorellales
        - Chlorellaceae
          - *Chlorella pyrenoidosa*
          - *C.* sp S01
          - *Ankistrodesmus* sp 163
          - *Ankistrodesmus braunii* 750
        - Scenedesmaceae
          - *Scenedesmus* sp S02
          - *S.* sp 308
          - *S.* sp S08
        - Uncertain: S03, S04, S05, S09
- Chrysophyta
  - Chrysophyceae
    - Vancheriales
      - Botrydiaceae
        - *Botrydium becherianum*
        - *Botrydium stoloniferum*
  - Bacillariophyceae
    - Pennales
      - Nitzschiaceae
        - *Nitzschia palea*
      - Unknown: S06

2 Characterization of the metabolic response of Chlorella SOla (a locally isolated high-oil producer) toward nitrogen starvation. Experimentally demonstrated that high rates of biomass production can be maintained for 5-7 days after severe nitrogen starvation even though cell division as measured by cell density of the culture stopped during this period. Furthermore, during this stage of no reproductive growth, 80-90% of the newly produced algal mass appears as intracellular storage oil and lipids (Figure 1). Morphologically, the algal cells are characterized by a large change in their cell volume (Figure 2) and a great reduction in the complexity of their intracellular membrane structures. Exclusive accumulation of triglycerides by the nitrogen-starved Chlorella is demonstrated by thin-layer chromatographic analysis of the extracted algal lipids (Figure 3) and by GC analysis.

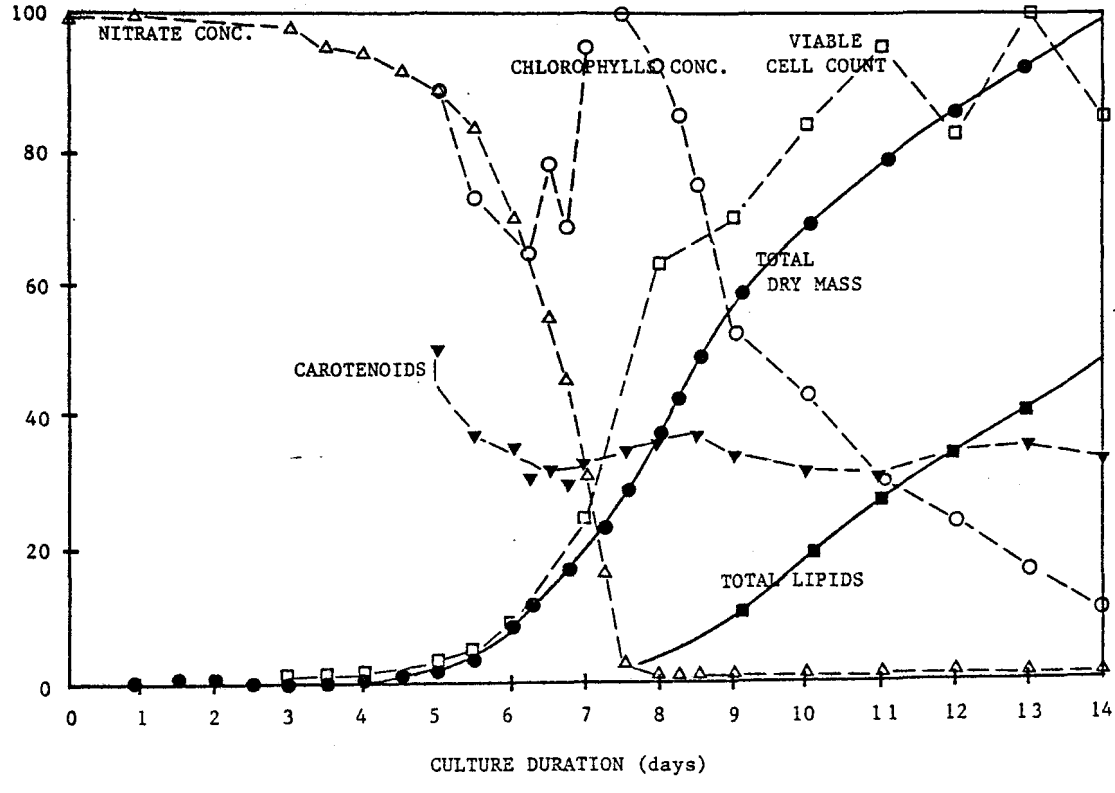

Figure 1. Physiological Responses of Chl. SOla to N-Limitation. The cells were grown in a fermentor on Bold's Basal Media (with 0.1 M NaCl) at 30°C under 20 $W \cdot m^{-2}$ of fluorescence light.

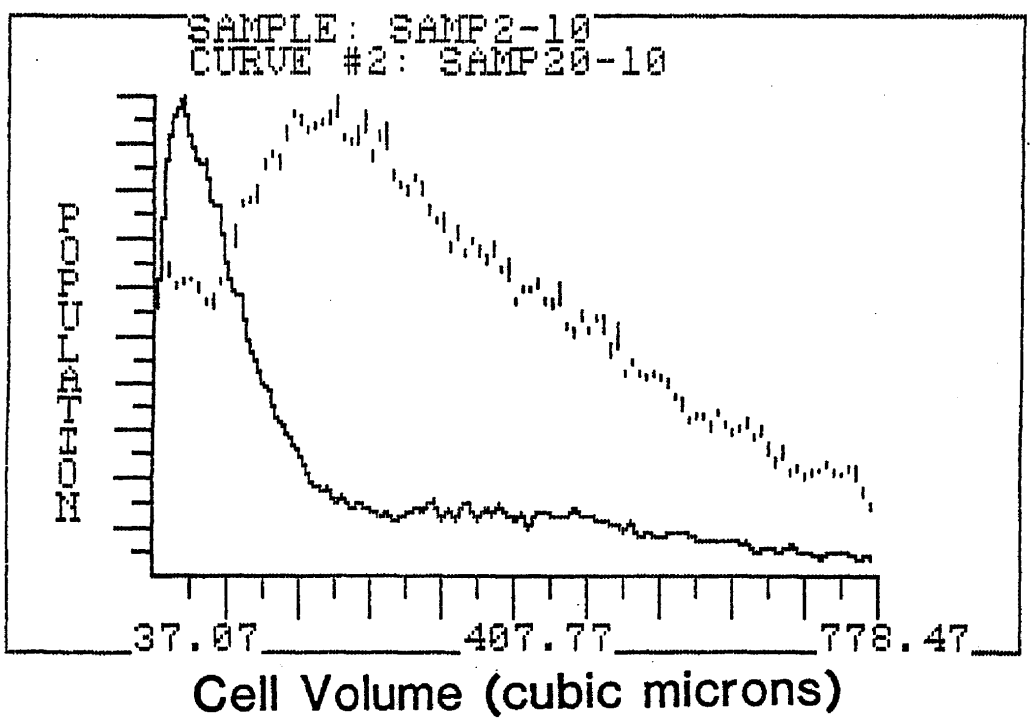

Figure 2. Cell Volume Increase in Chl. SOla Induced by N-Limitation. Size of cells during growth study. Sample 2-10 is at exponential growth phase (4 days before N-depletion). Sample 20-10 is stationary phase culture (7 days after N-depletion).

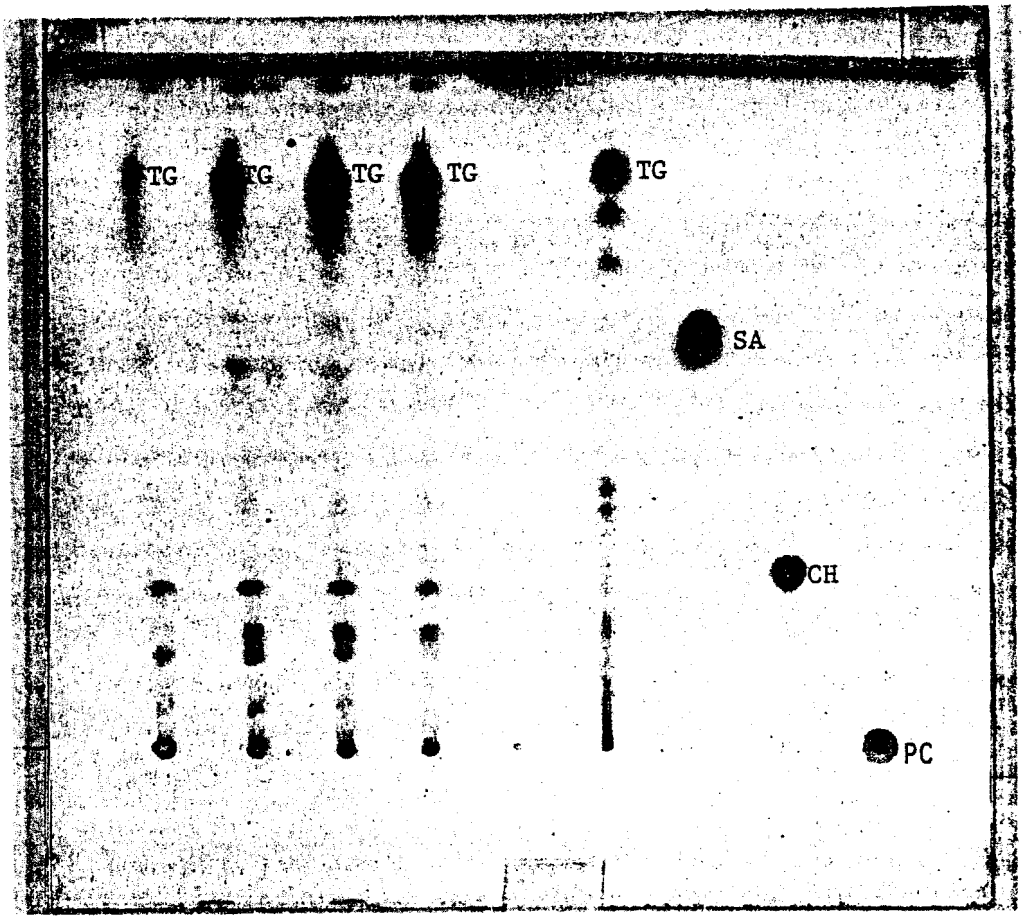

Figure 3. Triglycerides Synthesis Induced by N-Limitation on Chl. SOla (30°C under continuous light). Silica gel chromatograph developed with hexane:ether:formic acid (75:25:1). Lanes 1 to 4 (from left) are the ethanol-extract of total lipids from algal cells obtained at: 72 hr before to N-depletion (Lane 1); and 24, 96, and 262 hrs after N-depletion (Lanes 2, 3, and 4, respectively). Lane 5-9 are 30 µg of standards: HC = Hydrocarbon (C16-C22), TG = Triglycerides; SA - Stearic acid; CH = Cholesterol; PC = Phosphatidyl Choline Diolyl.

2.3 Characterized the responses of oleaginous microalgae toward salinity stress:

The following results are obtained:

(1) Several of SERI's current oleaginous microalgal strains can tolerate up to 0.3 M of NaCl (Table II).

(2) Chlorella SOla can be adapted to grow at its normal growth rate in salt-containing media after an initial lag period of varying duration (Figure 4).

(3) During the initial lag period following an artificially imposed salinity stress, an enhanced level of storage lipids accumulation was observed for Chlorella SOla, even though the nitrogen supply has not become exhausted. Experimental evidence was obtained indicating that nitrate reductase activity of Chlorella SOl is subjected to strong modulation by salinty stress (Figure 5). These data provided the first possible biochemical link between nitrogen-limitation and salt-induced enhancement of lipid accumulation by the oleaginous microalgae.

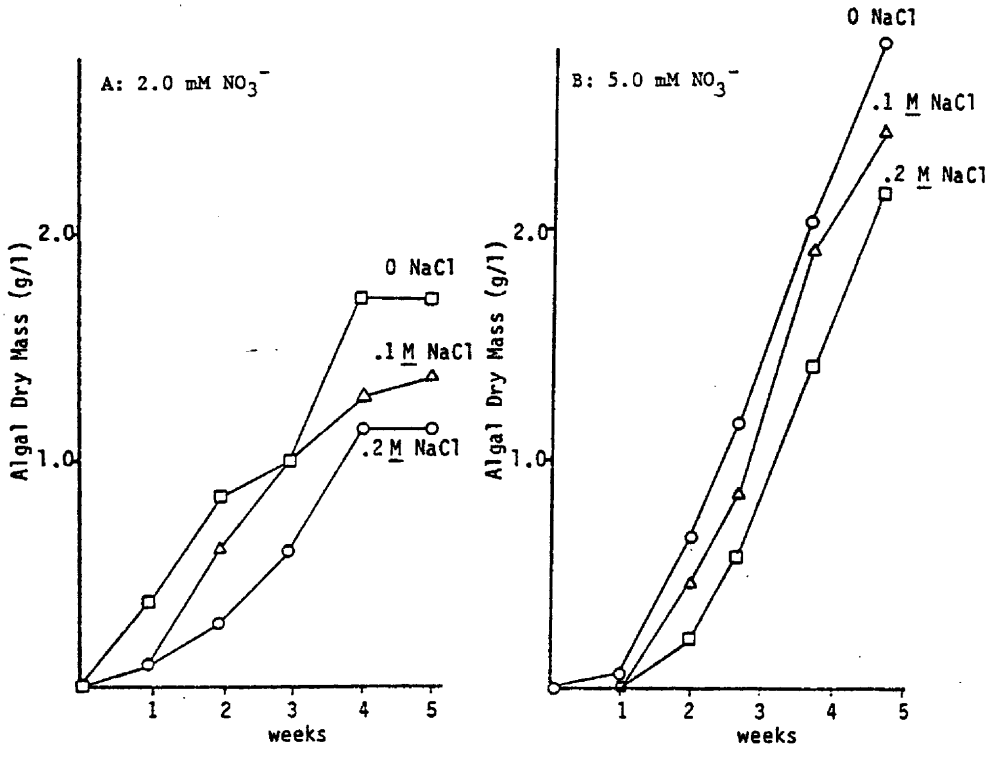

Figure 4. Adaptation of Chl. SOla to Salt-Containing Media.

Table II: Effect of Salt on Mass Yield of Oleaginous Microalgae

Organism \ M NaCl	0 (.2 ppt)	0.1 (6.0 ppt)	0.3 (17.5 ppt)
Ankistrodesmus sp.	1.8	0.05	.06
braunii	2.0	.07	.06
Chlorella SOl (a)	3.9	2.1	.9
(b)	3.3	2.2	.1
(e)	3.1	2.0	.2
(g)	3.9	2.3	.5
Neochloris oleoabundans	1.1	2.1	1.8
Chlorococcum pinguideum	2.2	0	0
refringens	4.6	0	0
Scenedesmus S02a	1.5	3.1	1.2
308a	3.3	2.4	1.9

Note: All strains of algae are grown in standard BBM-8 medium with indicated concentration NaCl. The dry algal masses were determined at the end of four weeks of growth under continuous illumination. Mass yields are given as g (dry wt.)/liter.

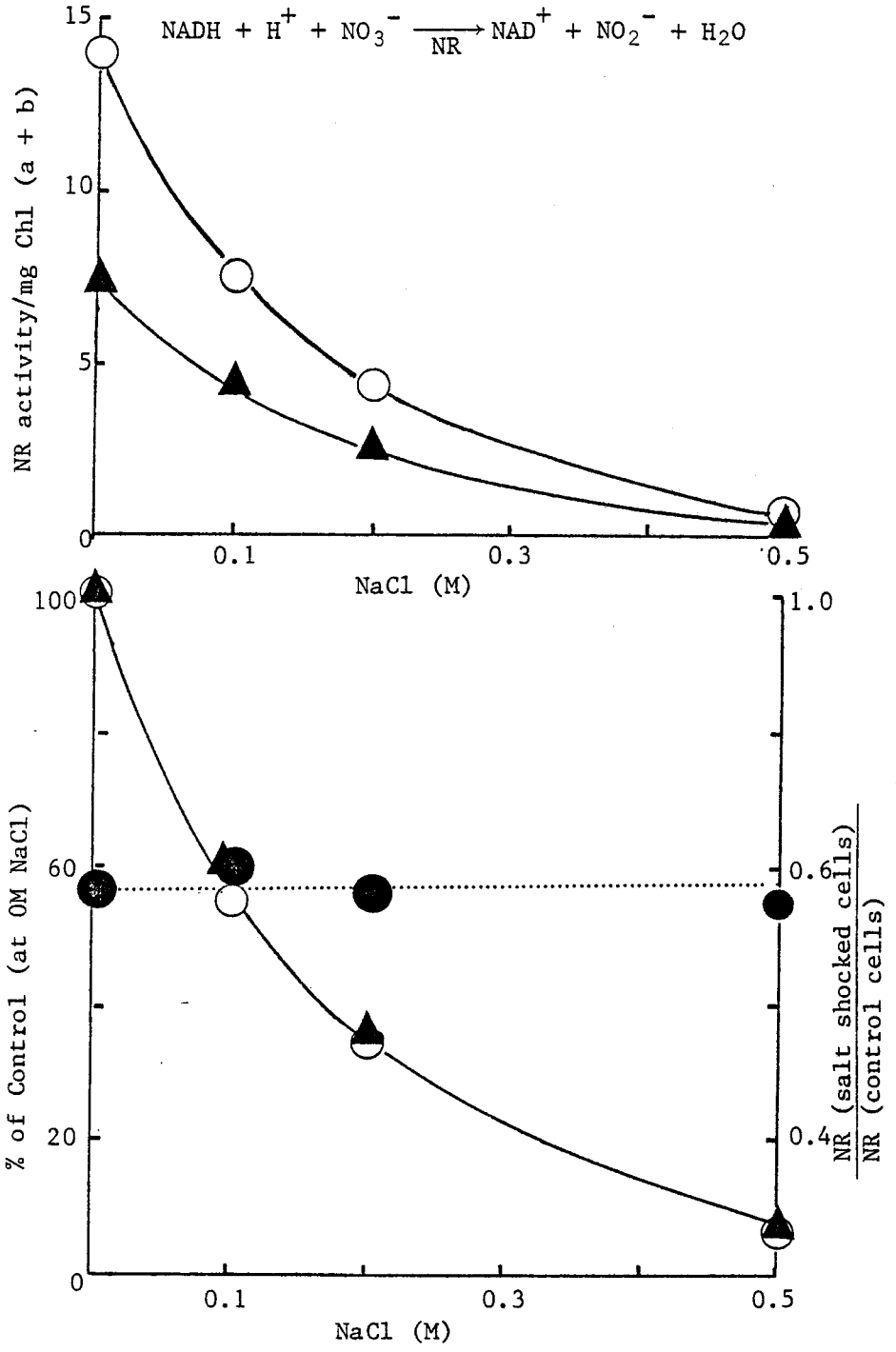

Figure 5. Modulation of Nitrate Reductase Activity by Salnity Shock.

2.4 Studies on the Metabolization of Stored Oil by Algal Cells.

The utilization of storage lipid by an oleaginous microalga (Chlorella SOla) during dark aerobic incubation has been characterized as a function of its nitrogen supply. The experimental results summarized in Table III and Figures 6 and 7 are suggestive that: (1) dark utilization (or remetabolization of the storage lipid) requires a reduced nitrogen substrate (such as NH_4^+). Nitrate is not a good nitrogen source for dark remetabolization of the storage lipids. In spite of the fact that storage lipids are a potentially excellent source of reductant, they are not coupled to the reduction of nitrate into ammonia in the dark. These observations are important information useful for the optimization of oil-lipid accumulation by algae under natural conditions where nightly dark periods are unavoidable.

Table III: Effect of Nitrogen Source on the Remetabolization of Storage Lipid by Chlorella SOla

Conditions	Lipid %	Dry Mass (g/ℓ)	Avg. Cell Size (fl)	Cells/ℓ ($X10^{-9}$)
STARTING	33.0	--	303	9.62
DARK				
no N	35.6	1.08	300	9.61
2mM NH_4^+	23.3	1.10	312	9.40
2mM NO_3^-	36.8	1.07	303	9.30
LIGHT + DCMU (3 μM)				
2mM NH_4^+	24.4	--	319	9.93
2mM NO_3^-	31.9	1.02	283	11.4
LIGHT				
no N	35.6	1.26	303	8.97
2mM NH_4^+	31.3	1.48	329	11.5
2mM NO_3^-	32.8	1.46	334	10.2

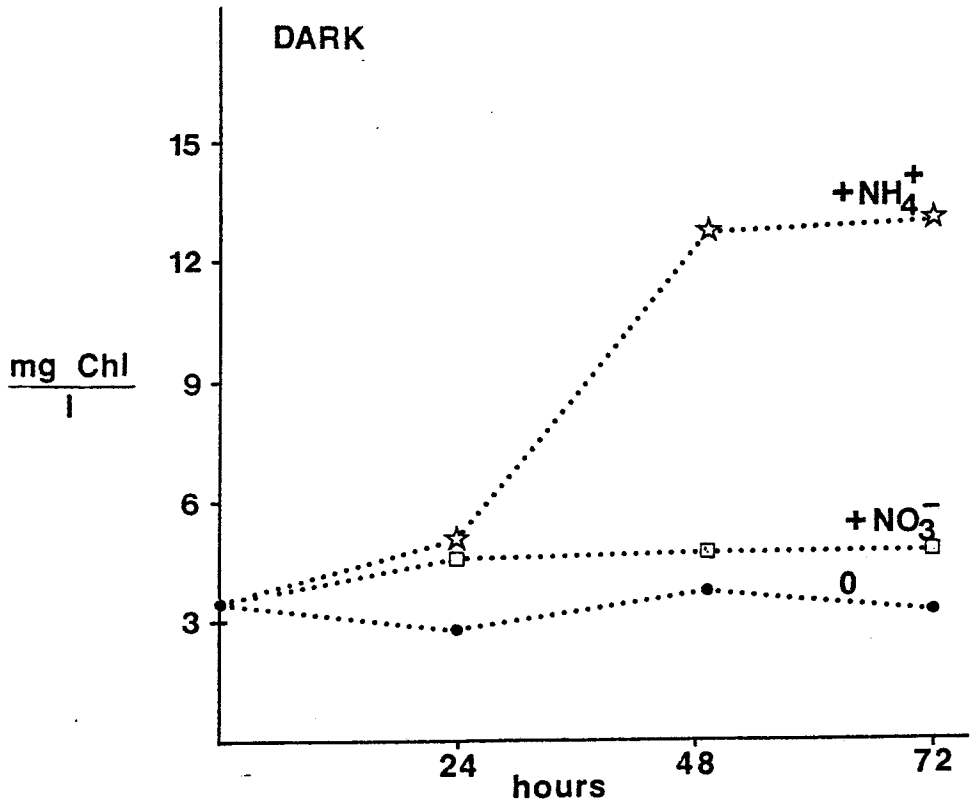

Figure 6. Effect of Nitrogen Source on the Chl-Biosynthesis (Dark)

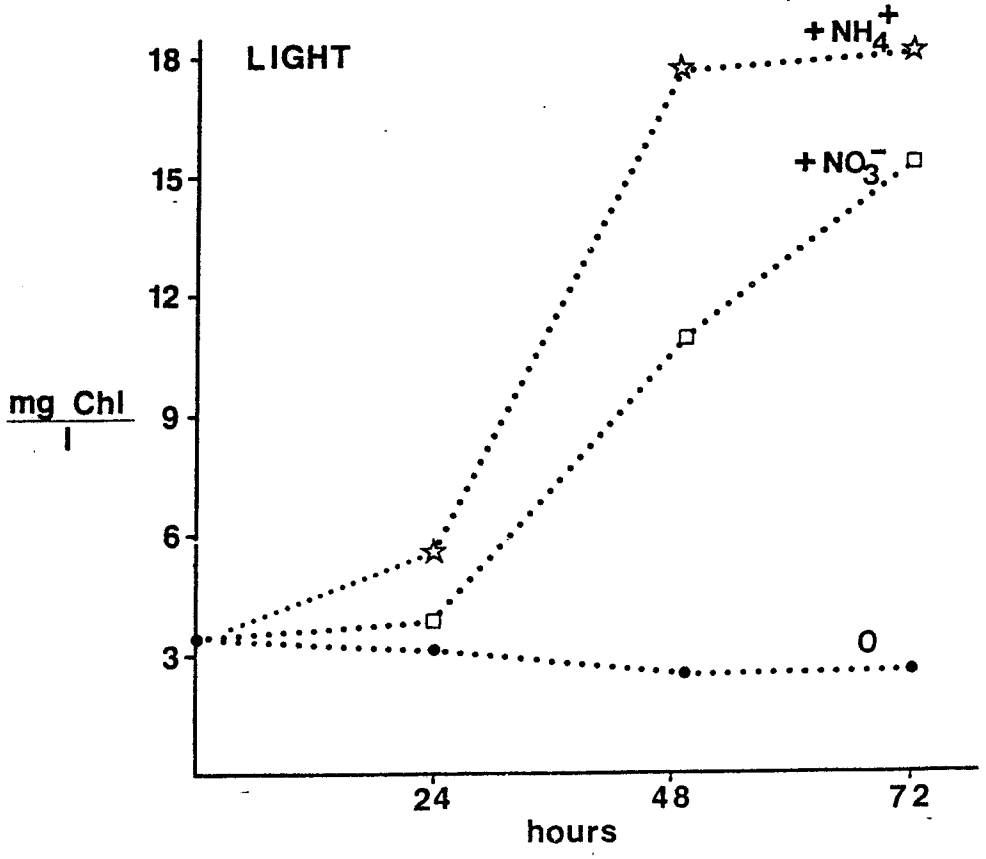

Figure 7. Effect of Nitrogen Sources on the Chl-Biosynthesis (Light)

2.5 Developed an efficient extraction and quantitation method to monitor the synthesis and accumulation of the structural and storage lipids using semimicro samples of microalgae. The procedure is based on quantitative liquid extraction and TLC/scintillation quenching estimates of the lipids [1-3].

3.0 Future Plans

Specific research plans for FY83-FY84 will follow the research objectives as outlined under Section 1.1 through 1.3 with special emphasis on:

A. The compilation of laboratory data needed to complete the first descriptive catalog of SERI's oleaginous algae collection detailing the growth characteristics, oleaginous capacity in liquid and solid media. Targeted publication date of the descriptive catalog is tentatively set for the first quarter of FY84.

B. Studies on the phenomenon of salt-tolerance in oleaginous microalgae will concentrate on: (i) identification and selection of more salt-tolerant strains, and (ii) continue the in vivo studies and cell-free experiments on the response of the liquid metabolism and nitrogen metabolism pathways toward artifically imposed salinity stress.

C. Studies on biochemistry and photophysiology of lipid metabolism with emphasis on ^{14}C-labelling experiments and in vitro analysis of the enzymatic systems to better characterize alteration in the biosynthetic pathways following nitrogen-limintation and resupply of nitrogen compounds.

4.0 Problems and Variances: None

5.0 Literature Cited

1. Tornabene, T. G., Benz-Amotz, A., and Hubbard, J. S., "Isolation, Analysis and Identification of Lipids", SERI Contract XK-2-02149-01. Report 1982.

2. Selvam, R., and Radin, N. S. "Quantitation of Lipids by Charring on Thin-Layer Plates and Scintillation Quenching," Analytical Biochem. 112, 338-345 (1981).

3. Shand, J. H., and Noble, R. C., "Quantification of Lipid Mass by a Liquid Scintillation Counting Procedure Following Charring on Thin-Layer Plates," Analytical Biochem. 101, 427-434 (1980).

6.0 Publications and Presentations

1. Lien, S., and Spencer, K. G. "Microalgal Production of Oils and Lipids." Proceedings of IGT Symposium on "Energy from Biomass and Wastes VII," in press.

2. Spencer, K. G., and Lien, S. "Utilization of Stored Oil in a Strain of Chlorella," abstract submitted to Plant Physiology.

3. Lien, S., and Spencer, K. G. "Production of Fuels and Chemicals by Microalgae: Its Biological Fondatin and Limitation," abstract for invited Keynote speech submitted to the XI International Seaweed Symposia.

4. S. Lien made three invited lectures at:

   a. University of Colorado, February 18, 1983, Boulder, CO. "Product Accumlation and Metabolism of Lipids by Algal Cells."

   b. Louisiana State University, February 9, 1983, Baton Route, LA. "The Biological Aspects of Fuels and Chemical Production by Microalgae."

   c. IGT Symposium on "Energy from Biomass," January 27, 1983, Lake Buena Vista, FL. "Microalgal Production of Oils and Lipids."

PRODUCTION OF LIQUID FUELS AND CHEMICALS BY MICROALGAE

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ABSTRACT

This project has for its goal to investigate the feasibility of microalgae cultivation for production of liquid fuels. The general objective of this subcontract is to initiate operations of the experimental outdoor facility partially constructed under a predecessor subcontract (XB-9-9253-1). Specific objectives for this reporting period (August 1, 1982 - January 31, 1983) included making four growth ponds operational (two 200 m^2 and two 100 m^2 ponds), carrying out preliminary inoculations with natural blooms of microalgae, studying effects of pond operations and nitrogen limitations on culture growth and lipid content, investigating the hydraulics of the ponds, and selecting appropriate species and operating conditions for a more detailed study during 1983. This report presents the results obtained to date and some preliminary conclusions as well as future research plans.

OBJECTIVES

The long term objective of this project is to develop microalgae cultivation techniques using open, mechanically mixed ponds to produce a high lipid (oil) containing biomass - suitable as a feedstock for liquid fuels and chemicals extraction.

The alternative technologies for microalgae production have been reviewed previously (1), they range from very complex systems, for example the "cascade" system extensively used in Eastern Europe (2), to very simple, unmixed, open ponds of various depths such as used in most sewage treatment and some aquaculture projects. This project focuses on the "high rate pond" design (3) - characterized by channels of varying width, mechanical mixing (preferably with paddlewheels), and moderate depths (20-40 cm).

The high rate pond (HRP) design has been extensively used in sewage treatment research (4) and some applications (5), as well as many algae production systems for high value products (health foods) (6), and has been proposed as a suitable design for the production of microalgae fuels (7, 8, 9). The authors have recently completed an engineering and economic analysis of microalgae fuel production and

concluded that - using the most optimistic assumptions - oil production costs as low as $60/barrel could be extrapolated using this design (10, 11). These results suggest that microalgae production, although too expensive under present circumstances for fuel alone, can be considered suitable for production of some commodity chemicals with fuels as by-products of such operations. Thus, at least on a preliminary basis, the economic feasibility of microalgae production appears favorable.

The technical feasibility of the production of microalgae biomass for fuels and chemicals must still be demonstrated. Our engineering and economic analysis was based on several key assumptions about the performance of these systems: the ability to maintain and control desired algae species in the ponds using a small initial inoculum, achieving a relatively high productivity (30t/a/y) with a very high total content of extractable oils (40% of total dry weight); and being able to harvest the algae species through a simple process such as sedimentation in large settling ponds. Although these assumptions are reasonable - being based in extrapolations of laboratory and field data - they must still be demonstrated in practice. Indeed, achieving all these requirements simultaneously under the exacting conditions that would apply to the low cost production of microalgae for fuels and chemicals, will require a significant, long term R&D effort, whose success is not predictable.

The general objectives of this subcontract are to initiate the required R&D effort by operating the outdoor, experimental microalgae cultivation facility previously designed and constructed under a predecessor SERI subcontract (12, 13). This experimental system, briefly described below, will allow a close interaction and feedback between laboratory and outdoor experimentation. Most importantly it will avoid the limitations inherent in extrapolating data from laboratory or small scale systems. Thus, in terms of the key parameters of interest, microalgae species dominance, culture stability, harvestability, oil content, and productivity - this project should allow, over the next two growing seasons, development of sufficient data to reach some confident conclusions about the technical feasibility of this approach to biomass fuel production.

The specific goals of the current subcontract are to:

1) Complete the construction activity such that two 100 m^2 and two 200 m^2 ponds can be operated.

2) Carry out hydraulic studies in the ponds such as to describe areas of poor mixing, silt deposition, and mixing velocity profiles.

3) To inoculate the ponds with mixed cultures derived from other outdoor ponds and allow selection of the dominant microalgae species.

4) Carry out laboratory analysis and measurements characterizing the dominant microalgae species and comparing these to other, already studied, species.

5) Operating the outdoor pond system for several months with selected species of microalgae to obtain data on lipid production, harvestability, productivity, species dominance and culture stability.

The results obtained by this contract should allow a preliminary assessment of the problems and prospects of this approach to microalgae fuel production, and a better, more definitive, design of longer term R&D plans. It's significance to the SERI Biomass Program rests on the fact that it represents an experimental test of some key concepts of microalgae biomass production, that it will allow feedback to laboratory and supportive research projects, and that it will allow a comparative assessment of alternative biomass production concepts.

ACCOMPLISHMENTS

Experimental System

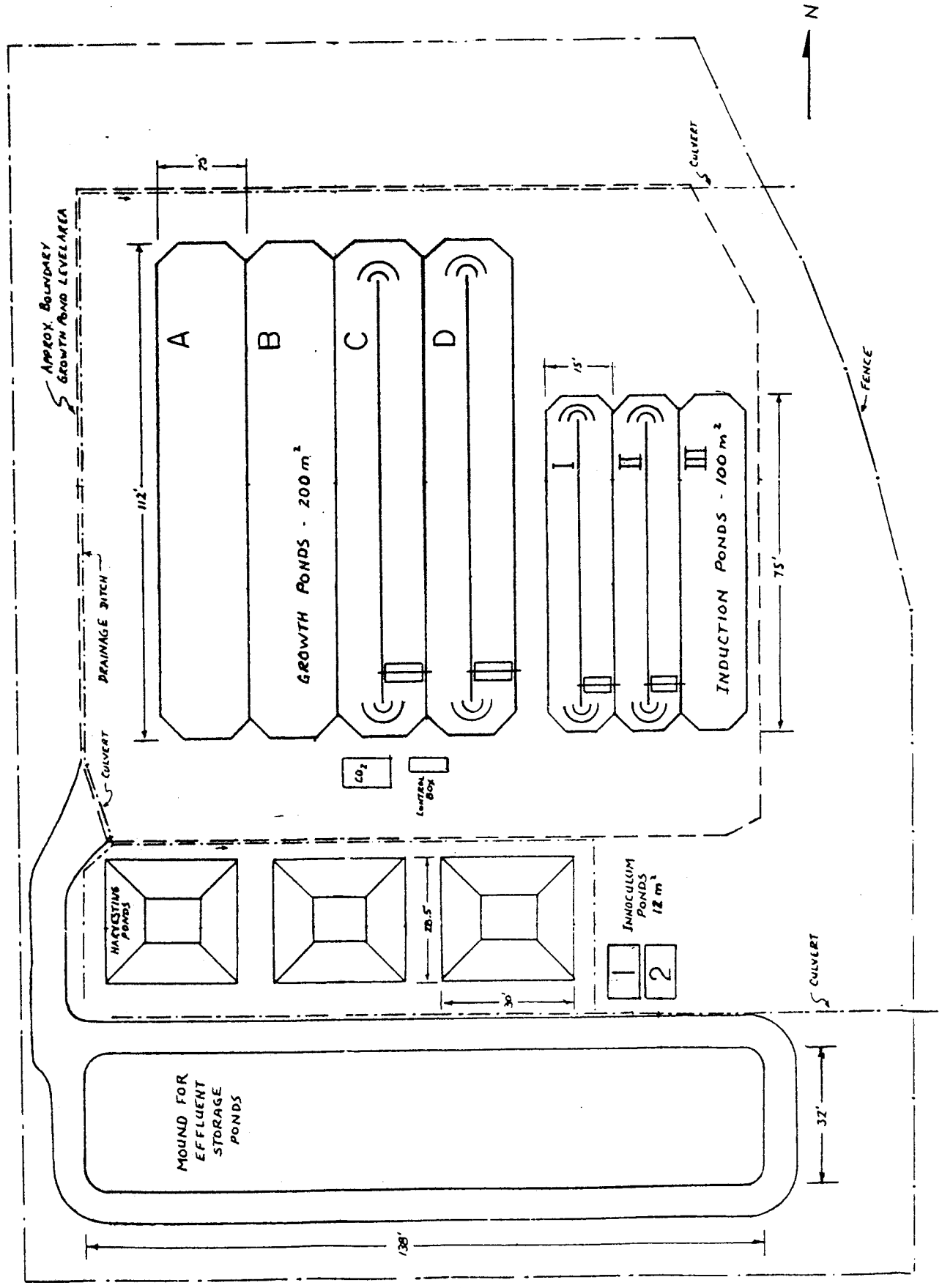
The experimental microalgae cultivation system (Figure 2.1) consists of four 200 m^2 (A-D) and three 100 m^2 (I-III) growth ponds, three deep settling or harvesting ponds (not used at present) and two 12 m^2 inoculation ponds (1 - 2). This system was designed based on observations by the investigators and others (Shelef, private communication) which suggested that to obtain kinetic and other process data extrapolatable to larger pilot scale systems (above 1 hectare), an experimental microalgae cultivation pond should be at least 100 m^2 in size.

To maximize the production of lipids, both microalgae species and nutrients - specifically nitrogen supply, must be controlled in these ponds. Nitrogen limitation has been recognized for about forty years as a powerful and relatively simple way of producing a very high lipid content in microalgae (14) although it has been assumed that this could only be achieved by a large loss in productivity (15). We have previously demonstrated that nitrogen limitation in starch storing microalgae does not necessarily result in a decrease in biomass productivity (in batch cultures) (16) and this may also apply to lipid producing species (17, 18, 19). This must still be unequivocably demonstrated.

The outdoor facility was designed to allow demonstration of the effects of nutrient limitation - specifically nitrogen - on lipid content and productivity. Short-term controlled periods of nitrogen limitation are to be the main technique used to produce a high lipid microalgae biomass at a high overall productivity.

Nitrogen limitation of the algae cultures has a further advantage: the N-limitation often results in microalgae cultures that exhibit strong tendencies to flocculate and settle (20). In some cases, particularly if lipids were to approach 50% of the dry weight, the algae may have a tendency to float. In either case, this greatly facilitates the harvesting of the culture, as settling or flotation are low-cost processes for biomass concentration. Also, it allows recycle of the liquid media back to the growth pond, after adding back nutrients removed by the algae harvest or lost during cultivation. The key

Figure 2.1. Experimental Pond System Schematic



"harvestability" parameter to be followed in this study is the sedimentation (or rising) velocity of the algae cells and colonies in the cultures.

Figure 2.2 shows a recently taken photograph of the operating growth ponds. A detailed description of the facility is found in the first Quarterly Progress Report and prior reports (21, 12). During this subcontract we have made two 100 m^2 (I and II) and two 200 m^2 (C and D) operational. This involved fabricating and installing a new baffle and paddlewheel in one of the 100 m^2 ponds (II) as well as installation of another paddlewheel, carbonation equipment, and other subsystems required for manual operation of the growth ponds. (Completely automatic operation is not required until all seven ponds are made operational, which is not scheduled for the present subcontract period.)

Pond Operations

All pond operations were carried out in a batch mode - either using a new inoculum or from a 20-25% seed culture left over from the prior batch. In all cases, the media used contained 16 ppm P (Na_2HPO_4), 12 ppm Mg and 16 ppm S (Epsom salts), 10 ppm Fe ($FeSO_4 \cdot 7H_2O$) 100 ppm Versene (EDTA, ethylenediaminetetraacetic acid) and 10 meq/ℓ of alkalinity ($NaHCO_3$). Minor nutrients were present in the irrigation water. CO_2 was added as pure CO_2 (from 65 lb. tanks) by diffusers, with CO_2 added when the pH increased above 9.0 and additions regulated manually by watching the pH decrease to 8.0. (pH increased due to algal growth normally to 9.5 and occasionally 10.0.) Ammonia was added when NH_4^+-N in the ponds fell below 3-5 ppm and was added at about 10 ppm (ammonia was measured from pond samples). Sampling of the ponds was daily during periods of rapid growth, less frequently when growth slowed down. Analysis included dry weights (VSS), NH_4^+-N, alkalinity, microscopic observations, and chemical compositions (protein, carbohydrate, lipids).

The first inoculation was made on August 13, 1982, when a mixed Micractinium - Scenedesmus culture grown on sewage at the University of California Richmond Field Station was inoculated into a 100 m^2 (I) and a 12 m^2 (1) pond. The 100 m^2 culture settled out almost completely due to the lack of a flow deflector which resulted in very poor hydraulics in this pond, Selenastrum became the dominant algae in the supernatant, however biomass concentration remained quite low (below 50 mg/ℓ) even after one month of operation.

On September 21st this pond was equipped with two flow deflectors at the far end (from the paddlewheel). It was re-inoculated on Oct. 1st with the culture from the 12 m^2 pond which by then, was almost unialgal Scenedesmus. The flow deflectors eliminated most of the eddying and dead spots allowing complete suspension of the cultures, and no sludge accumulation was noticed. This marked the beginning of successful algae growth experiments. On the same date a similar inoculum into the 200 m^2 pond (D) equipped with similar deflectors resulted in severe algae settling (see the Hydraulics section below).

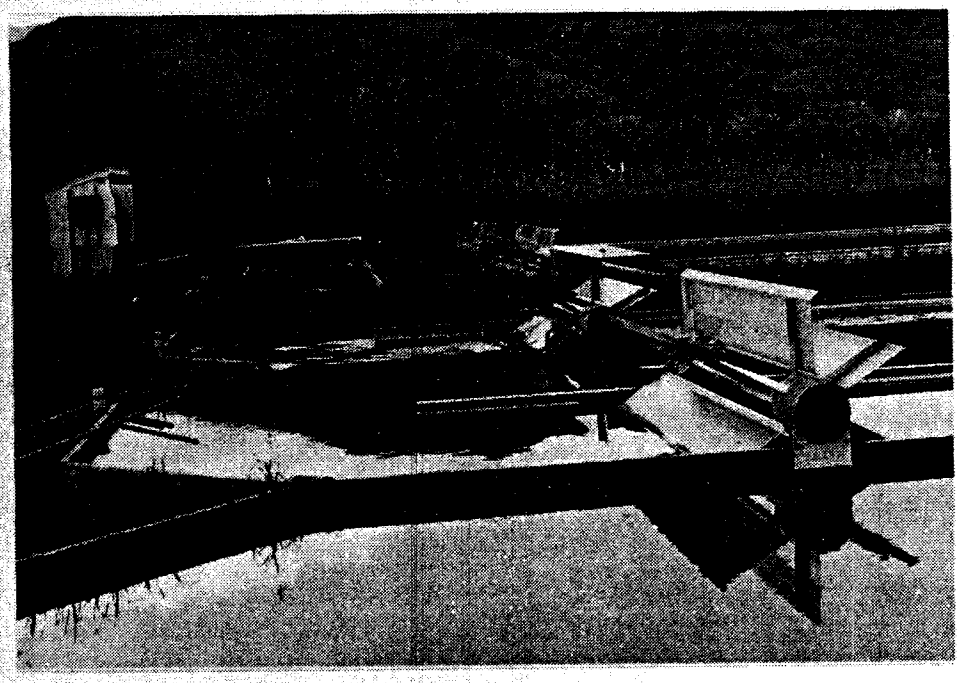


Figure 2.2A. View of the Four Operating Ponds (100 m^2 in foreground)

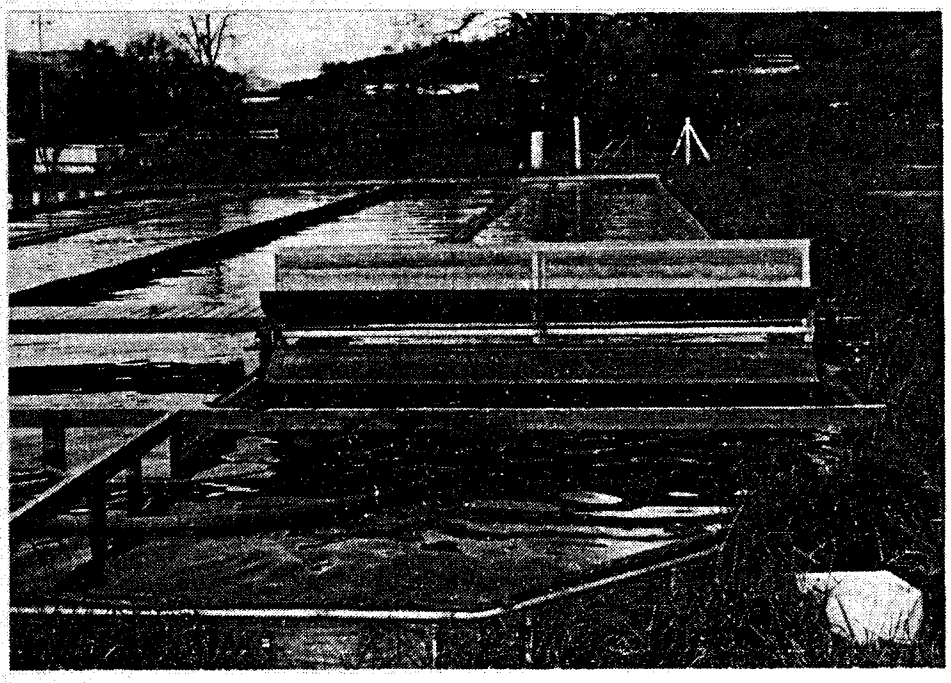


Figure 2.2B. View of 200 m^2 Pond (D) Showing Flow Deflectors

Three batches of the Scenedesmus inoculum were grown in the 100 m^2 pond (I) during October, the second two from inoculum left from the prior batch (Table 1). In the first batch, lasting October 1st to October 14th, ammonia was added in sufficient amounts to allow the culture to be N-sufficient. After cell density had increased to 430 mg/ℓ (from 50 mg/ℓ initially) about 80% of the culture was pumped out and new media added containing 18 mg/ℓ NH_4^+-N. After a doubling of the culture density (from 75 mg/ℓ to 150 mg/ℓ) the NH_4^+-N was exhausted and the algae started to change color (from deep green to olive green). The culture, however continued to grow and productivity (both maximal and average) was the same as in the previous batch under N-sufficient conditions.

For additional comparison, the culture was also inoculated into a 200 m^2 pond (D) which received sufficient ammonia in the first week and it was not allowed to exhaust it until a 300 mg/ℓ cell density was reached. Its color remained deep green throughout. Despite the different regimines, both cultures exhibited no significant differences in average or maximal productivity, or doubling rate. (Lipid productivity also increased, however it must be noted that the lipid assay was not yet standardized at that point - thus this data should be considered on a relative basis only.)

Within the limitations of this single preliminary data point, it appears that this Scenedesmus species increases in lipid content soon after nutrient exhaustion. The culture which used up its N at lower density (more light per cell) induced further in terms of lipid production. However, the extra days of N limitation led to diminishing returns, giving overall the same results as the culture limited for N for a shorter period of time but at a higher density.

When the N-limited culture in the 100 m^2 pond (I) was diluted (about four fold, from 365 mg/ℓ to 95 mg/ℓ) and allowed to grow in the absence of NH_4^+-N - productivity was very low (although, possibly the bad weather also influenced the decrease in productivity). The optimal time and density for lipid induction by nitrogen limitation will be dependent on the algal species and solar irradiation. This must be a major objective of future research.

By the end of October and during November - the weather - unusually wet - limited productivity of the cultures. The Scenedesmus culture in the 200 m^2 pond (D) was diluted on 10/24 to 80 mg/ℓ and allowed to grow for about ten days with sufficient NH_4^+-N, during which time it produced only 4.5 g/m^2/day on average. Diluted again (to 125 g/ℓ) on 11/5 and allowed to grow for 8 days, average productivity fell to 2.6 g/m^2/d. A further N-limitation experiment was carried out when the culture was diluted on 11/14 to 77 mg/ℓ and no further NH_4^+-N was added. Productivity did not change much - but lipid content decreased - calling into question the validity of the lipid analysis.

Thereafter no further experiments were carried out in the 200 m^2 pond (D) although the pond was kept operating over the December-February period to determine whether the inocula would remain over the winter

months. This was indeed the case. Despite essentially negligible productivities during December-February the culture is still essentially unialgal Scenedesmus.

While the above described experiments with Scenedesmus were ongoing in the 100 and 200 m^2 ponds, the other ponds (II,C) were made operational. (Pond II has, however, not yet been used.) The 100 m^2 pond (I) was again inoculated on October 31st with a culture of, essentially, unialgal Micractinium (obtained from Richmond's high rate sewage fed pond). Over a two week period the culture increased in cell density from 23 to 170 mg/ℓ , producing, on average, 2.4 g/m^2/day. By the end of this period about 20% of the algal cells were Scenedesmus. A two fold dilution carried out on 11/14 resulted in relatively little growth over the next ten days, with a slight increase in Scenedesmus population. Since then - during December and January - the culture has slowly changed over to be almost exclusively Scenedesmus.

The other 200 m^2 pond (pond C) contained rainwater in October and started to exhibit a layer of bright red, motile algae. These have been observed under similar circumstances and seasons over the years by project personnel in both Richmond and Vacaville. Laboratory culture has previously been unsuccessful. It was decided to see whether this algae species - identified as a Chlamydomonas species, could be cultured in the pond. Nutrients were added and mixing started on 11/6. Within a week the cell density increased from 65 mg/ℓ to 168 mg/ℓ . On 11/14 the culture was diluted to 86 mg/ℓ , but no nitrogen was added. Productivity was 3.0 g/m^2/d for a couple of days and then dropped to zero. Carbohydrate - not lipid - content increased dramatically, from 20 to 47% after a week of N limitations. This culture has also been successfully maintained in the outdoor ponds over the December-January period.

Hydraulic Studies

The initial operation of the 100 m^2 pond (I) in August 1982 was described above: Without any flow deflectors the ponds exhibited large eddies at each end of the pond and poor circulation. The eddies which formed at the far end of the paddlewheel were so severe that the water in the return channel exhibited - over almost one third of its width nearest the baffle - a counterflow current. This caused the settling of algae biomass in this area. A second eddy - and settling zone - also formed right behind the paddlewheel near the baffle. Overall hydraulics was so poor that about all the biomass (Scenedesmus) settled out. Interestingly in over one month of operation the density of the culture did not increase, even though a new species (Selenastrum) became dominant in the supernatant.

These results made imperative the installation of flow deflectors around the bends. Empirical observations suggested that two flow deflectors at the far bend would be optimal - to a first approximation. These were installed and by the end of September 1982 a new culture inoculated. Hydraulics improved considerably, most eddying was eliminated and then no sludge accumulated.

In contrast, a similar set of flow deflections installed in the 200 m^2 pond (D) were insufficient to keep the algae suspended. Within a few days of inoculation (on 9/21) with resuspended algae/sludge from the (hydraulically deficient) 100 m^2 pond nearly all the algae biomass was concentrated within a 2 ft^3 volume of sediment in the eddy formed behind the paddlewheel and just downstream from the bend. Again, as in the 100 m^2 pond, no suspended algae growth became apparent (within a week). The pond was drained and two more flow deflectors were installed at the end nearest to the paddlewheels to straighten out the flow. A second inoculation was made from the 100 m^2 pond on October 14th and led to satisfactory growth with improved hydraulics (see above).

The above observations were of a qualitative nature - based on visual inspection of the ponds. They demonstrated that going from 100 m^2 to 200 m^2 results in very significant changes in the hydraulic characteristics of the ponds, which was unexpected. To obtain a better understanding of pond hydraulics a detailed program of flow velocity measurements and their analysis has been initiated in consultation with Prof. V. Ingen and Mr. Greg Lawrence of the Department of Hydraulics, U.C. Berkeley. A detailed velocity profile vs depth was taken at a position about 20 ft downstream from the paddlewheel.

The results are intriguing as they are counter to the behavior expected for open channel flow: flow velocities are higher on the inside than outside of the channel. A much more detailed study, and computer analysis of the data is being carried out.

It should be also noted that in the preliminary growth experiments no significant difference was observed in the ponds mixed at 1 ft/sec vs 0.5 ft/sec (Table 2.1).

SIGNIFICANCE OF FINDINGS

The experimental work reported above was of a preliminary nature. Its key objective was to initiate experimentation - while making the pond system operational - so as to "debug" the system as well as establish self selected cultures which could be used in future work. Due to the relatively late start of the work, climate (sunlight, temperature) rapidly became the limiting factor and productivities were quite low. Also some of the analytical measurements (principally lipids) were not sufficiently well worked out to give reliable data. Within these limitations - however - a number of important conclusions can be drawn from the first six months of this project.

Species Dominance

One culture - a Scenedesmus sp - became dominant in the 12 m^2 pond over a six week period - replacing the dominant Micractinium. This species - comprised of large bodied cells, associated in small colonies of 2-4 cells, maintained its dominance throughout the batch cultivation

Table 2.1

Summary of October-November 1982 Pond Operations

Batch	Dates	Density, mg/ℓ Init.	Density, mg/ℓ Final	Nitrogen Status	Productivity $g/m^2/d$ Ave.	Productivity $g/m^2/d$ Max.*	Mix. Speed, cm/sec	depth cm
Pond I, 100 m^2 Scenedesmus sp Inoculum (No change in Species)								
#1	10/1-10/14	50	430	Suff.	6.5	9.0	30	20
#2	10/14-10/24	75	365	Lim.	6.4	9.0	30	25
#3	10/24 10/28	95	91	Starv.	3.1	-	30	25-30
Pond I 100 m^2 Micractinium Sp Inoculum (20-30% Scenedesmus at end of experiment)								
#1	10/31-11/23	23	170	Suff.	2.4	3.7	30	24
#2	11/14-11/24	86	88	Lim.	1.2	2.7	30	21-30
Pond D 200 m^2 Scenedesmus Sp Inoculum (no change in species)								
#1	10/14-10/24	57	380	Suff.	6.1	9.5	15	25
#2	10/24-11/4	80	240	Lim.	4.5	4.5	15	25-30
#3	11/5-11/13	125	225	Suff.	2.6	4.4	15	25
#4**	11/14-11/26	77	136	Lim.	2.2	5.5	15	25
Pond C 200 m^2 Chlamydomonas Sp (Self selected culture - no change in Species)								
#1	11/6-11/13	65	168	Suff.	1.4	2.7	15	10
#2	11/14-11/29	86	71	Lim.	0.4	3.0	15	15-30

* Maximum productivity is based on a minimum of two days.

** A small amount (5%) Micractinium introduced (accidentally) on 11/14 disappeared by 11/26.

experiments in 100 m^2 and 200 m^2 ponds during October and November and, also maintained its dominance throughout the winter months (December - February). Micractinium fared less well - Scenedesmus was able to invade the ponds and, eventually, completely overtake them. As expected, in a competitive situation, a particular species was able to take over dominance.

Of some significance, was the fact that Scenedesmus did not overtake the Chlamydomonas culture, which was essentially unialgal, until February. This example shows that under similar environmental conditions (nutrients, water resources, pH, temperature, sunlight) different species of algae can persist as unialgal cultures for significant periods of time.

Another important conclusion from the work is that cultures can be maintained in the ponds for several months during the wintertime. In a practical sense this is important as it should allow relatively rapid start-up of production and avoid the cost of building up the inoculum required for startup of large scale systems.

Nutrient Limitations and Productivity

The experiments indicate that short term periods of nitrogen limitation do not result in a significant decrease in productivity. Whether this result can be interpreted in terms of lipid production is uncertain - due to the limitations of the lipid analysis methods used during these experiments and because the Scenedesmus and Chlamydomonas cultures did not exhibit high lipid contents upon nitrogen limitation. Overall productivity also was relatively low during these experiments because of the lateness of the season. Thus, any interpretation of the significance of these findings must be tempered by these limitations of the data. Nevertheless, the fact that short term nitrogen limitation did not result in significant declines of productivity was encouraging and points the way to future experimentation.

Pond Hydraulics

The great differences in hydraulic performance of the 100 m^2 and 200 m^2 ponds and the unexpected gradient of mixing velocities in the ponds suggest that a more detailed hydraulic study is required. The objective will be not only to describe the existing system but also to allow extrapolations to larger scales.

PROBLEMS AND PLANS

The key problem encountered during the reporting period was the late start of the outdoor work which did not allow sufficient time to carry out a significant experimental plan. Productivities declined somewhat faster than expected during the October-November period and were, essentially zero during December and January. Thus any results

obtained up to now can only be considered as very preliminary, and can not be used for any conclusive analysis or assessment. The laboratory cultivation of the outdoor cultures has been successful only for Scenedesmus, the Chlamydomonas was (as before) not successfully cultivated. Lipid analysis have been a source of problems early in this project but have been improved through attendance at the SERI sponsored workshop at Georgia Institute of Technology in December of 1982. The current series of storms in California suggest that the outdoor experimental program will not be initiated until mid to end of March, 1983, about one month behind the original schedule.

Future plans involve a longer term series of experiments with the four currently operating outdoor ponds. Two different approaches could be followed for species selection: inoculation of proven lipid forming species, obtained from culture collections, and cultivation of naturally dominant species - such as the current strain of Scenedesmus. The competitiveness of the former and lipid producing ability of the latter are in question. Thus the primary objective of next season's operations is to select one or two species through outdoor cultivation with the greatest potential for lipid production. For this purpose the research plan will focus on species proven to be good lipid producers. The two 100 m^2 ponds will be used for inoculations of several laboratory microalgae. Three to five strains will be inoculated over a six to eight week period. The inoculum will be grown in 1.4m^2 ponds in the laboratory and 12m^2 ponds outdoors prior to transfer to the 100 m^2 ponds. Nitrogen sufficiency will be maintained - as the objective is to determine the competitiveness of these strains. Spiking experiments (e.g. a 3-5% inoculum of Scenedesmus) are planned to accelerate the initial invasion process. Based on these results one species will be selected for a series of nitrogen limitation - lipid production studies over the remaining six to eight weeks of the project. Harvestability will also be studied during these experiments. Productivity (total and lipid) will be determined throughout these studies.

The 200 m^2 ponds will be used to carry out a parallel series of experiments using as inoculum the cultures in the 100 m^2 ponds. These experiments will also be carried out for a 6-8 week period to maintain the inoculated species. If inversions take place the invading species will be tested for lipid productivity. Once one or two species have been selected they will be subjected to nitrogen limitation as above.

This subcontract activity is scheduled to end by June 30, 1983. A continuation proposal to allow operations for the remainder of this season and to continue this research next year is being prepared. The results of that research, together with results from other projects and analysis sponsored by SERI should allow an evaluation of the technical feasibility of microalgae fuel production.

References

1. Benemann, J.R. and Raymond, L.P., An Examination of Aquatic Biomass Production Final Report Solar Energy Research Institute, Golden, Colorado June 1981.

2. Necas, J. and Lhotsky, O., Ann. Rep. Algology Lab. Trebon Czechoslovakia Acad. Sci. Inst. Microbiol Trebon - 1967.

3. Oswald, W. J. in Developments in Industrial Microbiol. 4 112-125 (1963).

4. Benemann, J.R., B.L. Koopman, J.C. Weissman, D.M. Eisenberg and W.J. Oswald, Large Scale Freshwater Microalgae Production for Fuel and Fertilizer USDOE SAN-003-4-2 June 1978.

5. Oswald, W.J., A. Meron, and M.D. Zabot in Proc. 2nd Intl. Symposium for Wastewater Treatment Lagoons Kansas City, Missouri 1970.

6. Kawaguchi, K. in Algae Biomass (G. Shelef and C. J. Soeder eds.) Elsvier Biomedical Press, Amsterdam 25-73 (1980).

7. Oswald, W.J. and Golueke, C.G. Adv. App. Microbiol 2 223-262 (1960).

8. Benemann, J.R. P. Persoff, and W.J. Oswald Cost Analysis of Large Scale Microalgae Biomass Systems USDOE (1978).

9. Dynatech R&D Co. Cost Analysis of Aquatic Biomass Production (E. Ashare, ed) USDOE HCP/ET-4000-78/2) Vol. II July 1978.

10. Benemann, J.R., R.P. Goebel, J.C. Weissman, and D.C. Augenstein Microalgae as a Source of Liquid Fuels Final Technical Report to USDOE under Contract DEACO 81 ER 30014 EnBio, Inc. May 1982.

11. Ibid presented at the SERI-Aquatic Species Contractors Meeting Washington, D.C. June 1982.

12. Benemann, J.R. and R.P. Goebel and J.C. Weissman, Production of Liquid Fuels and Chemicals by Microalgae Final Report under Subcontract XB-0-9133-1 to the Solar Energy Research Institute, Golden, CO., Ecoenergetics, Inc. July 1981.

13. Ibid, presented at the SERI Subcontractors Review Meeting Aquatic Species Program, Washington, D.C. July 1981.

14. Burlew, T.S. (ed) Algae Culture: From Laboratory to Pilot Plant Carnegie Inst. of Washington Publication 600, Washington, D.C. (1953).

15. Fisher, A.W. in Solar Energy Research (Daniels and Duffie, eds.) University of Wisconsin Press, Madison, Wisconsin (1955).

16. Weissman, J.C. and J.R. Benemann Polysaccharide Production by Microalgae Final Report to NSF, Ecoenergetics, Inc. May 1980.

17. Opute, F.I. J. Botany 38 889-902 (1974).

18. Shiffrin, N.S. and Chisholm, S.W. J. Phycology 17 371-373 (1981).

19. Lien, S., Presented at the SERI Subcontractors Review Meeting Aquatic Species Program, Washington, D.C. July 1981.

20. Weissman, J.C., D.M. Eisenberg, and J.R. Benemann, Biotech. and Bioeng. Symp. No. 8, 299-316 (1978).

21. Benemann, J.R., J.C. Weissman, R.P. Goebel Production of Liquid Fuels and Chemicals by Microalgae, First Quarterly Report under Subcontract XK-3-03000-01 to the Solar Energy Research Institute, Golden, Colorado, EnBio, Inc., November 1982.

SHALLOW ALGAL MASS CULTURE SYSTEMS

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BACKGROUND AND OBJECTIVES

Microalgae are attractive as biomass producers because they generally exhibit higher yields and photosynthetic efficiencies than terrestrial plants. In addition, many species of microalgae produce high concentrations of intracellular oils as energy storage compounds. Microalgae are projected from laboratory scale experiments to be capable of producing over 100 barrels of liquid fuels per acre per year. Most pilot-scale mass culture systems, operated at depths greater than 20 cm, have shown peak average yields of around 15 dry ash free grams m^{-2} day^{-1}. This project is based on the premise that operation at less than 15 cm depth will result in significantly higher productivities.

Research on this problem began at the University of Hawaii under SERI subcontract on February 15, 1980 under the title "Research, Development, and demonstration of Algal Production Raceway (APR) Systems for the Production of Hydrocarbon Resources." Work during the first two and one-half years resulted in the collection of laboratory data on the performance of Phaeodactylum tricornutum; the design, construction, and operation of one 50 m^2 raceway; the development of predator control techniques and baseline performance characteristics for the raceway; and the construction of four small (9.2 m^2) experimental raceways. During the past four months these small raceways have been used to perform blocked fractional factorial yield experiments on eight operational parameters, in order to rank the effects of the parameters with respect to their impact on photosynthetic efficiency and biomass yield. This recently completed task will reduce the number of parameters which must be studied in detail in order to optimize P. tricornutum production in the outdoor flumes. Future experiments will determine how much algal production can be expected from shallow outdoor flumes and the sensitivity of the production function to changes in design characteristics, in order to determine whether shallow outdoor flumes are significantly more productive than conventional algal mass culture systems.

A critical feature of the shallow outdoor flumes has been the use of foil arrays to effect systematic vertical mixing of the culture. Work conducted in the 50 m^2 flume indicated that the placement of the foil arrays in the flumes increased production of P. tricornutum 2.2 to 2.4 fold. Solar energy conversion efficiencies averaged 3.8% over a three-month period, and five day means of solar energy conversion efficiencies reached as high as 10 percent.

FRACTIONAL FACTORIAL ANALYSIS OF OUTDOOR ALGAL PRODUCTION

Fractional Factorial Analysis -- Introduction

The effects of n factors on an experimental response can be preliminarily evaluated in a two-level factorial design of 2^n runs. In a two-level factorial design, each factor is assigned two values, high (+) and low (-) selected for maximum potential response effect. Analysis of the data permits an evaluation of the effect of each factor as well as of their effects in pairs, triplets, and so on. In fractional factorial designs, the number of runs is reduced below 2^n. Proper selection of the runs can prevent significant losses of information about the primary effects of the variables. For a detailed discussion of factorial analysis, see Box et al. (1).

The present experiments were designed to evaluate the effects on algal production of eight variables: culture depth, dilution rate, temperature (cooling compressor cut-in), culture circulation rate, blue or white light, pH (CO_2 supply cut-in), nitrogen source (ammonium or urea), and salinity. A 1/16 fractional factorial was designed to evaluate the effects of these factors.

Experimental

Cultures of Phaeodactylum tricornutum Bohlin (Thomas strain) were grown in four 9.2 m^2 cultures, comprising two channels 7.6 m long by 0.6 m wide by 0.15 m deep. The channels were connected by 20 cm recirculation pipes; one of the two recirculation pipes of each culture contained an airlift pump which drove culture circulation. Each culture was equipped with a CO_2 supply line and with a 1.5 hp chilling unit, which exchanged heat with the water through 6 m of 2.5 cm diameter titanium pipe. Temperature and pH sensors in the cultures supplied data to a Hewlett-Packard 9845B computer, which in turn controlled chiller function and the CO_2 supply solenoids.

The fractional design employed is shown in Table 1, and is given in terms of the factor values in Table 2. In Table 1, each variable is assigned the + value in 8 runs and the - value in 8 runs. For the 8 + values or the 8 - values for each factor, each other factor assumes 4 + values and 4 - values. Thus, the effects of all other factors, if additive, will cancel in the calculation of the primary effects of a given factor.

Details of the manipulation of each experimental factor are:

1. Depths were 5 cm or 10 cm mean depth over the culture. Depths varied as a function of the distance along the channel, with ranges of up to ± 2 cm about the mean. Six sets of mixing foils (2) were placed in each culture spaced at 1.5 m intervals to provide ordered mixing. Foil design was depth-dependent: at the 5 cm depth the foils were 5 cm wide with 5 cm gaps gaps between, while at the 10 cm depth these dimensions were 10 cm. (see reference 2 for a discussion of the imporatnce of this dimensional relationship.)

Table 1. A fractional factorial experimental design for eight factors in 16 runs (2^{8-4}_{IV}).

	LEVEL FOR FACTOR:							
RUN	1	2	3	4	5	6	7	8
1	-	-	+	-	-	+	-	+
2	+	-	-	-	+	-	-	+
3	+	+	+	+	-	-	-	+
4	-	+	-	+	+	+	-	+
5	+	-	+	-	-	-	+	-
6	-	-	-	-	+	+	+	-
7	-	+	+	+	-	+	+	-
8	+	+	-	+	+	-	+	-
9	+	+	-	-	-	+	+	+
10	-	+	+	-	+	-	+	+
11	-	-	-	+	-	-	+	+
12	+	-	+	+	+	+	+	+
13	-	+	-	-	-	-	-	-
14	+	+	+	-	+	+	-	-
15	+	-	-	+	-	+	-	-
16	-	-	+	+	+	-	-	-

Table 2.

Raceway Experimentation Schedule -- Fractional Factorial Analysis

	Raceway #	1 Depth cm	2 Dilution rate (d^{-1})	3 Night T°C[2]	4 Flow Rate cm/s	5 $CuSO_4$	6 pH[3]	7 Nutrient	8 Salinity 0/00
BLOCK #1	1	5	20	20	15	-	9.0	NH_4^+	35
	2	10	20	15	15	+	8.0	NH_4^+	35
	3	10	40	20	30	-	8.0	NH_4^+	35
	4	5	40	15	30	+	9.0	NH_4^+	35
BLOCK #2	1	10	20	20	15	-	8.0	urea	15
	2	5	20	15	15	+	9.0	urea	15
	3	5	40	20	30	-	9.0	urea	15
	4	10	40	15	30	+	8.0	urea	15
BLOCK #3	1	10	40	15	15	-	9.0	urea	35
	2	5	40	20	15	+	8.0	urea	35
	3	5	20	15	30	-	8.0	urea	35
	4	10	20	20	30	+	9.0	urea	35
BLOCK #4	1	5	40	15	15	-	8.0	NH_4^+	15
	2	10	40	20	15	+	9.0	NH_4^+	15
	3	10	20	15	30	-	9.0	NH_4^+	15
	4	5	20	20	30	+	8.0	NH_4^+	15

1 - after dilution 2 - compressor cut-in 3 - CO_2 cut-in

2. Dilution Rates are presented as the percentage of the culture removed each morning and replaced with fresh medium. Due to errors in measuring depth, dilution rates varied about the desired means (see below).
3. Temperatures given are the temperatures above which the chiller for each culture was activated. Average temperatures in the cultures differed between the two treatments by 2-4°C, and other variables also affected temperatures (see below).
4. Flow Rates were 15 and 30 cm sec^{-1}, and were adjusted by varying the volume of air supplied to the airlift pump.
5. Blue light was introduced by placing double-walled Plexiglas sheets filled with 5% w/v $CuSO_4$ solution above the cultures. The average light path through the $CuSO_4$ solution was about 1 cm.
6. The pH factor denotes the culture pH above which the CO_2 supply solenoid was activated to supply approximately 200 ml min^{-1} of 100% CO_2 gas.
7. Nitrogen sources were ammonium or urea, both supplied as commercial fertilizers. Concentrations were maintained at 0.5-1.5 mM N. Samples for NH_4^+ abd urea concentrations were taken daily. If visual comparison to a standard solution showed concentrations of 0.5-1.0 mM, sufficient nutrient was added to cause a 0.5 mM increase. If concentrations were less than 0.5 mM, enough nutrient was added to cause a 1.0 mM increase. Equimolar (N:C) amounts of sodium bicarbonate were added along with the ammonium sulfate to offset alkalinity changes due to ammonium uptake.
8. Salinity was altered by diluting the 35-40 o/oo well water with tap water to achieve 15 o/oo. For 35 o/oo, the well water was undiluted.

Since all 16 runs could not be executed simultaneously, "blocking" of the factorial design was employed to divide the 16 runs into four sets of four runs each (Table 2). In such a blocked design, there can be variability due to changes in uncontrolled variables between blocks. Effects of such variation cannot be distinguished from the effects of factors which have consistent signs within blocks. These factor effects are said to be confounded with the block effects. Table 3 shows the primary effects and two-factor interactions which are confounded with block effects. The table also shows the differences between the mean values for the + blocks and the mean value for the - blocks of the measured environmental variables air temperature and daily irradiance. If either of these variables, but particularly light intensity, significantly affect culture performance, substantial block effects would be expected. The block effects will be confounded with the effects of factors 7 and 8. Unmeasured variables such as well water quality could also have affected the cultures and have led to block effects.

Cell numbers of Phaeodactylum tricornutum and contaminant species were determined by direct hemacytometer counts. Particulate carbon and particulate nitrogen were determined by CHN analysis of the material collected on glass fiber filters. Dry weights were also determined

Table 3. Block effects of measured environmental variables.

FACTOR	SIGN IN BLOCK				TEMPERATURE EFFECT	LIGHT EFFECT
	1	2	3	4	°C	E m^{-2} day^{-1}
7	-	+	+	-	-0.25	-0.35
8	+	-	+	-	+0.35	+3.65
16,35,78	-	-	+	+	+0.35	+13.25
24	+	+	-	-	-0.35	-13.25

Table 4. Confounding of effects in the fractional factorial analysis.

EFFECT	EQUIVALENT TO:
1	
2	
3	
4	
5	
6	
7	BLOCK
8	BLOCK
12	(-37, -46, -58)
13	48, 56 (-27)
14	38, 57 (-27)
15	36, 47 (-28)
16	35, 78 (-24) BLOCK
17	45, 68 (-23)
18	34, 67 (-25)
23	(-17, -45, -68)
24	(-16, -35, -78) BLOCK
25	(-18, -34, -67)
26	(-14, -38, -57)
27	(-13, -48, -56)
28	(-15, -36, -47)
37	46, 58 (-12)

on glass fiber filters, and were corrected for blanks run at the culture salinity, but were not corrected for ash content. Lipid contents were measured by a modified Bligh-Dyer extraction. Culture ammonium concentrations were determined by the phenol-hypochlorite method. Urea concentrations were determined by the same method after the samples were digested with urease for 15 min. Chlorophyll a was determined spectrophotometrically.

Results

Fractional Factorial Analysis -- Sample Response

Table 4 lists the 22 distinguishable effects from the fractional design employed; while there were 36 total primary and two-factor effects, 21 of the second order effects were confounded in groups of three, leaving 15+7 = 22 distinguishable effects. (Second order effects are referred to here by combinations of the digits referring to the factors involved; i.e. 24 is the second order effect involving dilution rate (2) and flow rate (4)). Also, for each set of three confounded two-factor effects, there is one other two-factor effect which differs only with respect to sign (Table 4).

Only primary and two-factor effects were calculated; normally, higher-order effects become progressively smaller, and can be ignored. Primary effects were calculated as

$$E(I) = \sum_{n=0}^{16} R(n) \cdot \mathrm{Sign}(I,n) \qquad (1)$$

where E(I) is the effect due to factor I, R(n) is the measured response in run n, and Sign(I,n) is the sign of factor I in run n (+1 or -1). Two-factor effects were calculated as

$$E(IJ) = \sum_{n=0}^{16} R(n) \cdot \mathrm{Sign}(I,n) \cdot \mathrm{Sign}(J,n). \qquad (2)$$

To demonstrate the data analysis technique in an idealized case, we selected a culture response which should only have been affected by certain of the factors employed. The example presented here is culture temperature; other similar examples can be found in Table 5. The effects calculated according to equations 1 and 2 will ideally be distributed normally about their mean if all effects are random. The cumulative frequency distribution of these effects, when plotted on probability paper, should appear linear. Any effects at the tails of these curves which fall outside of this linear relationship are thus greater than would be predicted by probability. In the case of temperature, two factors had considerable effects on culture temperature, 3 (chiller cut-in) and 5 ($CuSO_4$ panels)(Fig. 1). These are, in fact, the variables which we would most expect to influence culture temperature. None of the other variables, with the possible exception of 1 (culture depth, hence volume) would be expected to significantly affect temperature. (Factor 1 was in fact the next most significant variable, Table 5.)

FIGURE 1

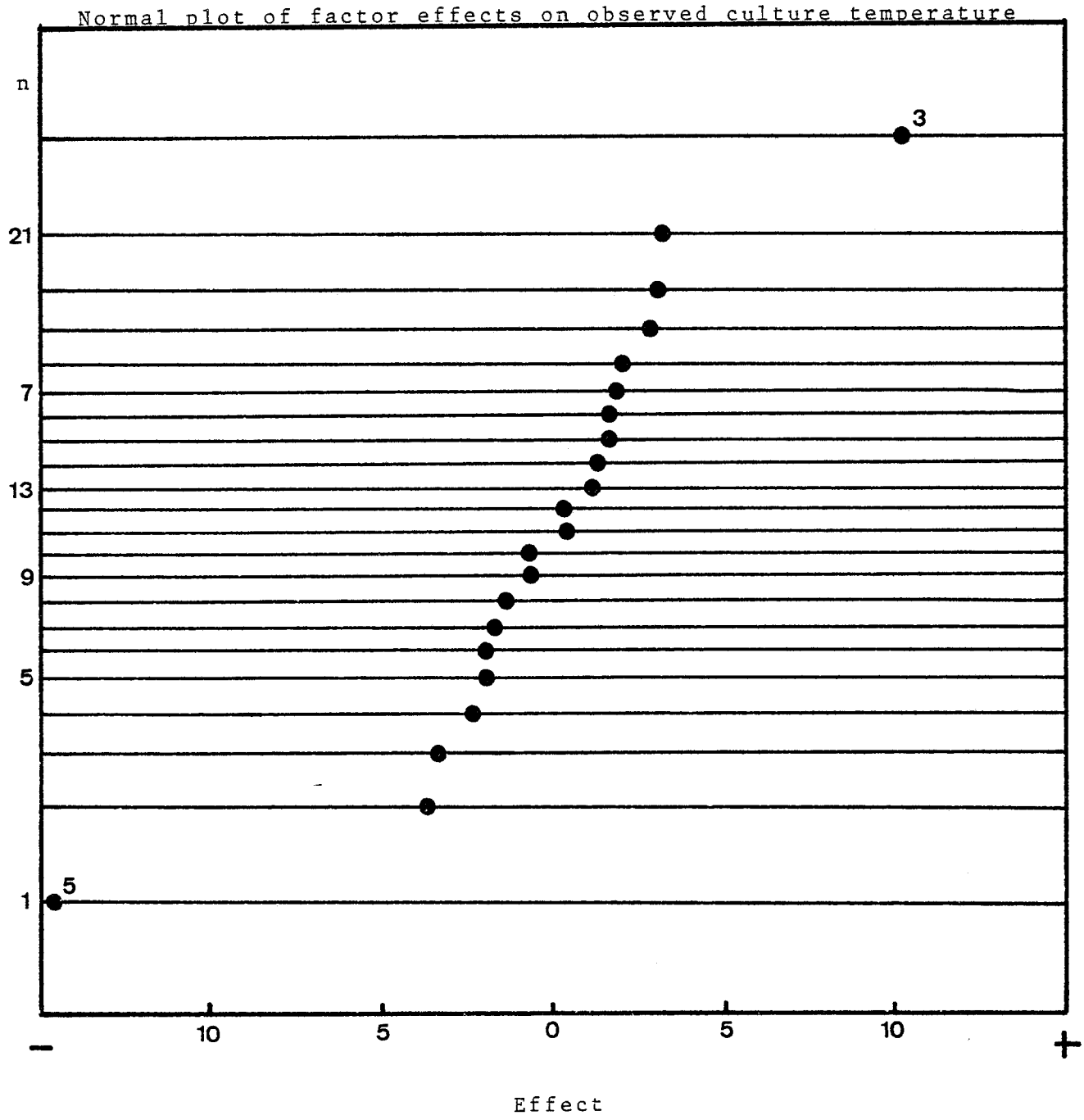
Normal plot of factor effects on observed culture temperature
n
21
7
13
9
5
1
3
5
10
5
0
5
10
−
+
Effect

Table 5. Summary of factor effects on various culture responses. Effects are given in standard deviations.

RESPONSE	EFFECT OF FACTOR: 1	2	3	4	5	6	7	8
1. Carbon Production	+2.16	-0.89	+0.56	+0.41	-0.56	-1.25	-0.27	-1.49
2. Dry Wt. Production	+2.08	-0.83	+1.04	+0.79	-0.46	-0.72	-1.05	+1.21
3. Photo. Eff. (C)[1]	+2.39	-0.84	+0.59	+0.25	-0.63	-1.66	+0.11	-1.88
4. Photo. Eff. (DW)[2]	+1.87	-0.64	+0.92	+0.56	-0.52	-0.90	-0.88	+0.86
5. Lipid Prodcution	+1.91	-1.14	+0.76	+0.03	+0.09	-0.77	+0.36	+0.11
6. Particulate Carbon[3]	-0.58	-1.95	+0.52	+0.36	-0.04	-1.03	-0.69	+2.48
7. Dry weight[3]	-0.44	-1.81	+0.66	+0.90	+0.09	-0.86	-1.27	+1.44
8. Monads[3]	+0.21	-1.62	-0.76	-1.97	+0.62	-0.21	+0.93	-1.41
9. % Non-*Phaeodactylum* C	+0.23	-0.90	-0.26	-1.79	-1.28	-0.21	+1.39	+1.39
10. Temperature	+0.68	-0.53	+2.39	+0.49	-3.42	-0.11	+0.12	+0.44
11. pH	+0.12	+0.04	-0.14	-0.50	-1.61	+2.31	-0.81	-1.54
12. Salinity	-0.69	-0.38	+0.47	+1.65	+1.14	-0.58	-1.26	-2.33
13. Chiller Running Time	-0.03	-0.46	-2.26	-0.05	+1.83	+0.14	+0.03	+1.45
14. CO_2 Running Time	+0.33	+0.24	+0.70	+0.77	-1.33	-2.42	+0.09	+0.54
15. Observed dilution	+1.21	+3.88	+0.17	-0.30	-0.69	-0.04	+0.83	-0.43

[1] - Photosynthetic efficiency calculated on the basis of carbon production.

[2] - Photosynthetic efficiency calculated on the basis of dry weight production.

[3] - Concentration units.

Fractional Factorial Analysis -- Biological Effects

The effects of the various factors on biomass and lipid production by the cultures, as well as other biological parameters, were not so simple to distinguish. Figure 2 shows factor effects on culture carbon production ($g\ m^{-2}\ day^{-1}$); no effect, with the possible exception of 1 (depth), lies outside of the predictions of probability. The second largest single factor effect was due to salinity (factor 8), but this factor is confounded with block effects, as are the largest two-factor interactions, 16 and 24. Photosynthetic efficiency effects, calculated on the basis of carbon production, showed a particularly tight linear relationship on a normal plot (Fig. 3). Not surprisingly, factor 1 and the block effects 8, 16, and 24 were also the most significant. Similarly, dry weight production pointed to factor 1 and the block effects 7, 16, and 24, although the normal plot suggests a somewhat bimodal distribution.

None of the biological effects showed significantly non-normal distributions; it does not necessarily follow, however, that the calculated effects do not represent real responses to the variable factors. The significance of the effects could be better evaluated if the variability of repeated runs under constant conditions was known. In the absence of such data, we have taken the standard deviation of the observed effects as a maximum estimate of the variability of the repeated runs, and normalized the effects to this standard deviation (Table 5). Effects of the various factors on biomass production were estimated from the means of the effects 1 - 4 (Table 5) the most significant variables are listed in Table 6. Lipid production (effect 5, Table 5), standing crop (effects 6 and 7, Table 5), and contamination (effects 8 and 9, Table 5) were treated similarly (Table 6).

Production Effects. By far the most significant factor affecting biomass production, and lipid production, was culture depth. Deeper cultures showed greater production, demonstrating that self-shading effects were more than offset by higher areal standing crops. Higher areal standing crops might also be achieved by increased culture density, but the latter variable is not always under the control of the culture operator. The second most significant factor affecting production was pH (CO_2 supply cut-in); cultures which did not receive CO_2 until the pH reached 9 showed less production. Increased dilution caused decreased production, presumably by reducing the standing crop to too low a level (see Standing Crop). Increased temperature gave increased production, the expected result as long as the temperature range of the species is not exceeded. The same four factors showed the greatest effects on lipid production, indicating that changes in lipid content were not significant, or responded to the same factors. The magnitude of the production effects was significant: the standard deviation of the effects was about 25% of the mean value. Thus, for example, the depth effect represents about a 50% increase in production.

Standing Crop Effects. Salinity had the greatest effect on standing crop expressed in concentration units, with higher standing crops at

FIGURE 2

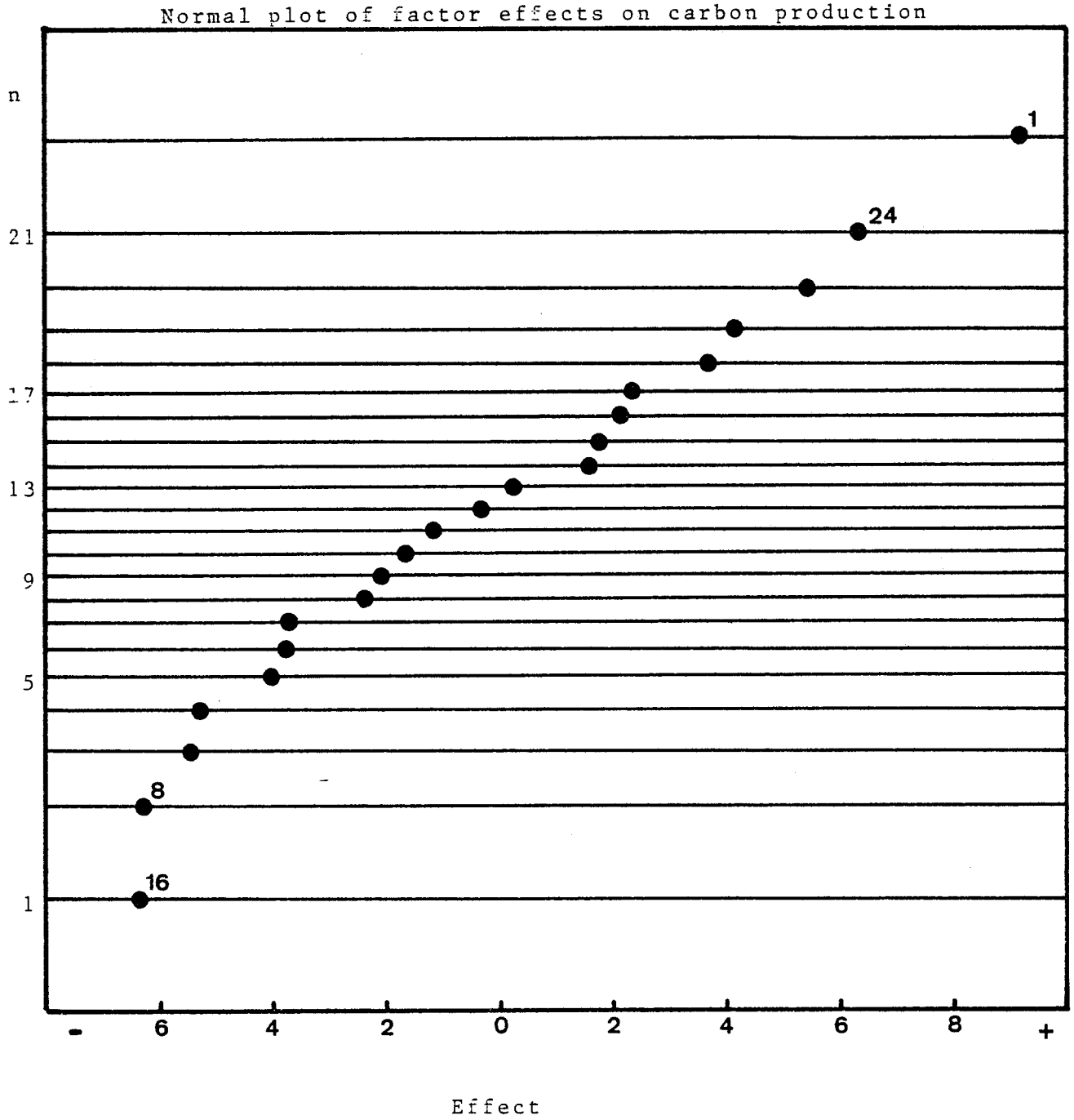
Normal plot of factor effects on carbon production
n
21
17
13
9
5
1
1
24
8
16
-
6
4
2
0
2
4
6
8
+
Effect

FIGURE 3

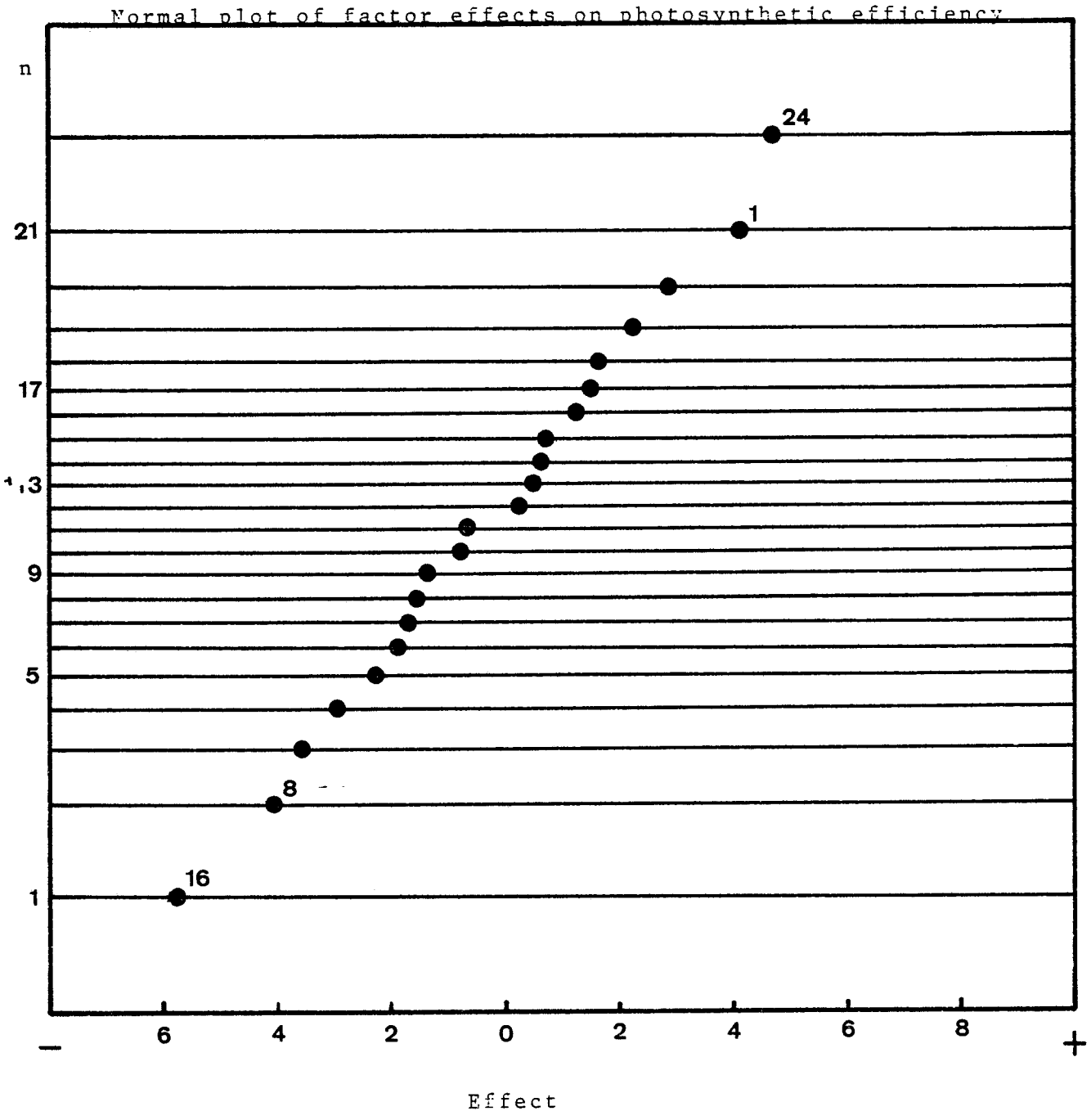
Normal plot of factor effects on photosynthetic efficiency
n
24
1
21
17
13
9
5
1
8
16
6
4
2
0
2
4
6
8
–
+
Effect

Table 6. Major variables determining biological responses.

Response	Factor	Effect (s.d.'s)
BIOMASS PRODUCTION	Depth	+2.13
	pH (CO_2 cut-in)	-1.13
	Dilution Rate	-0.80
	Temperature (chiller cut-in)	+0.78
LIPID PRODUCTION	Depth	+1.91
	Dilution Rate	-1.14
	pH (CO_2 cut-in)	-0.77
	Temperature (chiller cut-in)	+0.76
STANDING CROP (concentration)	Salinity (BLOCK)	+1.96
	Dilution Rate	-1.88
	Nitrogen Source[1](BLOCK)	-0.98
	pH (CO_2 cut-in)	-0.95
CONTAMINATION	Flow Rate	-1.88
	Dilution Rate	-1.26
	Nitrogen Source[1](BLOCK)	+1.16

[1] - + = urea.

higher salinity, but this conclusion must be treated with caution since the salinity effect is confounded with block effects. Increased dilution reduced the standing crop, as is expected. Standing crops were lower with urea as a nitrogen source than with ammonium, but again this result is confounded with block effects, and should be treated with caution. An increase in the pH threshold for CO_2 delivery resulted in a decreased standing crop, also the expected result.

Contamination Effects. Contamination, particularly contamination by a monad predator, is a significant concern in mass cultures of Phaeodactylum. The significance of such contamination in the present experiments was somewhat damped by artificial control of the monad with additions of an antimony salt. The greatest effect on contamination was due to the culture flow rate; there was less contami-ation at higher flow rates. It has been our observation that contaminant species frequently thrive in patches of settled material in the cultures. High flow rates, in the presence of mixing foils, tend to eliminate these patches, and thus may serve to reduce the significance of contamination. Increased dilution also served to reduce contamination, implying that cells at higher growth rates are more resistant to contamination, or that the contaminants cannot sustain high rates, and are washed out. Contamination was greater in the presence of urea than in the presence of ammonium; two reasons for this effect can be suggested. First, there is the demonstrated toxicity of free ammonium ion to the monad (2). Second, urea provides a carbon source as well as a nitrogen source, which may lead to increased bacterial production and subsequently to increased populations at higher trophic levels.

Selection of Variables for Further Study

Depth, and thus areal culture density, was the most clearly significant factor in determining production, and needs to be subjected to study in greater detail. Dilution rate was also of consistent importance. Temperature was also of importance, and since it significantly influences the economy, design, and location of future facilities, its further study is also recommended. Flow rate, which did not affect production, must be considered as a serious candidate for further study due to its effect on contamination. However, since the faster flow rate employed here is near the limit of our present apparatus to generate culture flow, it seems reasonable to accept the results of the present study and employ the faster flow rate in all optimization studies. Due to the slight negative effect of the copper sulfate panels on production (probably not significant), we choose not to apply these panels in the optimization experiments. Their effect on culture temperature (Figure 1), however, should be kept in mind for the design of future facilities where culture temperature increases are of concern. The pH threshold for CO_2 supply appears to deserve further investigation. Based on the present data, ammonium appears to be superior to urea as a nitrogen source, but the confounding of its effect with block effects makes conclusions difficult. Ammonium will be used for testing in the near future, but the lower cost of urea and its inertness with respect to alkalinity, make it a candidate for additional consideration at a later date. Salinity effects, which were confounded with block effects, were not conclusive, but we do not propose to study this factor further.

REFERENCES

1. Box, G.E.P., Hunter, W.G. and Hunter, J.S. Statistics for Experimenters, John Wiley and Sons, New York, 1978.

2. Laws, E.A., K.L. Terry, J. Wickman and M. Chalup. 1983. Preliminary results from a simple algal production system designed to utilize the flashing light effect. Submitted to Biotechnol. Bioengr.

THE MIDDLE ATLANTIC CONSORTIUM ON ENERGY RESEARCH (MACER) PROGRESS REPORT FOR DECEMBER, JANUARY, AND FEBRUARY

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OBJECTIVES

The objectives of the program are to identify species of plants containing high amounts of hydrocarbons that grow naturally and culturally in the four states of Maryland, Delaware, Pennsylvania, and New Jersey. To extract these compounds, measure the heats of combustion and determine the environmental conditions (particularly; soil, nutrients, water, stresses, etc.) to maximize the yield.

Purpose

The work above will provide the basis for future studies to investigate the feasibility of the cultivation and the processing of these plants to extract high-energy compounds. Also, we hope to develop an understanding of the metabolic pathways by which the hydrocarbons form in order to enhance the hydrocarbon content.

Data-findings could also be generated on economic aspects. The possibility also exists to provide a renewable source of fuel which could make the United States less dependent on foreign countries for its source of energy.

ACCOMPLISHMENTS

The first three months of this project has been concentrated on literature search at various libraries and the USDA in Beltsville, Md. A considerable amount of information has been obtained on published works on hydrocarbon producing plants. A list of plants which have been reported in the literature is being checked to verify which of them occur in the four states under study. See tables 1, 2, and 3. The results of this search will indicate which plants are indigenous to this area and will be collected in the field survey to be initiated this spring.

Method of Collecting Data

Bibligraphic material has been collected utilizing the libraries of the five colleges involved and the agricultural and general libraries at the University of Delaware, the Johns Hopkins University, and the USDA in Beltsville, Md.

Data Figures

Since the contract was initiated in December, no data is available at this time.

TENTATIVE PLANS

We plan to continue the library research, to begin field collections this spring, and to begin some cultures in our greenhouses. The heats of combustion of the plants will be measured and the metabolic processes studied.

LITERATURE CITED

See tables 1, 2, and 3.

ATTENDANCE AT PROFESSIONAL MEETINGS

All institutions in the consortium sent representatives to the February 24th and 25th DOE Meeting in Washington, D.C. This meeting was designed to apprise minority institutions of the areas of energy research DOE sponsors.

TABLE I - PLANT SPECIES IN SOUTHWESTERN AND WESTERN U.S. WITH POTENTIAL AS HYDROCARBON PRODUCING CROPS[+]

Rating: 1, poor; 2, fair; 3, average; 4, good; 5, excellent.

Genus Species	Common Name	Hydrocarbon Production Rating
Anacardiaceae		
Rhus glabra L.	Smooth sumac	4
Apocynaceae		
Apocynum cannabinum L.	Indian hemp	3
Asclepiadaceae		
Asclepias incarnate L.	Swamp milkweek	3
Asclepias syriaca L.	Common milkweed	2
Asclepias verticillata L.	Whorled milkweed	3
Caprifoliaceae		
Sambucus canadensis L.	Common elder	3
Compositae		
Ambrosia trifida L.	Giant ragweed	3
Aster novae-angliae L.	New England aster	3
Eupatorium altissimum L.	Tall boneset	4
Silphium Laciniatum L.	Compass plant	3
Solidago altissima L.	Tall goldenrod	3
Sonchus arvensis L.	Sow thistle	3
Euphorbiaceae		
Euphorbia cyparissias L.	Cypress spurge	4
Euphorbia suprina Raf.	Prostrate spurge	3
Labriatae		
Monarda fistulosa L.	Wild bergamot	3
Phytolaccaceae		
Phytolacca americana L.	Pokeweed	4

[+]From: Buchanan, R.P.; I.M. Cull; F.H. Otey and C.R. Russell. 1978 Hydrocarbon-and rubber-producing crops: Evaluation of U.S. Plant Species. Econ. Bot. 32: 131-145.

TABLE 2 - PLANT SPECIES IN SOUTHWESTERN U. S. WITH POTENTIAL AS HYDROCARBON PRODUCING CROP+

Genus Species	Hydrocarbon Production Rating
Aceraceae	
Acer saccharum	4
Campanulaceae	
Campanula americana	3
Caprifoliaceae	
Symphoricarpos orbiculatus	3
Compositae	
Cacalia atriplicifolia	1
Circum discolor	4
Silphium integrifolium	3
Silphium terebinthinaceum	3
Solidago graminifolia	2
Solidago rigida	2
Vernonia fasciculata	4
Euphorbiaceae	
Euphorbia dentata	4
Euphorbia heterophylla	4
Gramineae	
Phalaris canariensis	2
Labiatae	
Monarda punctata	3
Pycnanthemum incanum	2
Teucrium canadensis	2
Lauraceae	
Sassafras albicum	4
Pinaceae	
Pinus sylvestris	3
Rhamnaceae	
Ceanothus americanus	3
Rosaceae	
Prunus americana	4
Salicaeae	
Populus tremuloides	4

+From: Buchanan, R.A.; I.M. Cull; F.H. Otey and C.R. Russell. 1978. Hydro-carbon-and-rubber-producing crops: Evaluation of 100 U.S. Plant Species. *Economics Botany* 32: 146-153

TABLE 3 - PLANT FAMILIES IN THE U. S. SHOWING A POTENTIAL FOR HIGH RUBBER PRODUCTION[+]

Family	Number of Species Tested	% Mean Rubber Content	Max
Acanthaceae	15	0.66	(2.64)
Apocynaceae	21	0.81	(4.82)
Asclepiadaceae	33	1.04	(4.93)
Avicenniaceae	1	1.48	(3.49)
Balsaninaceae	3	0.59	(1.56)
Campanalaceae	6	0.72	(2.45)
Celastraceae	10	0.54	(2.37)
Combretaceae	2	1.02	(2.17)
Compositae	493	0.64	(11.26)
Loranthaceae	2	1.00	(2.81)
Melastomaceae	9	0.71	(3.30)
Pandanaceae	1	1.32	(1.32)
Punicaceae	1	1.30	(1.30)
Rubiaceae	39	0.70	(5.71)
Sapotaceae	11	1.28	(6.00)
Sauouraceae	1	0.69	(1.92)
Terminaliceae	2	0.82	(2.55)

[+]From: Polhamus, L.G. 1967. Plants collected and tested by Thomas A. Edison as possible sources of domestic rubber. Crops Research Division, ARS/USDA.

WETLAND BIOMASS PRODUCTION

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OBJECTIVE

The primary objective of the SERI-sponsored Wetland Biomass Production Project at the University of Minnesota is that of identifying, testing, and evaluating production practices necessary to capitalize on the considerable potential of emergent aquatic plants as sources of biomass. Wetlands dominated by Typha spp. (cattails) and other emergent vegetation, including Phragmites (reed) and Scirpus spp. (rushes), are one of the more productive natural systems in the temperate zone (1). Total plant yields often exceed 40 tonnes/ha (18 tons/ac) in natural stands of Typha (2,3); yields of 30 tonnes/ha (13 tons/ac) have been demonstrated in newly established managed stands (4). In addition to the high productivity of wetland species, natural monospecific stands of these plants have the advantages of being relatively free of pests and disease, and occurring on land unsuitable for traditional agriculture.

The potential of wetland vegetation for fuel and fiber production has been widely recognized in regions of northern and eastern Europe where research programs are investigating plant characteristics and production methods. Much of this research has focused on Phragmites australis which is a dominant wetland species in that area. In order to make use of existing European knowledge and technologies as well as develop biomass production practices suitable to North American wetlands, this project is directed toward work on the following four tasks.

- Stand Management Research

  Objectives: Continued investigations of establishment methods, yields, nutrient and water requirements, and other management practices; investigations of promising genotypes of Typha, Phragmites, and other emergent plants.

- Relevant Emergent Technologies Survey

  Objectives: Continued investigations of relevant research findings, management practices, and costs from other programs working with emergent aquatic plants; investigations of methods of incorporating appropriate existing technologies into Typha management and harvest research.

- Rhizome Harvest Research

  Objectives: Investigations of field characteristics in which harvesting equipment will need to operate; investigations of a variety of engineering conceptual designs for components of a belowground harvesting system for Typha rhizomes.

- Management Options Evaluation

  Objectives: Evaluation of production scenarios developed from information gathered in the first three tasks.

In addition to these SERI-supported tasks, the state-funded Bio-Energy Coordinating Office at the University of Minnesota is supporting associated work in areas of plant nitrogen fixation in wetlands, micropropagation of wetland species, economics, land use planning, and equipment development. This comprehensive approach to wetland biomass production research fits well with the stated SERI objective of timely development of technology in preparation for its transfer to the private sector. Ultimately, information gained from this project can be used to develop a bio-energy system that maximizes output while minimizing inputs, resulting in a renewable energy resource that is economically competitive and environmentally benign.

ACCOMPLISHMENTS

Accomplishments presented under stand management research are from establishment season results of new experiments and second season results of experiments begun in the preceding year. The SERI-supported research that began prior to this reporting period, and for which second season results are presented, involved 1) stand establishment and management, 2) Typha nutrient experiments, and 3) wetland species comparisons. Complete establishment season results from these studies can be found in the final report submitted under the previous SERI subcontract (5).

Progress Since June 1982

Stand Management Research

Second Season Results. During the second season of field trials, yields of Typha spp. increased dramatically in all experiments. Table 1 presents a comparison of mean establishment and second season aboveground biomass yields for various field experiments established and managed at different locations using different methods. Particularly encouraging is the second season yield for a large stand established from Typha angustifolia seed. This stand went from the lowest productivity of all experiments in the establishment season to the highest productivity in the second season. This demonstrates that stand establishment using seed is feasible and can result in second season biomass yield actually exceeding yields of stands established using the more costly methods of transplanting rhizome pieces or seedlings. Also, this result re-emphasizes the need to examine the impact of genotypic variation on productivity since, under identical field conditions, an adjacent stand of Typha latifolia flowered extensively and had an aboveground yield less than half that of Typha angustifolia.

In the experiments in which belowground biomass was sampled during the second season, increases in belowground yield occurred in all cases, but these increases were generally less dramatic than those for aboveground biomass. Table 2 presents a comparison of mean establishment and second season belowground yields as well as ratios of above to belowground biomass for Typha field experiments. Substantial variability exists between experiments in the ratio of above to belowground biomass. Whether this results from genotypic differences, establishment methods, or field conditions is still unknown, as is the stability of the ratios over time. Because of the significance of this ratio to various production scenarios, further studies of factors influencing this ratio are warranted.

Table 1. Mean Aboveground Dry Weight Comparisons for *Typha* Field Experiments.

Experiment	Planting Stock*	Mean AG Dry Weight (g/m^2) 1981	Mean AG Dry Weight (g/m^2) 1982	Mean AG Dry Weight % Change (1981-1982)
Fertilization Study	Rhizome	369	806	+ 118%
Establishment Study	Seed	49	1,385	+ 2,725%
	Seedling	69	616	+ 793%
Peatland Excavation Study	Seedling	190	465	+ 144%
	Rhizome	350	688	+ 97%

*Rhizome stock = *Typha* x *glauca*; seedling stock = *Typha latifolia*; seed = *Typha angustifolia*.

Table 2. Mean Belowground Dry Weight and Ratios for *Typha* Field Experiments.

Experiment	Planting Stock*	Mean BG Dry Weight (g/m^2) 1981	Mean BG Dry Weight (g/m^2) 1982	Mean BG Dry Weight % Change (1981-1982)	Ratio AG to BG 1982
Establishment Study	Seed	37	776	+ 1,997%	1.8
	Seedling	95	538	+ 466%	1.1
Peatland Excavation Study	Seedling	283	444	+ 57%	1.0
	Rhizome	450	733	+ 63%	0.9
Litter Removal Study	Rhizome	504	693	+ 38%	0.8

*Rhizome stock = *Typha* x *glauca*; seedling stock = *Typha latifolia*; seed = *Typha angustifolia*.

One factor influencing productivity of Typha and, possibly, the ratio of above to belowground biomass is that of plant density. Density has an impact on the plant canopy and, hence, the stand's efficiency as a solar collector; density also influences the degree of competition from other plants. A survey of natural Typha stands at 15 locations throughout Minnesota found a mean shoot density of $51/m^2$ (6). Table 3 presents a comparison of mean establishment and second season densities from several field experiments established with different planting stocks and initial densities. Most managed stands had achieved or exceeded the natural stand equilibrium density of $51/m^2$ by the end of the second season. It is too early to tell what an equilibrium density might be in a managed stand, and what effect various harvesting options would have on this density. It is, however, interesting to note that the stand of Typha angustifolia established from seed had the highest density in both seasons; it also had the highest productivity and ratio of above to belowground biomass in the second season. Density will be determined at the end of the third growing season for this experiment in an effort to better understand factors affecting density.

Table 3. Mean Shoot Density Comparison for Typha Field Experiments.

Experiment*	Planting Stock	Planting Density (per m^2)	Mean Density (per m^2) 1981	Mean Density (per m^2) 1982	Mean Density % Change (1981-1982)
1	Rhizome	9	28	41	+ 46%
2	Seed	700	42	111	+ 164%
	Seedling	5	20	50	+ 150%
3	Seedling	9	38	54	+ 42%
	Rhizome	9	28	38	+ 36%

*Experiment 1 = fertilization study; Experiment 2 = establishment study; Experiment 3 = peatland excavation study.

A component of production costs that could significantly affect the final cost of emergent aquatic biomass is that of nutrients required to attain high sustained yields. 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 emergent aquatic biomass production. Additionally, other macro- and micronutrients could prove limiting, especially in the anaerobic, low pH soils where Typha or other species will be grown. To address questions concerning nutrient requirements of Typha, a fertilization study involving several levels of nitrogen, phosphorus, and potassium was established at a field site in 1981. Results from the establishment season indicated that nutrients were not the limiting growth factor in the first season (4). High initial soil fertility and poor early season growth resulted in increased nutrient availability and decreased nutrient requirements, respectively. The fertilizer treatments were effective in significantly increasing tissue nutrient

concentration, nutrient standing crop (g nutrient per m^2 in plant tissue), and soil fertility for phosphorus, potassium, and in some cases, nitrogen. It was believed that the increased nutrient standing crop and soil fertility would carry over into the second season and have an effect on yield and density.

Preliminary examinations of second season results for this experiment have not supported this hypothesis. While aboveground yields more than doubled in the second season, statistical analysis indicates that carryover nutrients were not the cause of this increase. Interestingly, statistical analysis did indicate a significant relationship between tissue nutrient concentration and pre-establishment fertilizer treatment for nitrogen and phosphorus in the second season. This indicates the continued long term effect of the fertilizer treatment. Over all plots in this experiment, tissue nitrogen percent declined from 1.75% at the end of the establishment season to 0.91% at the end of the second season; tissue phosphorus percent declined from 0.22% to 0.15%. Soil nutrient levels and tissue potassium are currently being analyzed.

Another experiment begun in 1981 to assess the potential of using emergent aquatic species to reclaim mined peatlands and produce biomass for fuel was continued into the second season. In addition to assessing reclamation potential, the experiment was designed to evaluate the effects of different land preparation schemes on productivity, nutrient availability, and weed and water management. Figure 1 presents mean yield and nutrient results from both the establishment and second season for Typha plots established under three different land preparation schemes. Yields increased in all areas during the second season, although not as dramatically as those from other experiments. Extensive flowering occurred in seedling planted plots during the second season. This appeared to limit further vegetative growth of the plants as seen visually and quantitatively. For example, mean total biomass yield of rhizome planted plots in the 0.6 m (2 ft.) excavated area was 17.2 g/m^2 (7.7 tons/ac); that of seedlings was 7.6 g/m^2 (3.4 tons/ac). Significantly, in terms of possible management techniques, the two excavated areas remained free of weeds again in the second season, and water levels were maintained with no pumping in the 0.6 m (2 ft.) excavated area. Pumping was required into the unexcavated area and out of the 1.5 m (5 ft.) excavated area. Competition from weeds continued to be a problem in the unexcavated area although the problem was less severe during the second season as Typha plants became the dominant canopy.

Two new field experiments were established this year on two newly prepared paddies, one being 0.4 ha (1 acre) in area and the other 0.2 ha (.5 acre). Mechanically transplanted seedlings of Typha latifolia were used to establish the plots in the large paddy, and mechanically transplanted rhizomes of differing Typha genotypes were used to establish the smaller paddy. A small plot of Phragmites australis was also established. The primary objective of both experiments is to determine the rate of nutrient uptake over the course of two growing seasons and use this information to develop effective and efficient methods of nutrient application. Emphasis will be on nitrogen uptake since soil nitrogen can be rapidly lost from the system through denitrification.

Figure 2 presents establishment season yield increases and nutrient uptake as a function of time following planting. For both experiments, 50% of the plant's seasonal uptake of nitrogen occurred between 56 and 84 days after planting. Less than 20% of total uptake occurred prior to 56 days. If nitrogen had been applied prior to planting, significant losses of this nutrient from the system would have occurred prior to the time of greatest uptake by the plants. Between 84 and 140 days after planting, increases in aboveground biomass cease while belowground biomass continues to

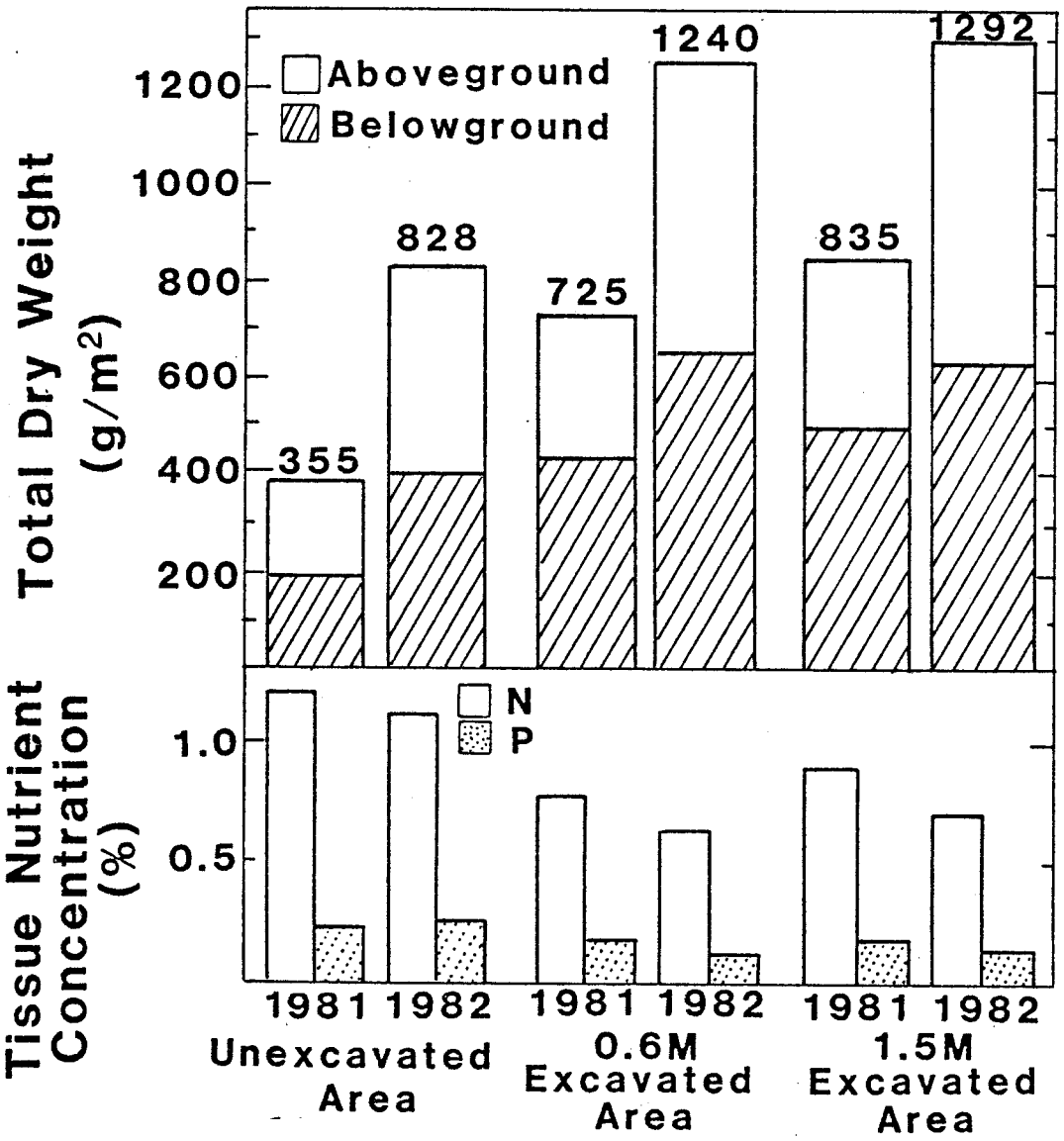

Figure 1. Peatland excavation study — two-year comparison of yield, and tissue nitrogen and phosphorus concentrations under three different land preparation schemes.

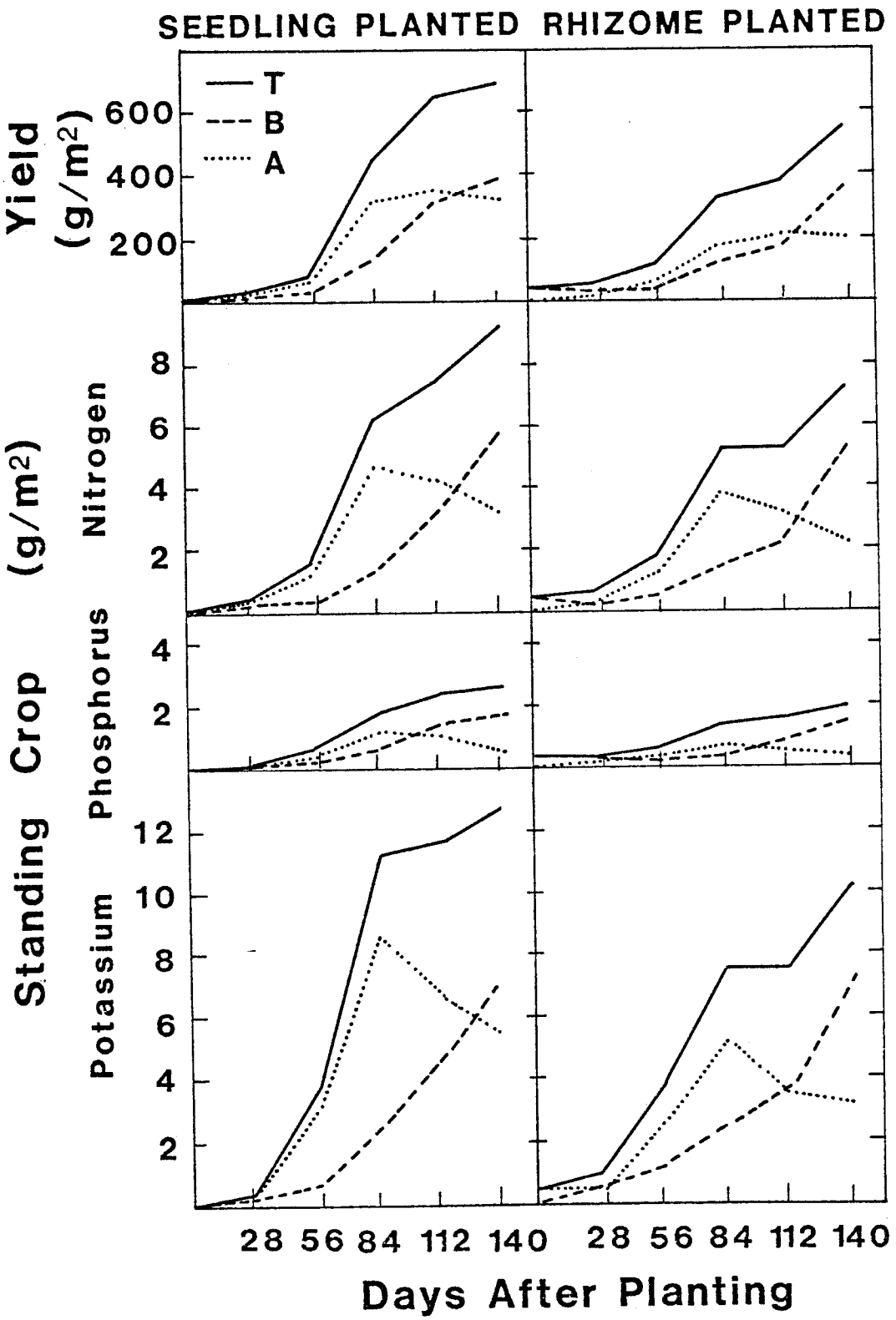

Figure 2. Establishment season trends in biomass production and nutrient uptake and partitioning. Planting date was June 7, 1982. Standing crop represents the amount of a nutrient removed with the harvested plant material on a unit area basis.

increase. During this same time period, it appears that significant amounts of nitrogen in the aboveground plant tissue are translocated to the belowground portion. At the end of the growing season, approximately 70% of the nitrogen taken up during the growing season is located in the belowground portion of the plant. It is interesting to note the changes in dry weight and nitrogen partitioning which continue to occur during October (112 to 140 days after planting) subsequent to the first frost date (81 days after planting; first hard frost on day 116). These late changes must be taken into account in deciding the optimum harvest date. Trends for phosphorus and potassium uptake are similar to those for nitrogen.

In addition to experiments involving Typha spp., several small paddy and field experiments have been set up to examine establishment methods and the potential productivity for other emergent aquatic species. A paddy study testing effective means of vegetative propagation of Phragmites australis (reed) found survival rates greater than 64% for methods using rhizomes and several shoot/rhizome combinations, but rates less than 10% for methods using cane sections. Further work on alternative methods is underway.

Table 4 presents a comparison of first and second year productivity and shoot density for five different wetland species. There is little consistency in species performance between the two years. Scirpus was highly productive both years in the paddies, increasing yield by approximately 35% in the second year. Sparganium, on the other hand, made no advances in the second year, probably due to extensive flowering and early senescence. Carex and Spartina both looked more promising after the second year when yield increased by nearly 65%. Productivity in the field was consistently lower than in the paddies, probably due in part to the fact that paddy planting density was 17 shoots/m^2 as opposed to 9 shoots/^{2} for the field plots.

Table 4. Productivity of Potential Wetland Biomass Crops.

Plant Genus	Experiment	Mean Density (Shoots per m^2) 1981	1982	Mean Aboveground Dry Weight (g/m^2) 1981	1982
Carex	Paddy	105	323	440	1264
Spartina	Paddy	80	264	560	1412
Phragmites	Paddy	26	134	50	723
Sparganium	Paddy	109	182	600	597
Sparganium	Field	67	80	480	530
Scirpus I*	Paddy	168	317	750	1256
Scirpus II*	Paddy	147	273	920	1229
Scirpus	Field	103	116	577	558

*Scirpus I and II were planted from different rootstocks.

Relevant Emergent Plant Technologies Survey. Work on this task has primarily involved 1) travel to several European projects concerned with emergent aquatic biomass research and production, 2) completion of a 700 citation bibliography covering research on Typha spp., and 3) information exchange with visiting scientists.

The European trip, undertaken in conjunction with a presentation at the European Communities Biomass Conference, allowed for meetings with engineers from the Seiga Harvester Company in Denmark. Seiga manufactures amphibious vehicles used in Denmark, Sweden and elsewhere to manage and harvest natural stands of emergent aquatic vegetation. Since the basic Seiga machine has been adapted by this project for use in fertilizing, planting (see Figure 3), and monitoring experimental areas in newly established and natural stands of emergent plants, these meetings proved quite valuable for visualizing additional applications for stand management.

A meeting was also arranged with a commercial venture using the Seiga equipment for Phragmites (reed) harvesting in Sweden. Information on the nature of the equipment and operation, possible equipment improvements, regeneration of reed beds following harvest, and harvest of species other than reed was obtained. The company sells the chipped reed to a forest products company to supplement the wood wastes that are used as its primary energy source. Reed chips have replaced more expensive residual oils as a back-up fuel in this case.

Several Swedish research institutions were visited. Discussions focused on propagation and utilization of reed and other species for energy. Field trips were taken to test plots in various parts of Skane including plots planted on mined peatlands in an attempt at reclamation. Greenhouse studies were also observed. Additional discussions concerned ecological impacts of energy farming activities and opportunities for solving environmental problems by using possible pollutants such as ash and other industrial wastes as nutrients on energy plantations.

As mentioned earlier, a comprehensive bibliography of research on Typha spp. has been completed (7). This project provides a detailed reference source for researchers or others interested in specific characteristics of Typha spp. and the wetland habitats that this genus occupies. The bibliography is indexed by species, physiological and ecological characteristics, as well as utilization studies.

Rhizome Harvest Research. Information is being gathered from various sources to address the technical questions concerning the harvesting of Typha rhizomes. Contacts with equipment manufacturers, including the one mentioned in the previous section, have provided a better understanding of the currently available equipment that might be modified for rhizome harvesting. Additional information has been obtained from associated agricultural engineering projects that have been assessing wetland characteristics relevant to machinery operation (8). Measurements of traction, flotation, and draft force requirements are also being made. Continued work on this task should provide information and potential engineering solutions to equipment development firms interested in harvesting the starch and sugar-rich rhizomes of Typha.

Methodology

Field Research

Establishment methods for Typha spp. experiments have consisted of 1) seeding using both dry and liquid seeding methods, and 2) transplanting using either rhizome material

or seedlings. Several seeding methods have been tested in an attempt to meet the physiological requirements (9) of Typha seed germination. Simple dry seeding on a mudflat and liquid seeding using a suspension of seeds pretreated with light in an agar solution have proven successful. A two-row mechanical transplanter, modified for use with Typha seedlings and rhizomes in a wetland (Figure 3), has been successfully used to establish the two large experimental stands (0.6 ha total area or 1.5 ac) reported on in the stand management research section of this report.

Fertilizer was generally applied and incorporated prior to planting at the rate of 75-150-300 kg/ha NPK (67-134-268 lb/acre NPK). Certain experiments examining nutrient relationships had different rates. This was the case in the newly established nutrient uptake experiment in which no nitrogen was applied. Nitrogen was applied in the form of urea to slow the denitrification process in flooded anaerobic soils. Micronutrients were applied to all organic soils; no soil pH adjustment was attempted. Water levels were maintained at approximately 15 cm (6 in.) throughout the growing season.

To determine plant dry weight and nutrient content, a 1 m^2 (10.8 $ft.^2$) area was sampled; a minimum border of 1 m (3.3 ft.) of undisturbed vegetation surrounded the sampled area on all sides. Where belowground samples were taken, the entire 1 m^2 area was excavated to a depth of 0.3 m (1 ft.) and rhizomes were removed by careful sorting. Plant samples were then divided into four sections for analysis: rhizomes, shoot bases, lower 15 cm (6 in.) of aboveground shoots, and upper shoots. The lower 15 cm was sampled separately to allow for a more precise estimation of harvestable aboveground biomass since a harvester would have to operate at some distance above ground level. A soil sample was also taken from each area for analysis.

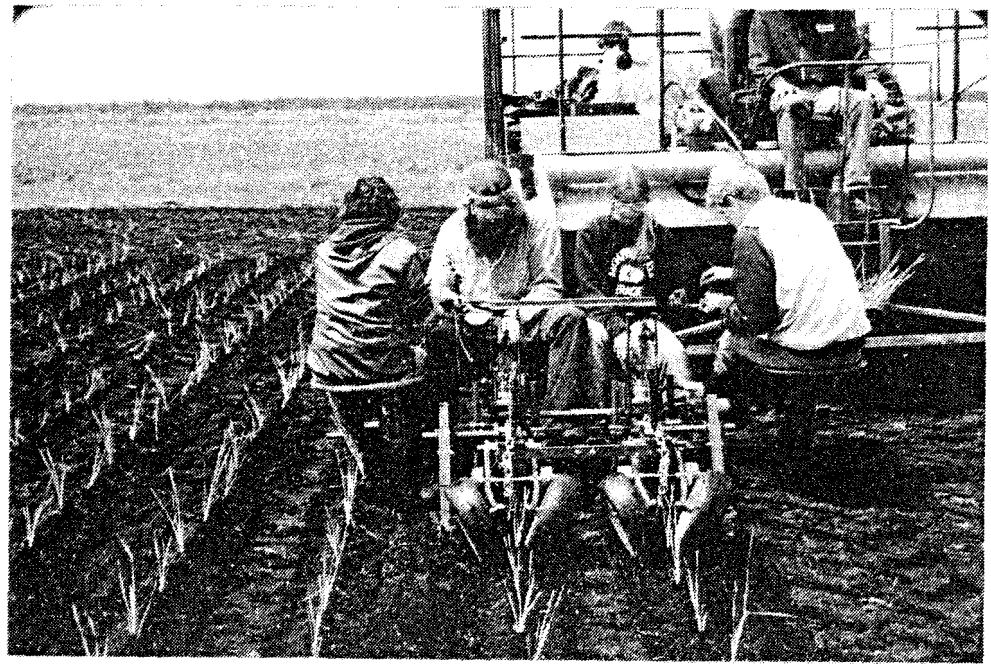

Figure 3. Mechanical transplanter being used for seedling planting in experimental field plots.

Analytical Methods

The following methods are used for analysis of plant tissue macronutrients.

- Nitrogen — a micro Kjeldahl technique is used for determination of total nitrogen. Following digestion, ammonia levels are measured with a NH_3 specific-ion electrode.

- Phosphorus — tissue is dry ashed at 500°C for 12 hours and phosphorus is then extracted with HCl solution. Concentration is determined by spectrophotometry at 882 nm using an ascorbic acid-molybdate blue assay.

- Potassium — tissue is dry ashed and potassium is extracted using same methods as for phosphorus. Concentration is determined by atomic absorption spectroscopy at 766 nm.

Available soil macronutrients and pH are determined using the following methods.

- Nitrogen — since both NO_3- and NH_4^+ levels are of interest, two methods are used. Nitrate levels are determined using a cadmium column to reduce water extracted nitrate to nitrite, reacting the nitrite with a red azo dye complex, and measuring absorbance of solution at 540 nm with a spectrophotometer. NH_4^+ is determined from a KCl soil extract using an NH_3 specific-ion electrode.

- Phosphorus and Potassium — Mehlich's extract (10) is used for determination of available P and K. Following extraction, concentrations are determined using the same procedures described after ashing P and K above.

- Soil pH — measured as both water pH and $CaCl_2$ pH, the latter being a closer approximation of the soil solution pH under actual field conditions. $CaCl_2$ pH is also less subject to variability from different soil:solution ratios.

FUTURE PLANS

During the upcoming quarter, work will focus on completion of plant and soil nutrient analysis from samples collected during the past field season, and planning will proceed for the upcoming season. In addition to planning for a new Typha seeding experiment on 0.4 ha (1 ac) of peat soil and several other new establishment and management option studies, decisions will be made regarding future use of field plots established in 1981.

The fertilization study, originally begun as a two-year study in 1981, was sampled for aboveground biomass only in 1982 to allow for sampling after the third season. This was done because of problems encountered during the establishment year (5). Based on second season results, a decision will be made on how this experiment should be monitored during the third growing season to obtain the most beneficial information. The excavation study, as originally designed, has been completed; it will, however, be monitored during the third season in order to better understand flowering characteristics, interspecies competition, and water management.

The nutrient uptake experiment established in 1982 will be followed during the second season to further identify and define seasonal patterns in nutrient uptake. A nitrogen treatment will be added as a factor in this experiment in the second year. The paddy experiment investigating methods of Phragmites establishment will also be monitored in 1983.

Work on rhizome harvesting and the relevant emergent technologies survey will continue through September 1983. Field conditions affecting harvester operation will be more thoroughly defined, and several conceptual designs for mechanical systems capable of performing the required steps in the harvesting process will be developed. The literature review will be expanded to include more information on emergent aquatic plant research other than Typha.

Following completion of tasks involving stand management research, relevant emergent technologies, and rhizome harvest research, several production scenarios will be developed incorporating information gained from these tasks. While these scenarios will be predicated upon early research findings, they should serve as a basis on which to formulate more refined models, define continued basic research needs, and provide a tentative basis, at least, for evaluating the economics of production.

PROBLEMS AND VARIANCES

No variances have occurred in terms of the SERI performance schedule. Two problems have, however, slowed progress on certain tasks. On several field plots, competition from various weed species, including Phalaris arundinacea (reed canary grass) and Glyceria grandis (reed meadow grass) has been troublesome. This appears to result from tillage of previously uncultivated land which produces favorable conditions for weed seed germination; it also appears to result from fluctuating water levels. The problem does not exist on excavated peat plots or plots that are continuously flooded to a depth of at least 15 cm (6 in.). During the second season, all plots were hand weeded; steps are being taken to develop practices that will reduce the problem in the future.

Another problem involves the analysis of soil nutrient concentrations for peat soils. Because of their structure, high organic matter, and water retention capacity, peat soils require different extraction ratios than those used for mineral soils. As measurements of available soil nutrients are very dependent on extraction ratios, it took some time to develop appropriate methodology for soil analysis. This situation has now been resolved, and analysis is proceeding rapidly.

LITERATURE CITED

1. Westlake, D.F. 1963. Comparisons of Plant Productivity. Biol. Rev. 38, p. 385-425.

2. Bray, J.R., D.B. Lawrence and L.C. Pearson. 1959. Primary Production in some Minnesota Terrestrial Communities for 1957. Oikos 10, p. 38-49.

3. Andrews, N.J. and D.C. Pratt. 1978. The Potential of Cattails (Typha spp.) as an Energy Source: Productivity in Managed Stands. J. Minn. Acad. Sci. 44, p. 5-8.

4. Pratt, D.C. and N.J. Andrews. 1980. Cattails (Typha spp.) as an Energy Source. In: Proceedings of "Energy from Biomass and Waste IV", Institute of Gas Technology, Chicago, Illinois. p. 43-63.

5. Pratt, D.C. et al. 1982. Emergent Aquatics: Stand Establishment, Management and Species Screening. Report to the Solar Energy Research Institute. 55 p.

6. Bonnewell, V. 1981. Typha Productivity, Mineral Nutrition, and Seed Germination. A Thesis submitted to the Graduate School of the University of Minnesota.

7. Penko, J.M. 1982. A Bibliography of the Biology, Ecology, and Utilization of Typha. A report published by the Bio-Energy Coordinating Office, University of Minnesota. 47 p.

8. Schertz, C., D.R. Dubbe and D.C. Pratt. 1983. Harvesting Cattail (Typha spp.) Rhizomes as an Alternative Feedstock for Alcohol Production: Modifications of Potato Harvester. Final report to U.S. Department of Energy, Alcohol Fuels Division. 19 p.

9. Bonnewell, V., W.L. Koukkari and D.C. Pratt. 1983. Light, Oxygen and Temperature Requirements for Typha latifolia L. Seed Germination. Can. J. Botany (in preparation for April 1983 publication).

10. Mehlich, A. 1978. Extractant for Soil Test Evaluation of Phosphorus, Potassium, Magnesium, Calcium, Sodium, Manganese, and Zinc. Comm. in Soil Sci. Pl. Anal., 9(6), p. 477-492.

PUBLICATIONS AND MEETINGS

The following reports and publications have been released during the past reporting period:

Pratt, D.C., N.J. Andrews, D.R. Dubbe, E.G. Garver, M. Penko, P.E. Read and E.S. Zimmerman. 1982. Emergent Aquatics: Stand Establishment, Management and Species Screening. Report to the Solar Energy Research Institute. 55 p.

Penko, J.M. 1982. A Bibliography of the Biology, Ecology, and Utilization of Typha. A report published by the Bio-Energy Coordinating Office, Univ. of Minnesota. 47 p.

Pratt, D.C., D.R. Dubbe and N.J. Andrews. 1982. The Development of Wetland Energy Crops in Minnesota, USA. In: Proceedings of "Energy from Biomass", International Conference on Biomass, 2nd European Communities Conference, Berlin, Federal Republic of Germany. p. 386-391.

Schertz, C., D.R. Dubbe and D.C. Pratt. 1983. Harvesting Cattail (Typha spp.) Rhizomes as an Alternative Feedstock for Alcohol Production: Modifications of Potato Harvester. Final report to U.S. DOE, Alcohol Fuels Division. 19 p.

The following meeting was attended during the past reporting period:

Energy from Biomass, International Conference on Biomass held in Berlin, Federal Republic of Germany, 20-23 September 1982. Sponsored by Commission of the European Communities. D.C. Pratt, attendee.

MICROALGAE PROGRAM ELEMENT COST GOALS

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Activities conducted under this project title are part of the general program management and evaluation responsibilities of the SERI Aquatic Species Program and specific task responsibilities associated with Task 1356.20 - Biomass Systems Cost Assessment.

OBJECTIVE

SERI is currently developing, under the direction of DOE/BET, a multiyear plan for the microalgae program element of the Aquatic Species Program. In addition to, and in support of, a detailed plan for R&D activities, designed to bring microalgae production systems to commercial feasibility, DOE/BET has requested that SERI develop cost goals for the microalgae program element. The objective of this task is to develop a concise, analytical framework that incorporates the comprehensive scientific and economic factors effecting the commercial feasibility of microalgae production systems into cost goals useful for guiding program planning, evaluation and execution. This analytical model is then to be applied to the data relevant to the microalgae program element and specific cost goals established.

ACCOMPLISHMENTS

A review of program evaluation techniques and cost goals used in other government R&D programs revealed considerable ambiguity in the basic terminology and concepts that underlie planning and evaluation. It is especially true that terms often used synonymously have entirely different conceptual bases, have different meanings to different people, and cause considerable confusion and misinterpretation when embodied in cost goals. For cost goals to be an effective tool for program planning and evaluation, they must be unambiguious, based on important, clearly defined concepts that are fundamental to planning and intelligible to those involved in all levels and areas of program activities. In order to create a logical, understandable basis for programmatic cost goals, key concepts have been identified and given precise definitions. Using these concepts as building blocks, assumptions are made and invoked to construct a methodology that facilitates setting numerical values for programmatic cost goals. The methodology then can be extended to evaluate the implications of the cost goals for R&D requirements for individual program components.

Basic Components of the Cost Goal Methodology

A comprehensive description of the methodology is currently in draft form and under review (SERI 1983). The basic elements and final methodology are briefly presented below, followed by preliminary estimates of microalgae programmatic cost goals.

Market Cost Goals

The fundamental objective of the microalgae program element is to select, manage and perform R&D activities sufficient to bring the technology to commercial feasibility. Commercial feasibility is defined as a commercial size facility producing lipid oils, in a competitive environment characterized by private capital investment and unsubsidized production, that are equal to or lower in price (on an equivalent product basis) than crude petroleum*. Thus, program planning efforts, especially the construction of cost goals, must recognize and take into account the economic and political forces that effect oil prices, the competitive standard to which the economic feasibility of lipid oil production is compared by private sector investors.

Market cost goals are defined as projections of competitive product prices that incorporate forecasted economic forces and how they effect crude oil price behaviour in the future. These forecasts set upper bounds, on a year-by-year basis, on the amortized cost for lipid oils - - microalgae systems must be able to produce lipid oils at least this cheaply to be competitive in production and attract private capital.

Market cost goals, in this case forecasts of crude oil prices, should be derived from reliable, general equilbrium forecasts of macro- and microeconomic variables, supply and demand conditions, and appropriate political and institutional assumptions.

Theoretical Cost Goals

Market cost goals represent constraints imposed on the program, in the form of the product cost that microalgae technology must achieve, by the political and economic environment. The program is further constrained in its R&D efforts by limits imposed by the physical world. The limits to which R&D efforts can increase productivity, reduce material requirements, etc. are bound by the physical and biological laws of nature and our ability to reach them. Therefore, the limit to cost reductions is also bound by these factors and this is another important factor to be considered in R&D planning.

Theoretical cost goals are defined as the minimum possible cost at which the product, lipids oils, can be produced. This value is calculated by applying the limiting physical or biological principles to the system concept to estimate the maximum achievable productivity and corresponding minimum cost of production.

State-of-the-Art Cost

To provide a meaningful perspective on the R&D challenge, the current cost of production, using available, proven process, techniques, materials, system designs, etc., is estimated to establish a reference point, or starting point, for program planning and evaluation.

Program Planning Horizon

In order to bound the multiyear plan and subsequent annual plans, a specific planning

*That is, if lipid oils are inferior to petroleum, on a physical basis, then per unit costs need not be equivalent as the market would improse a discount to represent the cost associated with upgrading the lipid oil to petroleum standards.

horizon is established. This gives a time dimension to the plan necessary to establish the required sequence of events, R&D activities and funding essential to moving the technology from the state-of-the-art to cost competitiveness, or better.

Program Milestones

An integral part of planning is transforming objectives into discernable, time dated measures of program performance and factoring in any fixed, anticipatable delays or time periods required between the occurance of significant events. As part of establishing the program planning horizon, an expected completion date is chosen, the date anticipated for delivery of a commercial feasible system to the private sector. This date, designated time t(SF) (system feasibility), marks the major milestone which will guide planning and evaluation. It establishes the interval over which R&D activities will be conducted.

A second milestone can be derived by recognizing that a technology typically moves through several stages of R&D development. The first stages involve basic R&D and laboratory experimentation. As parametric analyses isolate the nature of the yield response function (to changes in the level of factors governing output) data on specific components are developed. The next stage involves convoluting these components into an integrated, dedicated concept and ascertaining the feasibility of the resulting production. Once component technical feasibility (CTF) is verified and documented, the technology can be tested in a user's environment to further document performance data, reliability and overall economic feasibility.

Program activities between the point where the technology achieves component technical feasibility and when system feasibility is achieved generally involve the construction and operation of pilot or demonstration units. Further, experience should make it possible to anticipate the time required to perform these final demonstration effects and therefore it is possible to estimate the expected time between the point of component technical feasibility (t(CTF)) and system feasibility (t(SF)). Since t(SF) has already been established, it is also possible to give a specific date to another important milestone-component technical feasibility; the date that proceeds systems feasibility by the time estimated for demonstration and pilot activites.

The logic applied above gives two more constraints to the planning problem that, combined with the other concepts and the constraints they imply, can be used to clarify the planning environment and to set specific programmatic cost goals.

Preliminary Microalgae Program Element Cost Goals

The basic components described above comprise a unified, verifiable method for constructing programmatic cost goals. The methodology has been applied to data applicable to the microalgae program element to construct preliminary estimates of programmatic cost goals. The data used in the analyses are presented in Table 1. The data come from a variety of reports and analyses on the production of lipid oils from microalgae (see Literature Cited).

The principles established above to construct cost goals are more easily conceptualized graphically, as shown in figure 1. The milestones establish the appropriate time frame (horizontal axis) and product cost ($/bbl.) is measured (in constant 1982 dollars) on the vertical axis. The program element cost goal is found by constructing a verticle line from time t(SF), the year 1993, and reaching the intersection of this line with the market

Table 1. Data Used for Preliminary Microalgae Program Element Cost Goals

A. Program Milestones

Current Year, t(o) = 1983
Milestone for component technical feasibility, t(CTF) = 1991
Milestone for system feasibility, t(SF) = 1993

B. Market Cost Goals: Crude, Well-head Oil Price (1982 $/bbl.) Forecasts

	EIA [8] Forecast Scenario		
Year	Low-Price	Mid-Price	High-Price
1982 (Historical)	33	33	33
1985	34	43	49
1990	45	63	77
1995	63	86	112

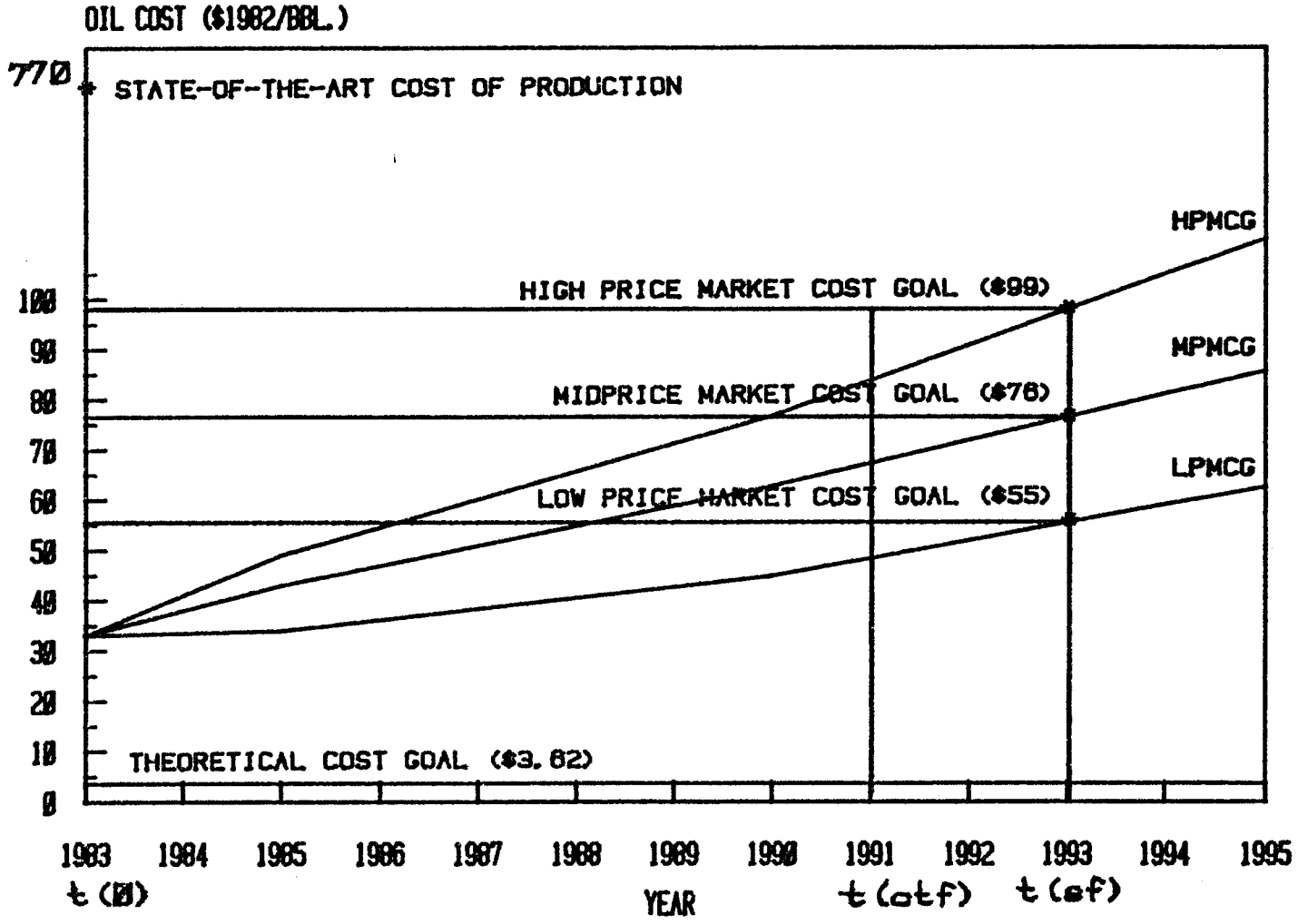
C. Parameter Assumptions for State-of-the-Art and Theoretical Cost Goals

	Value	
Parameter	State-of-the-Art	Theoretical Best
PAR	42%[a]	42%[a]
PSE (on PAR)	6%[b]	24%[c]
Oil Content	15%[d]	70%[e]
Harvest Efficiency	50%[f]	100%
Oil Yield	32bbl/ha (13bbl/a)	1638 bbl/ha (663bbl/a)
Annualized Cost	$24710/ha ($10,000/a)[g]	$5931/ha ($2400/a)[g]
Oil Cost	$769/bbl	$3.62/bbl

a Bassham [1], Richmond [7]
b Benemann [2], Raymond [6], JAYCOR [4]
c Bassham [1], Richmond [7], JAYCOR [4]
d Benemann [2], JAYCOR [4]
e Lien [5], Richmond [7]
f Estimated
g Benemann [2], Hill [3]

FIGURE 1. MICROALGAE PROGRAM COST GOALS

PRELIMINARY ESTIMATES



cost goal curve (MCG). Three MCG curves are drawn to correspond to the three price senarios used in Economic Regulatory Agency (EIA) crude oil prices. For programmatic purposes, it is suggested that the midprice market cost goal (MPMCG) be used since it represents "most likely" assumptions about the course of future economic and political events and corresponding effects on world and domestic oil prices. The result of this assumption is that the preliminary programmatic cost goal is $76/bbl. That is, the program will strive, through R&D efforts, to bring the cost of lipid oils from microalgae down from its current level of $770/bbl to $76/bbl by 1991. Based on current knowledge and economic projections, doing so would trigger commercial system feasibility by 1993 and result in achievement of the overall program element goal.

FUTURE PLANS

Activities to date have been directed toward refinement of the methodology and development of an overall program element cost goal for microalgae. Plans for the next period include extending the methodology to be able to derive performance goals for specific program components.

Peformance Goals

Performance goals are defined as goals, stated in the appropriate physical units, that assign to all system components a rate, yield, etc. that, if achieved individually, collectively would give a product cost equal to that of the program element cost goal. The basic idea is to look at potential improvements, from the state-of-the-art level to the theoretical best level, for individual components and then search through combinations and permutations of these levels to identify collective improvements consistent with the program element cost goal.

Examples of how this approach might be used are displayed in tables 2 and 3. The data in these tables are preliminary as refinements are requred in the modelling of the biological processes and in the calculation of capital and operating costs. It is anticipated that complete development of the methodology and a verified model of microalgae production processes and standard economic analyses will provide a powerful tool for planning and evaluating program element activities, both at the program level and for specific tasks and component R&D initiatives.

PROBLEMS AND VARIANCES

The methodology described above brings together concepts and data from diverse disciplines. The successful development and application of the methodology depends critically on review by and participation of all those involved in the program, including those involved in performing the R&D activities. The most urgent need is to involve those knowledgable in the basic biological sciences to review the data that are used to develop the state-of-the-art and technical cost goals. As work on the methodology progresses, the research community will be called upon to evaluate the performance goals developed to ascertain which represent achieveable and desirable R&D routes from the current sate of development to commercial feasibility.

ACKNOWLEDGEMENTS

The author gratefully acknowledges Andy Hill and Theresa Flaim for their contributions to this task while retaining sole responsibility for any errors in the text.

Table 2. A Comparison of Quartile Improvements in the Biological Engineering Parameters with No Improvements in the Capital and Production Cost Parameters

	Parameter	Unit	State-of-the-Art	% Improvement of Biological Parameters with No Improvement in Cost			Theoretical Best
				25%	50%	75%	
A.	Daily Solar Constant	mmj/m^2-d	21	21	21	21	21
B.	Photosynthetic Active Radiation	fraction	.42	.42	.42	.42	.42
C.	Photosynthetic Efficiency	fraction	.06	.105	.15	.195	.24
D.	Capacity Factor	d/y	300	315	330	345	365
E.	Algae Energy Content	mj/g	22.5	22.5	22.5	22.5	22.5
F.	Algae Biomass Yield	t/a-y	32	58	87	118	153
G.	Algae Lipid Content	fraction	.15	.28	.43	.56	.70
H.	Gross Lipid Yield	bbl/a-y	29	100	230	405	663
I.	Harvesting Efficiency	fraction	.50	.62	.75	.88	1.0
J.	Processing Efficiency	fraction	.90	.93	.95	.98	1.0
K.	Net Lipid Oil Yield	bb./a-y	13	58	164	351	663
L.	Capital and Production Cost	$/a	10000	10000	10000	10000	10000
M.	Lipid Oil Cost	$/bbl	769	172	61	28	15

Notes:

1. A, B, C, D, E, G, I, J and L are parameters.
2. F = A x B x C x D ÷ E x .0045 (constant converts $gm^{-2}d^{-1}$ to $ta^{-1}y^{-1}$).
3. H = F x G x 6.18 (constant converts $ta^{-1}y^{-1}$ to bbl. $a^{-1}y^{-1}$).
4. K = H x I x J.
5. M = L ÷ K
6. Percentage improvements in the parameters are calculated over the range defined by the (absolute) difference between the state-of-the-art and theoretical best parameter values.

Table 3. A Comparison of Quartile Improvements in Capital and Production Costs with No Improvements in Biological and Engineering Parameters

	Parameter	Unit	State-of-the-Art	% Improvement in Capital and Production Cost with No Improvement in Biology or Engineering			Theoretical Best
				25%	50%	75%	
A.	Daily Solar Constant	mmj/m^2-d	21	21	21	21	21
B.	Photosynthetic Active Radiation	fraction	.42	.42	.42	.42	.42
C.	Photosynthetic Efficiency	fraction	.06	.06	.06	.06	.06
D.	Capacity Factor	d/y	300	300	300	300	300
E.	Algae Energy Content	mj/g	22.5	22.5	22.5	22.5	22.5
F.	Algae Biomass Yield	t/a-y	32	32	32	32	32
G.	Algae Lipid Content	fraction	.15	.15	.15	.15	.15
H.	Gross Lipid Yield	bbl/a-y	29	29	29	29	29
I.	Harvesting Efficiency	fraction	.50	.50	.50	.50	50
J.	Processing Efficiency	fraction	.90	.90	.90	.90	90
K.	Net Lipid Oil Yield	bb./a-y	13	13	13	13	13
L.	Capital and Production Cost	$/a	10000	8100	6200	4300	2400
M.	Lipid Oil Cost	$/bbl	769	623	477	331	185

Notes:

1. A, B, C, D, E, G, I, J and L are parameters.
2. F = A x B x C x D + E x .0045 (constant converts $gm^{-2}d^{-1}$ to $ta^{-1}y^{-1}$).
3. H = F x G x 6.18 (constant converts $ta^{-1}y^{-1}$ to bbl. $a^{-1}y^{-1}$).
4. K = H x I x J.
5. M = L + K
6. Percentage improvements in the parameters are calculated over the range defined by the (absolute) difference between the state-of-the-art and theoretical best parameter values.

LITERATURE CITED

1. Bassham, J.A. 1980. "Energy Crops (Energy Farming)" in ed. San Pietro, Biochemical and Photosythetic Aspects of Energy Production. Academic Press, New York, Ch. 6, pp. 147-173.

2. Benemann, J.R. et al. 1982. "Final Technical Report: Microalgae As a Source of Liquid Fuels". EnBio Inc., Fairfield, CA., pp. 143-159

3. Hill, A.M. 1982. "Cost Budgeting for Microalgae Systems" in Proceedings of the SERI Biomass Program Principal Investigators' Review Meeting - Aquatic Species Program Reports. SERI/CP-231-1808. SERI. Golden, CO., pp. 17-30.

4. JAYCOR. 1983. State of Microalgae Energy Technology. JAYCOR. Alexandria, Va.

5. Lien, S. 1982. "Studies on the Production and Accumulation of Oil and Lipids By Microalgae" in Proceedings of the SERI Biomass Program Principal Investigators' Review Meeting - Aquatic Species Program Reports. SERI/CP-231-1808. SERI. Golden, CO., pp. 45-54.

6. Raymond, L.P. 1982. "Aquatic Biomass as a Source of Fuels and Chemicals" in ed. Yuan, S.W. Energy resources and Environment: Proceedings of the First U.S.-China Conference. Pergamon Press. New York., pp. 75-82.

7. Richmond, A.R. 1983. "Phototrophic Microalgae" in eds. Rehm, H.J. and Reed, G. Biotechnology. vol. 3, Verlag Chemie, Weinheim, Ch. 1d, pp. 109-123.

8. U.S. Department of Energy, Energy Information Agency. 1982. 1981 Annual Report to the President: vol. 3 Energy Projections. U.S. D.O.E./EIA-0173 (81)/3. Washington, D.C.

PUBLICATIONS AND MEETINGS

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CULTIVATION OF MACROSCOPIC MARINE ALGAE

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INTRODUCTION

Growth of the red seaweed Gracilaria tikvahiae in small, intensively-operated culture systems with strong aeration and over 20 culture-volume exchanges per day of enriched seawater resulted in biomass yields throughout the year that averaged 34.8 g dry wt/m^2.day (equivalent to 127 dry metric tons/hectare.year, about half of which is organic), Lapointe and Ryther[1]. Yields were found to be directly proportional to seawater exchange rate, between one and 30 culture volumes/day. Maximum yields occur at relatively low nutrient concentrations, 10-100 μmoles N/liter as NO_3^- or NH_4^+ and 1-10 μmoles/liter $PO_4^=$-P together with essential trace metals, and a starting seaweed density of 2-4 kg wet weight/m^2 culture surface area, harvested back to that density every one to 2 weeks.

Such high yields of Gracilaria are impressive, comparable to the best yields from the most productive terrestrial crops, e.g. Cooper[2]. However the highly intensive culture methods, requiring large volumes of pumped enriched seawater and air, are probably not economically viable, no matter how high the yields, e.g. Hugenin[3]. Research efforts have therefore since been directed towards attempts to grow Gracilaria using less energy-intensive culture methods but still with comparably high yields.

Effects of Aeration

Gracilaria is now routinely maintained in suspended culture by aeration along the long axis of the culture tank bottom. What purpose this serves is not known, but apparently does not derive from the air itself, since the same growth enhancement is obtained from seaweed kept in suspension by the action of a paddle wheel Neish et al.[4]. It may expose a larger density of seaweed to sunlight than would be possible in an unmixed culture, or it may increase the exposure of the plants to CO_2 and/or other nutrients. Whatever the function, aeration is a major cost and energy input that should be reduced to a minimum level consistent with high yield. Preliminary experiments showed that intermittent aeration, for as little as 6 hours per day, under 2 different periodicities, resulted

in the same yields of Gracilaria as does continuous aeration, but that yields decreased in cultures aerated for only 5 minutes per hour for a total of 2 hours per day. The effect of very brief aeration periods (ca one minute) with increasing intervals between aeration are now being investigated.

Nutrient Uptake and Storage

One of the major economic costs of large-scale seaweed biomass systems would be the supply and deployment of essential nutrients to the individual plants and the retention of the enriched water within the area of cultivation long enough for the nutrients to be assimilated by the seaweeds. The problem is further exacerbated by the need to rapidly exchange the seawater in the culture system, needed to achieve high yields of the seaweeds, as seen above. However, nutrient-deficient seaweed is capable of rapidly assimilating and storing inorganic nutrients which may then be drawn upon for normal growth for periods of days to weeks in the virtual absence of an external nutrient supply. Chapman and Craigie[5] have described how this system may operate in nature, and D'Elia and DeBoer[6] have demonstrated, in a controlled experiment, the extremely high affinity for ammonia of nitrogen-starved Gracilaria, far exceeding anything that could be associated with uptake kinetics for growth.

The phenomenon of rapid nutrient uptake and storage by starved Gracilaria and its relationship to subsequent growth of the seaweed was studied further Ryther et al.[7]

G. tikvahiae was grown for 2-4 weeks in running, unenriched seawater (one volume exchange of water per day) until the algae were a pale yellow color and growth had ceased. Five kilograms (wet weight) were then placed in tanks of seawater enriched with NH_4Cl and $Na_3PO_4 12H_2O$ to provide 2,000 μmoles N and 150 μmoles P/liter. The water was gently aerated to provide motion of the water through the seaweed. Water samples were then withdrawn at intervals and analyzed for ammonium-nitrogen. In one experiment, $KaNO_3$ was used in place of NH_4Cl.

In one control experiment, no seaweed was added to check any loss of NH_3 to the atmosphere during the course of the enrichment. In another control, recently-enriched, dark reddish-brown Gracilaria was used to measure uptake rates in non-nitrogen-deficient seaweed.

Samples of the seaweed were taken at the beginning and end of the enrichment period, usually 24 hours, and in one case midway through the experiment. These were oven dried at 80°C for 24 hours and analyzed for total C and N with a Perkin-Elmer Model 240 elemental analyzer.

No measurable amount of ammonia was lost to the atmosphere from the tank without seaweed. Thus any loss from the tanks containing the algae could be assumed to have been due to assimilation by the plants.

There was also no significant removal of nitrogen from the tank containing the well-nourished Gracilaria and held under the same conditions. However, the ammonia-nitrogen in the tank stocked with N-starved seaweed

decreased rapidly, from 16.8 to 3.0 mg N/liter in just eight hours, to nearly unmeasurable levels in 24 hours. That loss represents a total uptake of some 8.4 gN by the 5 kg wet wt (0.55 kg dry wt) of seaweed in 24 hours, a nitrogen-increase of 1.5% of toal dry weight or a 2.5 fold increase over the starting concentration of 1.0% of dry weight. The total measured increase in the plant tissues, sampled at 7, 11, and 24 hours agreed well with the increase calculated from the loss from the water, confirming the fact that the loss from the water was indeed due to assimilation by the plants.

The experiment in which nitrogen was supplied at the same concentration as $NaNO_3$ showed a much slower rate of uptake by the Gracilaria. In that case, only 37% of the available nitrate-nitrogen was taken up, a total of 2.7 g by the 5 kg wet wt or an increase of only 0.4% of the dry weight (e.g., from 1.0 to 1.4 of total dry weight).

In another series of experiments, the long-term effects of rapid nutrient uptake and storage on growth of the seaweed was examined. 2.5 kg (wet wt) portions of yellow, nitrogen-deficient Gracilaria were placed in 2500 liter aluminum tanks in seawater enriched with NH_4Cl or $NaNO_3$ (1000 µmoles N/liter), $Na_3PO_4.12H_2O$ (100 µmoles P/liter) and a mixture of trace metals and chelated iron. The tank was gently aerated to provide movement of the enriched seawater through the seaweed. The seaweed was held in the tank for various periods of time, from one to 48 hours, after which it was washed in clean seawater and placed in 700 liter concrete tanks, where it was grown in running, non-enriched seawater (one exchange per day), maintained in suspension by vigorous aeration. The experiment was run for 8 weeks during which the seaweed was taken from the tanks every 2 weeks, drained of excess water, weighed and harvested back to its starting weight (2.5 kg wet wt). The latter was then put into the enrichment tank again for the same length of time at each biweekly interval. A control was grown in the same manner but received no enrichment.

The results of this experiment showed that soaking Gracilaria in nutrient enriched seawater for as little as 6 hours every 2 weeks is sufficient to enable the plants to grow at the maximum possible rate under the conditions of the experiment. Increasing the exposure to the nutrients beyond the 6 hours, to a maximum of 48 hours, had no additional stimulatory effect. Reducing the exposure to one hour resulted in roughly half the yield of seaweed and the control showed no growth.

A companion experiment, in which nitrate rather than ammonium was the nitrogen source, showed a similar but less striking effect. Yields of Gracilaria soaked in the nitrate-enriched medium for durations from one to 24 hours were consistently lower than those exposed to ammonium treatment for the same period of time.

The results of the preceeding experiments led to the development of a new strategy for enriching cultivated seaweeds in which the smaller, unattached species like Gracilaria, Chondrus and other commercially valuable red algae may be removed from their culture system and soaked in a concentrated nutrient solution for only a few hours, during which they can more than double their nitrogen content. They may then

be returned to the culture system where they will grow with no additional nutrients added to the water until they double their biomass, thereby halving their nitrogen content again. Harvesting the new growth may then be accompanied by another session of nutrient-soaking of the standing stock to be returned again to the culture unit.

Not only is such a system highly efficient in nutrient utilization, with no loss of unused nutrients, but it also solves simutaneously one of the chronic problems in seaweed culture, the growth of undesirable epiphytes on the cultured seaweeds. With no nutrients added to the seawater in which the algae are grown, epiphytes do not have an opportunity to become established and the externally-fed target species has the competitive advantage.

Seawater Retention, pH and Carbon Dioxide

The one factor that has been found to date the most important in affecting the growth and yield of Gracilaria is seawater exchange rate (retention time). The reason is not obvious, but does not appear to be related to the mineral nutrient supply, since the effect is observed when nutrients are added at a constant rate separately from the seawater supply, or when nutrients are premixed and added at a constant concentration in the seawater supply, or when nutrients are supplied by soaking the seaweed periodically for a few hours in a static, enriched seawater reservoir, as discussed above.

Since pumping water is a (probably the) major cost factor in the culture system that is described here, clearly it would be desirable to achieve the high yields possible at very rapid exchange rates with much less water flow. First, however, it is necessary to understand the relationship between yield and water exchange.

The only essential nutrient not provided in the artificial enrichment normally used to culture Gracilaria and other seaweeds is carbon dioxide. Seawater of normal salinity (30-35 ppt.) contains about 2 m moles/liter of total CO_2 which exists in the equilibrium:

$$CO_2(\text{diss.}) \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- \rightleftharpoons CO_3^{=} \qquad (1)$$

Seawater one-meter deep thus contains 24 g C/m^2 which could theoretically support the growth of 48 g ash-free dry weight (50% carbon) or about 74 g total dry weight of Gracilaria, a potential yield of 74 g dry wt/m^2.day with a retention time of one day. However, removal of free CO_2 during the photosynthetic growth of algae increases the pH; the slower the circulation of seawater through the seaweed the higher the pH rises. At pH >9.0, there is almost no free CO_2 in seawater Harvey[8] and both its rate of dehydration from bicarbonate to maintain the equilibrium and its actual concentration are so low that they become rate limiting to photosynthesis and growth.

Some seaweeds are able to utilize bicarbonate directly in photosynthesis.

Measurement of photosynthesis (ΔO_2 by oxygen probe in a closed, recirculation seawater chamber) at 4 pH levels maintained with TRIS buffer indicates, however, that Gracilaria can use little or no bicarbonate, with greatly reduced photosynthesis at pH 9.0 compared to that at pH 7.5. Essentially the same results were reported by Blinks[9], who found Gracilaria the least able to photosynthesize at high pH of 22 species of seaweeds tested.

However, short-term photosynthesis measurements may not necessarily be directly translated in terms of growth, so additional growth studies were carried out to investigate the phenomenon further.

Gracilaria was grown in 700 liter (1.6 m^2) concrete tanks in aerated culture at a density of 3.0 kg wet weight/m^2. Once a week the complete culture was removed, weighed and incremental growth removed. The starting density was then soaked for 24 hours 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 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 no acid or pH control. One control received no additions. All 5 tanks were stagnant, with no exchange of seawater. A second control, with no additions, received 8 exchanges of seawater per day.

Yields were monitored weekly for 2 weeks. 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 8 exchanges of seawater per day, the only time that such high yields have been observed in stagnant seawater. Addition of acid alone, controlling pH at 8.0, apparently drove all the natural carbon in the seawater to CO_2 and bubbled it off to the air. No growth enhancement was observed. Addition of bicarbonate at uncontrolled, high pH provided more carbon in an unusable form, though growth was slightly enhanced in that culture, presumably by increasing the small amount of free CO_2 available under the new equilibrium.

The conclusion from these experiments is that the growth of Gracilaria, in culture or in nature, can indeed become carbon limited and that sustained high yields of the seaweed require replenishment of CO_2 by seawater exchange or by direct addition.

Recycling of digester residue as a nutrient source for seaweed production

While separate pulse feeding of seaweeds, as described earlier, has obvious economic advantages over continuous enrichment of the seawater flowing through the cultures, an enrichment medium made from commercial fertilizers or bulk inorganic chemicals still represents a major cost to the production system. However, where seaweeds are digested anaerobically to produce methane or when their polysaccharides are extracted, all of the essential nutrients remain in the residue and represent a potential enrichment medium that could be supplied to the production system at little or no cost.

An experiment was conducted in which the growth of Gracilaria in standard inorganic nutrient medium is compared with that in the residue from its anaerobic digestion as respective enrichment media.

The seaweed was grown in suspended culture in nine 700 liter concrete tanks. Three cultures were enriched each week, by the pulse-feeding method described above, using an inorganic nutrient solution. Three other cultures were similarly enriched with the liquid residue from an anaerobic digester that was fed weekly the incremental growth of those cultures. Three control cultures receive no enrichment.

Twenty-liter carboys were used as digesters. These were initially inoculated with 11.25 kg of seaweed, 11.25 liters seawater and one liter of liquid residue from another seaweed digester. They were run for 3 weeks in a batch mode with almost daily NaOH titration in an effort to stabilize pH to near 7.0. When the latter was accomplished, the digesters were fed weekly, a corresponding amount of liquid residue being withdrawn for the seaweed enrichment.

Growth of the cultures enriched in digester residue was essentially the same as that of the inorganic medium enriched seaweed, both being significantly greater than that of the unenriched controls.

The results of that experiment show that anaerobic digestion, for just one week in ambient temperature, unmixed digesters, is sufficient to break down the algae and mineralize their nutrient contents to the extent necessary for their reassimilation by the plants. At the time of this writing, new experiments were in progress to characterize chemically digester residues with respect to species and concentrations of respective nutrients as functions of digester residence time as well as species and nutritional condition of the seaweeds.

Growth of the Green Alga, Ulva sp.

In contrast to Gracilaria, the green seaweed Ulva sp. is apparently able to utilize bicarbonate readily and can maintain photosynthesis and growth at high pH. In short-term photosynthesis experiments, Ulva was found to produce oxygen at pH 9.1 at 71% the rate at pH 7.5 (vs. 19% for Gracilaria. In longer term growth studies in stagnant water, with no exchange of seawater, Ulva grew as well in cultures enriched with bicarbonate and with no pH control as in cultures with carbon added as CO_2 with pH held at 8.0 with a pH-stat. Growth of Gracilaria with bicarbonate-enrichment was only very slightly enhanced in stagnant cultures beyond that of controls with no carbon enrichment.

For the above reason, Ulva may be a better candidate species for biomass production in semi-stagnant situations with limited water exchange, an environment that may be characteristic of any large-scale, high density seaweed farm of the future. Similarly, in an artificial, land-based culture system (i.e. ponds or raceways) Ulva could be preferable to species like Gracilaria in requiring less pumping of seawater, a highly cost and energy intensive input. However, the advantage of a bicarbonate-using or high pH-tolerant alga must be kept in perspective. Stagnant conditions will very quickly result in carbon limitation, however, it

may be used by the plant, and it must be resupplied to maintain yields either with seawater exchange or by direct addition.

Ulva has other advantages over Gracilaria. A delicate algae only two cell layers thick, it is much more quickly and completely attacked and digested by methanogenic bacteria, and its mineral nutrient contents are much more readily recycled and made available for new growth. Further, in contrast to most plants, old nutrient deficient Ulva produces more methane more rapidly than do healthy, rapidly-growing plants, apparently because the carbonhydrate storage products of nitrogen-deficient, non-growing Ulva are, to a large extent, starch, soluble sugars and other easily attacked compounds in contrast to the highly resistent cellulosic storage products of higher plants and the complex polysaccharides (e.g. agar, carrageenin) characteristic of many other groups of algae.

Until recently, a persistent problem in the culture of Ulva has been its procilvity to become reproductive and shed a major fraction of its biomass as microscopic spores or gametes, as often as every week or two. This has now been circomvented by the acquisition of what appears to be a sterile population, but the latter unfortunately derives from New England and cannot survive Florida summers. A search is now underway for a sterile, tropical or semi-tropical clone of Ulva capable of year-round vegetative growth in Florida.

Conceptual Model of a Gracilaria Farm

Gracilaria may be grown in channels or raceways on land or in shallow coastal waters in tropical to semi-tropical latitudes. At an offshore site, the seaweeds would presumably be confined by a fence or other barrier. Within the enclosure, it is maintained at a density of approximately two kilograms wet weight per square meter, at which it is compacted well enough that normal wind and tidal action will not move it and cause it to drift and accumulate unevenly. At brief intervals during the day, it is mixed and rotated by compressed CO_2 through pipes distributed throughout the culture systems.

Well nourished Gracilaria exposed to full sunlight at such latitudes may be expected to double its biomass in one to 4 weeks depending upon season, water flow and other variables discussed earlier. After its biomass has doubled (i.e., from 2 to 4 kg/m^2) the incremental growth is harvested to return the crop to a starting density that will insure continued optimal yield.

If the Gracilaria were well nourished, as stipulated, at the beginning of each growth period, the doubling of biomass would be accompanied by the utilization of all stored nutrients and a reduction of elemental nutrients in the plant tissues to roughly half the initial concentration.

Enrichment of the new starting crop following harvest (i.e., 2 kg wet wt/m^2) could conceivably be accomplished on site in the seaweed farm, but the rapid uptake and storage of nutrients by depleted seaweeds, as illustrated earlier in this report, makes possible a simpler, more efficient enrichment process. This would involve harvest of the

entire crop, half of which is permanently removed for utilization while the remaining half is exposed to a concentrated nutrient solution for the 6-hour period that is needed for the plants to double their nutrient content.

The relatively small Gracilaria plants, which seldom exceed a diameter of 20-30 cm per individual clump and which readily break up into small, individual plants with any agitation, would be readily harvested by pumping to a central processing, utilization and enrichment plant located as close as possible to the farm site. A circular farm with the processing plant at its central hub would appear to be most efficient. Such a design would permit the farm to be subdivided into pie-shaped segments that could be routinely harvested at intervals of one to several days (depending upon the time required to carry out the complete operation), returning the enriched half of each harvest to the appropriate farm segment.

If the main objective of the culture is to produce biomass for conversion to methane, the remaining half of each harvest would be anaerobically digested. Alternatively, if the primary objective is chemical products, these would first be extracted and the organic residue then anaerobically digested. The residue from the digesters, with whatever additional processing as may be required to mineralize and/or otherwise make available the nutrients for assimilation and storage, would then serve as the enrichment medium for that portion of the harvest to be pumped back to the farm as the starting culture for the subsequent growth cycle.

The biogas generated from the anaerobic digestion of the seaweed would be utilized on site as process energy for chemical extraction and processing of the algal products or any other appropriate local industry. The waste gas from the anaerobic digestion and biogas combustion, primarily CO_2 would then be available for distribution to the seaweed farm, the yield of which would otherwise be CO_2 limited. The CO_2 may be mixed with air and the mixture used to provide both CO_2 and the necessary aeration/mixing of the culture.

In the preceeding conceptual design, all materials are perpetually recycled. Nutrients, including CO_2, needed as make-up for inefficiency in the recycling processes, can be provided by pumped seawater, for a land-based system, or by the normal tidal and non-tidal flow of seawater, through and ocean-based farm.

Preliminary Studies on the Lipid Content of Gracilaria

Preliminary results concerning the lipid content of Gracilaria tikvahiae indicate that this species displays lipid concentrations typically found in macroalgae (1-2%). A nutrient enriched Gracilaria sample contained 2.1% lipids by weight, while a nitrogen-starved sample contained 1.0% lipids. This result is atypical when compared to microalgae, which normally accumulate lipid under nutrient-limited conditions. However, this paradox may arise from the tendency of Gracilaria to synthesize proportionately more agar under nutrient stress.

Separation and elution of the lipid fractions from silicic acid columns with solvents ranging in polarity from hexane to methanol suggest that the majority of the lipids are associated with the acetone fraction. This is particularly evident for nutrient-starved Gracilaria. The greatest disparity between starved and enriched Gracilaria occurs in the chloroform fraction, which is well represented in enriched plants, but considerably reduced in content in starved thalli. Acyclic hydrocarbons, found in the hexane fraction, appear to represent a minor fraction of the Gracilaria lipid content. Youngblood et al.[10] analyzed the hydrocarbons in six genera of red algae, (not including Gracilaria), and in all cases except Porphyra, n-heptadecane strongly predominated. Thus, just on speculation, n-heptadecanes may also dominate the hydrocarbon component of Gracilaria. Four distinct components were resolved by TLC from the benzene fraction. One component co-chromatographed with the standards used. However, there is a possibility that one or more of these components could represent cyclic or unsaturated hydrocarbons. In nutrient enriched Gracilaria, the chloroform fraction contained components that co-chromatographed with triclyceride, 1, 2 diglyceride and fatty acid standards. Components co-chromatographing with monoglyceride standards were detected in the acetone fraction for both enriched and starved Gracilaria. Several sterols, including chloresterol, were also detected. Alam et al.[11] indicated that glycolipids represent the largest percentage of the lipid fraction in Fucas. Given the large number of components in the acetone fraction of Gracilaria, glycolipids may also be well represented in this red alga. In addition, some phosphate positive lipids were detected, along with at least one ninhydrin-postive component.

References

1. Lapointe, B. E. and J. H. Ryther. Some aspects of the growth and yield of Gracilaria tikvahiae in culture. Aquaculture 15, (1978), 185-193.

2. Cooper, J. P. Photosynthesis and productivity in different environments. The University Press, Cambridge. 1975. 378 pp.

3. Huguenin, J. E. An examination of problems and potentials for future large-scale intensive seaweed sulture systems. Aquaculture 9, (1976), 313-342.

4. Neish, A. C. et al. The cultivation of Chondrus crispus: Factors affecting growth under greenhouse conditions. Canadian Journal Botany 55, (1977), 2263-2271.

5. Chapman, A. R. O and J. S. Craigie. Seasonal growth by Laminaria longicruris: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. Marine Biology 40, (1977), 197-205.

6. D'Elia, C. F. and J. A. DeBoer. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. Journal Phycology 14, (1978), 266-272.

7. Ryther, J. H. et al. Nitrogen uptake and storage by the red algae Gracilaria tikvahiae. Aquaculture 26, (1979) 107-115.

8. Harvey, H. W. The chemistry and fertility of seawaters. The University Press, Cambridge, 1960. 240 pp.

9. Blinks, L. R. The effect of pH upon the photosynthesis of littoral marine algae. Phycologia 57, (1963) 126-136.

10. Youngblood, W. W. et al. Saturated and unsaturated hydrocarbons in marine benthic algae. Mar. Bio. 8, (1971) 190-201.

11. Alam, M., A. Chakravarti, and M. Ikawa. Lipid composition of the brown alga Fucus vesiculosus. J. Phycol. 7 (1971) 267-268.

THE SERI MICROALGAE RESEARCH GROUP

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MICROALGAE TECHNOLOGY RESEARCH AT SERI

SERI has been charged with establishing a national leadership role in the development of technologies for making fuels and oils from microalgae. This mission is led by the SERI Solar Fuels and Chemicals Division. The position of this Division within the organizational structure of SERI is shown in Figure 1, as are the relationships of the major research and operational components within the Division. The responsibility for program management of the Aquatics Program lies within SERI Biomass Program Office (BPO). Research and development is conducted by the SERI Photoconversion Branch, and the SERI Microalgal Technology Research Group (MTG), as well as by subcontractors.

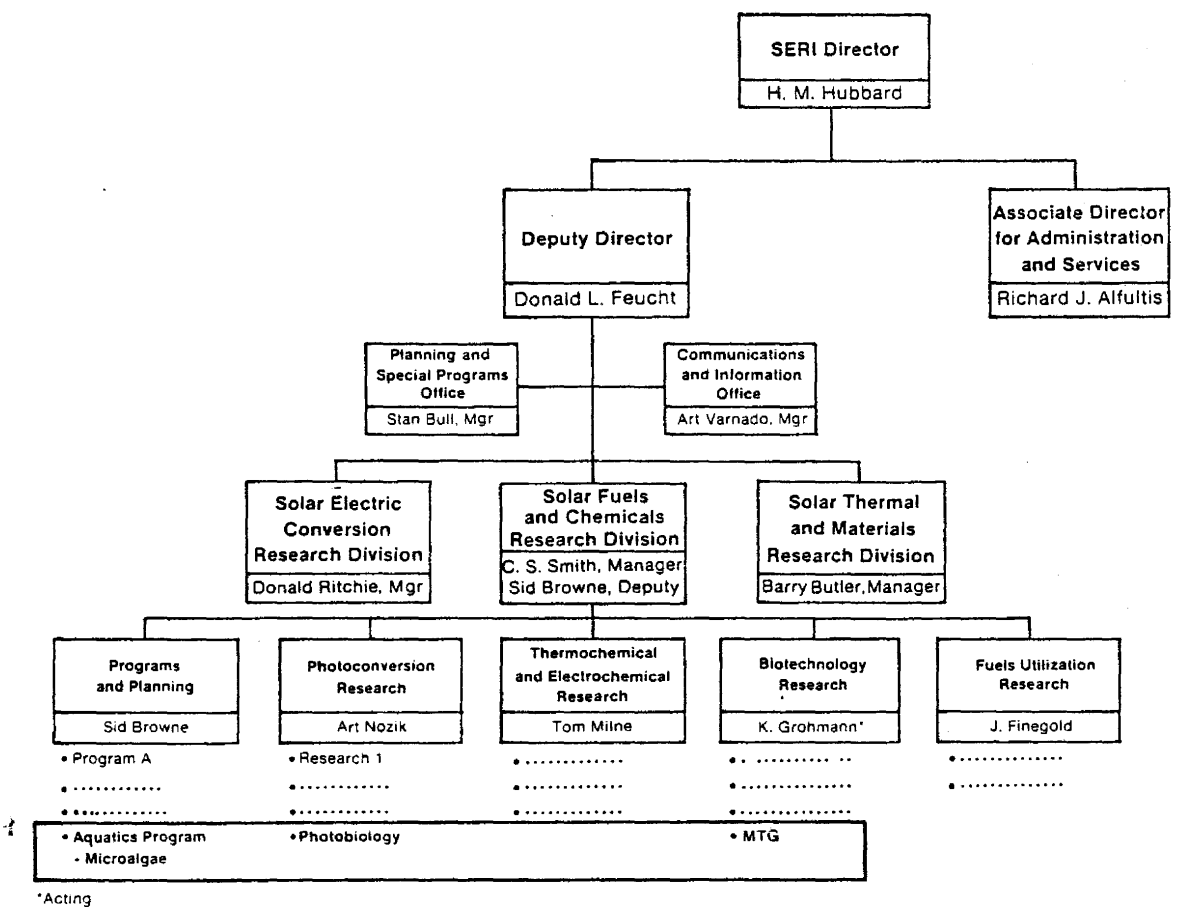

Figure I. SERI Organizational Structure

The SERI Photoconversion Branch conducts basic research appropriate to advancing scientific understanding of processes involved in the conversion of light into chemical energy. Included in this research is the development of photobiological principles acting on 1) primary photoexcitations induced by photoabsorption; 2) the transfer of excitation energy within and between photosystems; 3) the establishment and maintenance of a cross-membrane energy potential sufficient to develop high energy compounds as well as the reducing power required to drive anabolic reactions; 4) elucidation of the water splitting reactions leading to charge separation, proton transport, and oxygen evolution; and 5) the process manifestations brought about by specific environmental conditions. This research leads to establishing the basic principles upon which innovation of practical concepts rests, but it is not oriented towards preconceived and specific applications, other than photon conversion processes in general.

The SERI/MTG is part of the Biotechnology Research Branch and funded through the Aquatic Species Program; it functions to provide program support and to conduct research as appropriate to the program mission. It serves as both a technical resource and an active research center.

As a resource, the MTG serves to centralize technical data, literature, and species for use throughout the program and for ultimate distribution to the private sector. It is responsible for 1) providing advice to the SERI/BPO on microalgal R&D needs and directions, 2) developing and exchanging insights, ideas, findings, and innovations for use by scientists within the program, and 3) guiding training for students and professionals interested in learning the technology.

As an active research center, the MTG conducts investigations appropriate to the development of high-yield, capital intensive systems for producing mesophylic halophiles in arid highland habitats. Principal research includes aspects of 1) species collection and characterization, 2) species development, 3) process development, and 4) documentation of technology for transfer. Species collection and characterization involves locating species of diatoms and chrysophytes in the field, isolating and purifying them into culture, and identifying fundamental physiological tolerance and capability limits. Species development involves developing and applying the methodology necessary to realize maximum productivity of species that possess highly desirable chemical compositions, as well as assuring that species have the tolerances appropriate to sustain high yields under varying environmental conditions. Process development entails the integration of biological and engineering principals that lead to the reliable manufacture of high quality fuels and oils from these microalgal species. Technology documentation for transfer involves all that is appropriate for assembling and maintaining the records and species necessary for microalgal technologies to be ready for transfer to the private sector.

Figure 2 places the research work of the SERI/MTG in perspective with other researchers within the program. The MTG is committed to investigations of high yield systems. Others are looking at moderate yield systems, adapting existing systems, or focusing on a specific example of high yield microalgal production. The MTG is coordinating the development of and centralizing a comprehensive literature and data file, contributing specifically that literature pertaining to photosynthesis, algal physiology, and high-yield systems engineering. Other

laboratories are contributing considerations specific to other engineering approaches, aspects of species chemistry, species physiology and tolerances, and species characteristics. The MTG is collecting species of diatoms and chrysophytes from Colorado, Utah, and New Mexico that are suitable for production in highland habitats. Other laboratories are collecting from the oceans and deserts, from estuaries and streams, from hot springs and transient pools. They are collecting other classes of microalgae, and at the same time, are broadening the full resource base.

	Literature Review	Preliminary Performance Criteria	Species Collection and Culture	Species Screening	Species Selection	Species Biochemistry	Production Biology	Quantify Operational Species Improvement	Engineering Limits	Engineering Criteria	Outdoor Production R&D	Results Verification Testing	Centralize Cultures and Data
SERI/MTG	Photosynthesis, Physiology, High Efficiency Systems	•	Highland Colorado, Utah, New Mexico	Halophylic and Mesophylic	Diatoms, Chrysophytes	•	•	•	•	•	•	•	•
En Bio	Moderate Raceways	-	-	Fresh Water	Greens	-	•	-	•	•	•	•	-
University of Hawaii	Shallow Raceways	-	Marine	Marine	Diatoms	-	-	-	•	•	•	•	-
Israel	Moderate Raceways	•	Desert	Halophylic and Thermophylic	Any	-	•	-	•	•	•	•	-
Georgia Tech	Species Chemistry	•	Georgia, Florida, Texas	Estuarine, Halophylic	Any	•	•	•	-	-	-	-	-
University of California at San Diego	Deserts and Desert Species	•	Desert, California, Arizona, Nevada	Marine, Desert Lowlands	Any	-	-	-	-	-	-	-	-
New Contractors	•	•	•	•	•	•	•	•	•	•	•	•	-

Figure 2. Interrelationships Between Program Contributors to Further the Development of Microalgae-Oil Technologies. SERI's Microalgal Technology Research Group is actively investigating options in each research category, leading the technological side of systems development as part of the DOE/SERI Aquatic Species Program. The Group's activities are distinguished by their focus on maximizing photosynthetic and physiological adaptation to high yield systems, producing diatoms and chrysophytes in systems engineered for arid highlands operations.

SERI/MTG RESEARCH

Any microalgal production system consists of growth or cultivation "ponds"; harvesting; and processing of algae to products. Key research issues related to microalgae cultivation include:

- o selection of appropriate specie(s)
- o contaminant control
- o temperature control
- o nutrient supply
- o CO_2 supply
- o balance of yield and capital investment.

Species Selection

Species selection depends upon overall production system requirements and varietal characteristics. SERI MTG will develop an appropriate type collection, characterize these strains and evaluate their suitability within system constraints.

Contaminant Control

Contaminants can destroy microalgal populations overnight or render feedstock unsuitable for processing. This has been identified as the number one research issue by several investigators in the field based on observed effects of predators, pathogens, and other toxic agents. Biological and engineering methods will be developed to place this risk at an acceptable level.

This will be addressed through the study and development of natural and engineered approaches. Natural approaches include selecting highly tolerant, adaptive, and productive species that have unique features and/or growth requirements, such as specific nutrient preferences, ability to generate extracellular antimetabolites effective against a broad spectrum of potential competitors, and tolerance to specific ions present at high concentration. Engineered approaches include deploying cellular disruptors, such as sonic transducers, that might be effective on contaminants but not the preferred species, selecting the light wave lengths that enhance production of desirable microalgae while inhibiting contaminants, and production stream manifolds that separate microalgae from contaminants on the basis of size, density, or electroattraction.

Temperature Control

Covered systems are likely to be needed for mega-scale microalgal production for several reasons, including 1) evaporation control, 2) protection against solid debris accumulation, and 3) control of salinity levels. The disadvantage is that evaporative cooling is eliminated as an effective means of temperature control in hot desert climates. However, in the cooler climates of highland regions, this heating may prove advantageous. The cost of temperature control in either instance is almost certain to place some limits on the types of algae and products that can be produced practically in either of these regions. Thus cost-effective, energy efficient methodologies will be sought for maintaining desired production temperature regimes.

SERI/MTG approaches to this challenge will include studies of convective cooling, use of heat pumps, and use of highly humid enclosures. Computerized networks will be developed to monitor and control temperature over a specified but rather broad range. This requires locating and culturing species with broad temperature tolerances, or using different species at different times of the year.

Nutrient Supply

The quantities of nitrogen, phosphorous, potassium, and other nutrients required from microalgal production are enormous when viewed on a mega-scale. Microalgae typically contain 6% nitrogen, 0.6% phosphorous, and about 1.0% potassium. Thus, the production of one ton of microalgae would be expected to

require 0.07 tons NH_4, and 0.04 tons of KH_2PO_4. The yield objective for high yield systems is on the order of 60 tons $acre^{-1}$ $year^{-1}$, and about 1×10^6 acres is within the expected range for the land area requirement for one quad production. Annual requirements calculate as 4.2×10^6 tons NH_4, and 2.4×10^6 tons KH_2PO_4. An enormous amount of commercial nitrogen fertilizer would be required to fulfill this demand. Without nutrient conservation through recycling, and the development of technologies for fixing nitrogen from the atmosphere or concentrated deposits, impacts upon supply, availability, and transportation systems for these resources would be enormous. Consideration will be given early to developing the techniques necessary to supply the nutrients required for future production efforts.

This will be accomplished by developing methods for conserving and recycling nutrient resources, concentrating on providing products composed entirely of carbon, hydrogen, and, perhaps, oxygen. Theoretically, all other elements could be placed back into the system. Some will be lost however. Analyses will be made to assess and mitigate the impacts of such losses.

Carbon Dioxide Supply

Carbon is one of the most abundant elements on earth, yet it is bound up in a delicate cycle that influences global climate. Depending on which sources are consulted, increasing atmospheric carbon dioxide content will result in either global heating or global cooling, leading to boiling tropical sun, or to a return to the ice age; either way the effect is less than desirable.

One reason given in support of biomass technologies is their inherent capacity to fix atmospheric carbon into organics which, upon combustion, return and balance carbon supply. Yet carbon has been demonstrated limiting to biomass productivity, a fact which demands carbon be supplied in some concentrated or enriched fashion in order to maximize productivity.

Carbon dioxide is a major industrial waste product, readily and inexpensively procured for today's uses. As a result, there is virtually no incentive to developing technologies for concentrating CO_2 from the atmosphere. Such methods exist, but have not been applied, except on very small scales. Potential supplies of the CO_2 high-yield microalgal technologies will be evaluated.

Yield vs. Capital Investment

This question is the basis of present day research on microalgae technologies. Current systems can sustain productivities on the order of 20-25 tons per acre year without considerable operating expense. However, harvesting, dewatering, and processing costs are so high that unit price is forced beyond reach, except for speciality purposes or products.

One alternative is to decrease costs by accepting the higher initial investments required to improve yield with respect to cost, add to the capital equipment and materials to decrease operating cost, and improve material lifetimes and stability to amortize investment over a longer period. The objective is to realize a disproportionate increase in product yield with respect to associated increases in cost, thereby driving unit prices downward. This work will be undertaken to provide the framework for making microalgal technologies practical in large markets.

Research will focus initially at increasing the yield of microalgal species able to tolerate temperature ranges typical of highland regions and thrive in saline groundwaters. This will be done through analyses of species requirements, engineering design requirements, and development of appropriate environmental controls. Single variable laboratory-scale experiments will be conducted to identify candidate species that perform well and produce the products desired. Engineering designs, covering a wide variety of options, shall be examined under outdoor conditions. Trade-offs involved in implementing concepts, as well as optimizing species performance will be conducted utilizing a multi-factorial design. A number of one m^2 outdoor units will be designed to permit maximum flexibility in design testing. This will permit the simultaneous examination of up to four species. The outputs of these studies will include data specific to material and operational energy costs, especially as they relate to achieving the required environmental controls. These data will not be taken as absolute with respect to yield, growth rates, or photosynthetic efficiency because of edge and other scale effects. However, the relative performance measures should be representative and provide the information necessary to design larger-scale systems that will be more costly to construct, operate, and maintain.

Research objectives are shown in Table I.

SERI/MTG ORGANIZATION AND MANAGEMENT

Figure 3 shows the organizational structure of the Microalgae Technology Research Group as well as its position within the Solar Fuels and Chemicals Research Division of SERI. The MTG is one of two research groups comprising the Biotechnology Branch; the other group is the Fermentation Research Group which focuses on improving the fermentative capacity of microbial species, principally to produce alcohol, methane, organic acids, and chemical intermediates. The capabilities and interests vested in the reseachers within the Branch and throughout the Division are highly complementary, permitting in-depth technical exchanges and collaboration which enhances the quality of their R&D results.

The manager of the MTG plans, implements, directs, and performs the R&D of microalgae-based technologies, integrating scientific and engineering disciplines to produce high-value fuels and chemicals. This development-oriented research includes 1) application of algal physiology and biochemistry to chemicals and fuels production, 2) collection, selection, characterization, and manipulation of microalgal species for this production, 3) definition and implementation of culture and systems requirements, and 4) integration of algal physiology, environmental requirements, and engineering specifications that define the technical options for potentially profitable applications in this area.

Table 1. Research Objectives of the MTG for the Period FY 1983 - FY 1987 Inclusive.

1983	1984	1985	1986	1987
o Formulate R&D plan for MTG o Initiate species development effort o Construct 1 m^2 engineering R&D design test units o Conduct R&D on harvesting technology o Examine alternate methods for fabricating systems coverings	o Identify and characterize 10 species for development o Identify trigger for lipid synthesis other than nitrogen limitation o Conduct multifactorial studies of growth response surfaces o Investigate biological methods for species development o Expand and revise engineering criteria o Conduct R&D on pretreatment technology o Examine new materials for coverings	o Define growth tolerance for microalgal species o Establish fundamental design criteria for production system o Develop physical methods for controlling contaminents o Identify suitable low-cost resin mixtures for possible in-field cover fabrication o Examine techniques for fabricating cover materials o Establish a listing of preliminary criteria for verifying process system test data	o Complete analyses of media formulations for collected species o Identify target oil/fuel products for microalgal technologies o Conduct process design trade-off studies o Develop capacity for utilizing autoflotation as a preharvest concentrator o Conduct cost trade-off analyses between factory and field fabrication of covering materials o Finalize design criteria for first process verification studies	o Complete characterization of collected halophylic microalgal species o Decide whether species characteristics are compatable with program goals and objectives o Identify options for control of production synthesis o Develop highly productive strain containing >50% w/w oil o Determine and select promising options for production system operations o Conduct R&D to separate cellular components into process flow streams o Complete laboratory testing of candidate materials o Initiate outdoor material lifetime studies o Examine structural support requirements for cover materials o Establish design specifications for verifying first process test data

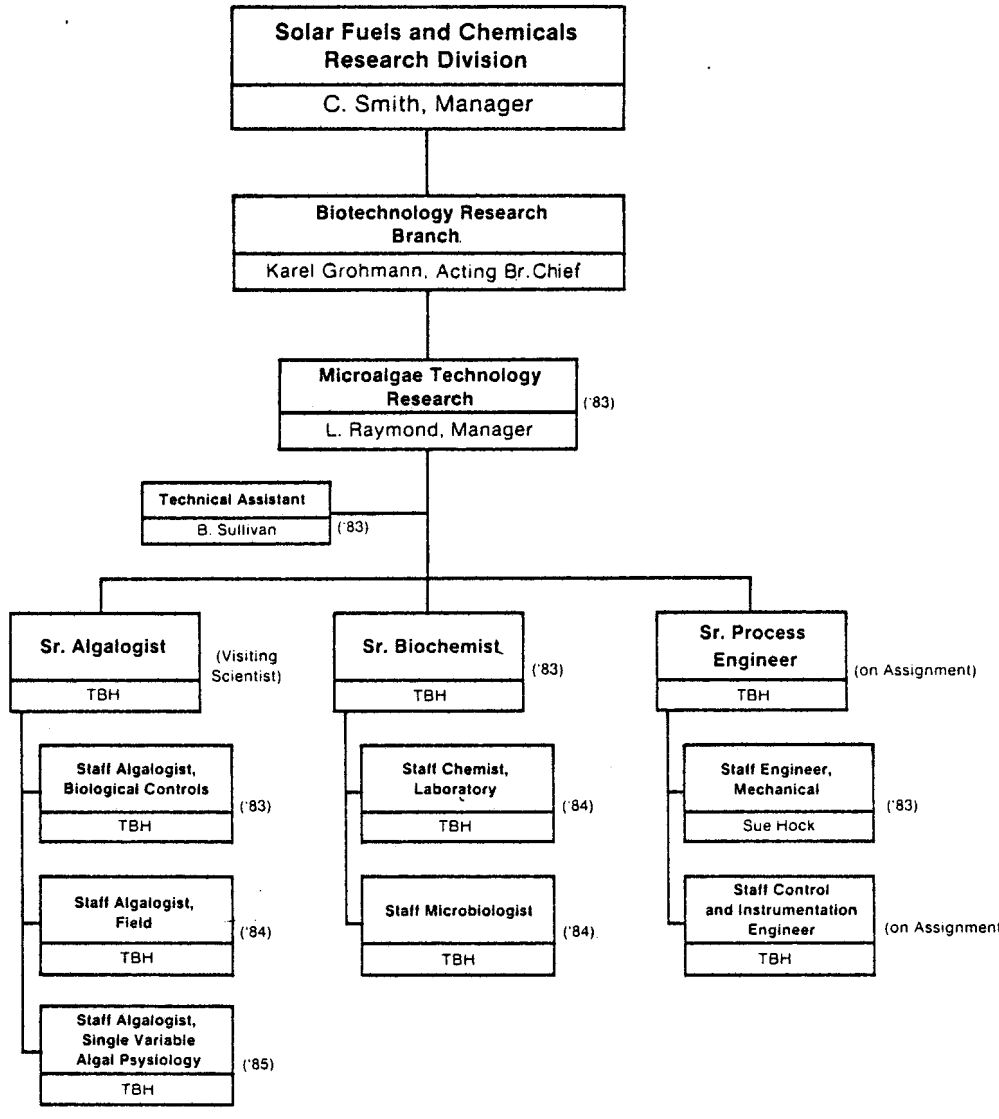

Figure 3. Organizational Structure for the SERI Microalgae Technology Research Group

SELECTION OF HIGH-YIELDING MICROALGAE FROM DESERT SALINE ENVIRONMENTS

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1.0 ABSTRACT

The purposes of this investigation are to isolate microalgae from desert saline water sources in eastern California and western Nevada; to analyze these waters for their major and minor constituents; to carry out preliminary selection experiments on growth in artificial media and temperature, salinity and light requirements; and to carry out experiments to maximize yield. Various microalgal strains have been isolated from waters brought back from field trips. Some 25 strains are unialgal and another 25 have some algal contaminants. Most isolates are diatoms, green algae, and blue-green algae. These waters have been analyzed commercially. Most waters have a moderate salinity of < 10,000 mg liter^{-1}, are high in carbonates and bicarbonates, and have a high pH (>9.0). Trace metals are low and these waters contain appreciable amounts of boron and fluoride. Preliminary media formulation experiments show that dense cultures can be obtained with nitrate as an N source, but, for one species, urea was a better source. No growth occurred when ammonium was supplied. Lipid contents of centrifuged cells were about 20-30% of the dry weight; protein ranged from 60-75%, and carbohydrate was about 16-24% of the dry weight. Calculated rough yields ranged up to 23 gm m^{-1} day^{-1} with efficiences of light utilization of up to 14%. In the future the work will proceed sequentially through the several purposes of the work (see above). Various problems have been encountered - these are mainly associated with limited space and personnel.

2.0 OBJECTIVES

The American desert has abundant resources of land, sunlight, and saline water. The latter is not usable for conventional agriculture, but could be used to grow algae in outdoor installations (ponds). Various microalgal species occur in these saline waters.

2.1 Institute of Marine Resources-Phytoplankton Resources Group Objectives.

We are finding species of algae that grow well in saline desert waters and selecting those that will produce biomass at high yields. Our specific goals are as follows:

2.1.1 Preparation of a Bibliography on Desert Algae.

Since there had been a considerable amount of previous work on algae from the arid American Southwest, we gathered this literature for a SERI Technical Report, annotated it, and put it on microcomputer disk storage for easy retrieval by keywords. With knowledge of the previous work, we would be in a better position to carry out field trips for isolation of desert saline species of microalgae (see 2.1.2 and 3.2).

2.1.2 Field Trips to the Desert Areas of Eastern California and Western Nevada.

Our second goal was to carry out field trips to saline water sources in the desert areas of California and Nevada. More specifically we intended to sample these sources to isolate algae from them, and to bring back water for chemical analysis and for maintaining the algae until they could be transferred to artificial media. Originally we planned to do this work with a mobile field laboratory - a trailer or mobile home which we could drive to each sampling site. This proved to be too expensive to build and it would have been especially difficult to provide adequate electrical power for it at remote locations. Therefore we have used established field laboratories in the desert and these have proved to be much more adequate than a mobile laboratory.

2.1.2.1 Isolation of Algae. We initially planned to isolate the algae in the field by micropipetting of individual cells and agar plating from initial water samples and enriched cultures. We also planned to select for algae having high growth rates by continuous cultures set up in the field. We have used these techniques in the field and we have also brought water back to La Jolla and have completed isolations here.

2.1.2.2 Field Experimentation for Temperature and Salinity Requirements. Initially we planned to set up a temperature gradient block in the field, but we were unable to obtain enough algal material for such studies and the equipment is much too bulky to transport, so we have been doing such experimentation in La Jolla.

2.1.2.3 Chemical Analyses of Saline Waters. We originally planned to perform many of the analyses in the field and have detailed analyses done by a commercial laboratory. Some chemistry was done in the field, but commercial analyses have proved to be reliable and we are presently having most analyses done commercially.

2.1.3 Preliminary Laboratory Selection Experiments.

Prior to choosing promising candidate species for yield maximizing in our chemostat apparatus, we have planned preliminary experiments on media formulation, and on temperature, salinity and light requirements.

2.1.3.1 Media Formulation Experiments. In these experiments we initially planned to enrich natural water samples and see how well the algae could be "pushed" to high densities and yields. However, we have not been able to bring back enough "natural" water for these experiments and have found that artificial media formulated from the chemical data on each sample suffices. The use of artificial media is a selection process for high yielding species in itself.

2.1.3.2 Chemical Analyses of Cells. At the end of each medium formulation experiment, the cultures are harvested for analyses of their chemical constituents, particularly lipids. The samples are sent to Drs. Tom Tornabene and Ami Ben-Amotz at Georgia Tech for detailed analyses. However we are doing some analyses ourselves, on micro amounts of material, for total lipid, carbohydrate, protein, and ash. These chemical analyses are a further selection process for cells having a high energy and/or protein content.

2.1.3.3 Temperature, Salinity, and Light Requirement Experiments. We plan to carry out experiments on temperature and salinity in our home laboratory using a temperature gradient block. Growth in each of the 30 50-ml bottle cultures in the block will be monitored by measuring optical density in each bottle without taking samples. The bottle itself is the optical density cuvette.

For light intensity experiments we plan to use a compartmented plastic container illuminated with a 2000-watt tungsten-halide stage lamp. The highest intensity will be about 70% of La Jolla summer sunlight. Such experiments are just beginning.

2.1.4 Experiments to Maximize Yields.

We plan to grow dense cultures of a very few species that have been selected from preliminary experiments. Their yields will be maximized by varying temperature, light intensity, culture density, and culture thickness in factorial experiments in which interactions of two of the above four factors will be studied at a time. These experiments will be carried out in four chemostats operated simultaneously. Growth will be monitored frequently by optical density sensors connected to a microcomputer. The computer will also monitor temperature and control pH.

We hypothesize that the main factor affecting yield is the mean light intensity seen by individual cells. While this cannot be measured directly at the scale of the individual cell, mean light intensity can be varied by varying incident light intensity, culture density, and culture thickness.

2.1.5 Sequence of This Work.

Originally we planned to carry out many of these experiments simultaneously, but it has proved more desirable to proceed sequentially as follows; 2.1.1, Bibliography Preparation; 2.1.2, Field Work; 2.1.3, Preliminary Selection Experiments; and 2.1.4, Maximizing Yields.

2.2 Significance of These Studies.

The above studies should enable us to select microalgae from saline desert water habitats that are potential candidate species for growing in outdoor ponds in the desert at high yields. In addition laboratory experiments on maximizing yields will be directly applicable to outdoor cultures in that parameters such as light intensity can be varied by screening, by varying culture thickness, or culture density so that the mean light intensity seen by individual cells is optimal.

3.0 ACCOMPLISHMENTS AND RESULTS

This section describes what the Scripps-IMR Phytoplankton Resources Group has accomplished since the last SERI Subcontractors Review Meeting. The subsections that follow are organized within the context of the specific goals presented in Section 2.0. Methods that we have used are described within each subsection.

3.1 Desert Algae Bibliography.

This bibliography was prepared by computer and library searching of the literature and by correspondence and personal contact. Each paper was retrieved from the UCSD libraries, other University libraries, and by interlibrary loans. Some 300 references were collected, read, and abstracted. Each reference was xeroxed and the title, author, journal, date, etc. was entered onto computer disk storage along with the annotation and keywords. Keywords include "author, state, water type, habitat, algal taxa, etc." The bibliography was printed out via microcomputer connection to a printer, and the bibliography is searchable by keywords. Both author and keyword indices have been added. The bibliography has been extensively edited and a final copy has recently been sent to SERI for publication as a Technical Report.

Most papers just list the 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 and on soil algae. In Nevada, Pyramid, Walker, Big Soda Lakes and some springs have been investigated and there is a 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.

3.2 Desert Field Trips.

During September-October, 1982 and January-February, 1983 we carried out three field trips to the desert areas of eastern California and western Nevada to isolate desert algae and to bring back water for chemical analyses and

maintenance of the isolated species. These trips went to established field laboratories from which we went out into the adjacent desert for water sampling. Generally, only saline waters were sampled. The general locations of the areas and sites that we sampled are shown in Figure 3-1.

3.2.1 Field Trip Number 1 to the UC Sierra Nevada Aquatic Research Laboratory (SNARL) - September-October, 1982.

For the first field trip, we based at SNARL (for location see Figure 3-1). We took a lighted incubator, microscopes, specific ion electrodes, conductivity and pH meters and various other laboratory glassware and chemicals. We set up the equipment in one of the SNARL labs, and brought water back from various adjacent desert saline sources for processing. From SNARL we sampled two local ponds (A and B - Big Alkali Lake), Black Lake, Pyramid Lake, Walker Lake, a saline pond along a dry lake in the Saline Valley, Owens Lake, and Columbus Salt Marsh. We did not sample Mono Lake since Dr. David Chapman of UCLA had already given us cultures from that site. The locations of our sampling sites are shown in Figure 3-1 and in more detail in Figure 3-2. Field sampling of nearly saturated brine in the Saline Valley is shown in Figure 3-3.

After bringing the water back to SNARL, it was filtered, enriched with nutrients, and inoculated with raw water containing the algae. These cultures were then incubated for several days and examined periodically for growth. Isolation of single cells were done by micropipetting them into enriched media. Raw water was also plated on agar plates, but this was not successful in starting cultures. We also held raw water in dim room light and at room temperature for up to a week and started cultures from it. The algae seemed to persist and we showed that we could start cultures from water that was held.

Chemical analyses for Na^+, Ca^{++}, and Mg^{++} were performed with specific ion electrodes as time permitted. We also titrated a few samples for total CO_2 measurements. However, the chemical studies were of low priority, and we delivered samples to the E.S. Babcock and Sons commercial laboratory in Riverside, California for more extensive analyses of major and trace ions.

After three weeks at SNARL, we had successful cultures growing so we brought them back to La Jolla for maintenance and experimentation.

3.2.1.1 Field Data - Field Trip Number 1. Field data are shown in Table 3-1. Note that water temperatures varied widely and these were dependent on air temperatures; that is, Ponds A and B were sampled during a snowstorm and had low water temperatures and Owens Lake and Saline Valley samples were taken after a prolonged period of sunny weather, at lower altitudes, and had higher water temperatures.

The Saline Valley Salt Lake water was a concentrated brine (see Figure 3-3) and differed from all the other waters by having a lower pH and very much greater electrical conductivity and ionic concentrations. Generally ionic values and pH measurements done commercially agreed well with those obtained

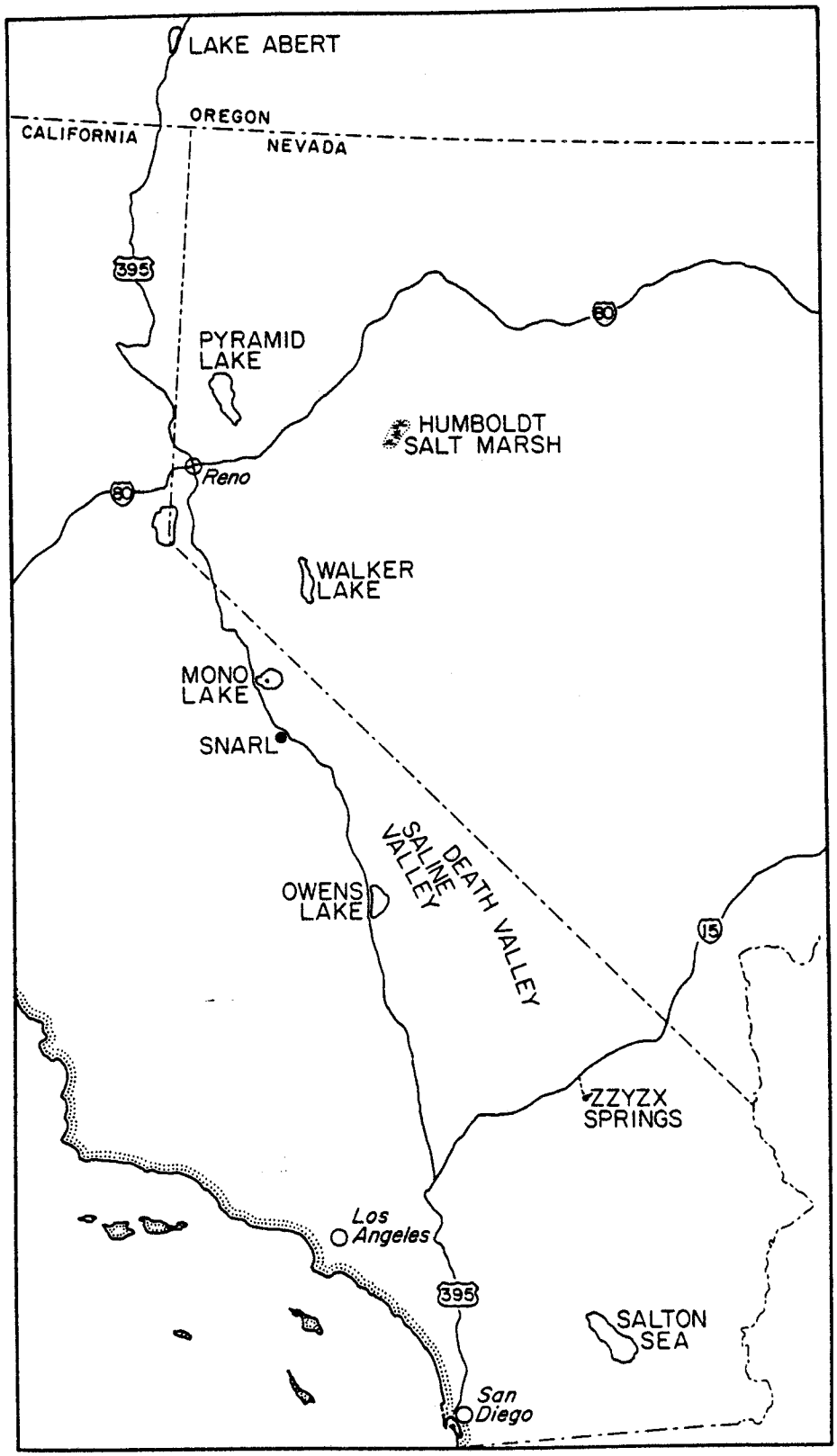

Figure 3-1. General locations of areas sampled in the desert of eastern California and western Nevada.

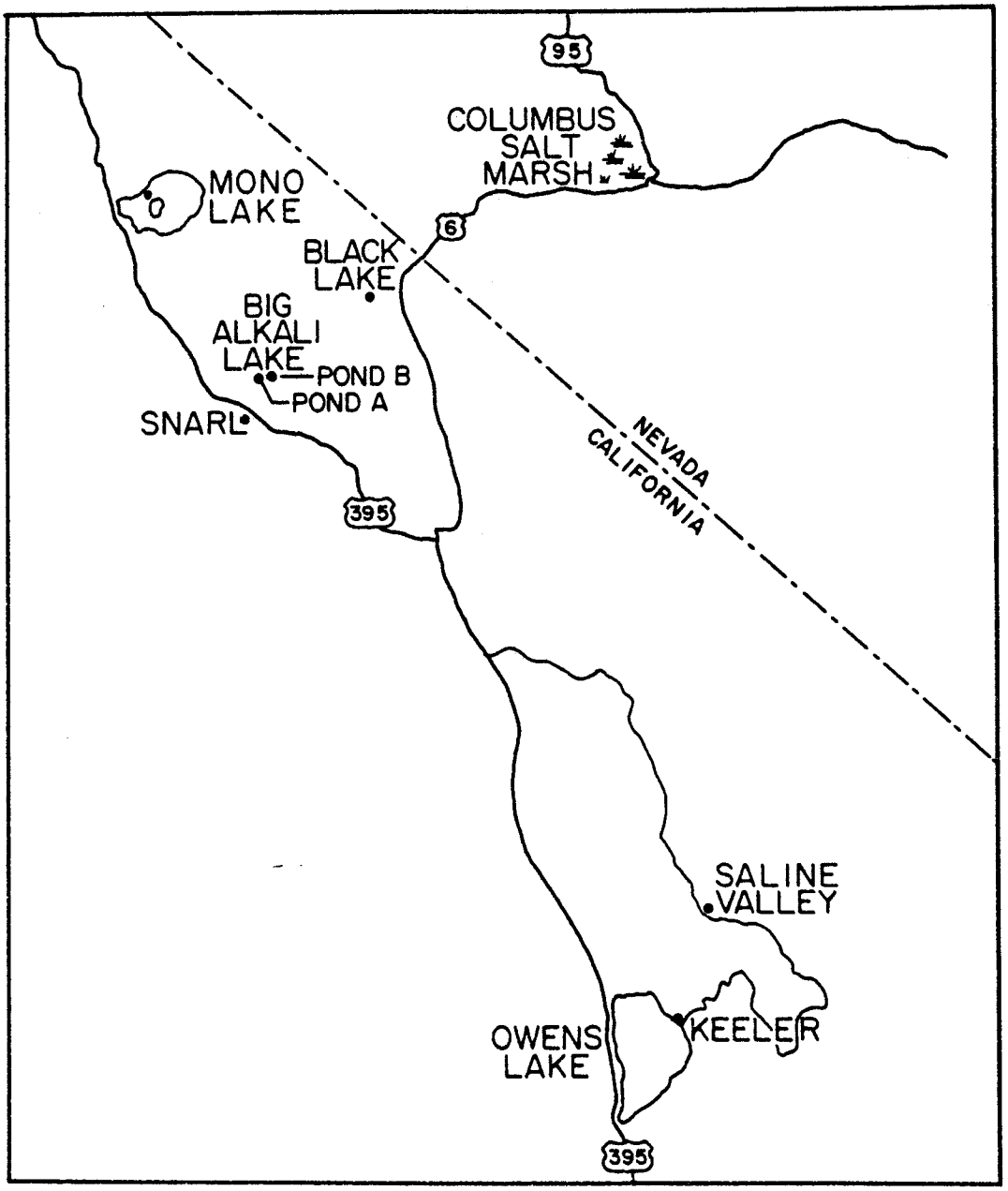

Figure 3-2. Specific locations where samples were taken — Field Trip Number 1.

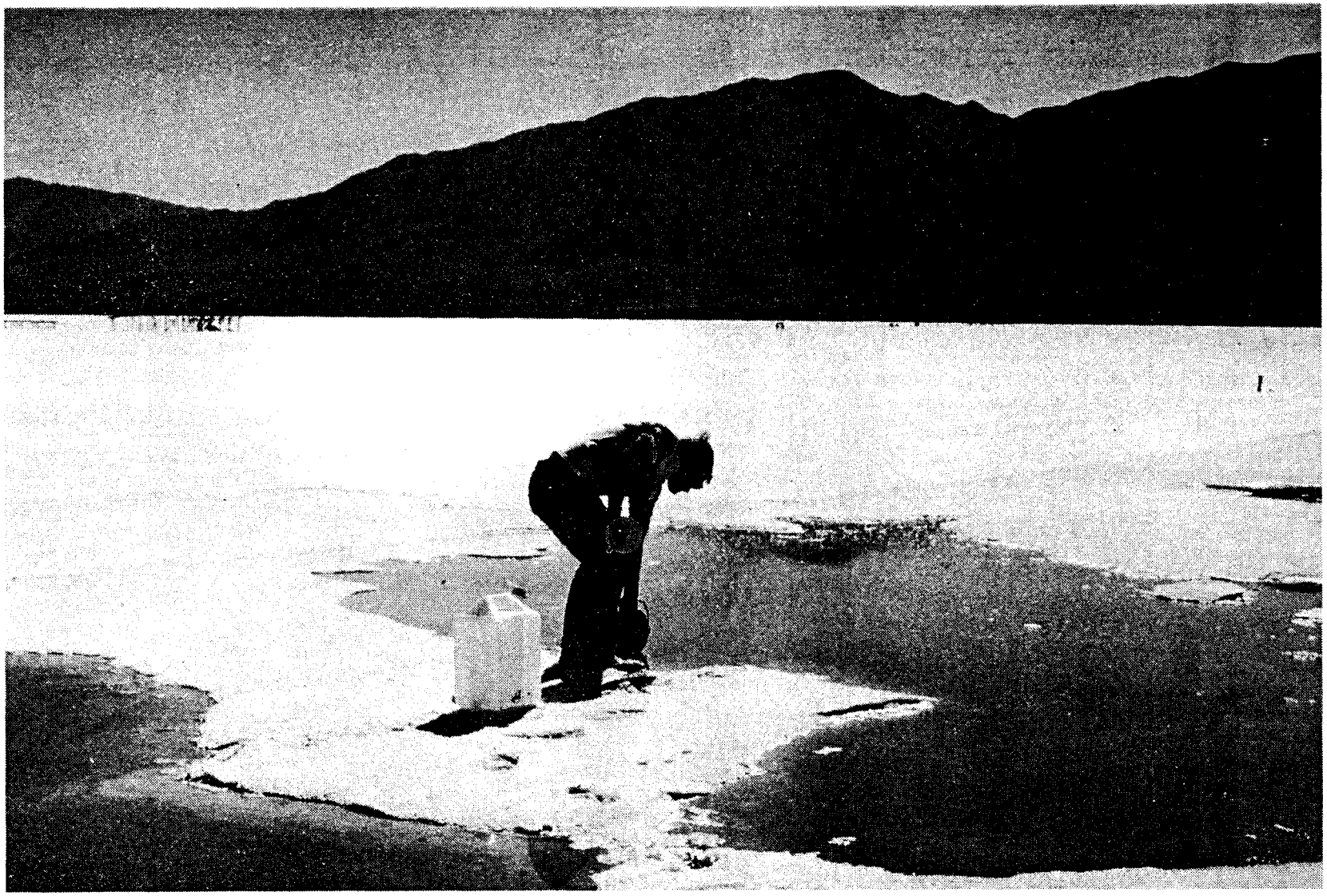

Figure 3-3. Sampling the Salt Lake in the Saline Valley.

TABLE 3-1. Physical and Chemical Parameters of Waters Sampled During Field Trip Number 1.

Location	Temperature °C	pH	Electrical* Conductivity	Na (mg ℓ^{-1})	Ca (mg ℓ^{-1})
Pond A	9.6	9.2 (9.6)	1.54 (1.875)	420 (450)	4.2 (5)
Pond B	2.0	9.1 (9.5)	4.8 (4.71)	1040 (1150)	4.4 (6)
Black Lake	13.2	9.7 (9.8)	32.0 (30.00)	9400 (8800)	1.8 (4)
Pyramid Lake	17.4	8.9 (9.1)	6.4 (8.03)	1540 (1775)	7.5 (10)
Walker Lake	18.0	9.1 (9.3)	15.4 (15.00)	3300 (3900)	6.1 (10)
Owen Lake	27.5	9.8 (9.8)	27.0 (15.60)	---- (4700)	--- (8)
Saline Valley	25.4	7.5 (7.5)	249.0 (209)	---- (100×10^3)	--- (220)

*Electrical Conductivity measured in mmhs cm^{-1}.

Note: Values in parentheses are those from later commercial analyses.

at SNARL.

3.2.1.2 Species Isolated - Field Trip Number 1. Twenty-two unialgal strains have been isolated and maintained from this field trip (see Table 3-2). An additional 20 cultures contain algae that may interest us, but have not been isolated into unialgal culture. The strains listed in Table 3-2 consist of diatoms, green algae, and blue-green algae. In addition we previously received two strains, Nitzschia and Coccomyxa, from Dr. David Chapman of UCLA.

3.2.1.3 Commercial Chemical Data - Field Trip 1. Commercial data for major chemical constituents of waters sampled during this trip are shown in Table 3-3 and for minor ions in Table 3-4. Note that the total major cation concentrations are equivalent to total anion levels. This gives us further confidence in the reliability of the commercial data. As described above, the Saline Valley data are very much different from the other data in that the ionic concentrations are much higher and the pH is lower. Of the other results, Black Lake sodium, carbonate, bicarbonate, sulfate, and chloride data stand out as being relatively high. Trace metals are mostly undetectable or low and the boron and fluoride levels are high in all of these waters. The As level for Owens Lake is much higher than that of other waters.

3.2.2 Field Trip Number 2 to the California State Universities' Desert Studies Center (Zzyzx Springs) near Baker, California - October, 1982.

In late October, 1982 we made a three-day trip to Zzyzx Springs. For location see Figure 3-1. This Center is just off the freeway to Las Vegas and consists of a number of resort-type buildings for housing and research. Drinking water is trucked in from Baker, and power is supplied from a generator and windmill-battery system.

There are two large saline ponds at the Center and a nearby saline spring. This area is the hydrological terminus of the Mohave River and a large supply of saline groundwater is found about 4 feet below the surface. The ponds contain an endangered fish species - the Mohave chub. Prior to our visit the ponds had never been sampled for algae.

When we sampled them we noted that the water was reddish which we mistakenly attributed to a high iron content. However, microscopic examination showed that the red color was due to a bloom of dinoflagellates of the genera Peridinium and Gymnodinium. Few other algae were seen. These algae are unique in that the group is generally marine - we had discovered a "red tide" in the middle of the desert. We transported the water and live algae back to La Jolla for isolation, but we have been unable to isolate these particular algae into successful cultures that we could maintain indefinitely. However, other algae were isolated from these samples. Water conductivities ranged from 4.3 to 7.5 mho cm^{-1} (estimated total dissolved solids of 2752 to 4800 mg $liter^{-1}$. The pH ranged from 8.5 to 9.5 and temperatures were 23-25°C. We have sent samples of the dinoflagellates to Dr. F.J.R. Taylor of the University of British Columbia for identification.

TABLE 3-2. List of Unialgal Strains Isolated and Maintained From Field Trip Number 1 — October, 1982.

Location	Strain
Pond A	Nitzschia (1) Nitzschia (2)
Pond B	Cymbella (82-1) (Large)
Hot Springs, near Pond B	Unidentified small, round, green alga (96-1)
Black Lake	Chaetoceros (76-2) Cymbella (11-15) (small)
Pyramid Lake	Anacystis Unidentified small, round yellow-green alga (Nannochloris?) (26-1A) Ditto (26-1B) Ankistrodesmus (91-1)
Walker Lake	Unidentified centric diatom (28-1-2) Anacystis (31-1A) Chroococcus (32-1) Cyclotella (33-1) Unidentified small, round yellow-green alga (34-1) Ditto (35-1)
Saline Valley	Dunaliella (orange) 10-2) Dangeardinella (12-1) Ditto (15-1)
Owens Lake	Unidentified blue-green (1-1) Selenastrum (1-24)
Columbus Salt Marsh	Anacystis (47-1)
Mono Lake Cultures	Nitzschia (S-16) Coccomyxa (S-9)

TABLE 3-3. Chemical Data From Field Trip Number 1 — Major Constituents.

	Pond A	Pond B	Black Lake	Pyramid Lake	Walker Lake	Owens Lake	Saline Valley
Major Cations (mg ℓ^{-1})							
Ca	5	6	4	10	10	8	220
Mg	5	5	4	60	77	20	320
Na	450	1150	8800	1775	3900	4700	100×10^3
K	43	124	825	128	225	525	1500
NH_4^+	1	1	2	1	2	0.6	0.6
Total Cations (me ℓ^{-1})	21.4	53.96	404.4	86.01	182.34	229.28	4423.99
Major Anions (mg ℓ^{-1})							
CO_3	240	420	5880	222	750	2640	0
HCO_3	268	1159	2526	854	1586	915	146
SO_4	20	140	3200	140	2600	1600	10.4×10^3
Cl	300	600	3200	2150	2600	2250	154×10^3
NO_3	1	1	1	1	1	1	2
Ortho PO_4	0.1	1.9	0.1	0.1	0.1	3.8	0.8
Total PO_4	1.2	1.9	18.5	0.7	2.3	7.9	3.0
Total Anions (me ℓ^{-1})	20.89	52.84	394.81	84.90	178.73	199.73	4557.14
Electrical Conductivity (mmho cm^{-1})	1.857	4.710	30.00	8.030	15.00	15.60	209
Equivalent TDS (mg ℓ^{-1})	1200	3014	19200	5139	9600	9984	133.8×10^3
Total Dissolved Residue (mg ℓ^{-1})	1350	3205	26460	4900	10580	11870	225×10^3
pH	9.6	9.5	9.8	9.1	9.3	9.8	7.5

TABLE 3-4. Chemical Data From Field Trip Number 1 — Minor Consituents.

Element	Pond A	Pond B	Black Lake	Pyramid Lake	Walker Lake	Owens Lake	Saline Valley
	mg ℓ^{-1}						
As	0.20	1	0.63	0.03	0.80	5.9	0.25
Ba	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
B	8.0	20.0	21.0	11.0	19.0	60.0	155.0
Cd	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
Cr	<0.01	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Cu	<0.02	<0.02	0.02	<0.02	0.02	0.04	<0.04
F	4	10.0	20.0	5.0	4.0	5.0	2.5
Fe	0.15	0.15	0.15	0.05	0.15	0.25	0.20
Pb	<0.05	<0.05	<0.05	<0.05	0.05	<0.05	<0.05
Mn	0.04	0.04	0.02	<0.02	0.02	<0.02	0.08
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Ag	0.01	0.01	0.01	<0.01	<0.01	<0.01	0.04
Zn	0.01	0.01	0.01	<0.01	<0.01	0.05	0.01

We also talked to Mr. Ted Laddo, the resident Bureau of Land Management biologist in Barstow. He suggested several other saline water sites to sample from the Center on subsequent trips (see 3.2.3).

3.2.2.1 Commercial Chemical Data - Field Trip 2. The BLM furnished us with commercial chemical data done previously at Zzyzx Springs so we did not bring back samples for analysis from this trip. These data are shown in Table 3-5. Generally these ponds are less saline than those sampled during Field Trip 1. The ions that were present in highest concentration were sodium, chloride, and sulfate. One pond was particularly high in iron.

3.2.2.2 Species Isolated - Field Trip 2. Five unialgal strains have been isolated and maintained from three waters at Zzyzx Springs. An additional four strains await purification. We were unable to isolate and maintain the dinoflagellates which colored these waters reddish. The strains are listed in Table 3-6.

3.2.3 Field Trip Number 3 to Zzyzx Springs and Environs - January-February, 1983.

This trip commenced January 31, 1983 and was finished on February 4, 1983. From the Zzyzx facility, three sites were sampled along highway 127: Sperry River, Salt Creek, and Tecopa Springs. Additional samples were taken from Saratoga Springs, Mormon Point, and Badwater in Death Valley; at the Zzyzx ponds and spring; and at Harper Dry Lake. These locations are shown in Figure 3-4.

Water was brought back to La Jolla for algae isolations and seven samples were delivered to Babcock Labs in Riverside for commercial analyses. Data collected in the field are shown in Table 3-7. Isolations are presently in progress.

3.3 Preliminary Selection Experiments.

During isolations and subsequent culture we noted visually that some desert algae grew more rapidly than others. Some of these have been selected for measurement of their growth in media formulation experiments and in temperature-salinity interaction experiments.

3.3.1 Media Experiments.

With regard to major ions, artificial media were formulated from the chemical analytical data on the various waters that were brought back. However, the Mono Lake Nitzschia culture was grown in an artificial media developed by Dr. David Chapman of UCLA who provided us with that culture. Cultures were grown in 6 liters of media contained in 9-liter serum bottles. The cultures were incubated at 21°C and illumination was supplied from below at an intensity of 0.056 cal cm^{-2} min^{-1} in the photosynthetically active spectral range with fluorescent lamps. These were operated on a light/dark cycle of 12 hours

TABLE 3-6. List of Unialgal Strains Isolated and Maintained from Field Trip Number 2 — October, 1982.

Location at Zzyzx Springs	Strain
Lake Tuendae	Unidentified yellow-green, round alga (50-1)
3-BATS Pond	Cosmarium (37-1-1)
	Colonial green alga (44-1)
NC Pond	Yellow flagellate (61-1)
	Chlorella

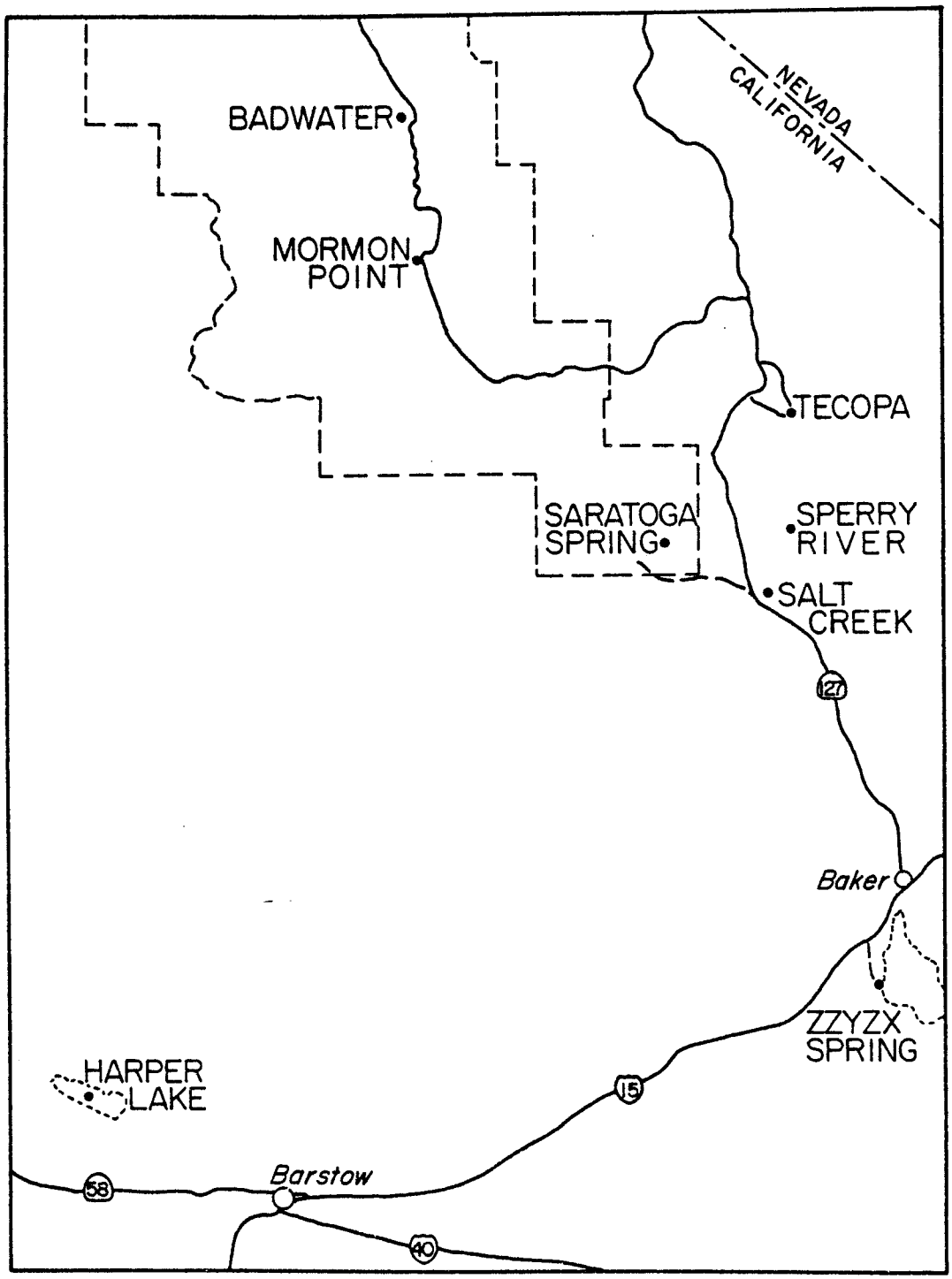

Figure 3-4. Specific locations sampled — Field Trip number 3.

TABLE 3-7. Physical and Chemical Parameters of Waters Sampled During Field Trip Number 3.

	Temperature °C	pH	Electrical Conductivity (mmhos cm^{-1})	Equivalent TDS (mg ℓ^{-1})
Salt Creek	14.9	8.0	7.9	5056
Sperry River	14.0	8.8	7.8	4992
Tecopa @ Amargosa	8.4	9.1	11.7	7488
Lake Tuendae	9.0	8.8	3.7	2300
3-BATS Pond	8.9	8.9	6.5	4160
Mojove R. @ Ofton	14.0	8.9	1.2	768
Harper Lake	9.0	8.6	22.5	14400

on/12 hours off. Nitrate, urea, and ammonium were tested as N sources and were supplied at 15 mg-at N liter^{-1}. Phosphorus was supplied as K_2HPO_4 at 1.5 mg-at P liter^{-1}. Trace metals and iron were added at the levels in Dr. Chapman's Mono Lake medium. After the cultures became visibly green, they were bubbled with 1% CO_2-in-air at 2 liters min^{-1}. Growth was assessed daily by measurements of optical density at 750 nm, cell numbers (Coulter counter or hemocytometer), and dry weight of cells filtered onto tared glass fiber filters. The dry weight filters were dried overnight at 60°C and weighed on a Cahn microbalance.

At the end of each experiment cells were filtered onto glass fiber pads for measurements of their lipid, carbohydrate, carbon content, and nitrogen content. Remaining cells were centrifuged and stored frozen for more detailed lipid analyses by Drs. Tornabene and Ben-Amotz.

3.3.1.1 Mono Lake NITZSCHIA Medium Experiment. Figure 3-5 shows increases in optical density with time for both nitrate and urea. Nitrate was a superior N source to urea and ammonium failed to support growth - probably because it was available only as NH_3 gas at the high pH (~9) of these media. Cell number changes paralleled optical density changes but dry weight changes were somewhat erratic except toward the end of the experiment. Final dry weight was 810 mg liter^{-1}.

From linear dry weight increases near the end of the experiment we caluclated rough yield values and photosynthetic efficiencies. The values are rough because light was supplied not only through the bottom of the serum bottles but to their sides as well. For nitrate-grown cells the yield was 21.6 gm mg^{-2} day^{-1} and the efficiency was 13.3%.

3.3.1.2 NITZSCHIA Cellular Chemical Data. For these analyses cells were filtered on glass fiber papers. Lipid was extracted with acidified water:methanol:chloroform. The extracts were washed with additional acidified water to get the lipids fraction into the chloroform phase. Aliquots of this fraction were then dried under N_2 and weighed on a microbalance. Carbohydrate was determined by the phenol-H_2SO_4 method and carbon and nitrogen were analyzed with a Hewlett-Packard 185 CHN analyzer.

Analyses performed in our laboratory showed that cells grown on nitrate contained 20% lipid andd 18% carbohydrate. Cells grown on urea contained 27% lipid and 16% carbohydrate. Protein and cell carbon values were very much too high (>100% of the dry weight) and we suspect that the filters were contaminated.

3.3.1.3 Pyramid Lake ANKISTRODESMUS Medium Experiment. Figure 3-6 shows optical density increases for both nitrate and urea. Urea was a superior N source. The ammonium culture failed to grow. Cell numbers and dry weight changes paralleled optical density changes. The final dry weight in the urea culture was 1247 mg liter^{-1} and in the nitrate culture, 710 mg liter^{-1}.

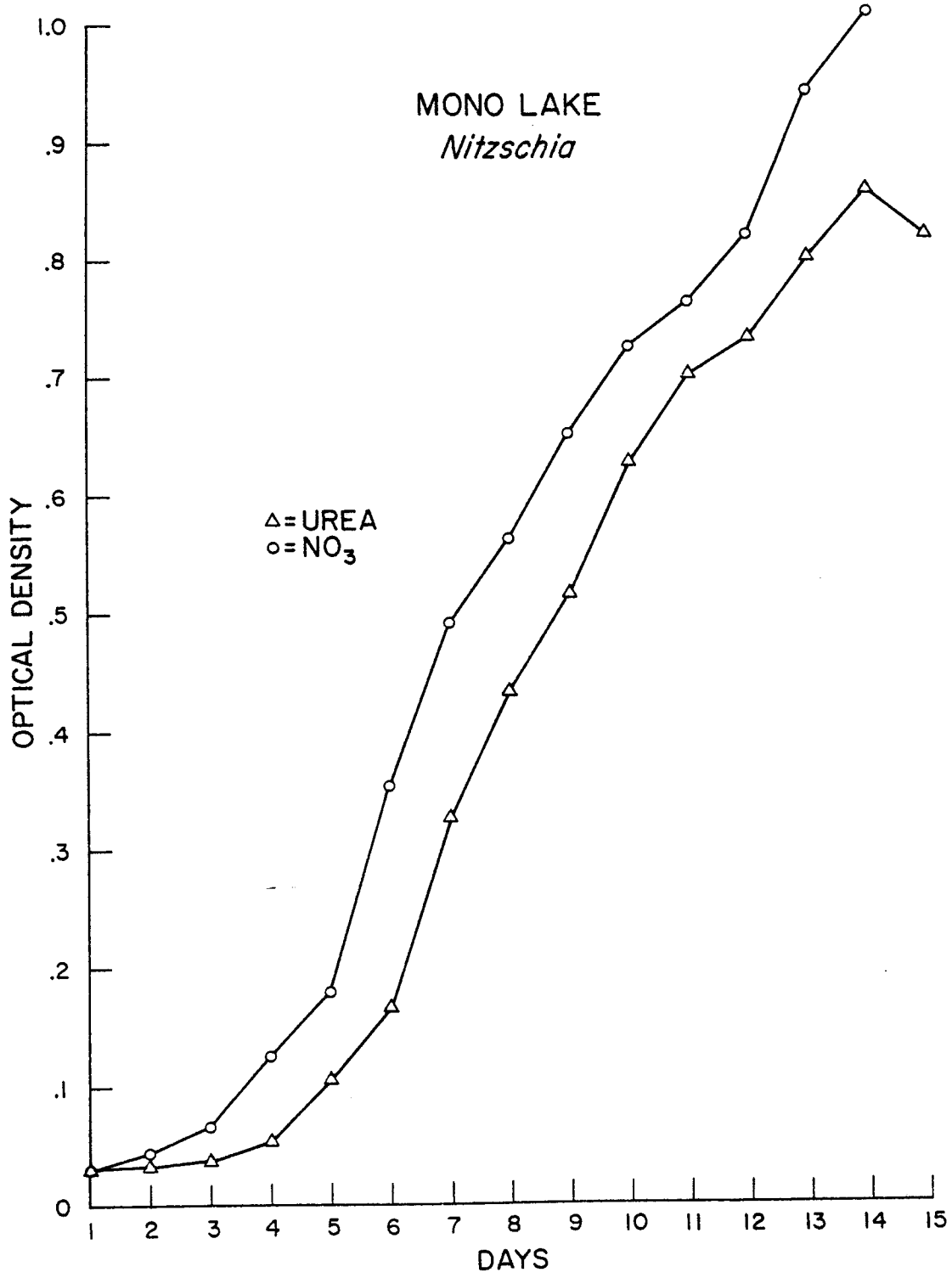

Figure 3-5. Increases in optical densities of Mono Lake Nitzschia cultures grown on nitrate and urea.

TABLE 3-5. Chemical Data from Zzyzx Springs Sites — Bureau of Land Management Data.

	Lake Tuendae	3-BATS Pond
Major Cations (mg ℓ^{-1})		
Ca	22	28
Mg	5	8
Na	920	1000
K	18	20
Total Cations (me ℓ^{-1})	41.96	46.09
Major Anions (mg ℓ^{-1})		
CO_3	108	111
HCO_3	119	228
SO_4	440	450
Cl	1007	1049
NO_3	0	0
Total Anions (me ℓ^{-1})	43.09	47.18
Minor Anions (mg ℓ^{-1})		
Ba	0	0
B	6.0	6.0
F	12	13
Fe	0.21	3.3
Mn	0.01	0.15
Se	0	0
Ortho PO_4	0.0	0.1
Total PO_4	0.3	0.6
Electrical Conductivity (mmho cm^{-1})	4.40	4.65
Equivalent TDS (mg ℓ^{-1})	2816	2976
Total Dissolved Residue (mg ℓ^{-1})	2615	2770
pH	9.4	8.7

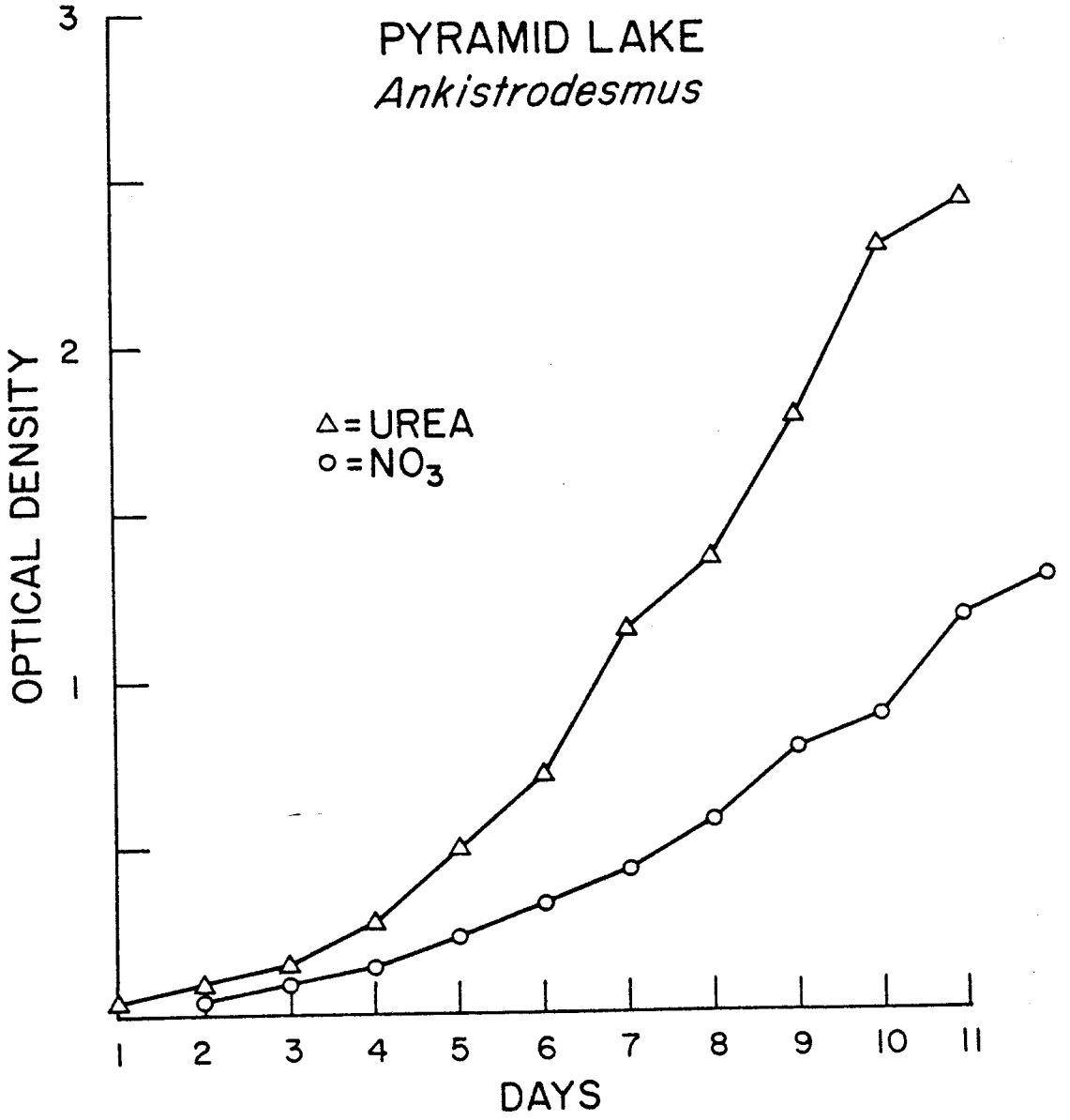

Figure 3-6. Increases in optical density of Pyramid Lake Ankistrodesmus cultures grown on nitrate and urea.

Rough yield values for nitrate-grown cells (calculated from linear dry weight increases) were 11.5 gm m^{-2} day^{-1} and the rough efficiency was 7.2%. For urea-grown cells the corresponding values were 17.4 gm m^{-2} day^{-1} and 10.8%.

3.3.1.4 ANKISTRODESMUS Cellular Chemical Data. Ankistrodesmus cells grown on nitrate contained 30% lipid, 24% carbohydrate, and 70% protein. Urea-grown cells contained 30% lipid, 17% carbohydrate, and 58% protein.

3.3.1.5 Pyramid Lake NANNOCHLORIS Medium Experiment. Figure 3-7 shows optical density increases for nitrate-grown cells. Growth on urea was very slow and the growth curve is not shown. No growth occurred with ammonium as an N source. Cell number and dry weight changes paralleled optical density changes. The final dry weight in the nitrate culture was 680 mg $liter^{-1}$. The rough yield was 23.2 gm m^{-2} day^{-1} and the efficiency was 14.4%.

3.3.1.6 Cellular chemical Data for NANNOCHLORIS. This species contained 21% lipid, 21% carbohydrate and 73% protein when grown on nitrate.

3.3.2 Temperature-Salinity Experiment - Mono Lake Nitzschia.

For this experiment, 5 ml of a dense cell suspension was inoculated in 45 ml of medium. The media were contained in 30 65-ml glass bottles. Six of these contained 45 ml of deionized water (0 salinity), 6 contained 0.5X the usual salinity of Mono Lake medium, 6 contain 1X, 6 contained 1.5X, and 6 contained 2X the usual salinity of the Mono Lake medium. Since this medium has a salinity of 86 m salts $liter^{-1}$, the actual salinities after inoculation were 8.6, 47.3, 86, 124.7 and 163.4 gm $liter^{-1}$. One bottle of each salinity set was then placed in an aluminium temperature-gradient block. The block had 30 two-inch holes so that 30 combinations of temperatures and salinity could be achieved. Each hole had a plastic window sealed with an O-ring so that deionized water could be placed around each bottle for proper heat transfer. Thermostated methanol-water at 0°C was circulated through the other end. Thus six rows of holes containing water at the following temperatures were set up: 10, 15, 20, 25, 29, and 34°C. Each bottle was illuminated from below with fluorescent lamps. The light intensity in each hole was measured with a Li-Cor irradiance meter and the intensity was quite uniform over the whole block because mirrors were placed under the lamp bank and along its sides. The mean light intensity was 0.178 $\pm$ 0.011 cal cm^{-2} min^{-1}. Optical density of the cell suspension was measured daily by placing the bottles in a photometer designed to hold one bottle. There was a hole on one side of the photometer which was illuminated with a 6 volt DC lamp. On the other side another hole contained the Li-Cor radiometer sensor. Thus by comparing the irradiance across the culture bottle with irradiance across a standard bottle containing deionized water, the optical density within each culture bottle cold be measured without taking samples from the bottles. The bottles themselves served as cuvettes in this non-invasive system.

The mean initial optical density among the 30 bottles was 0.03. After 6 days the highest optical density in any bottle was 0.52. Growth proceeded

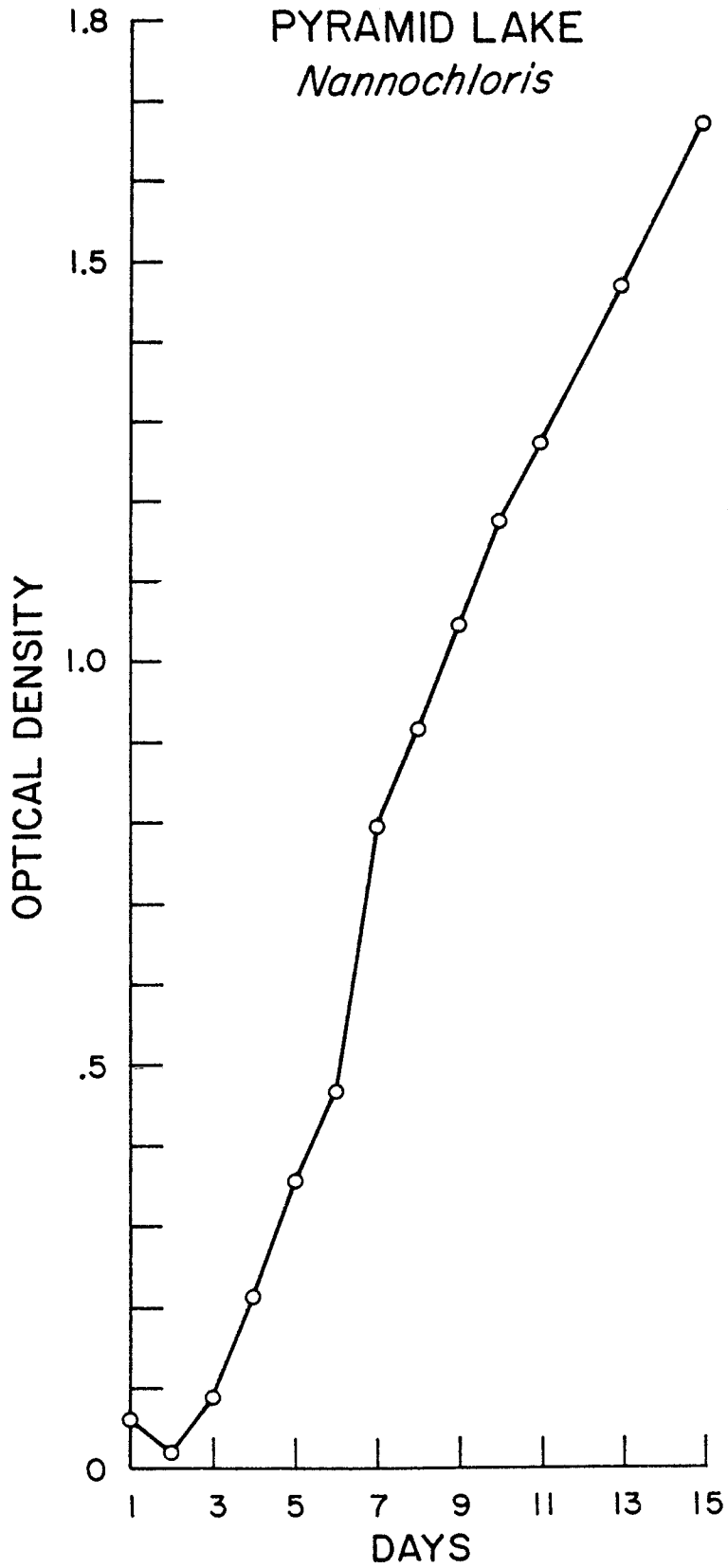

Figure 3-7. Increases in optical density of a Pyramid Lake Nannochloris culture grown on nitrate.

exponentially in this bottle for the first 5 days of the experiment and then leveled off. Optical densities in all of the bottles after 6 days are plotted in Figure 3-8 as a function of temperature and salinity. The optical densities are contoured to show equivalent growth. Little or no growth occurred at 10°C or at salinities of 9 and 163 gm liter^{-1}. Relatively good growth was found at 47 gm liter^{-1} from 15 to 34°C and the best growth occurred at 29°C and 47 gm liter^{-1} salinity. Growth was reduced somewhat at the present salinity of Mono Lake - 86 gm liter^{-1} - and this species seems to prefer warm water - 20 to 34°C.

This experiment was completed during the time of writing of this paper so the results could not be calculated in terms of exponential growth rates at each of the 30 combinations. However, the pattern of optical density contours show in Figure 3-8 appeared on the third day of the experiment and persisted until the sixth day, and we have confidence that Figure 3-8 correctly depicts temperature-salinity effects on this alga.

4.0 FUTURE PLANS

During the next six months we plan to continue Preliminary Selection Experiments on media formulation, temperature-salinity interactions and light requirements. Equipment for the latter remains to be set up. These experiments will allow further selection of promising species for experiments designed to maximize yields in chemostat cultures. The latter experiments will start in about six months and continue until the end of our contract period - July 31, 1984.

We also plan additional major field work to isolate new cultures in May and July, 1983 and probably in the fall of 1983. These trips will be based at SNARL and Zzyzx Springs. We may also make a few 1-2 day trips to the Salton Sea since it is so close. The Sea has not been sampled for algae for about 10 years. It has been known to contain largely marine species and these have not been included in our present emphasis. However, the Sea is becoming more saline - the salinity is presently up to 40,000 mg liter^{-1} total dissolved solids - and there may be some interesting species out there that we should know about.

5.0 PROBLEMS AND VARIANCES

We originally planned to perform all of the above types of work simultaneously. However, this has not been possible and we are doing the work more or less sequentially as follows: 1) Field work and isolation; 2) Preliminary selection experiments; 3) Experiments to maximize yields. The reasons for these variances are as follows:

a). It has taken and will take a great deal of time to set up the necessary equipment.

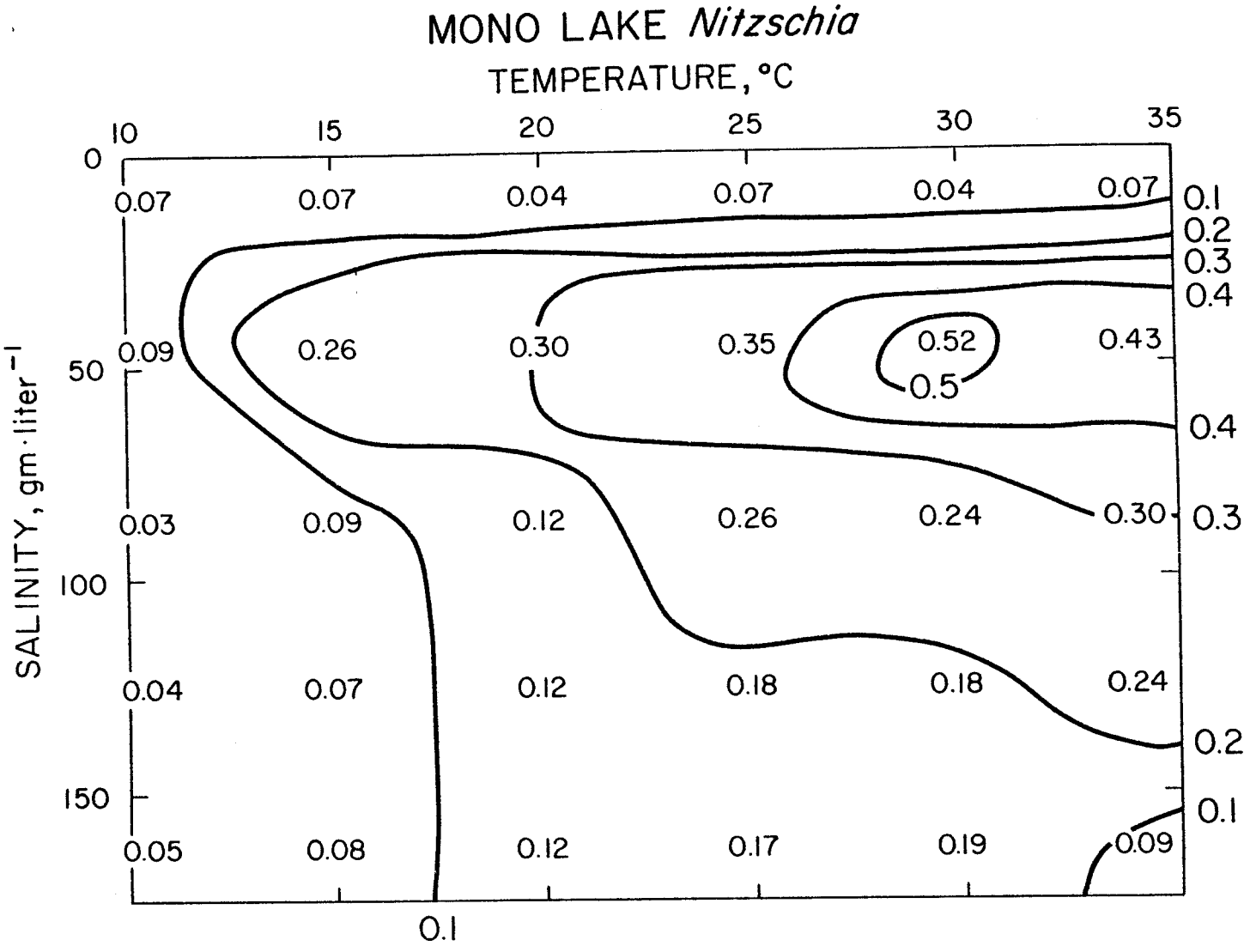

Figure 3-8. Optical density of Mono Lake Nitzschia cultures after 6 days of growth and plotted as a function of temperature and salinity.

b). There are only four of us working full-time on this project. A lot more could be accomplished if each of us were cloned, but then we would really get in each other's way, due to limited lab space.

c). The available laboratory space for this project is really severely limiting and there seem to be no immediate prospects for obtaining more space. Associated with the space problem are needs for additional electrical power in the right rooms. We may be able to tap into unused lines or run lines into our culture and cold rooms aas needed. This will require the services of an electrician.

d). When one or two of us go off into the field, experiments are delayed. They are also delayed due to the need for timely isolations and maintenance of microalgae from water that is brought back.

Despite these problems, our work seems to be progressing well and we believe we have accomplished a great deal.

6.0 PUBLICATIONS AND MEETINGS

The following reports have been delivered for publications as SERI Technical Reports:

1. Thomas, W.H., Seibert, D.L.R., Alden, M., Neori, A. and Eldridge P. 1983. Yields, photosynthetic efficiencies and proximate chemical composition of dense culture of marine microaglae. Final Rept. to SERI-Subcontract XK-09111-1.

2. Thomas, W.H. and Gaines, S.R. 1983. Algae from the arid southwestern United States: An annotated bibliography. SERI Technical Report.

Dr. Thomas attended two meetings:

Symposium on Algal Mass Culture
New Mexico State University
Las Cruces, New Mexico
September, 1982

Workshop on Algal Lipid Separation
Georgia Institute of Technology
Atlanta, Georgia
December, 1982

7.0 ACKNOWLEDGEMENTS

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CHEMICAL PROFILE OF ALGAE WITH EMPHASIS ON LIPIDS OF MICROALGAE

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ABSTRACT

Analysis of proximate chemistry composition with emphasis on the neutral lipids was carried out as a comparative study among different algae. Total lipid content varied from a high of about 50% lipids/organic weight in Botryococcus, 26% in Isochrysis, 19% in Chlamydomonas P202, 18% in Dunaliella, 12% in Nitzschia, 9.3% in Scenedesmus to a low of 2.0% in Gracilaria. Nitrogen deficiency caused lipid accumulation in Isochrysis, lipid elimination in Gracilaria, and no effects on the lipid content of Dunaliella. The distribution of the lipid classes were 60% neutral lipids and 40% polar lipids in Botryococcus; 30% neutral lipids and 70% polar lipids in Isochrysis; 15% neutral lipids and 85% polar lipids in Chlamydomonas P202; 10% neutral lipids and 90% polar lipids in Scenedesmus and about 2% neutral lipids and 98% polar lipids in Dunaliella salina, Gracilaria and Nitzschia. Dunaliella bardawil grown under the conditions of nitrogen deficiency accumulated about 50% neutral lipids most of it as β-carotene. Long chain aliphatic hydrocarbons (>C-30) have been identified in the hexane eluate of Botryococcus and Isochrysis. Medium chain aliphatic hydrocarbons (C-18 to C-20) have been identified in the hexane eluate of Nitzschia, and short chain aliphatic hydrocarbons ($\leq$ C-17) appeared in the hexane fractions of Chlamydomonas P202, Dunaliella, Gracilaria and Scenedesmus. The major component of the neutral lipid fraction in Isochrysis appeared in the benzene eluate and accounted for about 3% of the algal organic weight. This component has been isolated, purified and analyzed by TLC, MS, IR, and NMR.

INTRODUCTION

Lipids and fatty acids are ubiguitous to algae. There are significant differences, however, in quantity and nature of the lipids, in genera of algal classes as well as in strains of the same class [1]. Algal lipids construct a major part of the cellular membranes and they accumulate in many species as storage products. It is well documented

that ecological and physiological factors influence the chemical composition of algae. The lipid compositions are specifically responsive to a number of conditions. Some of these conditions can be controlled and manipulated experimentally. In this particular study our specific objective was to conduct lipid experiments that would challenge growth parameters of algae in an attempt to identify conditions that would alter the lipid chemistry of the algae toward higher production of neutral lipids.

MATERIALS AND METHODS

All methods were as previously described in the Aquatic Species Program for SERI Workshop held in December 1982 at the School of Applied Biology, Georgia Institute of Technology. Derivitized lipids were analyzed on Varian 3700 gas-liquid chromatograph connected to a CDS 401 Varian computer. Gracilaria was obtained from J.H. Ryther; Chlamydomonas P202 and Scenedesmus were obtained from J.R. Benemann; and Nitzschia sp. was obtained from W.H. Thomas.

RESULTS

Proximate Chemical Composition

The proximate chemical compositions of a few algae grown under nitrogen sufficient conditions are illustrated in Table 1. Botryococcus braunii grown on freshwater medium presented a slow growth rate of about 6 days/division. At the harvesting time the algal concentration was

TABLE 1

Proximate Cellular Composition of a Few Unicellular Algae Grown Under NO_3^- - Sufficient Conditions

Species	% Organic Weight					% Dry Weight
	% Protein	% Glycerol	% Carbohydrate	% Lipid	% Unknown	% Ash
Botryococcus braunii, Fresh water	16.5	>0.1	17.1	45.4	21	9.6
Botryococcus braunii, 0.5M NaCl	15.0	>0.1	13.3	46.3	25.4	59.6
Dunaliella salina, 2M NaCl	35.9	27.7	12.5	18.5	5.4	21.7
Isochrysis sp. UTEX #2307, 0.5M NaCl	37.0	>0.1	11.2	7.1	44.7	12.0
*Nitzschia sp.	16.8	>0.1	9.2	12.1	61.9	20.4

Growth conditions. Algae were cultivated in growth medium containing NaCl as indicated, 5mM $MgSO_4$, 0.3mM $CaCl_2$, 5mM KNO_3, 0.4mM KH_2PO_4, 1.5μM $FeCl_3$, 30μM EDTA, 50mM $NaHCO_3$, 0.1mM $Na_2S_2O_3$, 0.1mM H_3BO_3, 1ug/l Vitamin-B12, 0.2 μg/l thiamine, 1.0 μg/l biotin, and 20mM Tris, pH 8.0. Temperature, 25°C. Illumination, Cool-white and Agro-lite fluorescent lamps.

*Algae from W. H. Thomas.

close to 400 mg organic weight per liter. The same alga grown on 0.5M NaCl medium divided even slower with a growth rate of about 15 days/division, reaching a cell concentration at harvest of 150 mg organic weight per liter. The composition of both cultures of B. braunii was about the same exhibiting on the order of 50% lipids, 16% protein and 15% carbohydrates. No glycerol was detected in B. braunii cultivated in media containing different salt concentrations.

All other algae divided at a rate of about 1 division/day and were harvested at concentration of around 800 mg organic weight/liter. D. salina and Isochrysis contained about 36% protein and 12% carbohydrates. Lipid content in D. salina exceeded by about 10% that of Isochrysis and reached 18%. Glycerol content in D. salina was about 30% of the organic weight. Nitzschia sp., an alga grown at 1.38 M NaCl, contained 17% protein, 9.2% carbohydrates and 12% lipids. A major cellular fraction of around 62% of the organic weight of Nitzschia has not been identified by the routine procedures employed. The unaccounted weight difference must be further examined. Possibilities could be either certain complex carbohydrate derivatives or highly non-polar type waxes that are insoluble in solvent system employed. Table 2 illustrates the proximate chemistry of D. bardawil and Isochrysis grown on nitrogen deficient medium. The cellular carbohydrate content increased and the protein content decreased. The lipid content increased in Isochrysis while it did not change in D. bardawil.

TABLE 2

Proximate Cellular Composition of Two Unicellular Algae Grown Under NO_3^- - Deficient Conditions

Species	% Organic Weight					% Dry Weight
	% Protein	% Glycerol	% Carbohydrate	% Lipid	% Unknown	% Ash
Dunaliella bardawil 2.0M NaCl	9.7	16.4	40.4	10.4	23.1	14.7
Isochrysis sp. UTEX #2307, 0.5M NaCl	28.3	>0.1	20.5	26.0	21.2	52.0

Algae were grown in complete medium containing 0.5mM NO_3^- and the indicated concentration of NaCl.

Total Chlorophyll and Carotenoids

The contents of chlorophyll and carotenoid in the algae are illustrated in Table 3. All algae contained chlorophyll a; the green algae contained chlorophyll b in addition. The chrysophyte Isochrysis and the diatom Nitzschia contained chlorophyll c. The major carotenoids, β-carotene and fucoxanthin were analyzed spectrophotometrically and the carotenoid-to-chlorophyll ratios are given in Table 3. Dunaliella bardawil grown on nitrogen deficient medium was the only species with high content of β-carotene. Other species did not show significant pigment response to salt stress or nitrogen starvation.

TABLE 3

Pigment Content in a Few Unicellular Algae

Species	% Organic Weight		
	% Chlorophyll	% Carotenoid	Carotenoid / Chlorophyll
Botryococcus braunii, Fresh water, 5mM NO_3^-	1.76	0.53	0.29
Botryococcus braunii 0.5M NaCl, 5mM NO_3^-	0.69	0.22	0.32
Dunaliella bardawil, 2M NaCl, 0.5mM NO_3^-	0.46	2.71	5.89
Dunaliella salina, 2M NaCl, 5mM NO_3^-	4.34	0.95	0.22
Isochrysis sp, 0.5M NaCl, 5mM NO_3^-	1.83	0.64	0.35
Isochrysis sp. 0.5M NaCl, 0.5mM NO_3^-	0.49	0.25	0.51
Nitzschia sp. 1.38M NaCl	1.44	0.48	0.33

Lipid Extraction

Lipids were extracted by the Bligh-Dyer technique and were fractionated on Unisil silicic acid column with hexane, benzene, chloroform, acetone and methanol. Samples of each solvent were collected, dried and weighed gravimetrically for determining the lipid distribution along the polarity neutrality spectrum (Table 4). Neutral lipids are those found in the hexane, benzene, chloroform eluates while the polar lipids occur in the acetone, methanol eluates. B. braunii contained at least 50% of its total lipid weight as neutral lipids, most of it in the benzene fraction. D. salina and Nitzschia contained relatively low content of neutral lipids. Isochrysis contained about 30% of neutral lipids most of it in the benzene fraction. D. bardawil yielded β-carotene as the predominant fraction of its total neutral lipids [2]. Chlamydomonas and Scenedesmus comprised about 15% and 10% neutral lipids respectively (Table 5). Neutral lipid fractions of nitrogen sufficient or deficient

TABLE 4

Extraction and Fractionation of Lipids

of Unicellular Algae

Species	Total Lipids	% Total Lipid Weight				
	% Organic Weight	% Hexane	% Benzene	% Chloroform	% Acetone	% Methanol
Botryococcus braunii Fresh water, 5mM NO_3^-	45.4	10.5	50.1	19.4	12.3	7.6
Botryococcus braunii 0.5M NaCl, 5mM NO_3^-	46.3	5.2	46.0	28.5	9.3	9.7
Dunaliella bardawil, 2M NaCl, 0.5mM NO_3^-	10.4	0.4	49.8	14.8	24.1	10.8
Dunaliella salina, 2M NaCl, 5mM NO_3^-	18.5	0.2	2.1	28.2	55.9	13.6
Isochrysis sp., 0.5M NaCl, 5mM NO_3^-	7.1	1.4	27.4	32.1	26.3	12.6
Isochrysis sp., 0.5M NaCl, 0.5mM NO_3	26.0	2.2	28.4	18	26	25.3
Nitzschia sp. 1.38M NaCl	12.1	0.9	1.7	51.2	22.0	24.6

Proportions of lipid fractions recovered from Unisil column with the indicated solvents and determined gravimetrically.

TABLE 5

Analysis and Fractionation of Lipids Extracted

from Algae During the SERI Short-term Course

December 1982

Species	Total Lipids	% Total Lipid Weight				
	% Organic Weight	% Hexane	% Benzene	% Chloroform	% Acetone	% Methanol
Chlamydomonas, P_{202}	19.4	0.1	15.5	16.0	60.5	7.9
Scenedesmus	9.3	0.2	9.5	10.4	72.6	7.3
Gracilaria, NE	1.8	0.8	1.3	18.3	46.0	33.5
Gracilaria, ND	0.8	0.3	1.9	4.8	62.3	30.8

Proportions of lipid fractions recovered from Unisil column with the indicated solvent and determined by gas chromatograph for the Hexane samples or gravimetrically for the other samples.

grown Gracilaria were quite low, not exceeding 2.5% of the total lipid fraction. After analyzing the data, it was clear that B. braunii and Isochrysis contained the highest fraction of neutral lipids among the algae tested. Conversely, Gracilaria, Chlamydomonas P202 and D. salina contained the higher fraction of polar lipids.

Hexane and Benzene Eluates

The components of the hexane eluates were further analyzed on a OV-17 stainless steel capillary column. B. braunii contained long chain aliphatic hydrocarbons that were in accordance with previous observations [3]. The predominant hydrocarbons of B. braunii in the hexane eluates were C29:0, C30:0 and C31:0 (Fig. 1). Isochrysis contained predominantly C31:0 hydrocarbons (Fig. 2).

Relatively short aliphatic hydrocarbons were detected in D. salina (Fig. 3), in Scenedesmus (not shown) and in Gracilaria(Fig. 4). When the same algae were grown on nitrogen deficient conditions, a shift in the distribution of the hydrocarbon toward longer chain length was observed. Most of the neutral lipid fraction appeared, however, in the benzene fractions and not in the hexane fractions. Thin layer chromatography of the benzene fractions showed a variety of different compounds (Fig. 5) with major spots observed in B. braunii, Isochrysis and D. bardawil. The major spot in D. bardawil was clearly identified as β-carotene. The major spot of B. braunii was tentatively identified as C-34 botryococcene. The major spot of Isochrysis which accounted for about 3% of the nitrogen deficient algal organic weight has been isolated from the chromatogram and purified by preparative TLC. The purified component showing one spot by TLC was dissolved in chloroform and analyzed by MS, IR and NMR. Preliminary results indicate that it is a C-37 isoprenoid chain with one substituted six-membered ring, two double bonds and one tertiary hydroxyl group.

REFERENCES

1. Pohl, P. and F. Zurheide, Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Marine Algae in Pharmaceutical Science, H.A. Hoppe, T. Levring, Y. Tanaka, eds. Walter de Gruter, Berlin, 1979, pp. 473-523.

2. Fried, A., Tietz, A., Ben-Amotz, A. and Eichenberger, W. Lipid composition of the halotolerant alga Dunaliella bardawil, Biochim. Biophys. Acta, 713, (1982) 419-426.

3. Tornabene, T. G. Microorganisms as hydrocarbon producers. Experientia, 38, (1982), 43-46

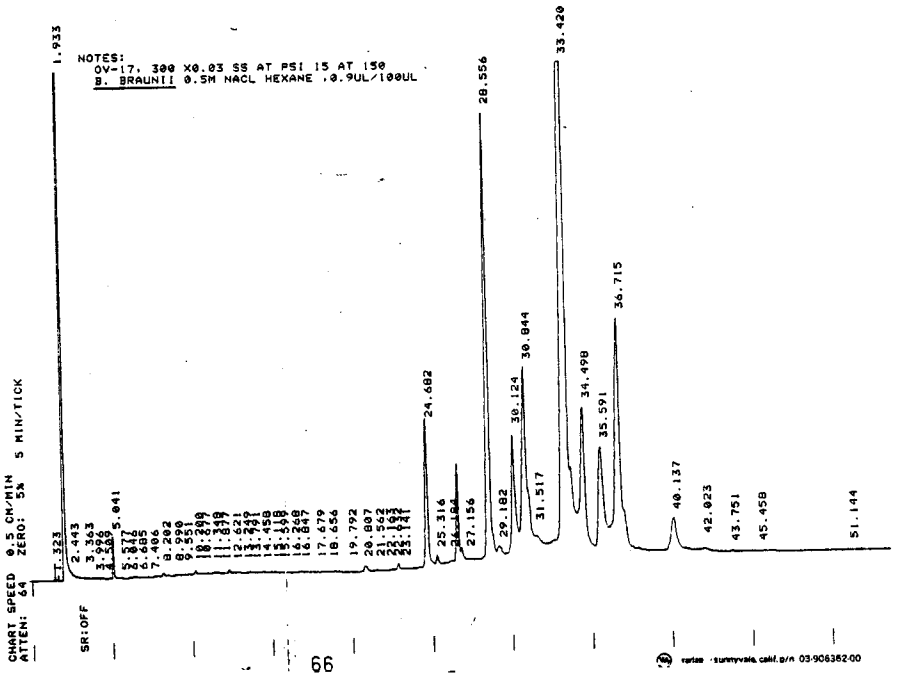

FIGURE 1. GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS IN THE HEXANE ELUATE OF B. BRAUNII GROWN ON 0.5M NaCL MEDIUM

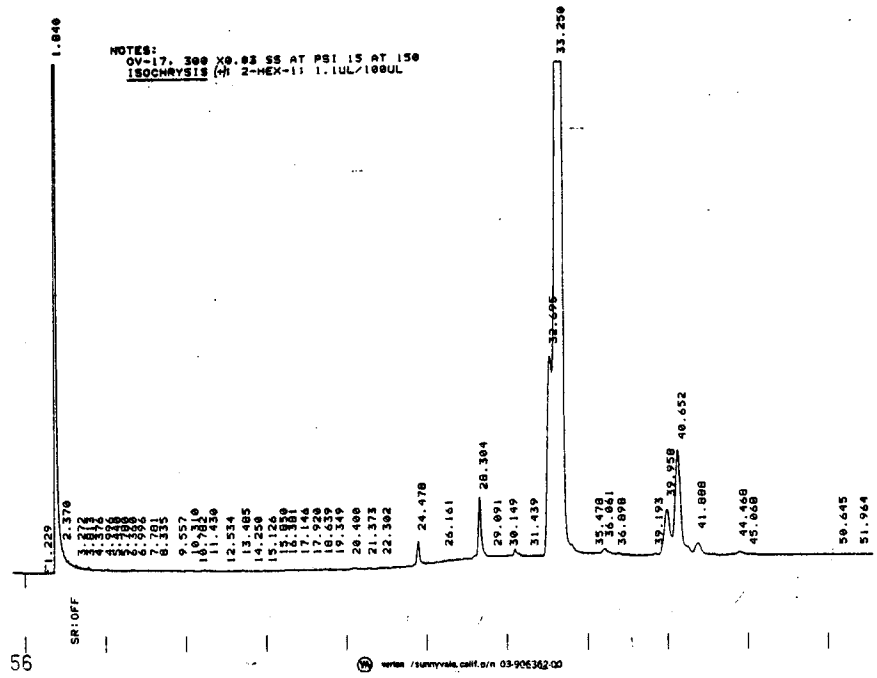

FIGURE 2. GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS IN THE HEXANE ELUATE OF ISOCHRYSIS

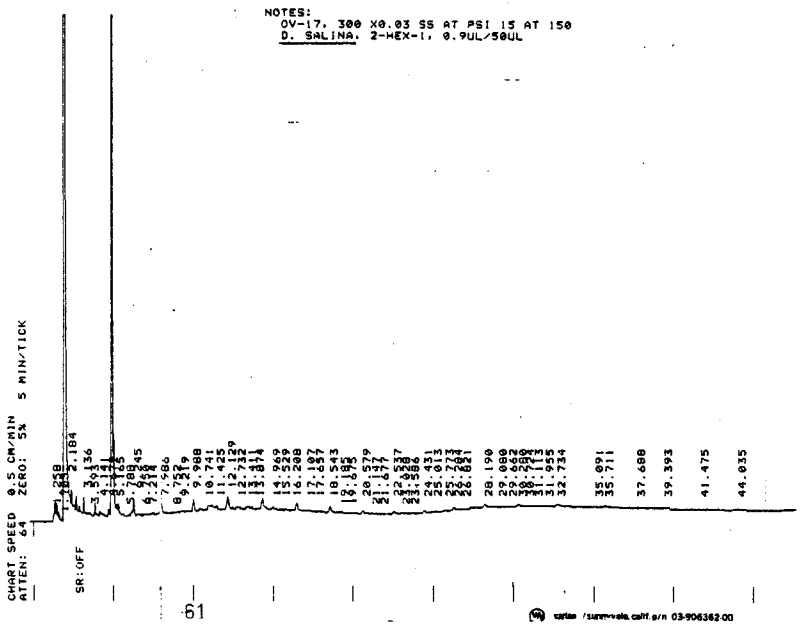

FIGURE 3. GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS IN THE HEXANE ELUATE OF D. SALINA

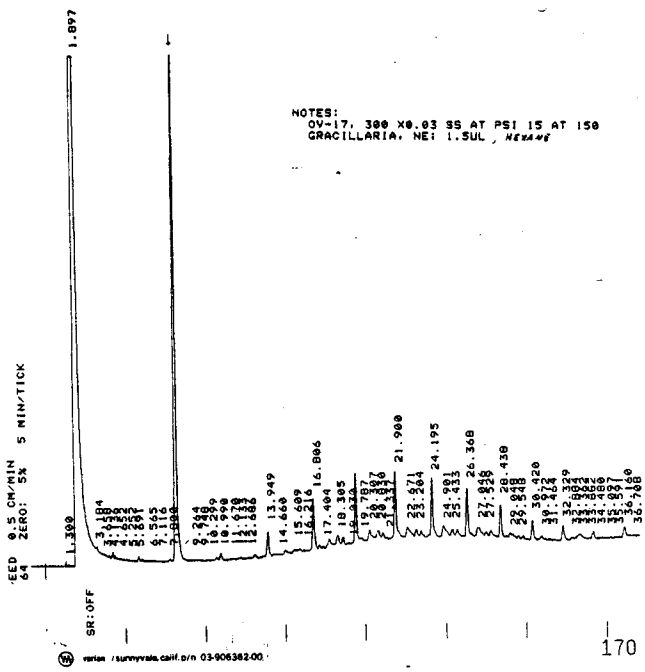

FIGURE 4. GAS CHROMATOGRAPHIC SEPARATION OF HYDROCARBONS IN THE HEXANE ELUATE OF GRACILARIA GROWN ON NITROGEN SUFFICIENT MEDIUM

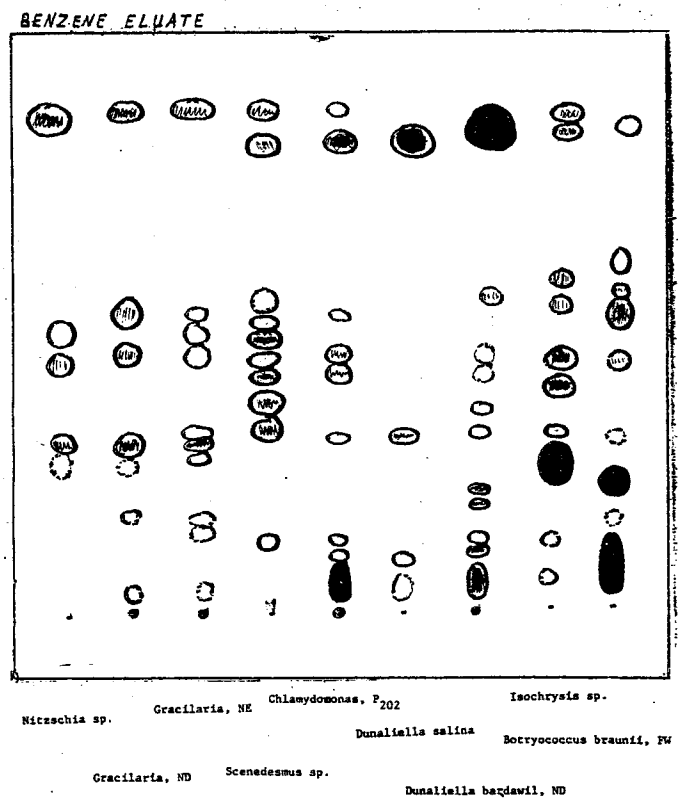

FIGURE 5. TLC OF THE FRACTIONS ELUATED FROM UNISIL COLUMN WITH BENZENE. SOLVENT SYSTEM: HEXANE: DIETHYLETHER (96:4). SPOTS WERE DETECTED BY IODINE VAPOR

PROCEDURES FOR STRATIFICATION OF THE U.S. SOUTHWEST FOR MICROALGAE PRODUCTION SYSTEMS BASED ON CLIMATE, WATER, AND LAND RESOURCES

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RESEARCH OBJECTIVES

Significance of the Research

If the technology of aquatic microalgae production is to make a significant contribution as a renewable source of fuels and chemicals, then systems must be scaled up from the laboratory bench to outdoor facilities encompassing thousands or even hundreds of thousands of hectares. This prospect does not appear outlandish if the existing areas devoted to conventional biomass production (i.e., agriculture and forestry) are considered.

The feasibility of developing large-scale microalgae production systems (MPS) is dependent upon the availability of adequate climate, water, and land resources. Successful design and operation of such systems will depend upon their performance under existing natural resource and environmental conditions. Laboratory and pilot-scale research should, therefore, be guided by the prevailing natural resource and environmental conditions of the areas judged to be optimal for siting MPS. Numerous questions pertaining to natural resources and the environment must be answered in coordination with species selection, culture, process engineering, and economic studies if the technology is to be developed in an effective and expeditious program.

Task Objectives

Resource evaluation for MPS can be viewed as an exercise of defining the interface between this developing bio-energy technology and the resources upon which it will depend. This can be accomplished by deducing resource (including environmental) requirements from the current state of the technology, identifying appropriate resource data and data sources, and analyzing the data to determine the resource potential. To the extent that the assessment technology and/or available data are inadequate, additional research may be required. Following the above approach, the objectives of the resource evaluation research from mid-FY1982 to mid-FY1983 have been as follows:

- o To identify and characterize data and data sources pertinent to the evaluation of climate, water, and land resources affecting the design, siting, performance, and cost of MPS;

- o To collect readily available data for some of the more critical parameters in order to stratify the southwestern United States into areas of varying potential (i.e., high, medium, and low) for operation of MPS; and

- o To develop a plan for improved resource evaluation and site selection for MPS.

RESEARCH ACCOMPLISHMENTS

As of this date, all three task objectives are close to completion. Appropriate resource data and data sources have been identified and examined. Data pertaining to selected resource and environmental parameters have been mapped and are being composited for a preliminary stratification. A draft plan for resource evaluation and site selection, which identifies critical research needs, is nearing completion.

This progress report emphasizes the resource stratification since it is central to the overall task and comprised much of the effort during the past nine months. Types and sources of resource data are mentioned here; though they are described in greater detail in the proceedings of the last contractor review meeting of the Aquatic Species Program [1], which was held shortly after the project was initiated. Discussion of the resource evaluation and site selection plan is included later under the heading of "Future Plans". This meeting is timely since reviewer comments on the process and preliminary results of the resource stratification are welcome inputs to the refinement of both the stratification and the plan.

Microalgae Production System Concept

These resource investigations were directed to examine the U.S. Southwest due to the high insolation of the region and the assumed availability at relatively low cost of saline groundwater and low-value desert land. The validity of these assumptions remains to be determined. The region of investigation consists of Colorado, Utah, New Mexico, Arizona, and parts of Texas, Oklahoma, and California between the 100th and 120th meridians (Figure 1).

While a variety of MPS have been described in the literature [2,3,4], there has been very little development of concepts and designs for large-scale commercial facilities such as might be sited in the southwestern United States. Figure 2 shows an artist's rendition of MPS under southwestern conditions. While much technical detail is obviously omitted, the drawing conveys a concept of a variable array of site-adapted culture ponds connected by a central water supply system all subject to the prevailing climate conditions.

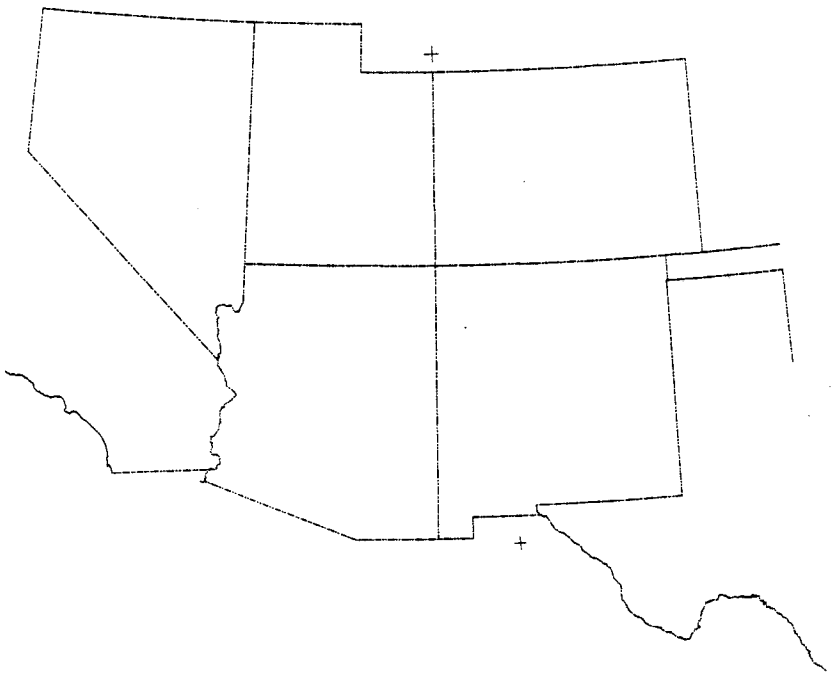


FIGURE 1. BASE MAP OF THE REGION.

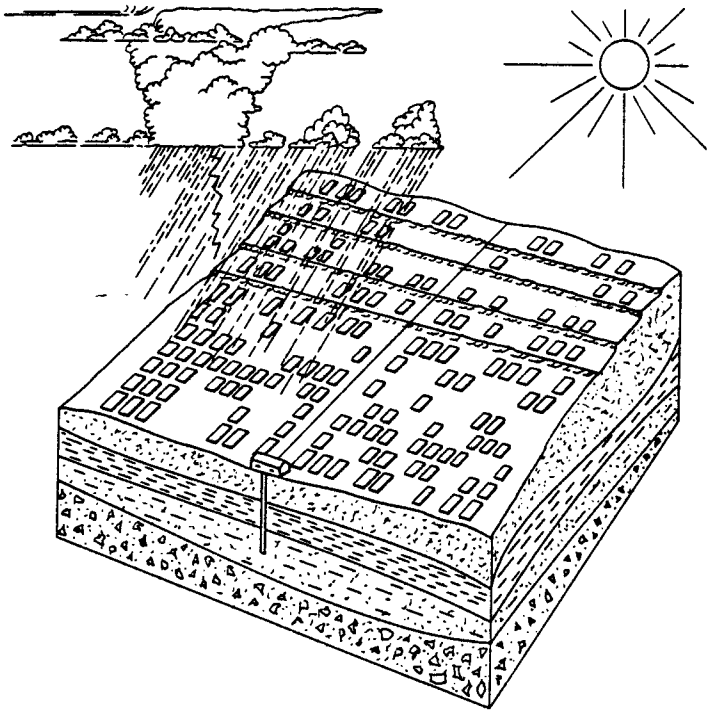


FIGURE 2. MICROALGAE BIOMASS PRODUCTION SYSTEM (ARTIST'S CONCEPT).

Figure 3 shows a familiar diagram of the basic inputs, processes, and products of microalgae production modified to highlight the inputs of climate, water, and land. Resource and environmental parameters were identified for climate, water, and land based upon microalgae system requirements and Southwest conditions. Solar radiation and temperature conditions are important for their influence on algae growth and biomass production. Evaporation and precipitation are important for their influence on make-up water requirements. Sporadic violent storms and flash floods, as are common in the West, are potentially damaging or devastating. Assured availability of a sufficient supply of water to sustain the system over its lifetime is clearly of vital importance. The salinity and chemical character of the water are significant to culture growth conditions and design requirements. The physical and institutional characteristics of land will determine its suitability and availability.

Sources and Characteristics of Resource Data

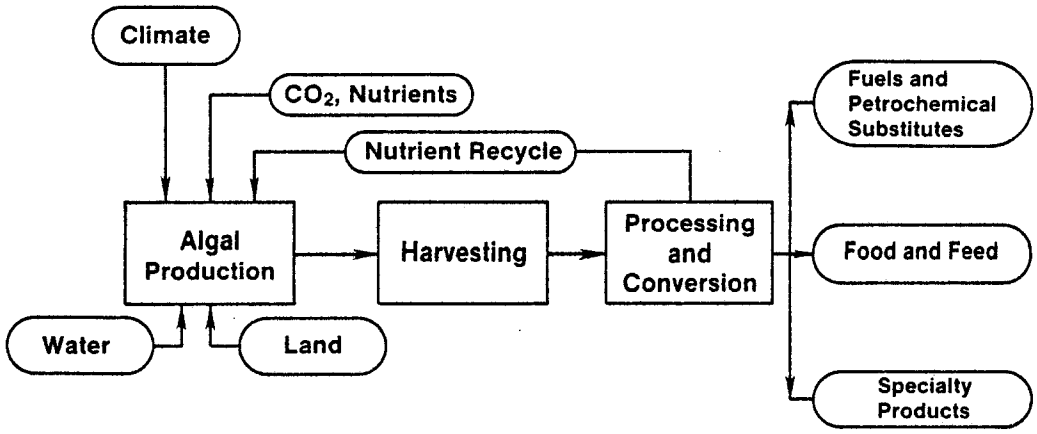
The resource and environmental data available for the stratification vary widely in resolution, quality, and accuracy. For example, insolation data based upon SERI's Solar Atlas [5] (Figure 4) and other climatic data obtained from the National Climatic Center are the best available and are generally of very low resolution. A manual effort to convert U.S. Geological Survey 1:500,000 state topographic maps to a regional map of areas of greater or less than 10 percent slope resulted in a map of considerable complexity and moderate resolution (Figure 5). In the case of saline groundwater resources, the best available source of region-wide data [6] is dated and incomplete due to lack of wells. Using this source, groundwater resources of salinities estimated to be 3,000 mg/l or greater were mapped as shown in Figure 6.

Stratification Process

The objective of the stratification process is to identify zones of varying suitability for MPS based on overlaying of selected geographically-referenced resource data (Figure 7). The data parameters used for this initial stratification are: insolation, freeze-free period, precipitation, evaporation, thunderstorm days, saline groundwater, 10% slope, land ownership, and land use. Maps at a scale of 1:2,500,000 were prepared for each parameter using photographic reduction or enlargement and light-table tracing to a regional base map.

The Problems of Mapping Land Ownership and Land Use/Cover

The mapping of land ownership and land use/cover were particularly tedious and time-consuming. The checker-board mixture of public and private lands, such as shown in Figure 8, is prevalent throughout the western states. Complex patterns of land use result from physical land characteristics and resultant land values as well as from differences in land ownership. Water availability is a predominant factor governing use of much of the land in the generally arid Southwest.



Resource and Environmental Parameters

Climate	Water	Land
Insolation	Location	Topography
Temperature	Supply/Demand	Use/Cover
Evaporation	Salinity	Ownership
Precipitation	Chemistry	Soils
Severe Storms	Allocation	Geology

FIGURE 3. TYPICAL MICROALGAE PRODUCTION SYSTEM WITH PERTINENT RESOURCE AND ENVIRONMENTAL PARAMETERS FOR THE SOUTHWEST U.S.

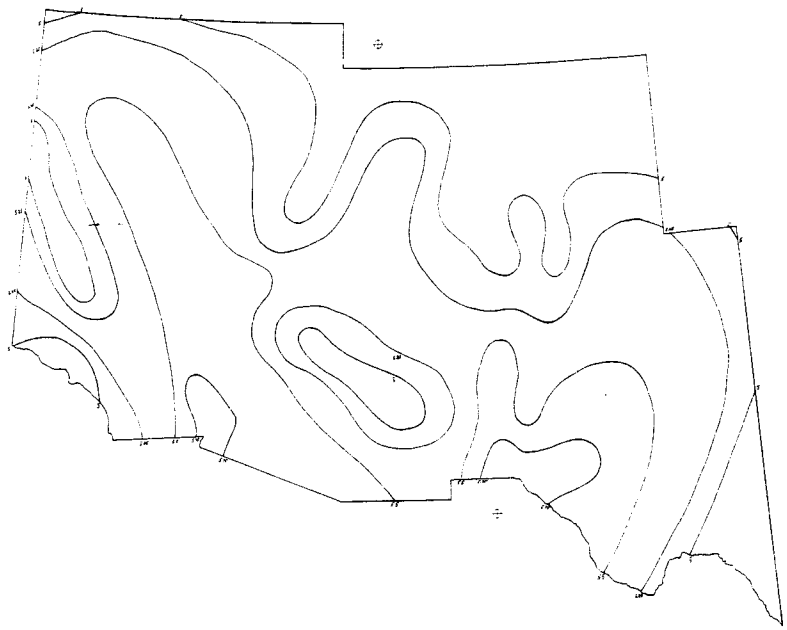


FIGURE 4. AVERAGE GLOBAL HORIZONTAL INSOLATION (KWH/M^2-DAY) (DERIVED FROM REF. 5).

FIGURE 5. AREAS OF GREATER OR LESS THAN 10 PERCENT SLOPE (DERIVED FROM USGS STATE TOPOGRAPHIC MAP SERIES).

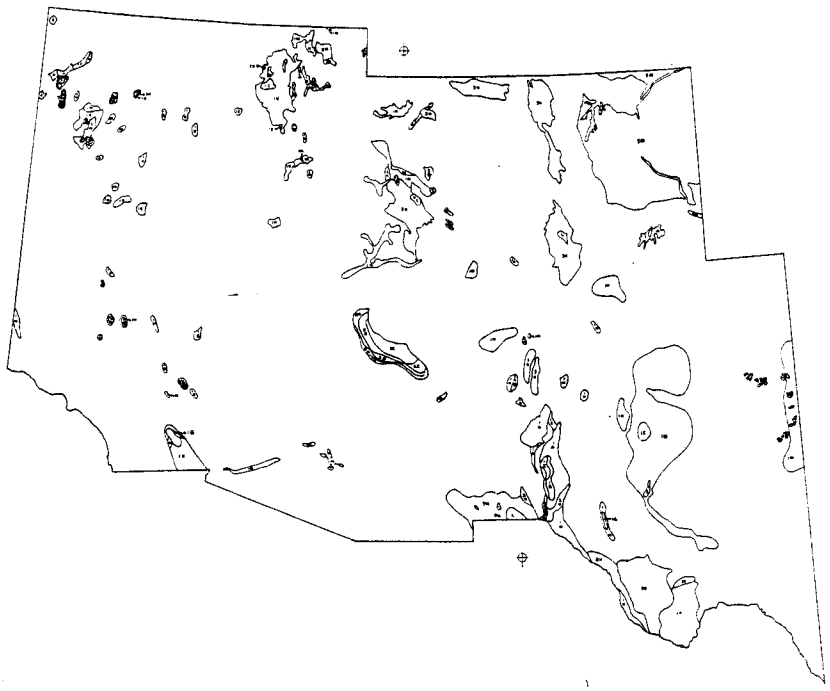


FIGURE 6. KNOWN GROUNDWATER RESOURCES WITH SALINITIES OF 3,000 MG/L OR GREATER (DERIVED FROM REF. 6).

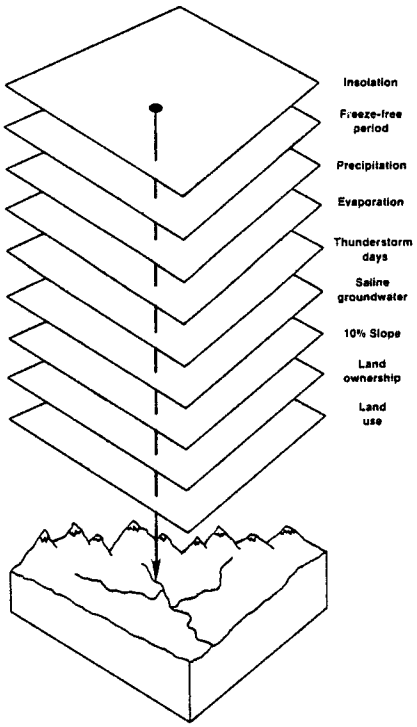


FIGURE 7. OVERLAY CONCEPT FOR RESOURCE STRATIFICATION.

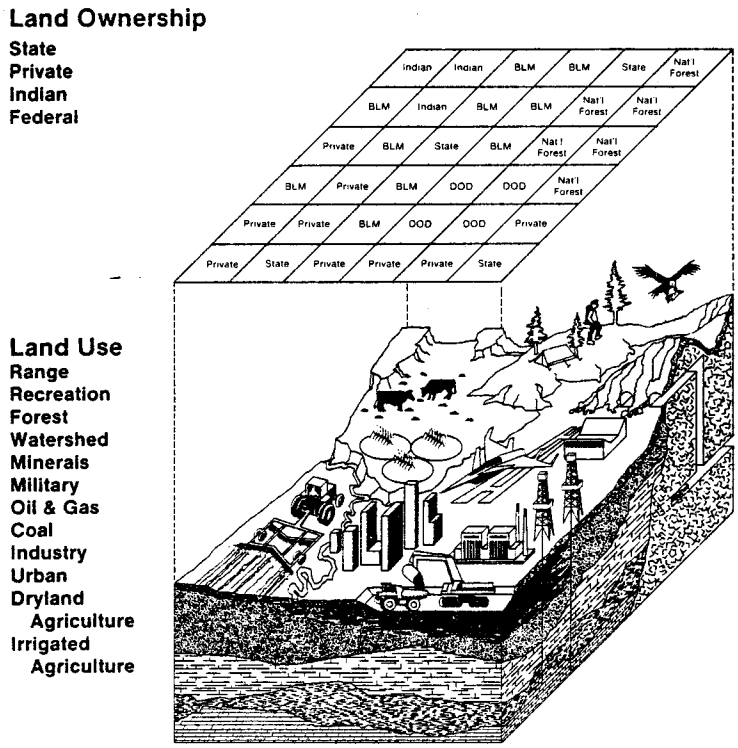


FIGURE 8. LAND OWNERSHIP AND LAND USE IN THE WESTERN U.S.

Land ownership maps at a scale of 1:500,000, such as that of Utah shown in Figure 9, are available from the Bureau of Land Management for all states in the region except Oklahoma, Texas, and California. The California map is at a smaller scale (1:750,000). Other sources were used for Oklahoma and Texas. The maps have a resolution of about 48.5 hectares (120 acres), which is one quarter of a section. (For perspective, Figure 8 shows a land ownership pattern of a hypothetical township, which consists of 36 sections.) In order to have consistent and usable information at a scale of 1:2,500,000, the maps were simplified by ocular identification of the predominant ownership of townships. Twelve land ownership categories, including a mixed category, were used. They are: state; BLM; Indian; military, national forests and grasslands; national parks, monuments, and recreation areas; national wildlife refuges and ranges; wilderness areas; urban areas; private; DOE; and mixed. The "mixed" category was defined as townships having less than two-thirds, but more than one-third, of their area in any one ownership category.

The land use/cover data were similarly complex. Selected Landsat false color composite imagery were ordered from the EROS Data Center of NASA and photo-interpreted using a slightly modified version of the classification system proposed by Anderson et al. [7]. The information was compiled for each state at a scale of 1:1,000,000 resulting in maps such as the one of Utah included as Figure 10.

Computer Storage of Mapped Baseline Data

The climate parameter maps were further reduced photographically to a scale of 1:5,000,000 in order to economize on the effort and costs of coding and digitization for computer data storage. The digitization was accomplished by overlaying the 1:5,000,000 scale maps with a grid of cells 1/8-inch (0.32 cm) by 1/10-inch (0.25 cm) in size, as depicted in Figure 11. Each of these cells represents an area just slightly less than 20,234 hectares (50,000 acres) on the ground. The size of the cell was selected to correspond to the size of a computer line-printer symbol.

The saline groundwater, land ownership, and land use/cover maps were coded and digitized at a scale of 1:2,500,000 because of their complexity and fine level of detail (each cell represents about 5,058 hectares or 12,500 acres). Even so, the land ownership map was further simplified for coding by combining the twelve categories to three broad groups based upon judgements of their relative availability for MPS use and only selected information was extracted from the state land use/cover maps. All the maps were made comparable at a scale of 1:2,500,000 by applying a computer algorithm which doubled the scale of the climate parameter maps. Three examples of the computerized baseline maps are shown as Figures 12, 13, and 14 of insolation, 10% slope, and land ownership, respectively.

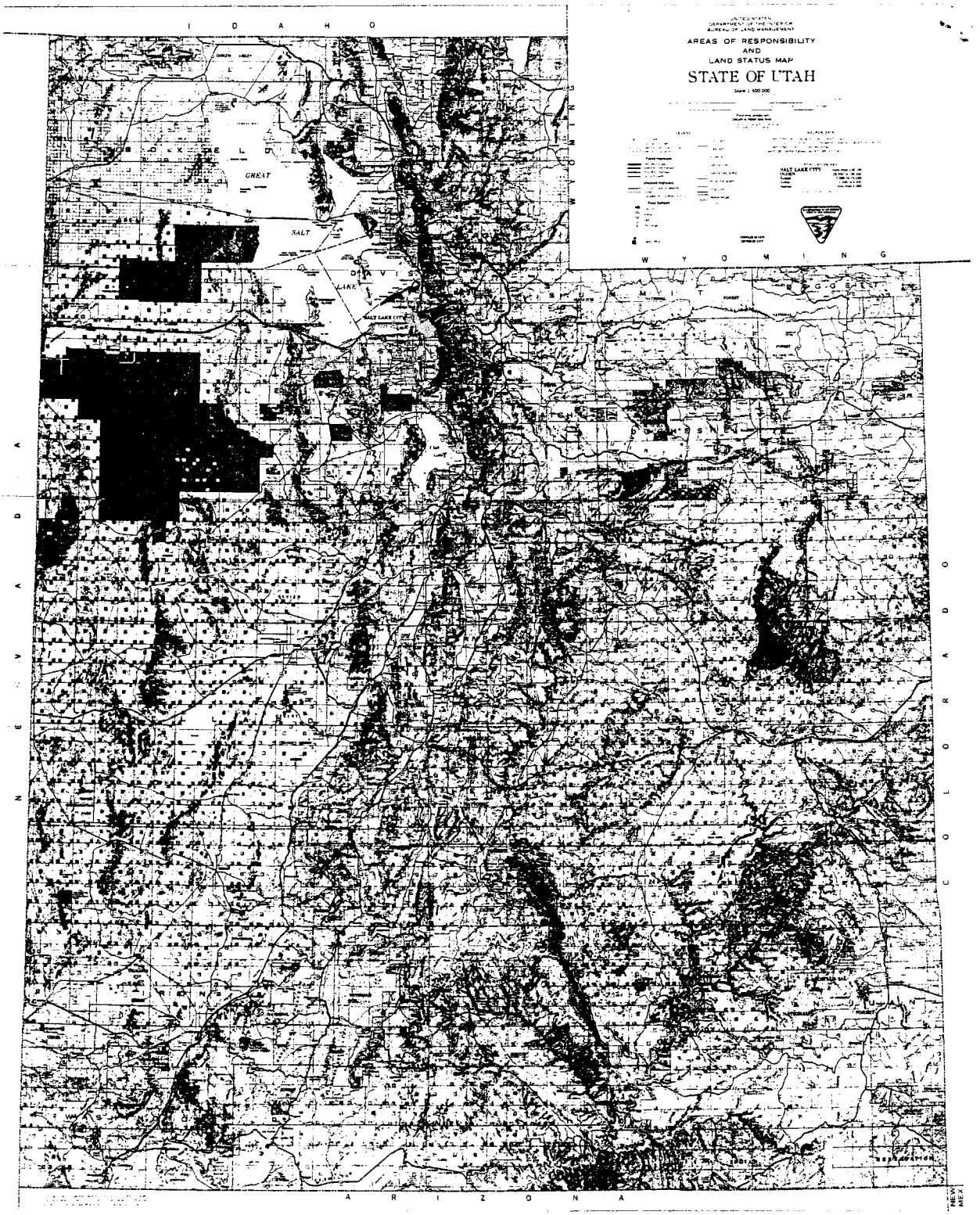


FIGURE 9. LAND OWNERSHIP MAP OF UTAH.

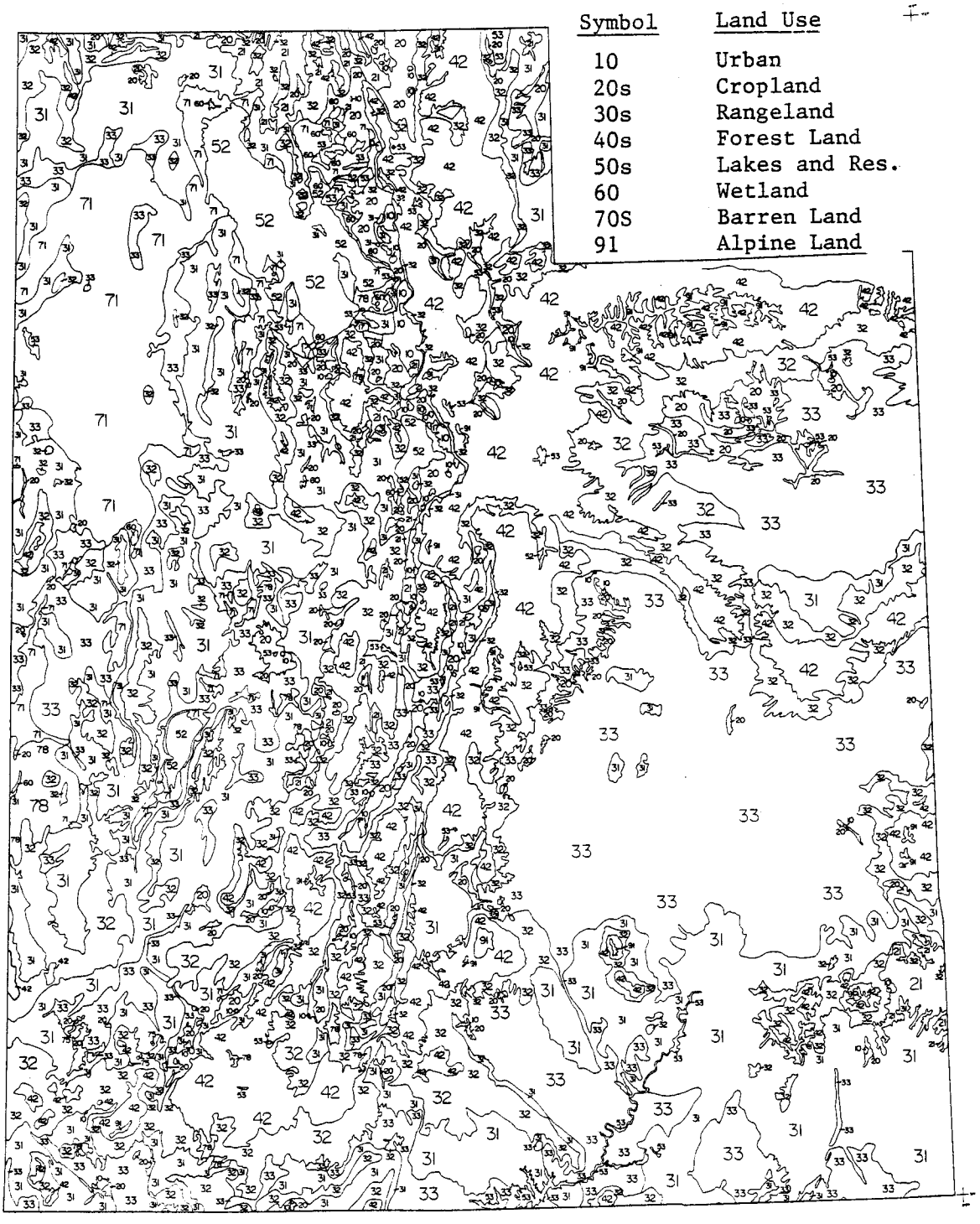


FIGURE 10. LAND USE/COVER MAP OF UTAH.

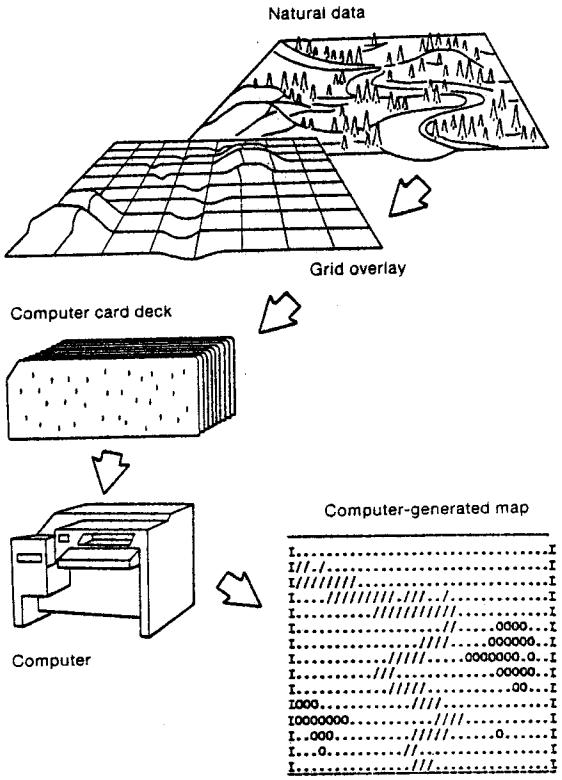


FIGURE 11. CELLULAR MAPPING DATA BANK DEVELOPMENT (FROM REF. 8).

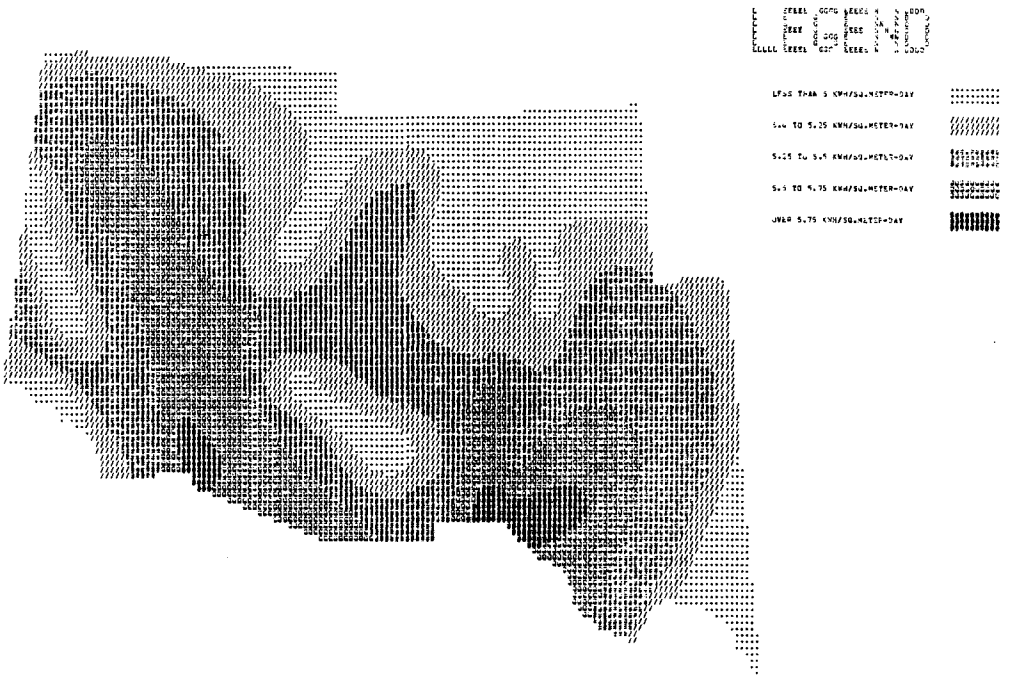


FIGURE 12. COMPUTER BASELINE MAP OF INSOLATION.

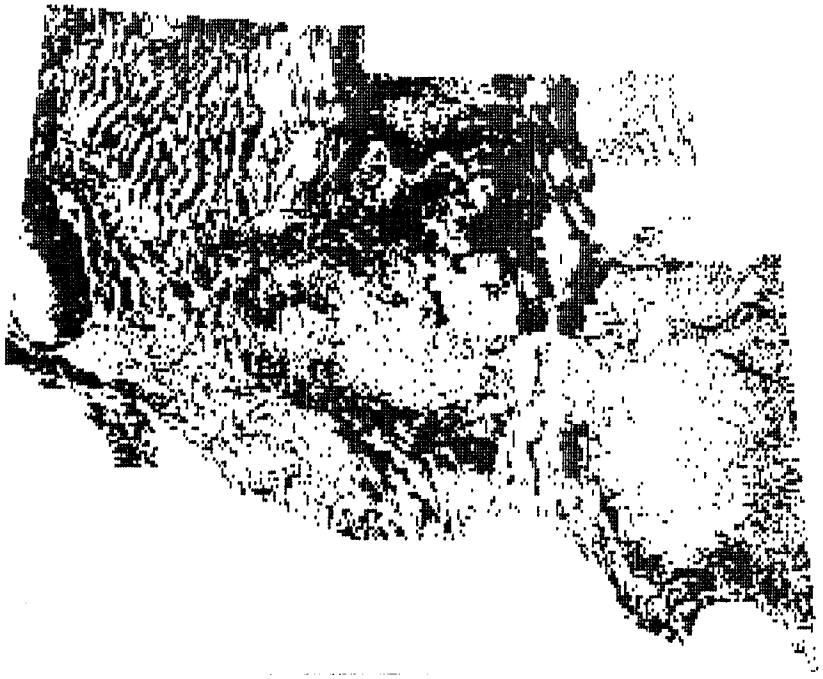


FIGURE 13. COMPUTER BASELINE MAP OF AREAS GREATER OR LESS THAN 10% SLOPE.

FIGURE 14. COMPUTER BASELINE MAP OF LAND OWNERSHIP.

Computer Compositing of Stored Data

In order to stratify the region into zones of varying suitability for MPS, the baseline resource parameter maps were composited through use of algebraic operations and variable weighting coefficients which reflect the relative influence of each parameter on siting, design, performance, and/or costs of MPS. The process used for developing preliminary composite stratification maps is illustrated diagrammatically by Figure 15. The Generalized Map Analysis Planning System (GMAPS) developed by A. K. Turner of Geometronics, Inc., in Golden, Colorado, was employed.

The weighting scheme utilized by GMAPS involves a system of internal and external weights for combining the information contained in the baseline parameter maps. Internal weighting factors (numbers between 0 and 99) are applied to different data categories within a map to reflect their relative significance. External weighting factors (numbers between 0 and 99) are applied to each parameter map involved in a particular composite to reflect the relative importance of each parameter. The values for the composite map are obtained from the sum of the products of internal and external weights and are then rescaled to values between 0 and 9. The rescaled values are represented as gray levels on the composite map using computer line-printer symbols.

The weighting schemes used for generating the composite maps are preliminary at this time. The results are representative, but should not be quoted or reproduced since they will be revised for final publication in the next few weeks. Internal weights are assigned to the data categories of each of the baseline parameter maps on the basis of costs, technical relationships, or some other measure of relative suitability. External weights were assigned to the parameters being composited based upon judgement of their relative importance. A computer program was developed at SERI to test alternative combined weighting schemes in order to ensure that the resulting composites exhibit the desired hierarchy of values.

Generation of Composite Maps

Climate. A map of make-up water requirements (Figure 16) was generated by combining the evaporation and precipitation maps (evaporation minus precipitation). A map of relative productivity (Figure 17) was prepared by combining the parameters of insolation and freeze-free period. A composite map of overall climatic suitability (Figure 18) was then obtained by combining the two preliminary composite maps of make-up water requirements and relative productivity plus thunderstorm days. In this composite equal weights were designated for make-up water and relative productivity; thunderstorm days were weighted at one-quarter the value of the other two.

Water. A composite map of water suitability (Figure 19) was obtained by combining maps of water salinity and water depth below the surface, which were derived from the original parameter map. Equal weights were

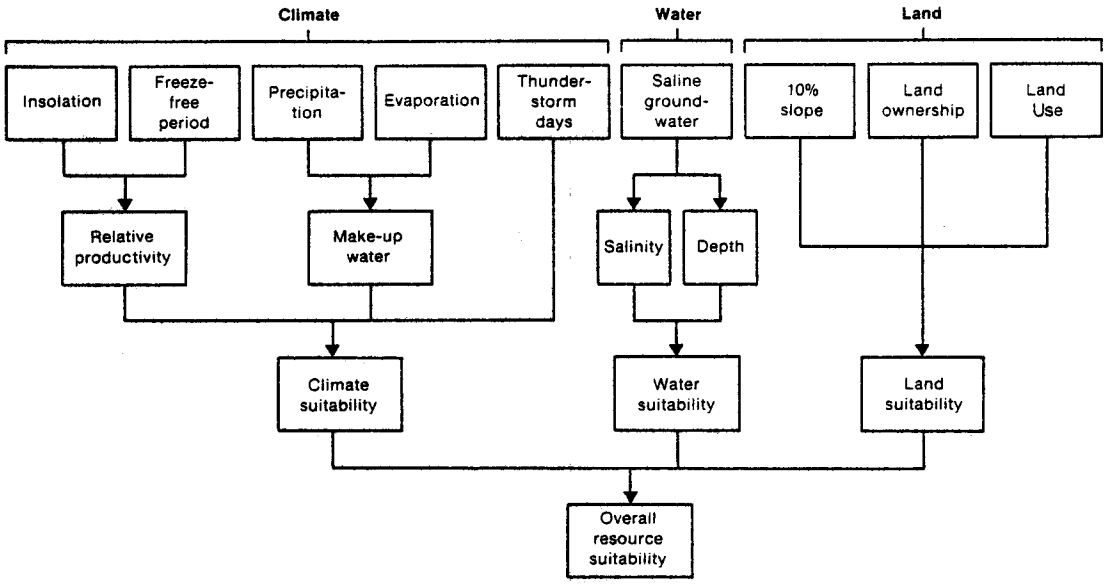


FIGURE 15. RESOURCE DATA MAPPING FROM BASELINE PARAMETER MAPS THROUGH INTERMEDIATE COMPOSITE MAPS TO THE FINAL COMPOSITE STRATIFICATION MAP.

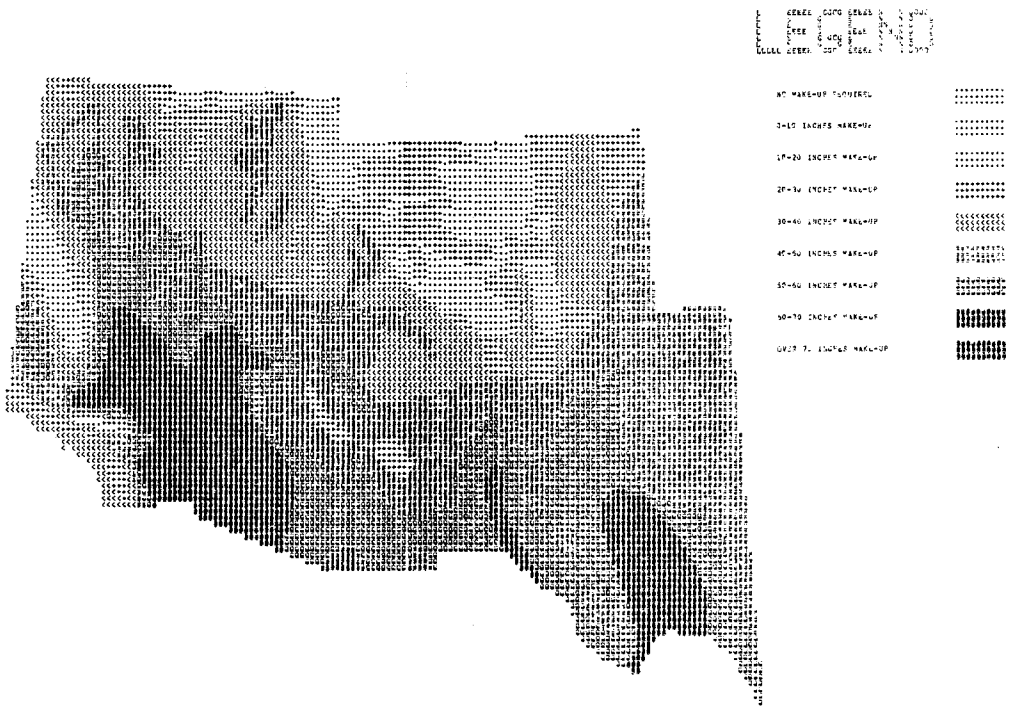


FIGURE 16. COMPUTER COMPOSITE MAP OF MAKE-UP WATER REQUIREMENTS (INCHES/YEAR). DARKER AREAS INDICATE GREATER WATER REQUIREMENTS.

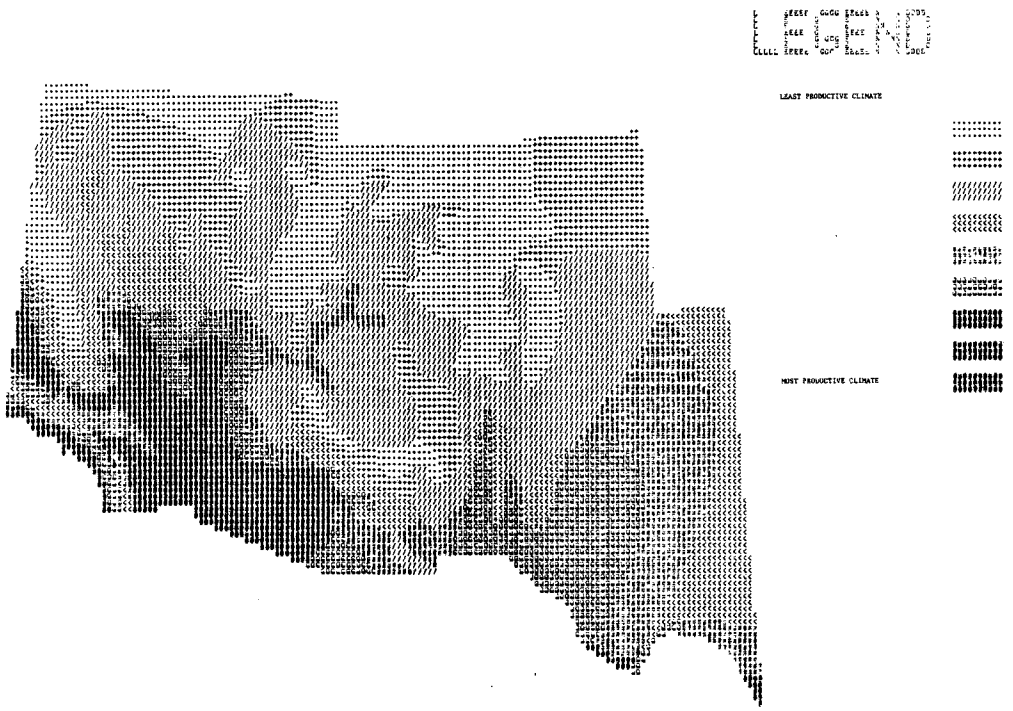


FIGURE 17. COMPUTER COMPOSITE MAP OF RELATIVE PRODUCTIVITY. DARKER AREAS INDICATE MORE FAVORABLE CLIMATIC CONDITIONS.

FIGURE 18. COMPUTER COMPOSITE MAP OF CLIMATE SUITABILITY. LIGHTER AREAS INDICATE GREATER SUITABILITY.

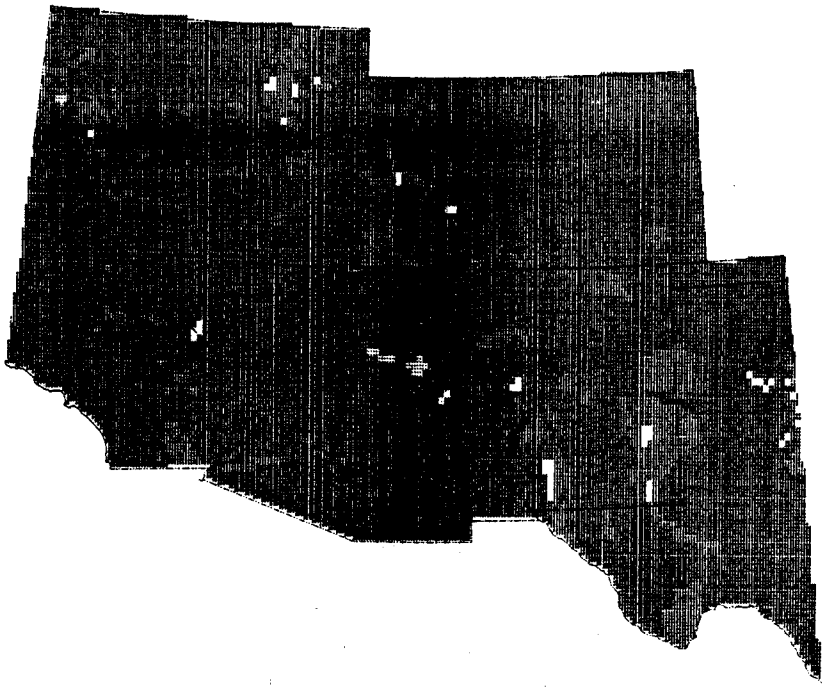


FIGURE 19. COMPUTER COMPOSITE MAP OF WATER SUITABILITY. LIGHTER AREAS INDICATE GREATER SUITABILITY.

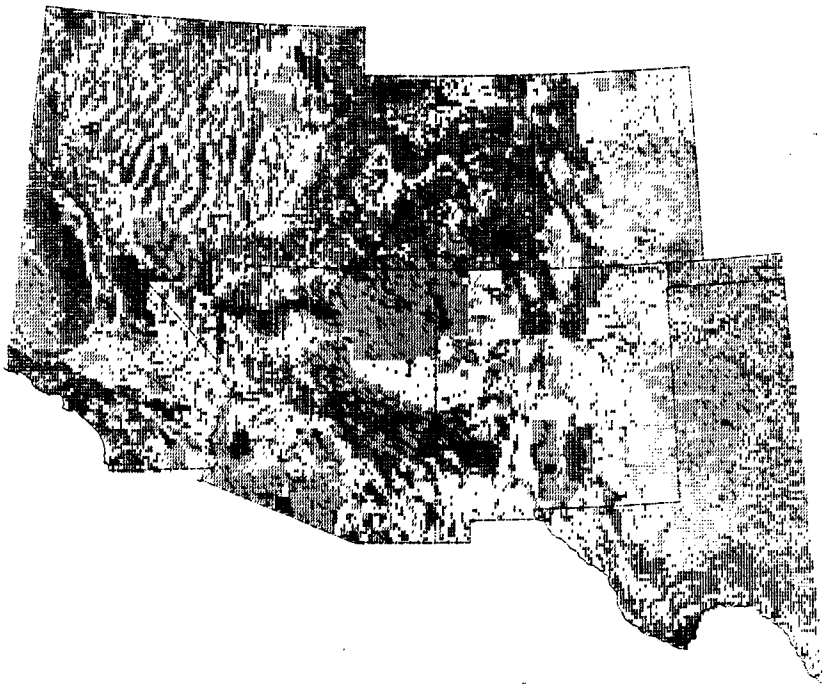


FIGURE 20. COMPUTER COMPOSITE MAP OF LAND SUITABILITY. LIGHTER AREAS INDICATE GREATER SUITABILITY.

assigned to the ranges of values for salinity and depth such that various combinations of these conditions were ranked in a hierarchy of desirability.

Land. The three maps of 10% slope, land ownership, and land use/cover were composited to obtain a map of relative land suitability (Figure 20). Equal weights were assigned to land ownership and land use/cover; the 10% slope map was weighted at 40 percent.

Overall Resource Suitability. Based upon the three composite maps of climate suitability, water suitability, and land suitability, a final composite map was generated to show overall resource suitability for MPS (Figure 21). For this very preliminary composite, the three component maps were given equal weights.

Discussion of Results

Comparison of the three composite maps pertaining to climate, water, and land with the final composite suggests that land parameters are the least restrictive to MPS and that water is the most restrictive resource. This may not be a correct representation since the water suitability composite strongly reflects the sparseness of available water data. Furthermore, the land suitability composite was derived from a modified land ownership map in which state, private, and Bureau of Land Management (federal) land were combined to comprise a category of highest availability. In reality it is likely that there would be differences among these ownerships which would affect relative availability of land for MPS. It should be noted that the steps between levels of suitability (i.e., gray levels on the maps) are not necessarily linear. No attempt has yet been made to quantify the degree of suitability implied.

FUTURE PLANS

Refinement of the Stratification

It is perhaps worth reiterating that the results of the stratification shown here are preliminary and should not be reproduced or cited except with very careful qualification. During the next several weeks, the composite stratification maps will be refined by testing alternative weighting schemes with the aim of producing as realistic a picture as is feasible and practical.

Resource Assessment Plan

Also forthcoming during the next quarter are an executive summary report and an accompanying background report which describe a plan for resource

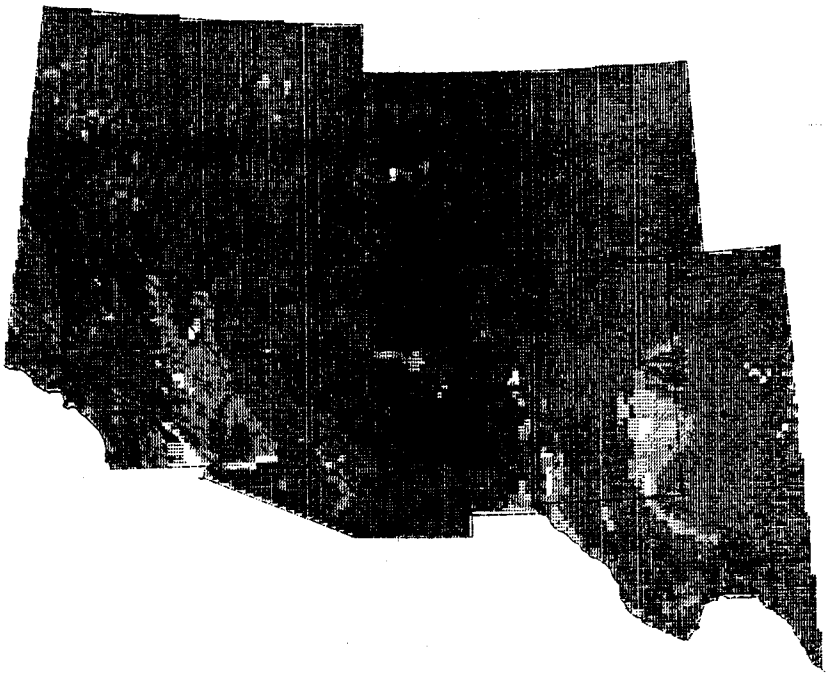


FIGURE 21. COMPUTER COMPOSITE MAP OF OVERALL RESOURCE SUITABILITY. LIGHTER AREAS INDICATE GREATER SUITABILITY.

evaluation and site selection for MPS in the Southwest. These reports incorporate the findings of the stratification process, but their intent is to describe requirements and an approach for evaluation of resource availability. Sources of existing resource data which can be utilized are identified and described in terms of requirements for access. Additional resource data requirements and data gaps are discussed with recommendations provided for obtaining the needed information. In some instances, the specification of additional resource and environmental data needs is contingent upon further research. These critical research needs are described in the reports and are summarized below.

Selection of Prime Test Sites

The SERI/DOE Aquatic Species Program is at a stage in the research and development of microalgae production technology that field experimental facilities should be developed. The stratification and the plan are both directed at providing guidance to selection of areas and sites for such facilities. As outlined in the plan, continuation of the resource evaluation effort would entail development of detailed information for areas identified as having the greatest potential for siting MPS.

Critical Research Needs

In the process of performing this resource evaluation task, many uncertainties and information gaps have become apparent. Following is a list of what are perceived to be the critical resource and environmental research needs for developing MPS in the Southwest. While these topics have been grouped according to climate, water, land, and general needs, no order or prioritization is implied.

- o Length of Growing Season in Shallow Algal Ponds
- o Wind and Wind Transport of Small Particles
- o Severe Storm Studies
- o Improved Estimates of Saline Water Resources
- o Estimates of Sustainable Water Yields
- o Water Law Constraints on Water Availability
- o Soil Physical Property Analyses
- o Environmental and Political Constraints on Land Availability
- o Opportunities for Integrated Systems
- o Procedures for Selection of Specific Sites
- o Carbon and Nutrient Supply Problems
- o Interaction of Extensive MPS with Environmental Systems

PROBLEMS AND VARIANCES

None

LITERATURE CITED

1. Maxwell, E. L. and Folger, A. G. "Resource Assessment for Saline Aquatic Biomass Production Systems". Proceedings of the SERI Biomass Program Principal Investigators' Review Meeting - Aquatic Species Program Reports. SERI/CP-231-1808 (1982), p. 55.

2. Goldman, J. C. "Outdoor Algal Mass Cultures - I. Applications". Water Res. 13 (1979), p. 1.

3. Shelef, G. and Soeder, C. J. (eds.) Algae Biomass: Production and Use. Elsevier/North-Holland Biomedical Press (1980).

4. Benemann, J. R. and Raymond, L. P. An Examination of Aquatic Biomass Production. Final Report to the Solar Energy Research Institute (June 1981).

5. SERI. Solar Radiation Energy Resource Atlas of the United States. SERI/SP-642-1037. U.S. Govt. Prtg. Ofc., Washington, D.C. (1981).

6. Feth, J. H. amd others. "Preliminary Map of the Conterminous United States Showing Depth to and Quality of Shallowest Ground Water Containing More Than 1,000 Parts Per Million Dissolved Solids". U.S. Geol. Survey Hydrol. Inv. Atlas HA-199 (1965).

7. Anderson et al. "A Land Use and Land Cover Classification System for Use with Remote Sensor Data". U.S. Geol. Survey Prof. Pap. 964 (1976).

8. Turner, A. K., Weber, J. C., and DeAngelis, M. A. A Geographic Market Suitability Analysis for Low- and Intermediate-Temperature Solar IPH Systems. SERI/TR-733-1194. U.S. Govt. Prtg. Ofc., Washington, D.C. (1981).

PUBLICATIONS AND MEETINGS

Maxwell, E., Folger, G., and Szwarc, V. "Climate Impact on the Siting of Biomass Production Facilities". Paper presented at the session on Climate Aspects of Renewable Energy Alternatives of the Conference on Climate/Energy Interactions at the 63rd Annual Meeting of the American Meteorological Society, January 10-13, 1983, New Orleans, LA.

ACKNOWLEDGEMENTS

We wish to thank David Hildbold and Bill Larson of Colorado State University for their assistance on cartography and imagery interpretation, the Biomass Energy Technology Division of DOE for providing funding support, and Larry Raymond for conceiving the idea which originated the study.

SYSTEMS OVERVIEW OF ALGAL MASS CULTURE

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OBJECTIVE

Algal mass culture systems built to produce energy will have certain definable characteristics. The allowable cost for such a system will be driven by the cost of liquid fuels which is currently around 10-15¢/lb. Although the price of energy may well rise it will undoubtedly not reach the price range of health food (at $10.00/lb) or specialty chemicals. Thus, algal systems built to produce energy will, from necessity, require low capital, operating and energy costs and will need to produce high yields of energy producing algae on a sustainable basis. Achieving this goal will require a thorough understanding of the technical issues related to yield and cost. Gaining the understanding required will come from fundamental and applied research in the areas of biology, chemistry and engineering as well as supporting systems and design studies.

This study is designed to review and analyze the technical issues, particularly related to energy involved in mass culturing algae in outdoor systems. Our study has three major parts. First, to set out to review algal systems built throughout the world and compare their characteristics with systems likely to be built to produce energy. Second, we have tried to review the technical literature on algae including topics such as light utilization, predation, genetic engineering, lipid production and nutrient requirements. Third, based on the previous reviews and analyses, we will attempt to recommend R&D tasks which need initiation or additional support to move algal mass culture systems for energy closer to feasibility. Analysis from a systems perspective is important to achieve a comprehensive overview of a complex technical program simultaneously advancing on several scientific fronts. The perspective provided to SERI in our reports can become one tool in their technical decision making processes.

ACCOMPLISHMENTS

Our accomplishments during the previous reporting periods are:

- Algal Mass Culture Technology Review of Patents. The patent search includes abstracts of all the patents issued by the U.S. Department of Commerce Patent and Trademark Office in the area of algal culture from October, 1867 to September, 1982. Additionally, the front end of the report is a brief analysis of patent activity in this field, and what it indicates, and listings of patents by company, country and topic. Seven patents of particular importance to the ASP are copied in full. There are several important reasons for reviewing the patent literature. It allows one to

learn what innovative ideas are being applied to algal technology today, as well as to get a historical perspective on algal culture since patents inherently present a time line of a technology's development.

- State of Microalgal Energy Technology. This paper is a condensed version of a microalgal state-of-the-art report to be sent to SERI in the next reporting period. It contains, in addition to technical information, suggested program goals for the ASP.

- Technical White Papers. Technical white papers have been prepared for SERI's use in developing program documentation.

- Review of Algal Culture Systems. Culture systems built throughout the world for various purposes, i.e., wastewater treatment and protein production, are being analyzed for their utility and applicability for energy production.

Data Collection

The data for the above studies was obtained from the original literature and discussions as well as from review articles. The importance of these documents and of the JAYCOR effort to the SERI program is the systems perspective we apply to the mass culture of algae. Rather than looking at algal culture systems from solely a biological, engineering or economic point of view, we try to understand how all these aspects of mass culture relate to growing algae as a source of energy.

Data and Figures

History. The current work in algal culture technology in the U.S. is traceable back to the work conducted by the Carnegie Institution of Washington in the 1940s and 1950s. The research conducted under the auspices of this group addressed issues in mass culture which remain important and, to a large extent, unsolved at the present time. The focus of the Carnegie research was not energy but protein production. Studies were initiated prior to the "green revolution" and during a period of world-wide protein shortages. Most experiments used Chlorella as the test organism because of its potential as a dietary supplement. It is of more than historical interest to review the work of the Carnegie Group; the innovative culture systems examined at that time may have application in energy systems. Two examples of these culture systems demonstrate this: one set out to grow algae in transparent shallow tubes, while a second employed a rocking tray for growth. Both systems were designed to increase utilization of available light, which has frequently been identified as a limiting factor in algal culture. Other groups have continued the tradition of carrying on basic research to improve the yield or economics of culture systems. In Czechloslovakia, a large experimental effort culminated in the construction of a production apparatus with a thin film cascading culture system in which the algae flowed by gravity from one trough to another. This system was also designed to increase light utilization efficiency. The

Japanese sponsored research programs at the same time the work at the Carnegie Institution was going on. The result of their research was the design and operation of ponds for the production of algae for health food both in Japan and Taiwan. Germany also had an extensive algal culture research program, which began even prior to World War II, to produce fuels. Spinoffs from the German research have been transferred to several third-world countries where German-designed ponds are operated for protein production or as part of integrated farm operations. SERI is continuing in this tradition by sponsoring and conducting basic biological and engineering research for algal mass culture, but focused once again on fuel as a product.

Comparison of Systems

Algal mass culture systems can be placed in groups based on different criteria. One way to group algal systems that has been used in the past depends on pond design. In this case, for example, shallow ponds may be placed in entirely different categories than deeper ponds although both these systems may share other important features in common. We have chosen to group algal mass culture systems on the basis of the type of end product produced. This classification seems appropriate because it is the value of the end product which determines how sophisticated and costly a production system can be built. Naturally, as with any classification scheme, the division between one group and another is not as clean and clear-cut as one could hope. Nevertheless, grouping algal production systems as to their end product makes sense conceptually and has relevance to the issue of growing algae to produce energy.

Figure 1 is a comparison of three systems analyzed by Benemann [1], with a hypothetical energy system. Comparisons between systems are difficult to make because of variations in assumptions in various engineering parameters. The Japanese process for health food production has high capital costs. The cost of algae from this system was calculated to be around $19.00/lb. The cost to produce algae in the Japanese systems can be assumed to have come down as the current value of health food algae is approximately $10.00/lb. The German system was built to produce single cell protein as a food for third-world countries. Much lower capital costs resulted in a cost of around $3.00/lb for the product. Part of the reason for the high cost of algae from the German system is the small scale of the pond, and lining the pond. Clearly the cost of producing algae in either of these systems is between one and two orders of magnitude higher than we have available, given the value of energy. The relatively high capital cost and low productivities of systems of these types are not feasible for energy production.

Benemann also analyzed a low-cost pond system he designed for its applicability to energy production. In this system capital costs were kept to a minimum and in the best case algae were produced for around 40¢/lb. In order for this system to produce energy at 10¢/lb, the current value, the yield of algae would need to be approximately 80 tons/acre/year. And this increase in productivity would necessarily have to be achieved with little or no additional

	Benemann Pond	German SCP Production	Japanese Health Food	Energy
Yield	30 tons/acre/year (65 bbl/acre/year at 40% lipid)	35 tons/acre/year	25 tons/acre/year	80/tons/acre/year (230 bbl/acre/year at 50% lipid)
Production Cost	$ .40/lb	>$ .50/lb	>$2.00/lb	$ .10/lb - .15/lb (price of energy)

Reference: Benemann, et. al., Microalgae as a Source of Liquid Fuels

FIGURE 1

COST OF PRODUCING ALGAE

capital and operating costs. Increases of this magnitude in yield can only be achieved by significant biological and engineering advances. Potential breakthroughs can be achieved in many ways, through classical and genetic engineering, by manipulation of biochemical pathways or through innovative bioreactor design.

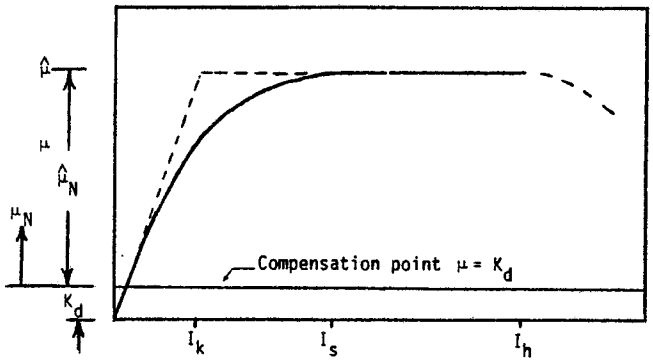
We have considered several approaches from a broad systems perspective to decrease cost or increase productivity. We will focus here on increasing yield by increasing light utilization efficiencies.

Light Utilization

Understanding the relationship between growth rates and light utilization is of critical importance in culturing algae. The response of algal growth rate (μ) in an optically thin culture to light intensity (I) is shown in Figure 2. Several features of this curve are important to outdoor algal mass culture. At low light intensities, there is a steep linear relationship between growth rate and light intensity. In physical terms, this initial steep slope of the light curve is controlled by the light reactions of photosynthesis [2]. Thus, this portion of the curve corresponds to algal growth conditions of maximum possible photosynthetic efficiency [3]. As the light intensity is further increased, at a certain light intensity (I_s), the light curve flattens out and additional available light does not result in any additional increase in growth rate. This portion of the curve corresponds to the region where the dark reactions (carbon cycle reactions) become rate limiting; these set up an upper bound to photosynthesis. In fact, if the light intensity is increased further, eventually there is a falling off in photosynthetic activity resulting from light inhibition [4, 5, 6, 7]. In some cases, light inhibition has been shown to start at intensities as low as 10% of full sunlight [7]. As a result, light inhibition may be an important factor affecting productivity in outdoor mass culture and cannot be ignored.

The above discussion deals in system-level or engineering measurements. It is often useful to relate these to more fundamental measures. One such critical biological parameter is the efficiency with which the available radiant energy is converted to stored chemical energy in the form of organic biomass, which is referred to as the photosynthetic efficiency. On a system-level scale, this efficiency is observed to correlate with the two phenomena shown on the light response curve (Figure 2): the initial slope of the curve and the magnitude of the intensity of light at saturation (I_s). Determination of the maximum possible photosynthetic efficiency has been the subject of exhaustive research among plant physiologists and its value still remains controversial [3, 8, 9, 10]. On a biological level, the photosynthetic efficiency of the algal cells themselves is dependent on:

- the amount of energy required to assimilate a molecule of CO_2. It is generally accepted that a minimum of 8 quanta are required per CO_2 assimilated in photosynthesis. More typically, 10-12 quanta are needed [10, 11, 12].

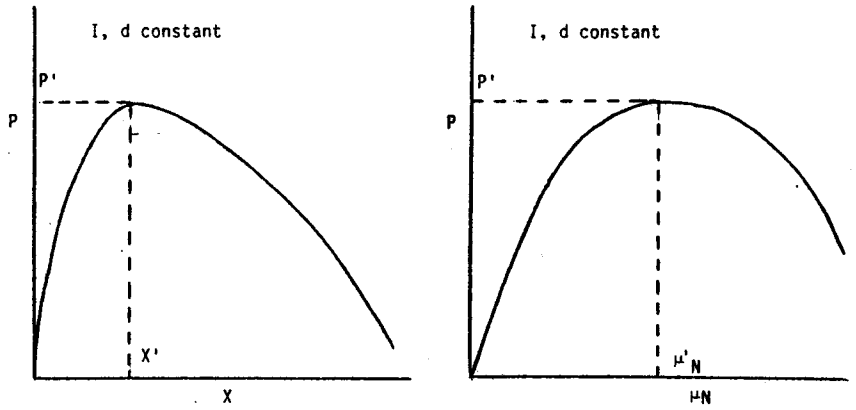


I_k is the light intensity at which the growth rate is half the maximum growth rate. I_s is the light intensity at saturation and I_h is the light intensity at photoinhibition. K_d is the compensation point where the growth rate of the culture is equal to the decay rate (death, lysis, etc). The assumption is made that K_d remains constant at all light intensities.

REFERENCE: 10

FIGURE 2

RELATIONSHIP BETWEEN ALGAL GROWTH RATE AND LIGHT INTENSITY



Dissipation of Light With Depth According to Beer-Lambert Law (10)

FIGURE 3

INCIDENT RADIATION (cal cm^{-2} min^{-1})

- wave length of light available. Photosynthesis does not occur with equal efficiency throughout the visible light spectrum. Photosynthetic absorption of sunlight occurs preferentially in the visible region (400-700nm).

The photysynthetic efficiency described above relates the conversion of incoming sunlight into chemical energy by a single algal cell. In practice, another efficiency which measures light utilization is the efficiency of the production system. This is a function of the attenuation of light in a vertical water column with no algae present. Figure 3 compares the dissipation of light with depth between low incident radiation (representing a cloudy day) and high incident radiation (full sunlight). Although light penetration is greater with the high incident light intensity, the relative fraction of light wasted is also greater. Thus, although, on a sunny day, more light is available for photosynthesis (as represented by the shaded area) and the yield of biomass is greater, in fact the efficiency of light use can be considerably reduced under sunny conditions [2].

Shelef has calculated a theoretical maximum photosynthetic efficiency using light with the spectral distribution of the sun under outdoor conditions. He found the maximum photosynthetic efficiency could rarely surpass 12% [12]. If it were possible to grow algae at that efficiency, conceptually, yields as high as 165 tons/acre/year would be possible. For a comparison, maximum short-term (i.e., over the peak growing season) photosynthetic efficiencies of some agricultural crops are shown in Figure 4 [13]. Sugar beets grown in the U.K. have the highest short-term photosynthetic efficiencies at 4.3% for the growing season.

Considering the various components of the light curves and characteristics of sunlight, Goldman has estimated the practical upper limit of outdoor system yields to be between 30-60 grams dry weight meter^{-2} day^{-1} [2]. This calculation does not consider decay reactions (cell lysis, death) or light inhibition at high light intensities. Goldman points out that yields of this magnitude have been achieved only for short periods in outdoor mass culture systems and sustained yields have been less. Figure 5 shows high yields reported for several outdoor mass culture systems operated to produce protein.

How then, considering the limitations of light utilization, is it possible to achieve the yields of 30-60 grams per meter^{-2} day^{-1} or higher on a sustainable basis, which are likely to be needed for a practical algae-based system?

It is important to realize that two major effects are occurring simultaneously in algal mass culture and that changes in either or both will affect yield. First, each individual algae can function at some given photosynthetic efficiency which depends upon intrinsic characteristics of the cell. These characteristics include the absorption spectra of the algal photosynthetic pigments, the number of quanta of energy it requires to fix CO_2 and the intensity of light at which its photosynthetic processes reach saturation (I_s). Improvements in these biological and physical characteristics of the cell

Crop	Country	$g/m^2/day$	Photosynthetic efficiency (% of total radiation)
Temperate			
Tall fescue	U.K.	43	3.5
Rye-grass	U.K.	28	2.5
Cocksfoot	U.K.	40	3.3
Sugar beet	U.K.	31	4.3
Kale	U.K.	21	2.2
Barley	U.K.	23	1.8
Maize	U.K.	24	3.4
Wheat	Netherlands	18	1.7
Peas	Netherlands	20	1.9
Red clover	New Zealand	23	1.9
Maize	New Zealand	29	2.7
Maize	U.S. Kentucky	40	3.4
Sub-tropical			
Alfalfa	U.S. California	23	1.4
Potato	U.S. California	37	2.3
Pine	Australia	41	2.7
Cotton	U.S. Georgia	27	2.1
Rice	S. Australia	23	1.4
Sugar cane	U.S. Texas	31	2.8
Sudan grass	U.S. California	51	3.0
Maize	U.S. California	52	2.9
Algae	U.S. California	24	1.5
Tropical			
Cassava	Malaysia	18	2.0
Rice	Tanzania	17	1.7
Rice	Phillippines	27	2.9
Palm oil	Malaysia (whole year)	11	1.4
Napier grass	El Salvador	39	4.2
Bullrush millet	Australia, NT	54	4.3
Sugar cane	Hawaii	37	3.8
Maize	Thailand	31	2.7

FIGURE 4

SOME HIGH SHORT-TERM DRY WEIGHT YIELDS OF CROPS
AND THEIR SHORT-TERM PHOTOSYNTHETIC EFFICIENCIES

Location	Organism	$g/m^2/day$ yield
South Africa	Chlorella sp	19[A]
Taiwan	Chlorella sp	30-40[B]
Thailand	Scenedesmus acutus	15[C]
Peru	Scenedesmus sp	20-25[D]
Israel	Unspecified mixed culture	11-37[E]

A. Algae grown in winter with temperature control. N and P added, minimum temperature 22°C.

B. Mixotrophic systems: CO_2 and organic carbon are added.

C. Yields remained low due to a parasitic infection.

D. Yields are measured during periods of high irradiation.

E. Mixed cultures of algae and bacteria - algal yields only reported.

FIGURE 5

REPORTED HIGH YIELDS FROM MASS CULTURE SYSTEMS

would result in higher photosynthetic efficiencies and therefore higher yields. For example, approximate values of the light saturation intensity are available for a few algal species. Based on the data shown in Figure 6 [2], the marine dinoflagellates have the highest light saturation value of the algae listed, thus permitting marine dinoflagellates to convert more light into chemical energy at high irradiances. However, marine dinoflagellates are difficult to grow in culture and are never the dominant species in outdoor mass cultures of marine algae [14, 15]. What is needed in a mass culture system then is an organism which couples the high light saturation values found in marine dinoflagellates with the hearty growth characteristics found in other organisms such as Phaeodactylum tricornutum. Such an organism might be obtained through selection or genetic manipulation.

The second major effect in mass culturing algae is the efficiency of the overall production system. The challenge is to design a system which makes the unused light present at the system's surface available to grow algae at greater depths. As was shown in Figure 4, increases in incident light do not necessarily translate into proportionately higher yields because more of the light may be wasted and not utilized by algae. A number of systems have been envisioned which distribute this unused light into the culture vessel. One example, from the patent literature (illustrated in Figure 7) shows a meandering algal culture in which alternating bands of light and dark have been established throughout the depth of the culture and perpendicular to the flow. This design was based on using an artificial light source, but mechanisms for gathering natural sunlight could be substituted [17].

One issue in mass culturing algae which has received some attention but seems far from being completely resolved is the effect of mixing the algal culture on light utilization. It is known, for example, that the light utilization efficiency of algae can be increased by exposing cells to alternating periods of light and dark [18]. Experiments have shown that flash periods on the order of 10-70 msec may result in significant enhancement of photosynthetic efficiencies [19, 20]. However, such short flash periods would seem to be impossible to achieve by randomly moving cells through the algal culture. Nevertheless, it is observed that adding turbulence or mixing to any outdoor system will enhance system yield [19]. The question which remains to be addressed is how much of the increased yield due to mixing is a result of increasing system efficiency (nutrient mixing, prevention of cell settling and thermal stratification) and what portion of the increased yield is due to actual improvement in photosynthetic efficiencies.

Mechanisms for Increasing Light Utilization Efficiency

Even without focusing on the flashing light effect, there are some general observations that can be made on light utilization based on the nature of the generic light curve shown in Figure 2. The microalgae growing near the pond surface are likely to be exposed to light in excess of I_s which will be wasted by absorption as heat, or through scattering. In the worst case, light inhibition will decrease the growth rate in those regions. The microalgae at

Species	Temperature (C)	I_s Illuminance (ft-c)	I_s Irradiance (cal cm^{-2} min^{-1})
Freshwater			
Chlorella pyrenoidosa	25		0.036
	25	500	0.025
	26		0.057
Chlorella vulgaris	25	250	0.013
Scenedesmus obliquus	25	500	0.025
Chlamydomonas reinhardti	25	500	0.025
Chlorella pyrenoidosa	25	500	0.025
(7-11-05)	39	1400	0.070
Marine			
Green algae (7)	20	500	0.025
Diatoms (3)	20	1000	0.050
Dinoflagellates (4)	20	2500	0.125
Phaeodactylum tricornutum	18		0.057

FIGURE 6

SUMMARY OF LIGHT SATURATION INTENSITIES (I_s) FOR DIFFERENT FRESHWATER AND MARINE MICROALGAE REPORTED IN THE LITERATURE

Reference: 2

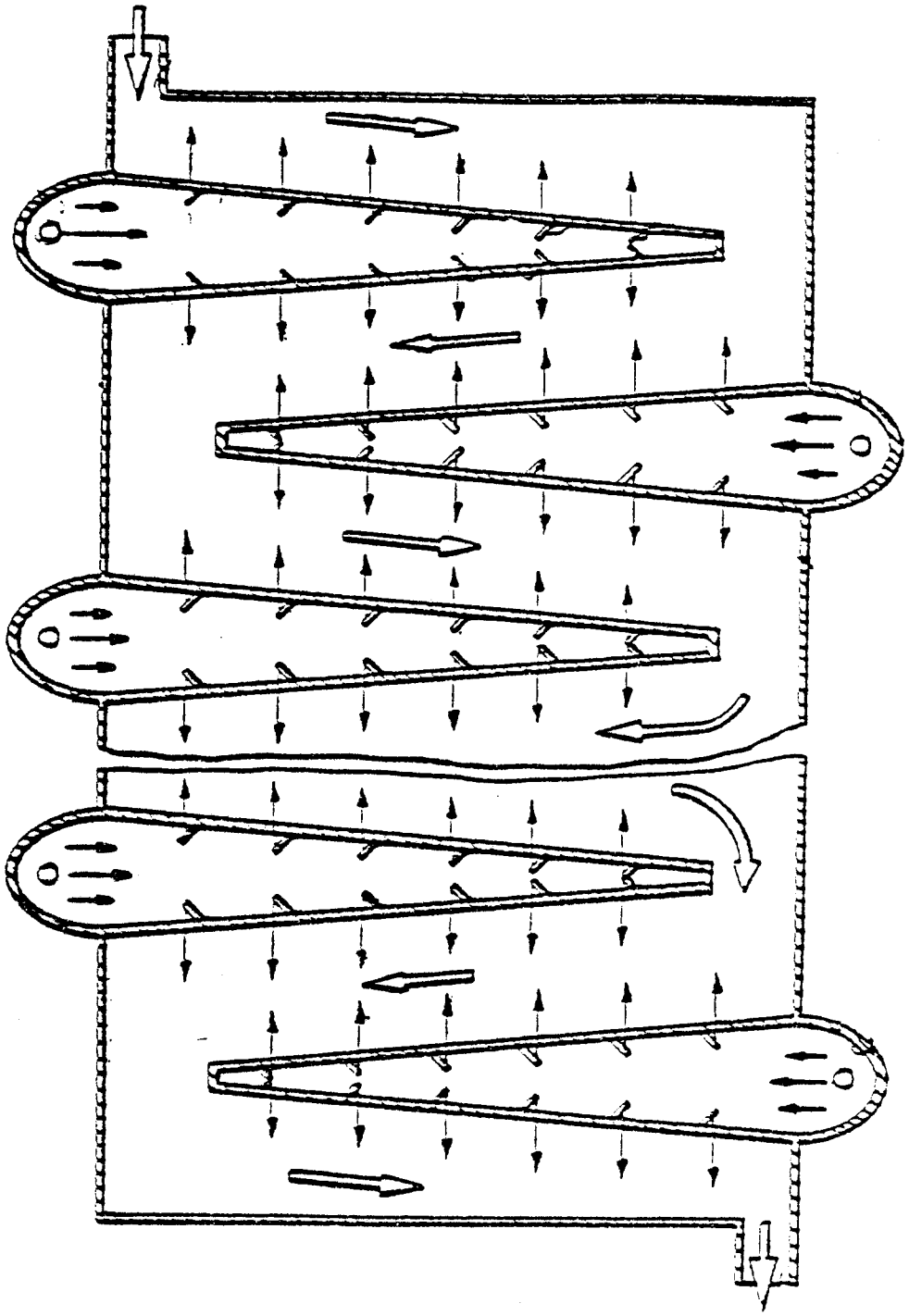


FIGURE 7

ALGAL CULTURE WITH ALTERNATING BANDS OF LIGHT

Reference: 17

the bottom of the culture, in contrast, will receive insufficient light intensity and, while perhaps operating at high photosynthetic efficiency, will have reduced growth rates. In qualitative terms, it is clear that the productivity when integrated over the depth of the pond is not maximized in terms of light utilization.

A quantitative estimate can be made of the potential benefit of such enhanced light utilization. The theoretical maximum photosynthetic efficiency of conversion of sunlight to biomass by microalgae has been estimated to be about 12%. If it were possible to grow microalgae in ponds at this efficiency, their yields would be on the order of 165 tons/acre/year. In practice, the best sustainable yields in outdoor ponds have been of the order of 22 to 44 tons/acre/year [10]. In laboratory experiments growth rates corresponding to productivities as high as 74 tons/acre/year have been measured [21]. In practical terms, then, we are talking about increasing pond yields by up to factors of five over current productivities.

In general, it is clear that one wants to decrease the average light intensities seen by the microalgae in the top regions of the culture and to increase the average intensities seen at the bottom. One potential way to accomplish this is by channeling light throughout the pond by some combination of plastic lenses, light pipes and the like. A particular example might be to utilize hollow vertical plastic cones standing at various points within the pond. Light striking the larger tops of the cones would be channeled to depths in the pond and then pass out of the cones into the culture.

A second general approach is based on the observation that the microalgae tend to float independently, carried along in regions of the fluid flow. Thus, one can continuously redistribute the microalgae to different points in the flow (and thus to different light intensities) by continuously mixing the flow in a controlled fashion. One way to do this is by introducing "airfoils" or other obstacles into the flow, an approach being studied at the University of Hawaii. A third general approach is based on the observation that one may need different light intensities at different phases of the algal growth cycle. Thus, for example, one might flow the algae through ponds of varying depths or ponds utilizing light in different ways. The algae would then pass into a sequence of ponds as they pass through phases of chlorophyll production, biomass growth, lipid production and harvesting.

Finally, one could look for ways to enhance the flashing light effect by exposing the bulk of the culture to alternating periods of light and dark. If the duration of the desired light is really on the order of a fraction of a second this poses a significant engineering challenge for an outdoor pond. One possible approach we have devised, however, is to use an inexpensive plastic pond cover which would serve as a series of cylindrical Fresnel lenses. This would concentrate the sunlight at the surface into alternate bands of light and dark perpendicular to the direction of pond flow. If one then introduced "airfoils" or other obstructions into the flow, one could also produce periodic vertical mixing of the microalgal culture flowing through this

portion of the pond. By adjusting the flow rates and periodicities of the incident light and the vertical mixing one could, in principle, create "beat frequencies" with each portion of the microalgal culture being exposed near the surface to short intense bursts of sunlight followed by relatively longer dark periods.

Some of these various design approaches have been suggested in the literature. However, there has not been any systematic way of evaluating the potential effects of various approaches, short of the expensive and time-consuming process of constructing ponds. One approach to do this would be to adapt some of the many fluid dynamic and ray tracing computer codes that have been developed over the years in other fields to look at the complex steady state behavior in microalgal ponds. If this were successful, these sophisticated computer codes would be of enormous utility in future algal systems design work.

FUTURE PLANS

Plans for the next quarter are to complete the review of algal mass culture systems and the review of the technical parameters and synthesize the information into a set of research recommendations for SERI's consideration.

REFERENCES

1. Benemann, J. R., Goebel, R. P., Weissman, J. C., and D. C. Augenstein. 1982. Microalgae as a source of liquid fuels. Final Report, DOE.

2. Goldman, J. C. 1979. Outdoor Algal Mass Cultures - II Photosynthetic Yield Limitations. Water Research. 13: 119-136.

3. Kok, B. 1960. Efficiency of photosynthesis. Handbuch der Pflanzenphysiologie. Ed. Ruhland, W. Springer, Berlin, 566-663.

4. Abeliovich, A. and H. Shilo. 1972. Photo-oxidative death in blue-green algae. J. Bact. 111: 682-689.

5. Eloff, J. N., Steinitz, Y. and M. Shilo. 1976. Photo-oxidation of cyanobacteria in natural conditions. Appl. Envir. Microbiol. 31 119-126.

6. Myers, J., and G. O. Burr. 1940. Studies on photosynthesis: some effects of light of high intensity on Chlorella. J. Gen. Physiol. 24: 45-67.

7. Ryther, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. Limnol. Oceangr. 1: 61-70.

8. Emerson, R. 1958. The quantum yield of photosynthesis. A. Rev. Pl. Physiol. 9: 1-12.

9. Ng., K. and J. A. Bassham. 1968. The quantum requirement of photosynthesis in Chlorella. Biochem. Biophys. Acta. 162: 254-264.

10. Rabinowitch, E. I. and Govindjee. 1968. Photosynthesis. John Wiley, N.Y.

11. Kok, B. and R. Radmer. 1976. Mechanisms in photosynthesis. Chemical Mechanisms in Bioenergetics. Ed. Sanadi, D.R. Amer. Chem. Soc. Monograph 172: Washington, D.C., 172-220.

12. Shelef, G., Moraine, R. and G. Oron. 1978. Photosynthetic biomass production from sewage. Arch. Hydrobiol. Beih. Ergebn. Limnol. 11: 3-14.

13. Hall, D. O. 1979. Solar energy use through biology - past, present and future. Solar Energy. 22: 307-328.

14. Goldman, J. C. 1977. Biomass production in mass cultures of marine phytoplankton at varying temperatures. J. Exp. Mar. Biol. Ecol. 27: 161-169.

15. Goldman, J. C. and J. H. Ryther. 1976. Temperature-influenced species competition in mass cultures of marine phytoplankton. Biotechnol. Bioengr. 18: 1125-1144.

16. Shelef, G., and C. J. Soeder. 1980. Algae Biomass Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam.

17. Selke, W. 1976. Equipment for growing algae. Patent No. 3,959,923.

18. Laws, E. A., Terry, K. L., Wickman, J. and M. S. Chalup. 1982. Preliminary Results from a Simple Algal Production System Designed to Utilize the Flashing Light Effect. Report to SERI.

19. Phillips, J. N. and J. Myers. 1954. Growth rate of Chlorella in flashing light. Plant Physiol. 29: 152-161.

20. Sager, J. C. and W. Giger. 1980. Re-evaluation of published data on the relative photosynthetic efficiency of intermittent and continuous light. Agricul. Meteror. 22: 289-302.

21. Shifrin, N. S. 1980. Phytoplankton lipids: Environmental influences on production and possible commercial applications. PhD Thesis. Massachusetts Institute of Technology.

MICROALGAL SYSTEMS PRODUCTION AND PROCESSING SIMULATION

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OBJECTIVE

The purpose of this subcontract is to develop a model which simulates the production, harvesting, and processing of microalgae in large-scale production systems. The model is to incorporate a wide degree of flexibility and adaptability so that a variety of production system concepts and configurations can be analyzed. The model will incorporate all biological, physical, engineering, and operating cost submodels which apply to the processes and species to be studied.

This work is being accomplished to provide a means for performing parametric sensitivity analyses of production and the operating portion of production cost of microalgae. It will provide an analytical testbed for the examination of new ideas prior to field development. It will provide a common basis for comparing systems which differ widely in form and function.

Analytical outputs will be prepared for use in the SERI TEP planning and R&D prioritization task for the Aquatic Species Program.

Specific research objectives for the present contract period are:

a. complete the debugging of the model and perform a parametric sensitivity analysis for the Hawaii APR, and a second system if data is received in time;

b. incorporate and debug new submodels to provide for the upgrading of harvested material; and

c. perform additional sensitivity calculations using the new processing submodels.

ACCOMPLISHMENTS

Work was begun on this model in mid-GFY82 and the initial development was completed at the end of GFY82. The present subcontract was started in December 82. Since that time there have been some revisions to the original formulation, owing to feedback from reviewers, other new information, and continuing code development. This work has improved the utility of the model and produced improved correlations between prediction and experiment.

Input data for the model has been obtained from several open literature sources and from the Hawaii Annual Progress Reports for GFY's81 and 82. The data collection and review task being performed by

another subcontractor is providing important uptake rate and other kinetic data. We have exhaustively analyzed Hawaii APR data to establish "baseline" values for the biological kinetic submodels incorporated in the code.

Representative sets of output data are included as an Appendix to this paper. Calculations have been performed for 5, 30, and 360 days of problem time. The 5-day run shows the precise details of internal pool growth; early, exponential growth of the algae; and the fine structure of solar input, ambient temperature variation, and energy balance in the culture vessel. The 30-day run follows growth into the linear phase and shows the time-dependent behavior of growth rate, nutrient uptake, and other variables in this region. The 360-day runs demonstrate the full range of system operation and controls, including harvester operation, nutrient injection, dilution and culture volume makeup, and cost components. The 360-day runs have been made for two levels of harvesting production in order to demonstrate the differences in system operation and explore for meaningful variations.

FUTURE PLANS

Over the next quarter, a parametric matrix will be developed and the model will be used to perform an exhaustive sensitivity analysis of (at least) the Hawaii APR. This will complete one of the tasks scheduled for the present contract and will provide necessary information to the TEP planning activity.

Following this work, the rest of the contract period will focus onto incorporating the processing models and performing additional system analyses.

PROBLEMS AND VARIANCES

No big problems exist at this time and no variances from schedule or cost are presently anticipated.

PANEL DISCUSSIONS

This section presents the participants and topics of each of the three panels which convened on Thursday afternoon, March 10. Summaries written by each of the panel moderators are also included. Since the discussions lasted approximately two and one-half to three hours, the summaries are intended to only briefly describe the salient points and the extent of consensus among the participants.

PANEL PARTICIPANTS

Panels

A. Biological Issues	B. System/Production Issues	C. System/Resource Issues
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Lien	Arad	Nelson
Krauss (R)	Browne	Taylor
Neushul (R)	Hubka	Wright
Ben-Amotz	Hinman (R)	Fox
Pryfogle	Levine	Neenan
		Chapman (R)
		Hollomon

(R) = Reviewer

* Panel Moderators

Unassigned People

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BIOLOGICAL ISSUES

I. Products

a) What products should be emphasized in the research on microalgae, macroalgae, and emergent plants?

b) In light of these product potentials, what ideal traits should be used to guide research activities?

c) If federal involvement in the ASP were to end in 1998, is the current emphasis on species screening and environmental manipulation appropriate? What advantages might be realized if classical genetic manipulation and genetic engineering techniques are included in the research? How costly is a productive effort in genetic research likely to be?

d) Recent research results indicate increasing lipid content per cell through nitrogen starvation results in decreasing overall lipid yield. What might this imply for research alternatives?

II. Photosynthetic Efficiency

Do you agree with published values of 24% and 6% for theoretical and practical limits, respectively, for photosynthetic efficiency? What do these limits imply for productivity in large-scale systems? What research would prove most beneficial in defining and improving the practical limits to photosynthetic efficiency?

BIOLOGICAL ISSUES SUMMARY

I. Products to be investigated in aquatic species.

The storage products of aquatic plants consist of a wide variety of carbohydrates and lipids all of which are more or less highly reduced carbon compounds representing stored energy sources for the plant. Accordingly, these reduced compounds also represent potential sources of energy to man.

Generally, the more highly reduced the compound, the greater its potential value, both because it contains more energy and because it presumably may be more effectively and economically converted to fuel or a petroleum replacement product. This, however, has not been well defined or documented, and the exact nature of the aquatic plant products that should be the target of a SERI research program is not clear.

It should be recognized that the lipids produced by aquatic plants are, with a very few exceptions, not straight-chain hydrocarbons. The more normal triglycerides and other similar lipid storage products of the algae are not directly useful as fuels, and cost analyses or production models that equate such products with petroleum should be used with caution.

The panel therefore recommends that the SERI Aquatic Species Program include consideration of as broad a spectrum of plant storage products as possible but that any practical limitation to restricted classes or groups of products be decided on the basis of well defined rationale.

II. Research needs and priorities.

The panel recommends that the SERI Aquatic Species Program consist of a three-pronged approach to be undertaken simultaneously, as follows:

A. Screening

The products, defined as recommended above, should be the object of an extensive screening program involving unicellular algae, macroscopic algae, and higher plants. Existing knowledge and experience is inadequate to exclude any major group of plants from such a survey. Marine organisms would appear to deserve particular emphasis because: (1) they include a much greater diversity of taxa, and (2) they have received relatively little attention to date. Such groups as nitrogen-fixing bluegreen algae and others that occur in nutrient impoverished environments under stressed conditions should be emphasized.

B. Development of mass culture technology

Certain chronic problems such as harvesting, species control and predator control in unicellular algae culture, remain s continuing constraints to economical biomass production. Research programs specifically directed towards these programs are needed.

The panel agrees unanimously that both theoretical and practical llimits to photosynthetic efficiency are in the range of 3-4% of total incident radiation (6-8% PAR). We unanimously agree that the importance of photosynthetic efficiency and yield have been overemphasized and that efforts to increase these parameters are unlikely to be successful and will probably not be cost-effective.

We feel that research emphasis should instead be placed on reliability, reproducibility and predictability of culture performance and on optimization of economic and energy cost-effectiveness.

C. Stock improvement

The selection of organisms from the screening program should be accompanied simultaneously with efforts towards their improvement with respect to yield, product quality and quantity, and adaptabililty to mass culture practices. The improvement should be approached through classical genetic manipulation including selective breeding, induced mutation, and similar traditional methods that hvae proved successful in terrestrial plant genetics but that hae been barely attempted with aquatic plants.

The new technology of genetic engineering promises still greater rewards, but is still in its infancy and has yet to be successfully applied to aquatic plant species. Technology development in this area should be encouraged now, however, and should accompany research efforts involving classical genetic manipulation. Neither require especially high cost equipment or facilities and rewards may be substantial from a modest investment of effort and research funding.

In all of the above areas, continuity is at least equally, if not more, important than the magnitude of research support, for interrupted programs are highly costly and inefficient.

SYSTEM/PRODUCTION ISSUES

I. Harvesting

What harvesting techniques indicate the greatest promise to minimize production costs in mass culture systems?

II. Predation

What is the predation risk for outdoor cultures? What control techniques promise to be the most cost effective in large outdoor facilities?

III. Processing

What after harvest processing is necessary to achieve final, saleable products? What might be the "best" system for processing, given opportunities for multi-products, recycling, etc.?

IV. Systems

What system size for proof of concept is appropriate for attaining reasonable estimates of scale-up costs?

SYSTEM/PRODUCTION ISSUES SUMMARY

The System/Production Issues Panel considered topics related to the engineering of algal culture systems yielding oil-type products. Discussions focused on four main issues, namely - 1) Harvesting Technology - status, problems, and research needs associated with development; 2) Predation Control - status, importance, and research needs to reduce risks related to contamination; 3) Processing Options - technological needs, development directions, and priorities; and 4) System Size - requirements for verification of yields and/or cost. Members of the panel were; Drs. Lawrence Raymond, John Benemann, Edward Laws, Shoshana Arad, Sidney Brown, William Hubka, Charles Hinman and Leslie Levine. The first four members have done, and are currently doing, extensive research on the issues addressed by this panel. The following summarizes the panel's discussions, conclusions, and recommendations.

Harvesting

The central problem is that large amounts of water must be handled to obtain relatively small amounts of biomass from present microalgae production facilities. This leads to high costs in both dollars and energy.

Several technologies were discussed at some length, including the use of centrifuges, vibrating screens, inclined screens coupled with backwash, sedimentation enhanced by autofloculation, or chemical floculation, and flotation enhanced by bubbles and/or surfactants. Each was found to have advantages and disadvantages specifically tied to the particular properties of different species and production systems. For example, screens seemed acceptable provided the algal species were larger than 100 microns; flotation seemed acceptable for use with the dense cultures characteristic of shallow systems.

The principal issue is to separate product from media in a cost-effective manner. This can be accomplished by separating the algae from water, followed by processing algae to product, or, alternatively, by concentrating and removing prospective products directly without handling the algae at all. This latter consideration arose from observations of species that excrete lipids directly into the media, sometimes without apparent damage to the algae or disruption of conversion efficiencies. The technology for accomplishing this may be drawn from the petroleum industry, where a variety of compounds and approaches are used to separate liquid products into specific and distinct flow streams.

The panel concluded that:

1) the choice of harvesting approach depends upon the species grown, the way its grown, and the product desired,

2) no existing harvesting approach meets the need for producing low-cost fuels.

Recommendations were as follows:

1) develop an expanded understanding of algal growth, lipid production,

cellular strength, and species properties to derive new concepts,

2) examine a range of alternative harvesting approaches to test and narrow options,

3) regardless of approach, exercise caution to avoid contamination.

Predation

Predation was defined as disruption of the desired population by either grazer or contaminent species. This problem was regarded as among the most serious deterents to sustaining high-yield from outdoor algal culture practices. Each researcher presented the experiences and approaches taken to reduce or resolve the impacts they encountered.

The approaches could be categorized into - 1) selection of resistant species; 2) control through culture condition; 3) physical methods; and 4) chemical methods. Species selection involved using 'weed' - type species that almost always out compete other species, and/or predators. Phaeodactylum tricornutum was given as a specific example, appearing in nearly all seawater culture systems, and dominating under conditions of higher temperature (24-26° C) and pH (7.95). The species is known to produce antibiotic(s) effective against fungi and bacteria, does not require silica or vitamin additions contrary to all other known diatoms, tolerates elevated NH_4 concentrations contrary to most golden phytoflagellates, and utilizes green light efficiently, contrary to green phytoplankton. Yet, even with all these special conditions, Phaeodactylum cultures are plagued by a highly cosmopolitan, tolerant, and very hungry protozoan, able to eliminate the population overnight. Therefore, selection of specialized, tolerant species helps to reduce the problem, but does not solve it.

Control of culture conditions, to the extent that only one species is favored, has been practiced for Spirulina culture in Israel. This works pretty well and allows relatively pure cultures to be maintained. However, the conditions employed lie outside the limits optimal for Spirulina, reducing growth rate and yield.

Physical control, such as screening out larger herbavores, inducing turbulent waters, or applying sonic bursts, have been met with varying success, depending on species and contaminent. Sonication may work as a control for Phaeodactylum cultures, and has proved effective in the laboratory. This has not been attempted outdoors to our knowledge.

Chemical controls, such as antibiotics, salts, or specific toxins, have met with limited success, although antimony has been proven effective in controlling - perhaps eliminating - the predator in Phaeodactylum cultures. These techniques appear to be very species and condition specific.

The conclusion was that all techniques were specific to species and conditions. Since neither parameter is well defined at this time, the recommendations are:

1. It probably is too early to devote a disproportionate amount of time or money to this problem until questions of species and conditions are better resolved.

2. Concentrate research on defining and selecting species and conditions for outdoor culture.

3. Limit research on predators to that level necessary to avoid confounding data and results obtained from outdoor experimental units.

Processing

Processing was regarded as that set of actions required to transform microalgal feedstocks into products. It assumes that the feedstocks are removed from their production environment.

The present status of microalgal processing technology is that methods are limited to preparing whole-cell products; only very small efforts have been directed to obtaining chemical products from them, including viatmins, pharmaceuticals, and organic dyes. Much of this technology may be readily adapted from current industrial practice, however. The characteristics and properties of the feedstock must first be known to some reasonable extent before this can be attempted productively.

The panel recommended that this remain a wide open question but focused towards a fully integrated agribusiness. Product refinement, separation, extraction, and direct recovery should be considered with no approach favored at this time. Monocultures should be established for the production of specific product sets. The properties of those species and products will direct the study of suitable processing techniques.

Systems

The panel focused on two specific aspects of this question, namely:

1. What size system could be regarded as giving reliable data with respect to extrapolable yield.

2. How large should a system be in order to accurately predict the capital and operational costs associated with a commercial facility.

General consensus was reached with respect to the first but not the second question. An algal production system having 200 m^2 of culture surface area should provide reliable yield data that can be extrapolated with reasonable safety to larger, commercial systems. System cost, however, was judged to be so closely dependent upon design, materials, and operations that questions appear to be unresolvable at this time.

SYSTEM/RESOURCE ISSUES

I. Carbon Supply/Demand

a) Identify and discuss alternative supplies of carbon for algae. What research should be initiated to minimize the need to import a carbon source to the algae facility?

b) What information should to be generated to facilitate analysis of the problem?

II. Nutrient Demand

What are potential sources and magnitude of demand for nitrogen, phosphorous and potassium, especially during start-up of a full-scale facility? How will these demands affect the existing markets for these commodities?

III. Support Infrastructure

What support infrastructure will be needed for any large-scale algae facility? What significant impact will these facilities have on the environment?

IV. Siting

Discuss the effects of the above on potential siting of algae facilities.

SYSTEMS/RESOURCE ISSUES SUMMARY

The topics addressed by the Systems/Resource Panel were issues of resource availability, siting of algal culture systems and existence of an infrastructure to support the development of algal ponds. Members of the panel were: Kimon Byrd, David Chapman, Pat Fox, Gene Maxwell, Bernie Neenan, Sam Nelson, Pete Pryfogle, Marilyn Ripin, Julius Taylor, and Lynn Wright. Two members of the Panel have studied resource questions in detail and their work should be consulted for a more complete analysis of these issues. Dr. Sam Nelson of Argonne National Laboratory has prepared a report on CO_2 avavilabililty entitled, "An Investigation of the Availability of CO_2 for the Production of Microalgal Lipids in the Southwest." Mr. Gene Maxwell's group at SERI has done an extensive study of land, climate, insolation and water characteristics of the arid Southwest. This study will be available in the report, "A Plan for Resource Assessment and Site Evaluation for Microalgal Pond Systems." The Executive Summary will be available in April with the full report released in May.

The Panel discussed availability in the Southwest of carbon, water and nutrients to support large-scale algal mass culture. Also considered were the environmental and legal aspects of mass culture. A synopsis of the Panel's discussion follows.

Carbon Supply

Due to the high cost of organic carbon and low value of energy, organic chemicals are not an economic cabon source for algal mass culture. Carbon will need to be supplied as inorganic carbon, either as CO_2 or as mineral salts. The two major ways of delivering CO_2 to algal ponds are: (1) flue gas from power plants, or (2) as pure CO_2 from underground reserves, coal gasification plants or purified from natural or flue gas.

Several problems exist in using flue gas as a CO_2. First, the cost of transporting flue gas any appreciable distance is prohibitive. Second, the presence of NO_x in the flue gas can poison microalgae and will need to be removed. In addition, to ensure a reliable source of CO_2, two power plants per algal pond will be needed in order to provide a continuous supply of CO_2 during power plant shutdowns.

The use of calcium carbonate as a carbon source was suggested since it is estimated that 20-40% of the composition of some arid land soil is limestone. Problems associated with using calcium carbonate for a carbon source include pH control and cost. If large quantities of calcium carbonate are added to the ponds, the pH will have to be carefully controlled since precipitation of minerals may occur. In addition, calcium carbonate costs $19.00 per ton undelivered and at that price adds $24 to the price of each barrel of microalgal oils produced.

The preferred choice of carbon for extensive outdoor systems therefore appears to be pure CO_2. The availability of CO_2 for the envisioned large pond systems has been a subject of discussion in the ASP. If massive pond systems (large enough to produce two to three Quads of energy) were built today, they would have to compete with Enhanced Oil Recovery (EOR) operations for CO_2. On an economic basis, EOR would be able to bid the CO_2 away from the algal systems. Realistically, however, pond systems of this size are not going to be built in the near term and when they are built (between 1995 and 2020) the availability of carbon dioxide will be influenced by several factors:

- Gas separation technology is becoming more efficient and less expensive and will result in future supplies of low cost CO_2.

- Natural gas may be available from undiscovered or unexplored fields.

- Development of the synfuel industry will result in production of copius amounts of CO_2 which should be available essentially free.

- Demand for CO_2 for EOR operations will have decreased (unless the price of oil drops and oil companies slow down their present rate of EOR and extend their operations into the future).

Water Availability

The major impediment to developing an algal energy industry will be the availability of water. No concerted effort has been made to search for and characterize saline water in the arid Southwest. The result is that little is known about the size and number of saline aquifers and their sustainable yields. At the present, water containing 3000 mg/l of dissolved solids is being used for irrigation and stock (and in some places, personal use); therefore, water considered for microalgal cultivation will probably contain at least 10,000 mg/l dissolved solids.

One important issue which needs to be studied with respect to water is whether pumping water from saline aquifiers will in some way affect the fresh water in the region. Questions such as the affect of pumping saline water on the water table and ground water and the method of used water disposal need to be studied.

Nutrient Supply

The amounts of nitrogen and phosphorous (and other elements such as iron) necessary to support growth in pond systems built for producing quads for energy will not be trivial. The simple solution of adding agricultural fertilizer will not be feasible. It is clear, however, that most water is too N and P limited to support growth of algal cells to the concentrations being discussed in the ASP. One solution to nutrient availability is recycle - that is, returning the algal cell mass to the culture after extraction of the lipids and/or chemicals. However, problems with recycling cells exist. They include:

- Continued recycle can result in the build-up of heavy metals which become toxic to algae and are a potential environmental problem.

- Addition of cell debris to the pond will decrease light penetration into the pond and may reduce production efficiencies.

Infrastructure Issues

Initially, ponds should be built near an existing city or town to take advantage of the roads and services available. Siting algal ponds near synfuel plants has the advantage of readily available CO_2 and piggy-backing on the development of roads and town built to serve the synfuel industry.

Dealing with state and local governments having jurisdiction over the areas in which algal ponds will be built has highlighted a potentially a serious problem. Water use in the Southwest is a very sensitive issue and environmental and legal constraints over its use are likely to be significant, although not insurmountable.

Recommendations

The panel thought that the focus of the ASP clearly needs to remain on solving the technical biological and engineering probems of algal culture. However, the panel believed that issues of resource availability, environmental impact and legal constraints are also important and need to be studied concurrently. Even if all technical problems in algal mass culture were solved, environmental and legal restrictions could prevent the development of an algal-energy industry unless answers to questions of nutrient, carbon and water availability and mpact of algal systems on the land and water supply have been addressed.

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