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SERI Aquatic Species Program 1984 Annual Report

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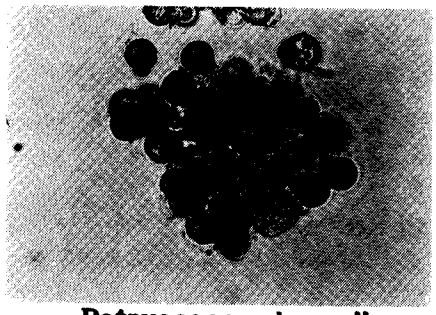
SPECIES AVAILABLE FROM THE
1984 MICROALGAE CULTURE COLLECTION

Ankistrodesmus falcatus



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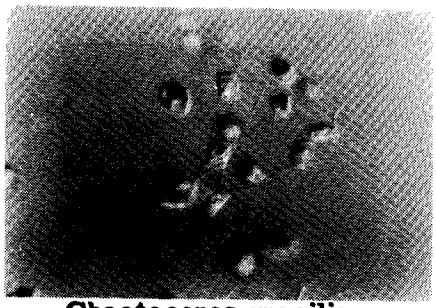

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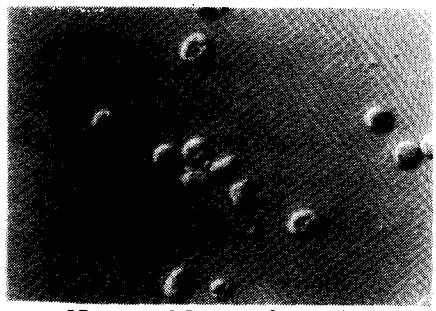
Chlorella sp. (SO1)

Isochrysis galbana



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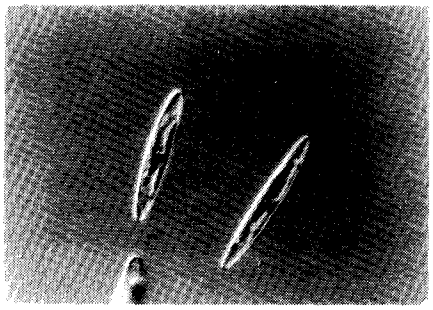

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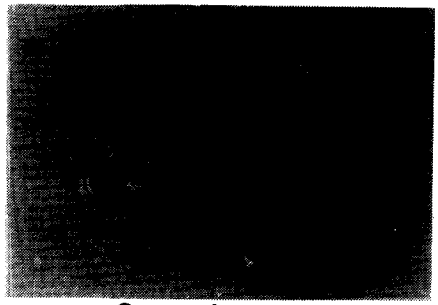

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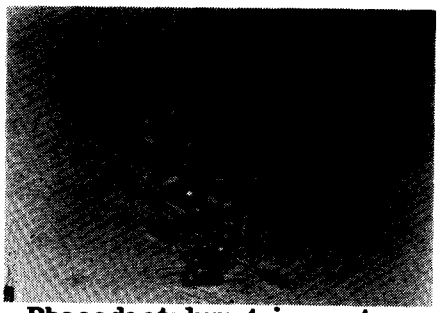

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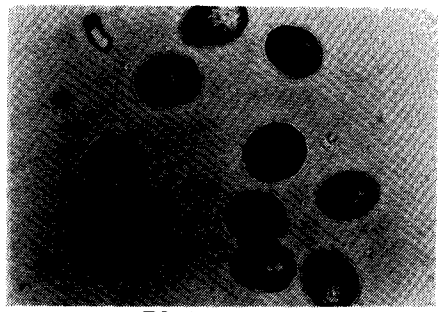

Platymonas

SERI

PREFACE

This report summarizes the progress and research accomplishments of SERI's Aquatic Species Program during FY 1984. This program is funded through the Biomass Energy Technology Division of the U.S. Department of Energy (Beverly J. Berger, Director). For further details, contact the SERI Biofuels Program Office (Robins McIntosh, Aquatic Species Coordinator, (303) 231-1472).

Robins P. McIntosh
Aquatic Species Program

Approved for

SOLAR ENERGY RESEARCH INSTITUTE

Stanley R. Bull, Acting Director
Solar Fuels Research Division

SUMMARY

Program Objective

To improve the productivity, conversion to fuels, and cost efficiency of aquatic plant species cultivated for energy. The emphasis of the program is on developing oil-yielding microalgae that will grow in the saline waters of the desert in the American Southwest.

Discussion

During 1984 research was carried out under three tasks: biological, engineering, and analysis. Biological research was aimed at improving photosynthetic efficiencies and lipid yield of species that can be cultivated using mass culture technologies operated in the American Southwest. Emphasis has been placed on screening for productive species, developing culture and management techniques for growing desirable species, and understanding photosynthetic and lipid physiology as it applies to increasing yields. Engineering research focused on the development and analysis of harvesting schemes applicable to species of microalgae that grow in saline waters. Three system designs and analyses were initiated in 1984, and these designs will be completed in 1985. The analysis task is designed to support technology development through the determination of cost goals, assessment of resources, and evaluation of emerging technologies. A comprehensive technical and economic evaluation was completed during 1984. This analysis and assessment provided insights into where program emphasis should be placed for the next ten years.

Conclusions

The major accomplishments for 1984 in the Aquatic Species Program were as follows:

- Three-hundred-twenty strains of microalgae were isolated. Of these, 46 strains were classified as good growers.
- A screening protocol was established that most efficiently selects for strains that do well in outdoor mass culture.
- Five strains of microalgae were identified that accumulate over 40% lipids.
- Outdoor yields were improved 40% by growing thermophilic strains and developing better management techniques.
- Lipid yield was found to be highest for a period of three to five days after the onset of nitrogen starvation in a green microalga.
- Microalgal cells that have been acclimated to low light that occurs in dense cultures were found to require less rapid modulations to enhance photosynthesis.
- Designs were commissioned for an advanced pond system, an advanced raceway system, and a closed continuous system.
- A technical and economic evaluation indicated that large-scale microalgae production for fuels is feasible, providing (1) lipid yields from microalgae can be improved, (2) there is sufficient saline water for large-scale development, and (3) microalgal lipids can be converted to conventional fuels.

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INTRODUCTION

The purpose of the Aquatic Species Program is to improve the productivity, conversion to fuels, and cost efficiency of aquatic plant culture technologies. The emphasis of the program is on developing a mass culture technology for cultivating oil-yielding microalgae in the American Southwest. A technical and economic analysis indicated that such a concept would be feasible if (1) lipid yields from microalgae are improved, (2) there is sufficient saline water for large-scale development, and (3) microalgal lipids can be economically converted to conventional fuels. It was determined that fuels from microalgal lipids presented better options than converting the microalgal biomass to either alcohols or methane. All lipids can potentially be catalytically converted to gasoline, or the fatty acids can be converted to substitute diesel fuels. The Southwest has the necessary low-cost resources available for this technology. These resources include large expanses of flat, underutilized land, and carbon dioxide is available from either natural deposits or flue gas from industrial plants. The amount of saline water available will probably determine how much fuel can be produced from aquatic species, and this question should be answered during 1985.

The largest constraint to this technology is the economical production of an oil-rich microalgal feedstock. In addressing this constraint, the Aquatic Species Program is concentrating research on (1) initially selecting the best species, (2) developing the best culture and management methodologies, and (3) improving the most promising species. An improved screening program has allowed for more efficient selection of species. A total of 46 new, fast-growing strains were added to the program this year for further characterization and future improvement. Outdoor studies resulted in a 40% improvement in biomass yields. Annualized yields of 70 tons/ac yr were sustained for one month in Hawaii. Major cost reductions resulted from recycling culture media and reducing the inputs of costly trace elements.

During 1984 a major initiative was started to upgrade present outdoor mass culture designs. Three new designs were commissioned: an advanced pond design, an advanced raceway design, and a closed continuous design. These projects will be completed in 1985. In conclusion, the Aquatic Species Program made significant progress in defining the constraints, developing meaningful research plans for the future, and further developing the technology toward a goal of enabling the private sector to successfully commercialize the technology. Next year promises even greater progress.

SECTION 2.0

AQUATIC SPECIES

2.1 BACKGROUND

The worldwide energy shortage and Arab oil embargo of the early 1970s encouraged many nations to look for new sources of oil, electricity, and gas. Renewable resources such as biomass were often highlighted as a long-term solution to the energy problem because of their nondepletable, renewable nature. While the first biomass sources considered were the readily available ones such as corn or wood, it was apparent that new biomass sources should also be developed, among them aquatic species.

Aquatic algae may be divided into two groups: macroalgae and microalgae. Macroalgae range in size from small filamentous forms to very large complex forms such as Macrocystis. Various concepts have been developed for culturing several species of macroalgae for biomass. The giant kelp Macrocystis is being cultivated in near-shore areas off California (less than 20 m of water). Floating or benthic species such as Sargassium or Gracilaria have been cultured in semitropical areas of Florida and Hawaii. The primary storage product of macroalgae is carbohydrate, which can be converted to either methane or ethanol.

In contrast to macroalgae, microalgae are small unicellular plants that range in size from 1 to 50 μm. Historically, microalgae have been grown in mass culture mainly for food production and waste treatment. Initial efforts at mass culture of microalgae were concerned with food production, but the hope of producing an abundant, low-cost source of protein has not yet been realized. However, the most promising early results of mass algal culture have been in the field of sanitary engineering, where microalgae are used to treat wastewater in oxidation ponds. This wastewater technology has been expanded to include protein production and treatment of irrigation water. More recently, the possibility of using algae as a source of energy received widespread attention as a result of the energy crisis during the 1970s.

The SERI/DOE Microalgae Program was initiated in 1979 with the benefit of several early technology assessments. Research initiated by the Carnegie Institute on growing microalgae in outdoor mass culture (for food) in the early 1950s resulted in one of the most comprehensive early reports on algal growth, physiology, and biochemistry. This work led to expanded efforts by German and Israeli researchers to commercially produce various species of microalgae for both wastewater treatment and animal feed protein.

Microalgae are presently being grown in Israel, Australia, Mexico, and the United States for high-value products for the health food market, including the alga Spirulina ($10,000/dry ton) and the vitamin beta-carotene ($60,000/dry ton). The main application of algal mass culture in the United States has been for oxidation ponds used in wastewater treatment. Of increasing importance is the cultivation of microalgae as a food source for culturing fish and shellfish and as a soil conditioner. Recently, a nascent industry has arisen in the southwestern United States that produced over 50 tons of algae in 1984. The value of these microalgae, which are converted to high-value health food products, exceeds $10/lb. Such an integrated system situated in the American Southwest is shown as depicted by an artist's conception in Figures 2-1 and 2-2. Use of microalgae as a fuel source was first proposed 30 years ago for the production of methane gas. This early interest in microalgae as a feedstock was due primarily to the projections made about the potentially high yields--from 30 to 300 metric tons/ha yr.

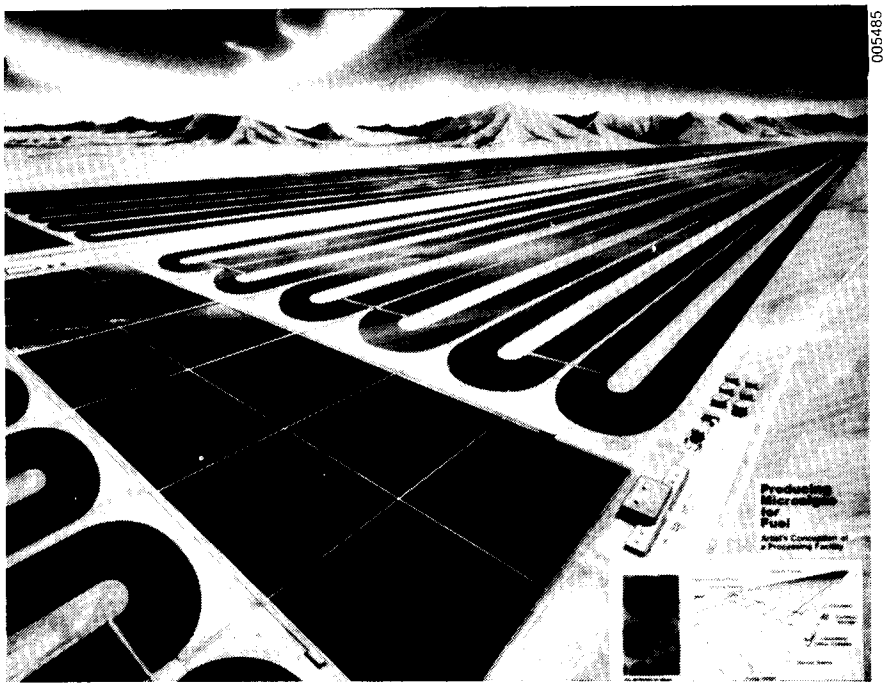

Figure 2-1. Artist's Conception of Microalgae Production Facility Located in the Southwest United States

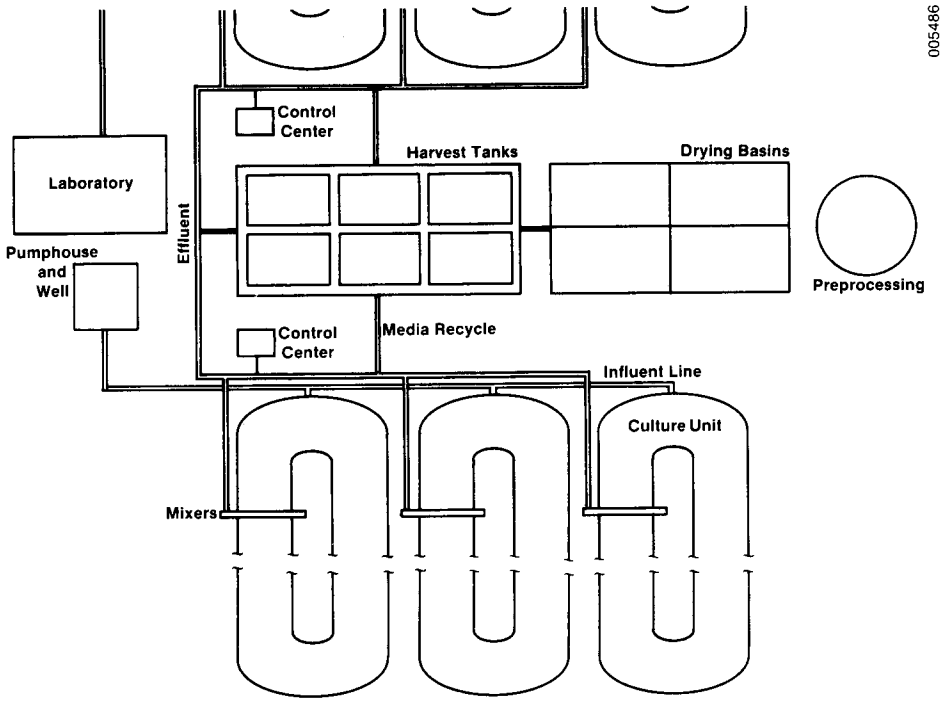

Figure 2-2. Schematic Layout of Microalgae Production Facility

Dubinsky (1980) extended the microalgal concept beyond joint wastewater cleanup and food or methane production by pointing out two other advantages of microalgae: microalgae will grow in desert saline waters, possibly making deserts productive, and certain microalgae are known to accumulate up to 70% of their dry biomass into lipids, which can be converted to substitute diesel fuels via transesterification processes or to gasoline via catalytic processes. Technology reviews and assessment studies by Goldman (1978) and Jaycor (1983) indicate that extensive world-wide initiatives have been pursued for the mass culture of microalgae for commercial purposes. From these reviews it becomes obvious that a mass culture system utilizing microalgae as a feedstock for fuels would require integration to make it cost-effective and efficient.

An integrated system for the extensive production of microalgae for fuels would require the following:

- Flat land with sufficient water
- A reliable supply of required nutrients (nitrogen, phosphorus) and carbon (carbon dioxide, bicarbonate)
- A source of power for operating the harvester, mixing systems, and pumps for water and nutrient distribution
- A mechanism for harvesting the lipid-rich cells from the culture
- A mechanism for dewatering or drying the biomass
- A method for preprocessing the feedstock for fuel production
- A mechanism for regenerating nutrients from the harvested biomass for recycle to the culture
- Microalgae feedstock production costs not exceeding $0.10/lb to produce competitive fuels.

These earlier efforts and concepts provide the basis for the SERI/DOE microalgae program and can be summarized as follows:

- Microalgae have the potential for tremendous biomass yields (30-300 metric tons/ha yr).
- Microalgae can potentially be cultivated on otherwise low-productivity desert lands utilizing nonpotable, saline waters.
- Microalgae are a potential source of high-energy lipids that could be converted into gasoline or diesel fuel substitutes.
- Microalgae can be cultivated in extensive outdoor mass culture systems and therefore are amenable to large-scale fuel feedstock production.

The SERI/DOE program has directed emphasis toward the production of lipids from microalgae for two reasons. First, microalgae are among the few photosynthetic organisms that directly produce and are known to accumulate storage lipids in great quantities. Second, plant lipids have been postulated to be among the best biomass feedstocks for production of renewable, high-energy liquid fuels. In 1945, it was first proposed that plant lipids could be refined to replace some petroleum-derived products.

Microalgae under normal growth conditions contain a high proportion of carbohydrate, polysaccharide, and protein. The lipid content in growing cells was generally reported to be between 5% and 20% of the total dry weight. Shifrin (1980) reported that microalgae

under nutrient stress could accumulate up to 72% of their weight as lipids. Even though large accumulations of lipids are found only under stress conditions (e.g., nutrient limitation or salinity stress), certain microalgae are obviously metabolically capable of producing these high-energy compounds in large quantities. The heat of combustion for a typical algal mass is in the range of 4.5-5.5 kcal/g (8,000-10,000 Btu/lb) dry weight, but it is strongly affected by the low heat of combustion of nonlipid products. The specific heat of combustion for algal oils and lipids is approximately 9 kcal/g, and, therefore, for the purpose of energy or fuel production by microalgae, it is desirable to increase lipid content of these organisms.

Microalgae lipids can be classified as polar (membrane lipids) or nonpolar (storage lipids, such as hydrocarbons and triglycerides) (Figure 2-3). Storage lipids, which accumulate when microalgae cells are stressed, offer the most potential since they more closely resemble petroleum-derived compounds. Imposing stresses, such as nitrogen limitation, increases the percentage of storage lipids.

Further initiative for emphasizing lipids for fuels was provided by reports of the direct synthesis of hydrocarbons by various microalgae. Specifically, large quantities of C-30 hydrocarbons were identified in the freshwater species Botryococcus, an organism that is postulated to be responsible for present petroleum reserves. It was initially assumed that hydrocarbons extracted from such organisms could be readily processed by the existing petrochemical industry and used to produce gasoline, although no process evaluations were performed to verify this hypothesis.

In summary, the SERI/DOE program has focused on microalgal lipids primarily because this feedstock represents a potential renewable resource that could be used to produce high-energy compounds that are similar to today's conventional liquid fuels (Figure 2-4).

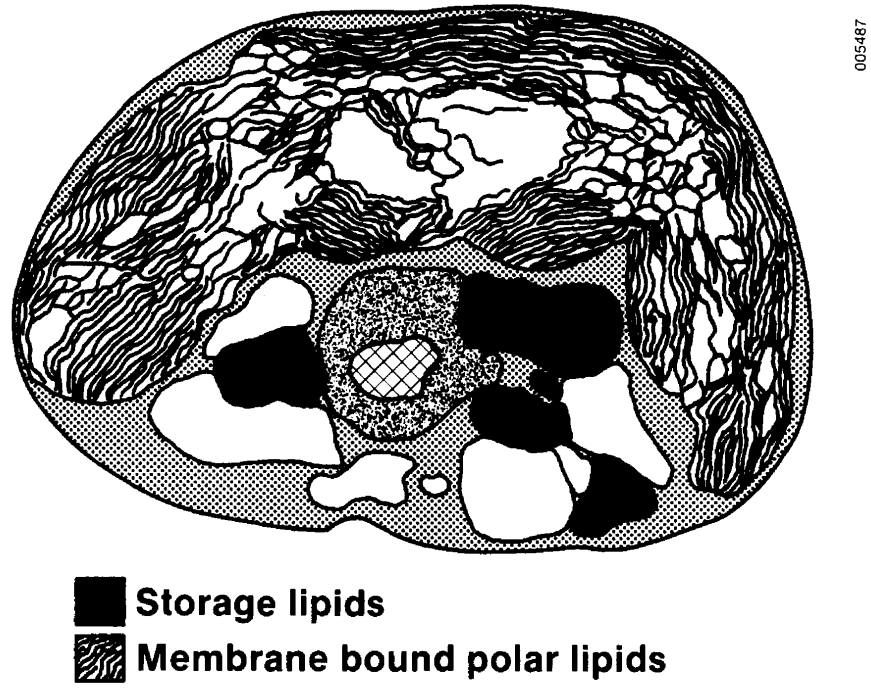

Figure 2-3. Micrograph of Algal Cell Showing Localization of Lipids

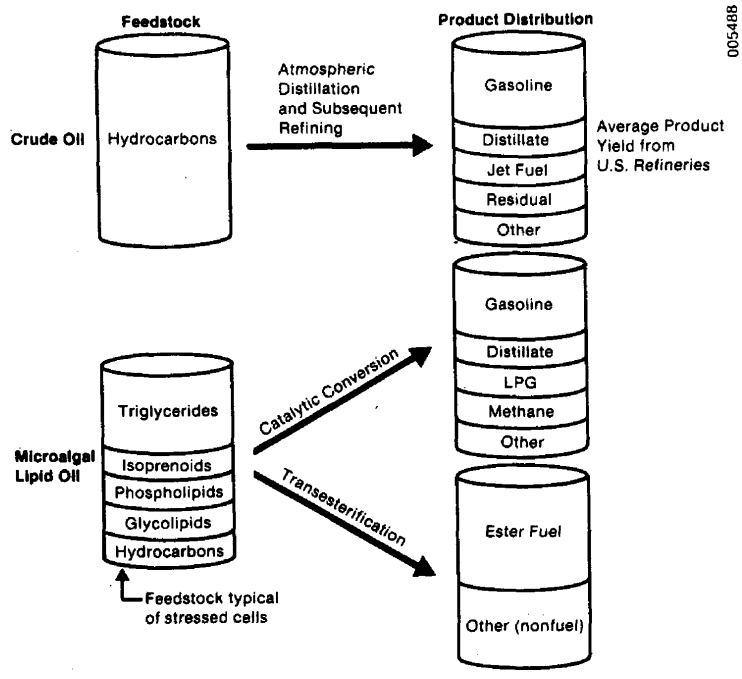

Figure 2-4. Feedstock Composition and Product Distribution of Petroleum and Microalgal Lipids

Most biomass production technologies require a large, inexpensive resource base to be economically competitive with conventional (mined) fuel sources. The advantages of microalgae are that they can grow in any climatic region of the world, since they require only light and nutrients, and that they are renewable and therefore not limited to fixed deposits like oil, coal, and natural gas. Development of a biomass technology that can exploit the underutilized, marginal resources of the Southwest—flat land, saline water, and high incident solar radiation--offers potential for the production of a high-value energy feedstock from microalgae; this concept has unique characteristics, few competitive impacts, and enormous potential for displacement of exhaustible conventional energy resources.

The American Southwest (Arizona, New Mexico, Southern California, West Texas, and parts of Nevada, Utah, and Colorado) have abundant lands that support relatively little conventional biomass productivity. Resource assessment studies completed in 1983 estimated that up to 33 million acres may be highly suitable for microalgae cultivation (Maxwell 1985), but the definition of criteria was too gross to provide definitive estimates of total resource availability (Figure 2-5). The abundance of available saline water has become an issue. While scarce fresh water in this region limits conventional agricultural possibilities, saline and brackish waters are known to exist in large underground aquifers, and large quantities of agricultural drainage waters are present in existing irrigation canals. These water resources are typically underutilized because of their marginal quality and characteristics; in some cases, such as irrigation runoff, these waters are an economic nuisance that is costly to alleviate.

A wide variety of microalgal species can grow in highly saline desert waters. Therefore, despite the fact that desert saline waters differ from marine waters due to different ionic ratios of biocarbonate, calcium, magnesium, and sulfate, these differences may not preclude adaptation of abundant marine microalgae to desert waters. Thus, the natural variability of both marine and desert species may prove to be useful in developing species for desert mass cultivation.

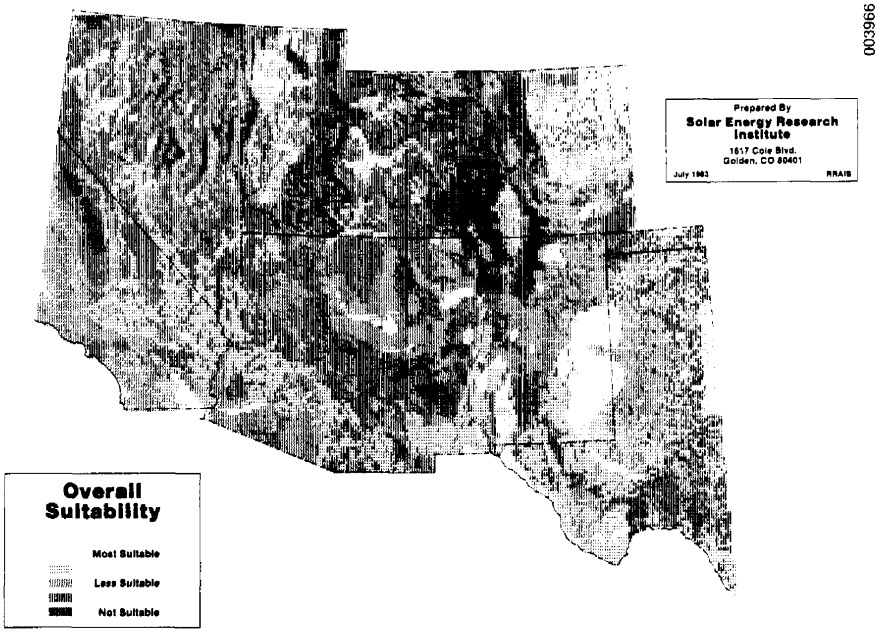

Figure 2-5. Computer-Generated Map of Overall Suitability for Microalgae Culture

There is no doubt that many areas of this technology will need long-term development before a barrel of lipid oil is economically produced and converted into a liquid fuel. The current estimate of the state-of-the-art cost is between $250 and $350 per barrel. To compete by the year 2000, it is estimated that the cost will need to be reduced to $60-$85 per barrel. Although the development of the technology is mid-term, it does provide a potentially valuable source of renewable high-energy liquid fuels.

2.2 PROGRAM ORGANIZATION

To meet the goal of producing cost competitive fuels from microalgae, the Aquatic Species Program is organized into three tasks: Biological Research, Engineering Research, and Technology Analysis.

2.2.1 Biological Research

The focus of the biological research is on increasing the lipid yield from cultured microalgae. To do this, the program is following a sequential strategy. Presently, species are being selected that have high productivities and/or lipid yields. These species will then be improved or domesticated through various genetic manipulation techniques and outdoor management culture strategies developed that maximize lipid yields.

2.2.1.1 Species Screening

In selecting the most promising microalgae, researchers are conducting a comprehensive survey of microalgae. Microalgae with the following characteristics are being considered the best species for fuel production.

- Grow rapidly in dense culture. Rapid division or accumulation of biomass in dense cultures results in the highest yields. Furthermore, dense cultures are required to reduce the cost of harvest.
- Grow in an unstable culture environment (fluctuating temperature and salinity). The culture apparatus will have a large surface area/volume ratio; thus, large temperature fluctuations will be expected. A range that might be encountered would be 20°-35°C in the summer. Winter temperatures would, of course, shift that range downward. Salinities would be expected to fluctuate with evaporation and rapid dilution by rainstorms. In regulating salinities, it is expected that higher salinities will decrease costs of pumping or reduce "blowdown" requirements.
- Grow in saline waters common to the desert Southwest. Presently, the program is concentrating on this resource base to support the proposed technology, thus ultimately the strain would have to perform in these waters or be developed to grow in these waters. Two basic desert water types have been identified, Type I and Type II, as shown in Table 2-1.

  Eighty-five percent of available saline water found at less than 200 ft in New Mexico is in the range of 3 to 10 g/L total dissolved solids (TDS). Type I water is the most prevalent, being found at 75% of the sites analyzed, and is characterized by relatively low monovalent to divalent cation ratios.

- Grow at high light intensity. The contemplated culture systems will be exposed to full sunlight, at least at the surface. Due to culture density, this intensity will quickly diminish. There are two considerations: the strain must not become photoinhibited in outdoor culture, and the strains must be photosynthetically efficient, converting more of the light energy into organic compounds.
- Accumulate large quantities of storage products (hydrocarbons, triglycerides, carbohydrates). The ability to accumulate a product rapidly indicates the genetic potential of the organism to rapidly synthesize that product. In the future it is hoped that this metabolic potential will be better controlled and linked with high photosynthetic efficiency.

Table 2-1. Ionic Composition of Two Common Water Types in Southwest as Compared to Seawater (mg/L)[a]

Ion	Type I	Type II	Seawater
Na	3,552	4,646	4,520
K	278	194	168
Ca	678	89	172
Mg	303	392	543
HCO_3	867	2,100	59
SO_4	2,973	805	1,137
Cl	6,349	6,775	8,128

[a]All normalized to a salinity of 15,000 mg/L TDS.

- Other desirable characteristics. The ability to utilize bicarbonate as a source of carbon (growth at higher pH), to require no vitamins, to exhibit predator resistance, to be highly competitive, and to not suffer growth inhibition by large concentrations of oxygen are all characteristics that are desirable for a species to be successfully mass cultured.

During the past year, a revised screening protocol (Figure 2-6) was implemented that enables the efficient selection of promising strains. Collection efforts are being expanded and focused on habitats throughout the United States. Habitats that fluctuate widely in temperature and salinity, such as ephemeral ponds, are being emphasized (Figure 2-7).

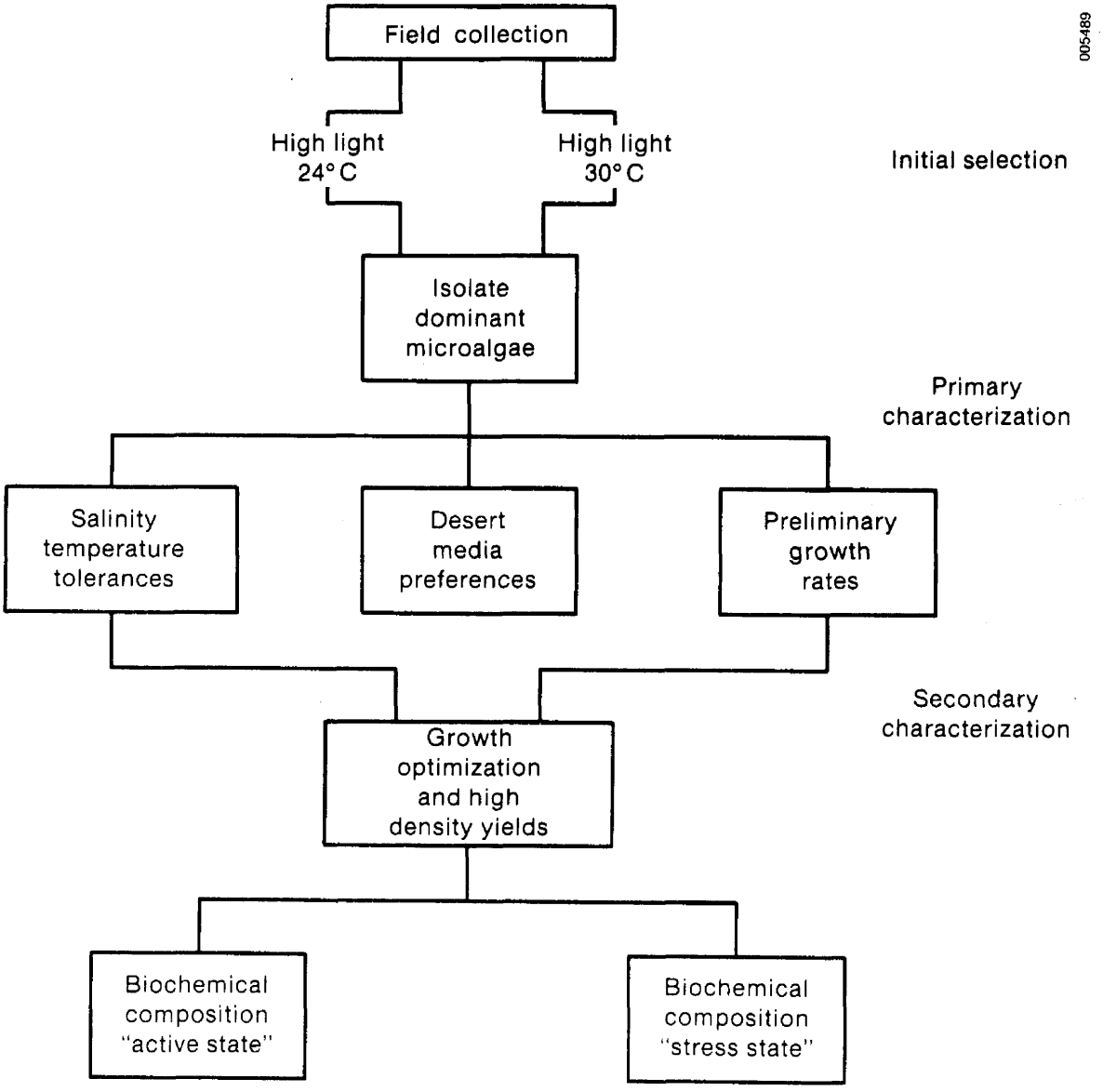

Figure 2-6. Protocol for Selecting and Characterizing of Microalgae

Figure 2-7. Typical Habitat from Which Microalgae Are Collected for Subsequent Screening

The best strains are then selected from the field collections by exposing nutrient-enriched cultures to various temperatures and high light intensities. Only 5%-10% of initial species are thus selected for further characterization (Figure 2-8). Initial characterization consists of defining the growth rate of the species for a range of temperatures, salinities, and water types that are common to the deserts of the United States. Secondary characterization includes maximizing growth of dense cultures in chemostats and the characterization of the lipids in both nonstressed and stressed cells.

2.2.1.2 Species Improvement

Species improvement involves developing the basic physiological and metabolic understanding that will enable the best species to be further developed. There are two components to species improvement: applied physiological studies and genetics.

Applied Physiological Studies. Applied physiological studies are directed toward understanding lipid metabolism and the effects of modulated light on increasing biomass yields from microalgal cultures.

Lipid Physiology. It is known that by removing nitrogen from the culture media, the cell begins to accumulate lipids. Several simultaneous metabolic events accompany this lipid accumulation: the cell may enlarge volumetrically, membranes break down, and photosynthesis declines. Newly synthesized lipids are almost entirely the desirable neutral lipids. Projects are currently examining the time course relationship of stress and lipid accumulation, as well as the effect of stress on the solar efficiency of lipid production in the cell.

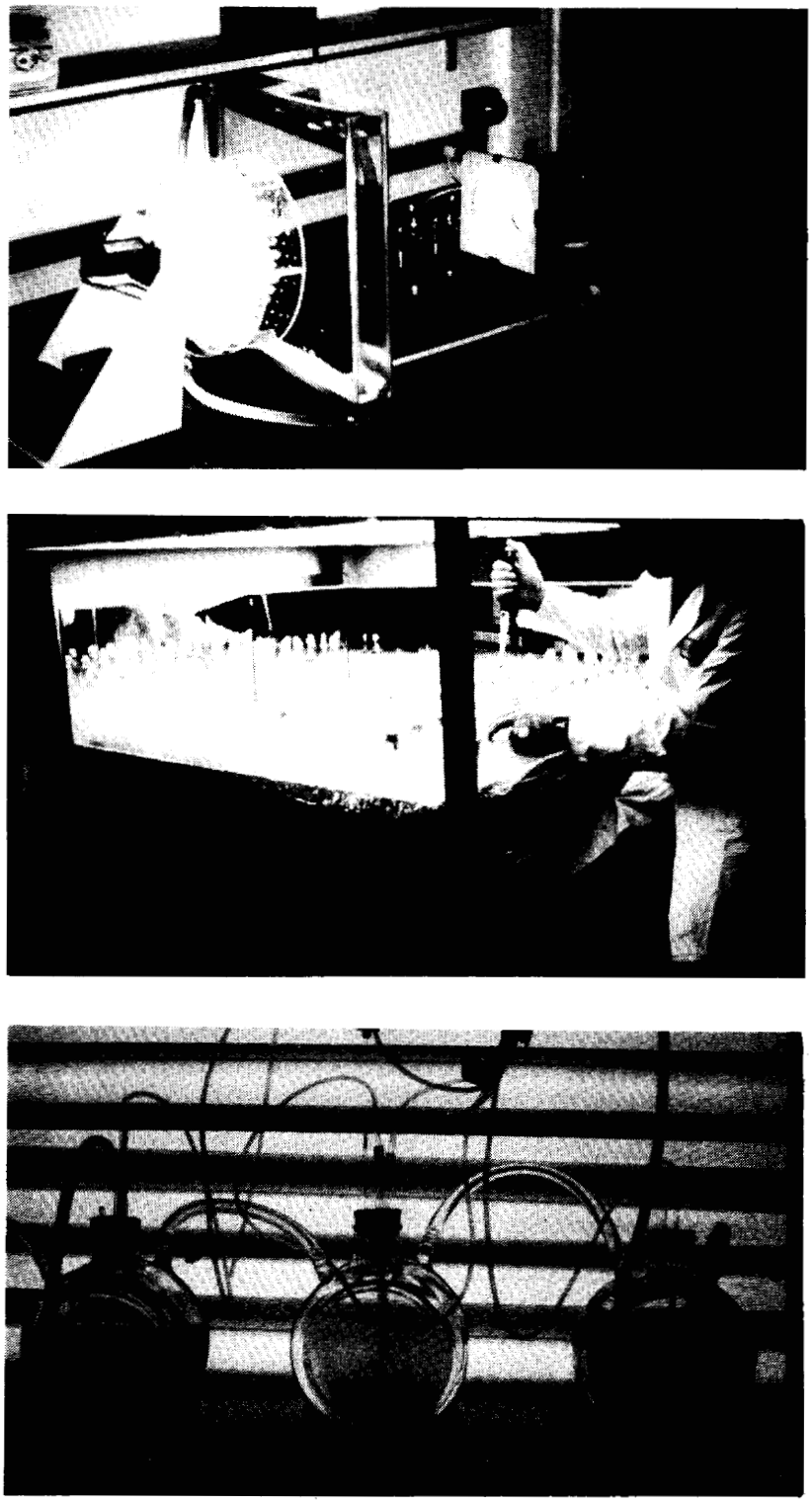

Figure 2-8. **Protocol for Species Screening.** (A) Initial Selection from Cultures Grown under High Light and Temperature; (B) Characterization of Growth Response at Different Salinities and Temperatures; (C) Maximization of Growth and Photosynthetic Efficiency in Dense Cultures

Growth Physiology. Individual cells of algae grown in dense cultures experience a fluctuating light environment by virtue of their movement into and out of the illuminated region near the surface of the culture. Cells that experience a fluctuating light environment can utilize high-intensity light much more efficiently than cells that receive the same light on a continuous basis. Since the sunlight received by outdoor mass algal cultures typically reaches intensities well in excess of the saturation level for photosynthetic growth, the use of flashing light or modulated light has been suggested as a technique for enhancing light utilization.

While the photosynthetic response of algae in flashing light has been studied extensively, the emphasis has been placed on flashes with a period of less than one second, frequently on the order of less than 100 milliseconds. In outdoor mass culture, the introduction of foils generates vortices with periods of one second and longer. Rapid cycle periods could be achieved with additional energy inputs. While different mechanisms used to induce vertical mixing in algal cultures can differ with respect to their energetic efficiency, for any given mechanism the turnover rate will increase only with increased energy input. Research is now being conducted to quantify the relationship between turnover rate and photosynthetic enhancement to determine the potential paybacks of increased energy inputs for vertical mixing.

Genetics. Once the best species are selected, genetic manipulations may be used to domesticate the microalgae in order to produce the greatest possible yield of lipid in mass culture. This process will be very similar to that used in modern agriculture to improve yields. To date, no substantial effort has been made to improve microalgal strains. In every major conventional crop, yields have been improved using genetics. From 1930, yields in U.S. crops have increased from 33% to 413%, largely due to the application of genetics (Table 2-2). Genetics will be used to develop strains that allocate larger portions of the fixed carbon into oils, are more competitive, and have higher tolerances to salinity. Presently the program is involved in assembling and characterizing a gene pool for such improvement efforts.

2.2.2 Engineering Research

The purpose of engineering research is to integrate biological concepts with engineering principles to develop a cost-effective microalgal culture technology. An example of this is developing culture systems that most efficiently utilize the flashing light effect.

2.2.2.1 Facility Design and Operation

The most critical microalgal production design considerations are the depth of the culture, the mixing strategy, and the enclosure configuration. The choices involve tradeoffs in algal productivity, environmental control, system capital, and operating costs. Currently, the program is supporting the operation of two systems of differing depth and channel configuration. A shallow (10 cm) raceway system (Figure 2-9) is being operated in Hawaii. Shallow systems have the advantage of developing higher cell concentrations, well above 1 g/L. This minimizes the volume that must be handled by the harvesting process, makes more efficient use of added nutrients, and increases the capacity for manipulation of the chemical composition of the cultured algae. Also, the flow characteristics of shallow systems are more easily manipulated. Foils placed in the Hawaiian system cause regular patterns of induced turbulence, which allows the culture to utilize a flashing light effect, thus substantially improving yields.

Table 2-2. Average Yield per Acre of Major Crops in 1930 and 1975

Crop	Average yield per acre		Units	Percentage Increase
	1930	1975		
Wheat	14.2	30.6	bushels	115
Rye	12.4	22.0	bushels	77
Rice	46.5	101.0	bushels	117
Corn	20.5	86.2	bushels	320
Oats	32.0	48.1	bushels	50
Barley	23.8	44.0	bushels	85
Grain sorghum	10.7	49.0	bushels	358
Cotton	157.1	453.0	pounds	188
Sugar beets	11.9	19.3	tons	62
Sugarcane	15.5	37.4	tons	141
Tobacco	775.9	2,011.0	pounds	159
Peanuts	649.9	2,566.0	pounds	295
Soybeans	13.4	28.4	bushels	112
Snap beans	27.9	37.0	cwt	33
Potatoes	61.0	251.0	cwt	129
Onions	159.0	306.0	cwt	92
Tomatoes:				
Fresh market	61.0	166.0	cwt	172
Processing	4.3	22.1	tons	413
Hops	1,202.0	1,742.0	pounds	45

Microbial Products, Inc., is operating a deeper (20 cm) pond system (Figure 2-10) in California. Deep systems have the advantage of natural temperature control, but the greater light limitation results in reduced cell concentrations, which increases the cost of harvesting the algae.

Microalgal cultures must be mixed to prevent cell settling or flotation, to prevent thermal stratification, and to ensure adequate contact between the algae and its nutrients. Mixing costs are the highest of the various power costs of these systems. Since the power required is proportional to the cube of the velocity for a given volumetric flow, it is important to minimize culture velocity while maintaining adequate mixing. Finally, major improvements in the reliability and cost of harvesting are required for a microalgae technology to develop for the purpose of producing fuels.

2.2.2.2 Harvesting Technology

The various techniques for harvesting microalgae include settling (or flotation), centrifugation, and filtration. These processes are aided by cell flocculation, either through the addition of chemical flocculants, or through culture autoflocculation (Figure 2-11). These flocculation techniques cause the cells to become aggregated into larger clumps that are easier to filter and settle more rapidly. Flotation is achieved through the introduction of small bubbles into the culture medium; algae are attracted to these bubbles and lifted to the surface for removal.

Figure 2-9. Experimental 50-m^2 Raceway Operated in Hawaii

The present slate of harvesting techniques for microalgae comprises processes that are costly both in capital investment and in energy input. It is not clear whether these techniques can be significantly improved, but the total engineering investment in developing these techniques to date is small relative to the potential scale of the technology. Continued innovation and research in the area of microalgal harvesting may offer significant improvements in harvesting costs.

Many of the procedures developed for the harvesting of algae from freshwater or wastewater systems have been based on the interaction between cells and various charged particles employed as flocculants. It has been demonstrated that the effectiveness of these flocculants is significantly reduced in saline waters. Normally, greatly increased doses

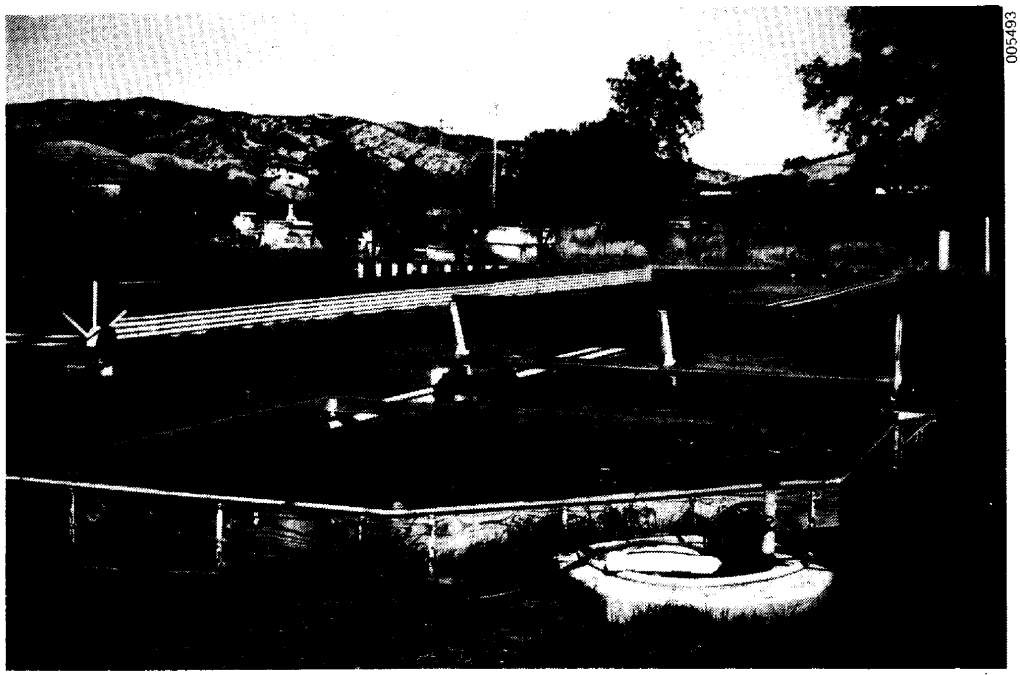

Figure 2-10. Experimental Pond System Operated in California

of flocculant are required to achieve the desired effect at high salinities. Research to optimize harvesting procedures for marine and brackish water species is presently being conducted by the Israeli project.

2.2.2.3 Culture Management

The development of culture management techniques is another area of engineering research. Culture management alternatives are needed to promote and sustain higher biomass yields. Two culture strategies are common: batch and continuous. In batch culture a pond is inoculated, the microalgae are allowed to grow, and at one point all algae in the pond are harvested. In continuous culture a portion is harvested daily and makeup water is added to the culture. Both strategies are being investigated by the program.

2.2.3 Technology Analysis

Data on all aspects of microalgae production have been unified in conceptual representations of commercial microalgal production systems. These models have allowed the necessary sensitivity analysis to be run, which shows the areas of research that will most benefit the technology development. Process evaluations were conducted to determine alternatives for converting microalgal feedstocks to useful fuel products at competitive costs with alternative renewable technologies. Attainable production costs were compared with fuel processing values, and it was determined that lipids provide the best fuel option from a microalgal feedstock. Either catalytic conversion to gasoline or transesterification to diesel fuels were found to be possible conversion routes for microalgal lipids.

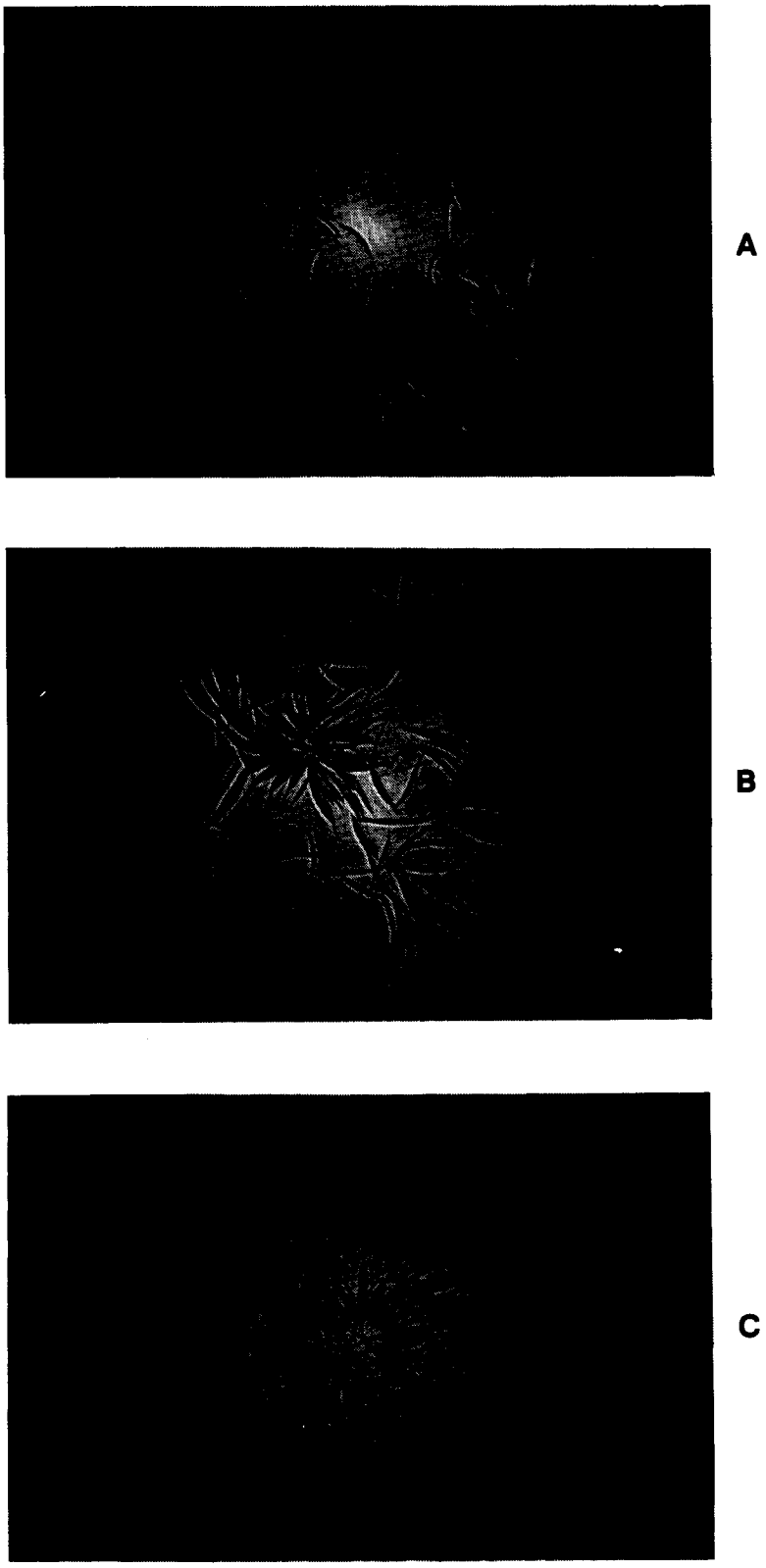

Figure 2-11. Flocculation Sequence for the Microalga Ankistrodesmus

In summary, the Aquatic Species program supports research on the development of microalgae and macroalgae as sources of renewable fuels. It is the objective of biological research to identify, culture, evaluate, and manipulate aquatic species to produce sustained yields of high-value energy products. The engineering research is concerned with design, evaluation, construction, and testing of the components and processes that will be required to produce these yields. The technology analysis efforts identify the availability of required resources and evaluate the economics of mass-production systems.

2.3 OBJECTIVES

The overall objective of the Aquatic Species program is to define and improve the productivity, conversion to fuels, and cost efficiency of aquatic plant species cultivated for energy. Table 2-3 lists the tasks, subtasks, and projects of the Aquatic Species program.

Table 2-3. Aquatic Species Program Tasks, Subtasks, Projects and Principal Investigators

Task I - Biological Research

A. Species Screening and Culture

Macroalgae

Harbor Branch Institute--Cultivation and Conversion of Marine Macroalgae--John Ryther.

Microalgae

Screening:

Alabama A&M—Screening for Microalgae in Southeastern United States--Mahasin Tadros

Georgia Institute of Technology--Chemical Profiles of Microalgae--Thomas Tornabene

Scripps Institution of Oceanography--Collection and Selection of Oil-Producing Desert Microalgae—William Thomas

Solar Energy Research Institute--Collection and Characterization of Saline Microalgae from Colorado and Utah—William Barclay*

Culture:

Ben Gurion University--Development of Outdoor Raceway Capable of Culturing Oil-Rich Halotolerant Microalgae—Shoshana Arad

Microbial Products, Inc.--Production of Liquid Fuels and Chemicals from Microalgae—Joseph Weissman

University of Hawaii--Productivity Optimization of Saline Microalgae Grown in Outdoor Mass Culture Systems—Edward Laws

B. Species Improvement

Solar Energy Research Institute--Effects of Light Modulation on the Productivity of Microalgae—Kenneth Terry*

Solar Energy Research Institute--Photosynthetic Efficiency of Lipid Production—Steve Lien*

Table 2-3. Aquatic Species Program Tasks, Subtasks, Projects and Principal Investigators (Concluded)

Task II - Engineering Research

A. Design Coordination

Aquaculture Associates, Inc.--Design of an Advanced Raceway System--Richard Spencer

Aquasearch, Inc.--Design of a Closed Continuous Culture System--Mark Huntley

Jaycor, Inc.--Evaluation of Immobilized Cell Systems for the Production of Fuels from Microalage—Marilyn Ripin

Microbial Products, Inc.--Design of an Advanced Pond System--Joseph Weissman

B. Component Development

No subcontracts.

Task III - Technology Analysis

Solar Energy Research Institute--Microalgae Analysis and Cost Evaluation--Bernie Neenan

*A part of the Microalgae Technology Research Group directed by Ken Terry.

2.4 RESEARCH STATUS

2.4.1 Biological Research

Biological research is divided into two subtasks. Species screening and culture is concerned with selecting the best species and developing cost-effective methods of culturing those species. Species improvement is concerned with enhancing the performance characteristics of the best species through culture manipulation, metabolic regulation, and genetic techniques. The program has been involved in the screening and culture of both macroalgae and microalage and is beginning to embark on species improvement.

2.4.1.1 Species Screening and Culture

Macroalgae. The production of macroalgae has been proposed for three different culture concepts: open ocean, near shore, and land based. The original concept of an energy farm for the production of macroalgae was the open-ocean farm, a suspended framework structure, buoyed and moored at depths of 700 m or more in open ocean, to which plants like the giant kelp, Macrocystis, would be attached. This concept proved to be economically infeasible. The Gas Research Institute has been sponsoring research into the development of near-shore cultivation of macroalgae such as kelp or the floating seaweed Sargassum. Near-shore culture would be in 20 m or less of water. SERI has been sponsoring, at the Harbor Branch Institute, research on the culture of Gracilaria and Ulva, either of which would be technically suitable for land-based raceway culture or bay-estuarine culture (Figure 2-12). In bay or estuary culture, the macroalgae would be

Figure 2-12. The Green Macroalga Ulva Cultivated at Harbor Branch

grown in shallow embayments confined by a fence or other barrier. In raceways, the culture is maintained at a density of approximately 2 kg wet weight/m^2. At this density, the algae are compacted so that normal wind and tidal action will not cause the algal mass to drift and accumulate unevenly. At brief intervals during the day, the culture is mixed and rotated by compressed CO_2 from pipes distributed throughout the culture systems. Preliminary economic analysis has indicated that land-based culture with Gracilaria would be as much as 4-5 times as expensive as bay-estuarine culture.

Well-nourished Gracilaria exposed to full sunlight in Florida or equivalent latitudes will double its biomass in 1 to 4 weeks, depending on the season, water flow, and other variables. 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 ensure continued optimal yield. The doubling of biomass can be accompanied by the utilization of all stored

nutrients and a reduction of elemental nutrients in the plant tissues to roughly half the initial concentrations. Enrichment of the new starting crop following harvest could conceivably be accomplished at the seaweed farm, but the rapid uptake and storage of nutrients by depleted seaweeds makes possible a simpler, more efficient enrichment process known as pulse fertilization, where the plants are enriched with nutrients only once a week. During this one enrichment, plants have been shown to assimilate and store enough nutrients in a few hours to support growth for the full week.

During the past year, research was directed at lowering the production costs of raceway culture by optimizing the amount of aeration provided the culture. Aeration was decreased twelvefold with a minimal impact on productivity (Figure 2-13). Aeration with a one-sixth duty cycle provided only during daylight hours was found to stimulate growth nearly as well as continuous aeration.

To determine if raceway culture of macroalgae could be adapted from coastal areas to less costly land areas such as the American Southwest, experiments were conducted to determine if Gracilaria or Ulva could be cultured in Southwest saline water Type I or II. In short-term experiments, Ulva grew better in Type I water than in seawater, but when growth was measured over more than two weeks, the growth rate slowed, and ultimately the plant died (Table 2-4). Work is continuing to determine why such water types are unsuitable for macroalgal culture.

Macroalgae are known to accumulate carbohydrates under stress. During the past year it was demonstrated that changes in culture conditions alter the chemical composition of macroalgae, thus effecting the quality of feedstock for conversion to fuels. Both changes in nitrogen levels and temperature result in a substantial change in carbohydrate content (Table 2-5). When Gracilaria was cultured with no nitrogen present in the medium, the soluble carbohydrate doubled. This could be very important in optimizing these plants for methane yield and ethanol production, which have been correlated with the soluble carbohydrate fraction.

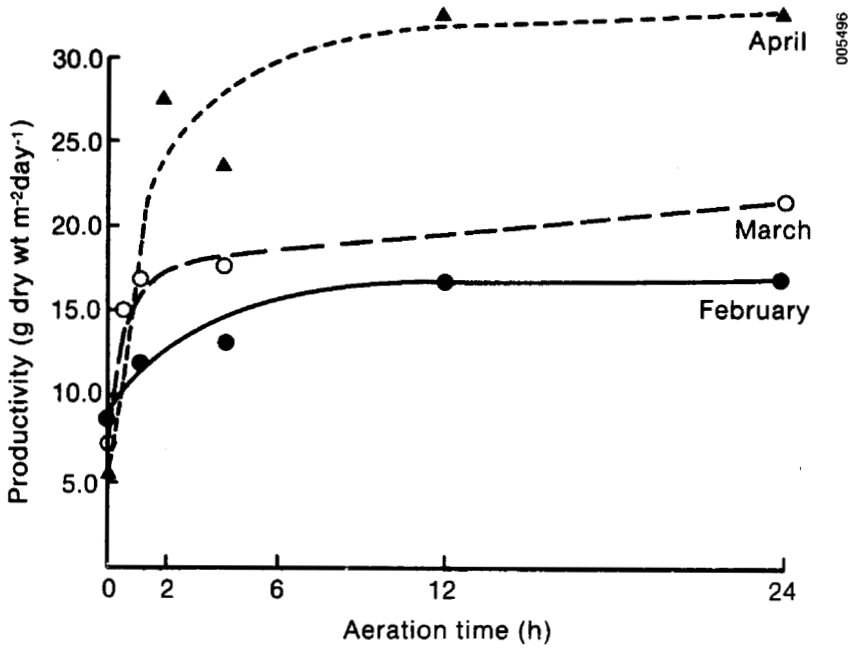

Figure 2-13. Effect of Culture Aeration on the Productivity of Gracilaria

SERI

Table 2-4. Specific Growth Rate of Ulva Cultured in Ocean and Desert Waters (doublings per day)

Water Type	Week 0[a]	Week 1	Week 2	Week 3
Ocean water	0.37	0.21	0.25	0.23
Artificial ocean	0.31	0.20	0.22	0.17
Type I desert	0.59	0.19	0.18	0.08
Type II desert	0.25	died	—	—

[a]Separate short-term experiment.

Table 2-5. Effect of Culture Conditions on the Productivity and Chemical Composition of Ulva and Gracilaria

	Ulva				Gracilaria			
	Nitrogen (mg/L)		Temperature (°C)		Nitrogen (mg/L)		Temperature (°C)	
	0	54	9	23	0	54	10	22
Productivity ($g\ dry\ wt\ m^{-2}\ day^{-1}$)	6.4	10.4	4.0	12.9	5.8	10.9	5.1	10.1
Protein (%)	4.5	8.3	11.9	7.6	4.5	10.7	7.3	7.3
Carbohydrate (%)	30.8	21.6	27.5	22.9	36.6	15.5	35.4	21.5
Lipid (%)	3.6	3.8	3.6	3.2	1.5	2.8	-	-
Ash (%)	17.2	30.3	25.6	29.2	36.9	51.3	37.4	47.4

Microalgae Screening. The objective of the screening activity is to efficiently select for microalgae with good characteristics for growing in outdoor mass culture and as a feedstock for fuel production. The screening protocol has been changed to concentrate on strains that grow well at higher temperatures and light intensities. Strains that have performed the best in outdoor culture have all been natural invader species. Of these invader species, a Platymonas that established itself in the Hawaii raceway has proven to be the most successful because it is an excellent biomass producer and is predator resistant. To validate the present selection protocol, this Platymonas was used as a control using the laboratory screening procedures. Platymonas was inoculated at 30°C and the equivalent of 20%-30% full sunlight with equal numbers of other marine species. At the end of eight days, it had established itself as the dominant strain and thus would have been the strain selected under the screening protocol. The results of a salinity-temperature growth experiment confirmed that Platymonas grows well at higher temperatures and over a broad salinity range (Figure 2-14). The best growth occurs at 28°-30°C and 35-40 g dissolved solids/liter.

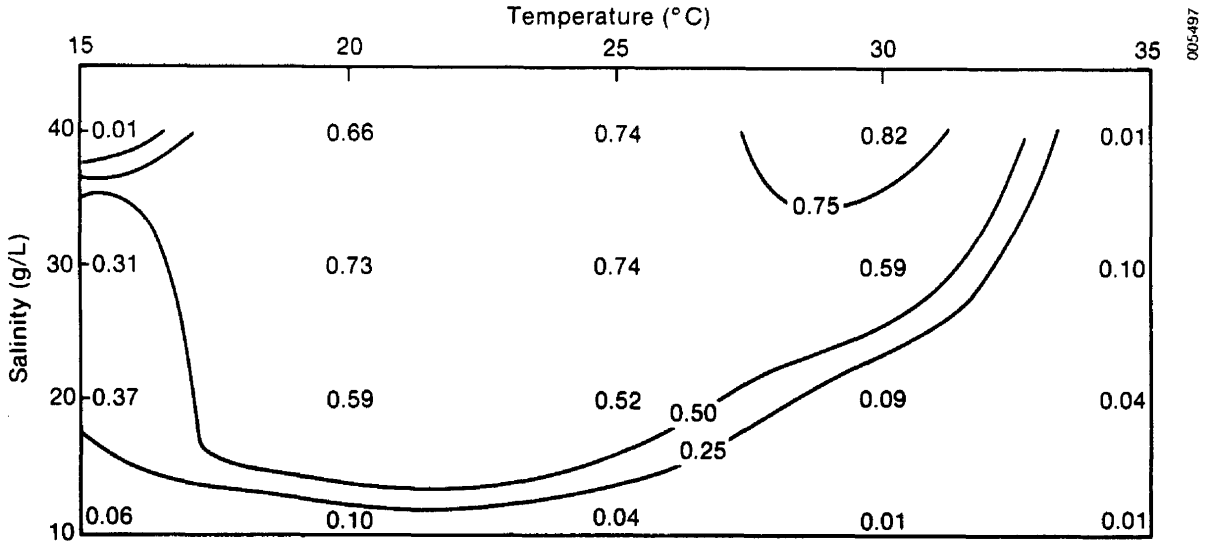

Figure 2-14. Growth Response of the Hawaiian Platymonas to Different Temperatures and Salinities (numbers = final optical density)

During 1984, microalgae were screened from locations in California, Nevada, Utah, Colorado, and the Gulf Coast of Alabama.

California and Nevada. From three collecting trips made to saline lakes during the summer of 1984, twenty strains were selected as good growers from a group of more than 60 that were tested (Table 2-6). Testing the growth response of these strains in the two types of desert saline media revealed that some strains perform equally well in both types, whereas others have strong preferences for either Type I or Type II desert saline waters. Marine species tested in desert marine waters also exhibited differential preferences (Table 2-7).

Yields were obtained for two species that had been initially characterized as promising for biomass production. A previously isolated Ankistrodesmus falcatus from Pyramid Lake yielded between 40 and 50 g/m^2 day of ash-free dry biomass. The Chlorella ellipsoidea BL-6 isolated from Black Lake yielded 30-40 g/m^2 day of ash-free dry biomass. The Chlorella strain also appears promising because of its wide temperature and salinity growth response (Figure 2-15). This Chlorella grows well from 10 to 40 g dissolved solids/L salinity and from 25° to 35° C. Work is continuing on identifying other promising species.

Colorado and Utah. During the summer collecting season, seven field trips in Colorado and Utah (Figure 2-16) led to the isolation of over two hundred strains of microalgae. These strains have passed preliminary screening tests for growth capacity and temperature and salinity tolerance. Based on these results, 23 strains have been selected for further characterization and evaluation. The genera to which these species belong are listed in Table 2-8; all these strains grow well at salinities greater than 5 g TDS/L and at temperatures of at least 30° C.

Table 2-6. **The Most Promising Microalgae Strains Selected from California and Nevada during 1984**

Culture	Temperature (°C)	Expontial Growth Rate (doublings/day)	Maximum Optical (density)	Rating
Oocystis, PA-2	24	0.43	0.74	+++
from Pond A, 4/83	30	0.2	0.52	++
Nannochloris, PB-4	24	0.50	1.02	++++
from Pond B, 7/83	30	0.28	0.72	+++
Nannochloris, Rt6-2	24	0.89	0.64	++
from Spring, Route 6, 9/83	30	1.08	0.62	+++
Chlorella, BL-6	24	1.31	1.07	+++
Black Lake, 5/84	30	2.48	1.02	++++
Green Round Cells, Mo2A	24	0.26	0.30	++
Mono Lake (D. Herbst)	30	0.88	0.76	+++
Chlamydomonas,	24	1.05	0.92	+++
China (R. Lewin)	30	0.58	1.07	++
Green Flagellate, HL-9	24	0.27	1.10	+++
Harper Lake, 5/84	30	0.26	1.02	+++
Chaetoceros, OL-12	24	1.23	0.30	++
Owens Lake, 5/84	30	1.29	0.44	++
Nitzschia, OL-10	24	0.73	0.21	++
Owens Lake, 5/84	30	1.51	0.46	++
Nitzschia, SS-1A	24	0.88	0.62	++
Salton Sea 4/83	30	0.39	0.40	++
Oocystis, PA-2	24	0.43	0.74	+++
from Pond A, 4/83	30	0.21	0.52	++
Nannochloris, PB-4	24	0.50	1.02	++++
from Pond B, 7/83	30	0.28	0.72	+++
Nannochloris, Rt6-2	24	0.89	0.64	++
from Spring, Route 6, 9/83	30	1.08	0.62	+++
Green Alga, Salton Sea,	30	0.65	0.33	+++
SS-4 4/83	24	0.46	0.50	++
Green Alga, Salton Sea,	30	0.95	0.48	++
SS-5, 4/83	24	1.12	0.47	+++

Table 2-6. The Most Promising Microalgae Strains Selected from California and Nevada during 1984 (Concluded)

Culture	Temperature (°C)	Expontial Growth Rate (doublings/day)	Maximum Optical (density)	Rating
Green oval cells, Salton Sea, SS-13, 8/84	30	0.84	0.60	++
	24	0.98	0.60	++
Chaetoceros, Salton Sea, SS-14, 8/84	30	1.03	0.61	+++
	24	1.14	0.61	+++
Green Flagellate, Pond A, PA-7, 7/83	30	1.08	0.41	+++
	24	0.85	0.39	++
Cryptomonas, Harper Lake, HL-1, 2/83	30	0.54	0.83	++
	24	1.16	0.53	+++
Green Flagellate, Rt. 80 Spring 56R80-3, 7/84	30	0.91	0.65	++
	24	1.04	0.49	+++

Table 2-7. Species Preference for Media Type

Species	Type I[a]	Type II[a]
Chlorella ellipsoidea BL-6	—	*
Chlorella SC-2	*	*
Nannochloris PB-4	*	*
Flagellate H2-9	—	*
Platymonas (marine)	*	—
Phaeodactylum (marine)	*	*
Isochrysis (marine)	*	—

[a] * = preferred water types

The Ochromonas (Chryso/Fl) appears to be extremely promising. Ochromonas showed satisfactory growth in all water types, but grew most rapidly in Type II, followed in order by seawater and Type I. The growth rate was high in all cases but particularly in Type II water, where it exceeded 2.0 day^{-1} under a variety of conditions. The maximum growth rate observed in Type II water was 2.38 day^{-1}, or about 3-1/2 doublings per day. The maximum growth rate in seawater was 1.79 day^{-1}, and in Type I was 1.9 day^{-1}.

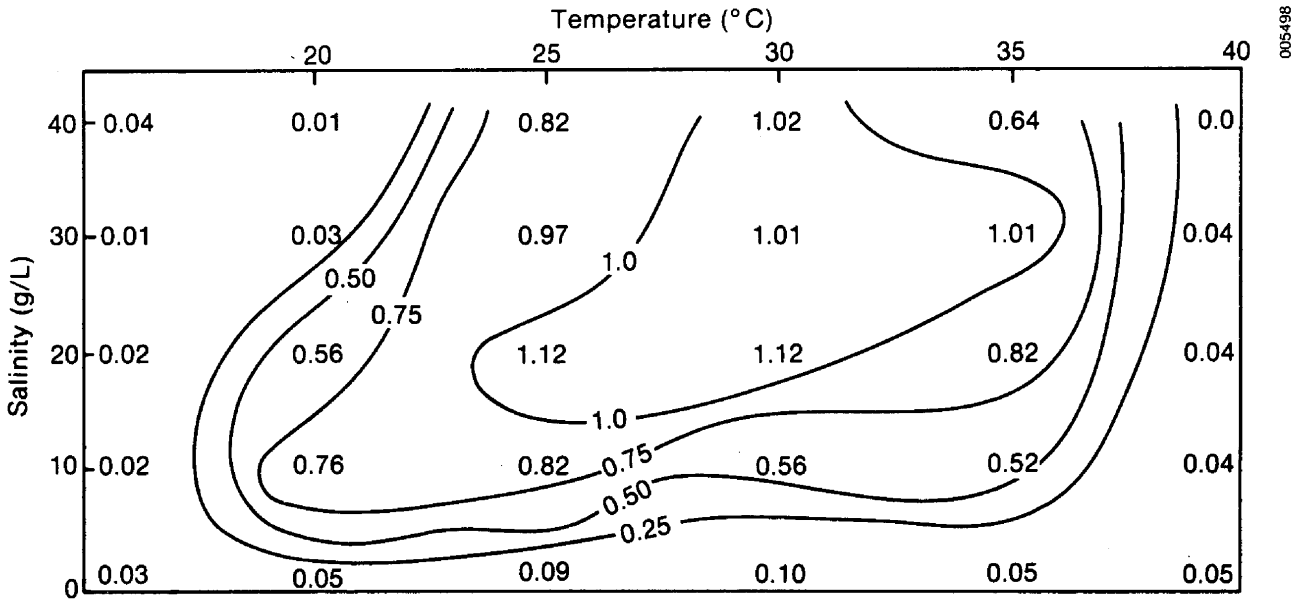

Figure 2-15. Growth Response of Chlorella ellipsoidea BL-6 to Different Temperatures and Salinities (numbers = final optical density)

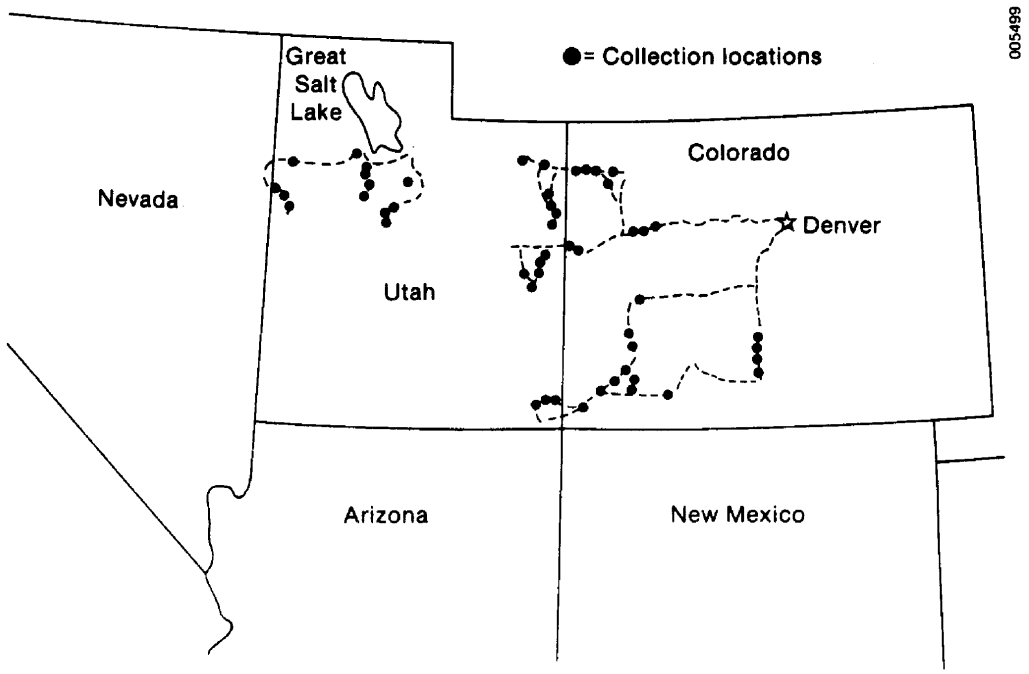

Figure 2-16. Collection Areas and Sites in Colorado and Utah

Ochromonas shows a wide range of salinity and temperature tolerance and is capable of good growth at temperatures above 30° C and salinities greater than 40 g/L. This strain is tolerant of low temperatures and salinities. The range of its tolerance is significantly enhanced in its most suitable water type, Type II. Lipid content ranges from 25% to 55%, depending on the nutritional state of the cell.

Alabama. Two collection trips were made to Dauphin Island, off the coast of Alabama, during the summer of 1984. Over sixty strains were isolated, and of these, six were ranked as good growers (Table 2-9). Two diatoms were isolated that are of particular interest due to their ability to accumulate lipids. Cyclotella grows well at elevated temperatures in media of 15-25 parts per thousand; with nitrogen stress it accumulates 42% of its dry weight as lipid. Hantzschia is a large diatom that also grows well at elevated temperatures and full strength seawater (Figure 2-17). Hantzschia accumulates as much as 66% of its dry weight as lipid.

Table 2-8. Promising Species from Collections in Colorado and Utah in 1984

Genus	Number of Strains	Genus	Number of Strains
Ochromonas	1	Hantzschia	1
Amphora	7	Diploneis	1
Cymbella	5	Chlorella	1
Amphipleura	1	Scenedesmus	1
Chaetoceros	1	Kirchneriella	1
Nitzschia	2	Chlorococcum	1

Table 2-9. Approximate Growth and Cellular Composition of Algal Species Collected in Alabama

Species	Cell Size (μm^3)	Growth Rate (doubling/day)	Growth Conditions[a]	% Organic Weight		
				Protein	Carbohydrate	Lipid
Cyclotella sp. DI-35	3-5	1.37	NE	12.2	37.5	13.2
			ND	16.4	10.2	42.1
Nitzschia sp. TR-114	10-15	0.84	NE	25.7	18.8	15.2
			ND	7.2	13.2	28.1
Chlorella sp. MB-31	2-3	0.92	NE	51.2	12.3	15.3
			ND	25.4	26.2	28.6
			NE	23.5	24.7	26.5
			ND	18.3	29.6	32.4
Scenedesmus sp. TR-84	5-6	1.79	NE	30.2	29.8	20.3
			ND	11.4	32.3	44.7
Ankistrodesmus sp. TR-87	5-7	1.11	NE	35.3	32.5	16.9
			ND	24.5	38.2	28.11
Hantzschia sp. DI-60	15-35	1.32	NE	20.2	29.4	26.3
			ND	12.6	9.3	66.0

[a]NE = nitrogen sufficient; ND = nitrogen deficient.

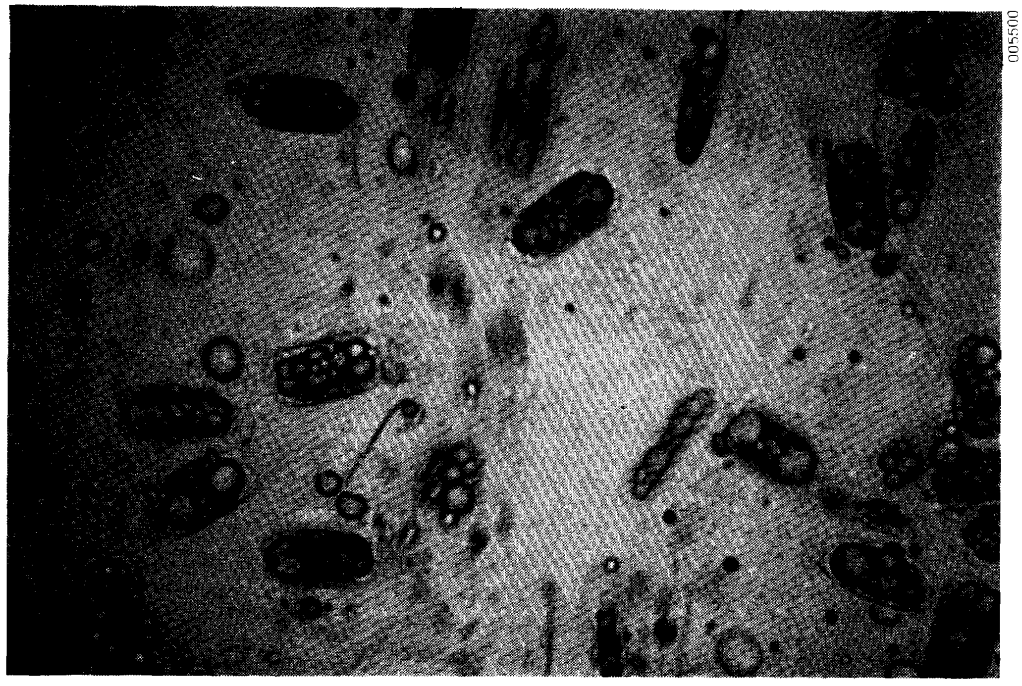

Figure 2-17. The Diatom Hantzschia, Collected from the Gulf of Mexico Accumulates up to 66% of Its Dry Weight as Lipids

Other Activity. Several strains of microalgae obtained from culture collections were measured for their ability to grow at elevated temperatures in order to select thermophilic strains for outdoor culture, since temperatures in the outdoor mass culture units typically reach 34°-35°C during summer months.

Most marine microalgae do not grow well at temperatures in excess of 30°C. For example, of seven tropical diatom isolates from Surinam, three exhibited no growth at 33°C, and the remaining four exhibited no growth at 37°C. However, some species exhibit remarkable thermal tolerance. A strain of Chaetoceros gracilis has been reported to grow optimally in the temperature range 23°-37°C and to be capable of growth in the temperature range 11°-41°C.

Figure 2-18 shows the results from laboratory studies for those species that were capable of growth at 32°C and 34°C. None of these species exhibited any growth at 36°C. The best overall growth at 32° and 34°C was displayed by Platymonas sp., the species which appeared as a contaminant in the Hawaii outdoor raceway during culture studies with Phaeodactylum tricornutum.

Sixteen strains of Chlorella were obtained for screening as potential candidates for culture outdoors. Temperature optima for the strains ranged from 20° to 30°C. Best growth was obtained with Chlorella minutissima, which has a temperature optimum of 20°C. The lipid content of various strains is shown in Table 2-10.

Screening - Lipids. A secondary step in screening is the determination and quantification of the chemical composition of selected microalgae grown under different conditions. These analyses are an important aspect of the screening process and are primarily carried out at the Georgia Institute of Technology. Chemical analyses are carried out in accordance with a standard procedure set up by the program.

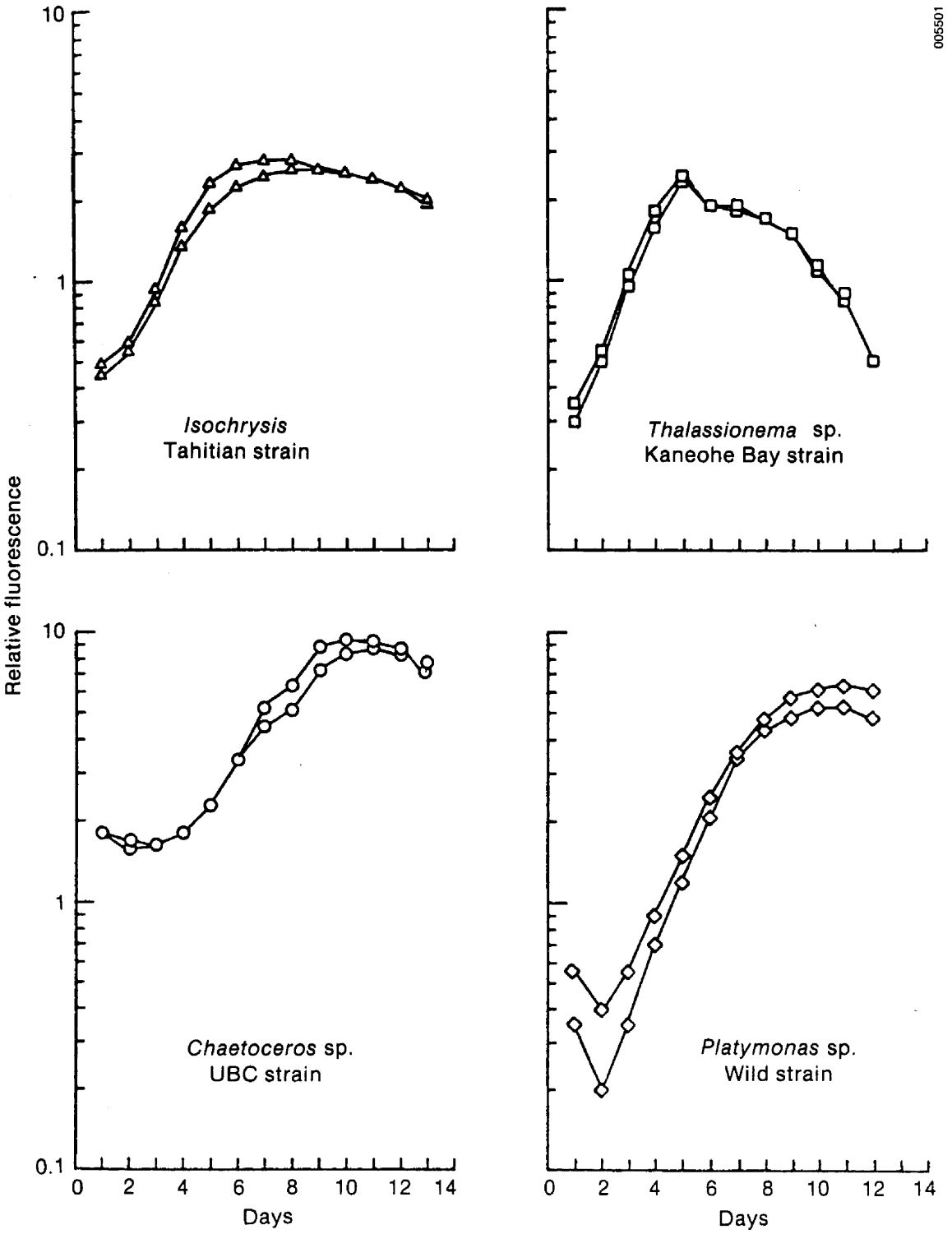

Figure 2-18. Growth Curves of Four Thermophilic Marine Microalgae at 34°C

Table 2-10. Lipid Content of Four Strains of Chlorella (%)

Strain	Nitrogen Sufficient	Nitrogen Deficient
luteovisidis	17.5	28.8
Sp. 2068	18.2	27.6
stigmatophora	16.2	14.9
minutissima	27.4	26.4

Microalgal lipids are typically esters of glycerol and fatty acids having carbon numbers in the range of C_{12}-C_{20}. Lipids can be subdivided according to their polarity, which depends on the nonpolar (lipophilic) carbon chains (fatty acids, long-chain alcohols) and on the polar (hydrophilic) moieties, such as phosphate and carboxylic groups, alcohols, sugar, or bases in each lipid (Figure 2-19). Nonpolar lipids (neutral lipids) are triglycerides, free fatty acids, hydrocarbons, and wax esters (only a few algae). The polar lipids are acyl lipids and mainly consist of phospholipids and glycolipids. The main phospholipids of algae are phosphatidyl choline (PC), phosphatidyl glycerol (PG), phosphatidic acid (PA), and diphosphatidyl glycerol (cardiolipin, DPG). The major algal glycolipids are monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), and sulphoquinovosyl diglyceride (sulpholipid, SL). A novel class of algal lipids is chlorosulpholipids, which are derivatives of N-docosane-1,14-diol and of N-tetracosane-1,15-diol disulphates and which have been found in Chrysophyceae and Cyanophyceae. Neutral lipids and triglycerides (fatty acids) are considered to have the most potential as fuels. It is well established that the types and quantity of lipids change with growth conditions and age of the culture; under nitrogen starvation, neutral storage lipids increase. Two species of microalgae whose lipid composition has been examined in detail are Ankistrodesmus falcatus from Pyramid Lake and Nannochloropsis salina (GSB Sticho) (Table 2-11). Both species accumulate lipids in excess of 40% when grown under nitrogen-deficient conditions.

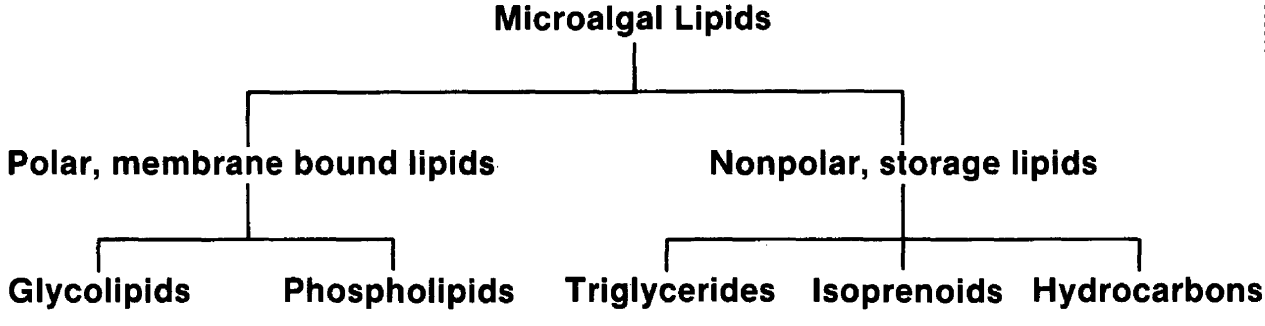

Figure 2-19. Classification of Microalgal Lipids According to Polarity

Table 2-11. Chemical Composition of Two Promising Species of Microalgae

	Ankistrodesmus		Nannochloropsis	
	Nitrogen Sufficient	Nitrogen Deficient	Nitrogen Sufficient	Nitrogen Deficient
Protein (%)[a]	28.6	14.3	43	20
Carbohydrate (%)	9.2	18.3	12	13
Lipid (%)	19.5	40.3	22	49
Hydrocarbon[b]	0.0	0.0	2	1
Isoprenoid	5.8	3.3	5	5
Triglyceride	16.4	13.5	5	25
Glycolipid	69.2	66.5	55	25
Phospholipid	12.0	12.1	33	27

[a]% of dry weight.
[b]% of total lipid.

Microalgae Culture Collection. Many of the advances during the last decade in the taxonomy, cytology, genetics, biochemistry, and physiology of algae have been made through the use of recognized clones or strains of algae maintained and distributed by culture collections. In order to promote strains of microalgae with potential for fuel production, a specialized microalgae culture collection was established at SERI. The collection presently consists of eleven strains that are available upon request (Table 2-12). Culture and composition data have been compiled on each strain maintained in the collection, and this information is presented in the catalog for the culture collection.

Table 2-12. The SERI Microalgae Culture Collection

Species	Source	Collection Site
Ankistrodesmus falcatus	W. H. Thomas	Pyramid Lake, Nev.
Botryococcus braunii	UTEX[a]	
Chaetoceros gracilis	R. York	
Chlorella sp. (SO1)	S. Lien	Golden, Colo.
Isochrysis aff. *galbana*	R. York	Tahiti
Nannochloropsis salina		Great South Bay, N.Y.
Nitzschia sp.	W. H. Thomas	Mono Lake, Calif.
Oocystis pusilla	W. H. Thomas	Walker Lake, Calif.
Phaeodactylum tricornutum TFX		Woods Hole, Mass.
Phaeodactylum tricornutum BB	W. H. Thomas	
Platymonas sp.	E. A. Laws	Honolulu, Hawaii

[a]University of Texas Culture Collection

2.4.1.2 Laboratory Culture Research Studies

Three organisms were identified by the Israeli research group as being model lipid producers. These species were Nannochloropsis, Isochrysis, and Chlorella. Research during 1984 focused on establishing the optimal culture conditions for maximal biomass and lipid yields from these three species. Temperature, salinity, pH, and nutrient optima were determined for Isochrysis and Nannochloropsis (Figure 2-20). The optimum growth for Isochrysis was at a temperature of 24°-28°C, a salinity of 15-40 parts per thousand, and a pH of 6-7. Nannochloropsis exhibited a narrower optimum range: a temperature of 28°C, salinity of 40 parts per thousand, and a pH between 8.0 and 9.0. The addition of supplemental iron increased the growth rate of both Isochrysis and Nannochloropsis.

Several factors were identified that affected the lipid content of Nannochloropsis. Lipid content was highest in cultures grown at a pH of 7.5, utilizing ammonia as the nitrogen source. It was also discovered that Nannochloropsis grown in a natural seawater media had a higher lipid content (45%) than when grown on an artificial seawater media (25%). When either Isochrysis or Nannochloropsis was grown outdoors, efforts to induce lipid accumulation failed; lipid contents from outdoor cultures were generally less than 20% of the dry weight. The reason for this is being investigated, and work is now focused on optimizing productivity for Isochrysis and Nannochloropsis in outdoor ponds.

2.4.1.3 Outdoor Culture Studies

Outdoor mass culture experiments are carried out to (1) compare the performance of various systems, (2) to develop strategies for mass culture management, (3) to scale up tests, and (4) to test selected microalgae strains in outdoor mass culture. Presently SERI supports

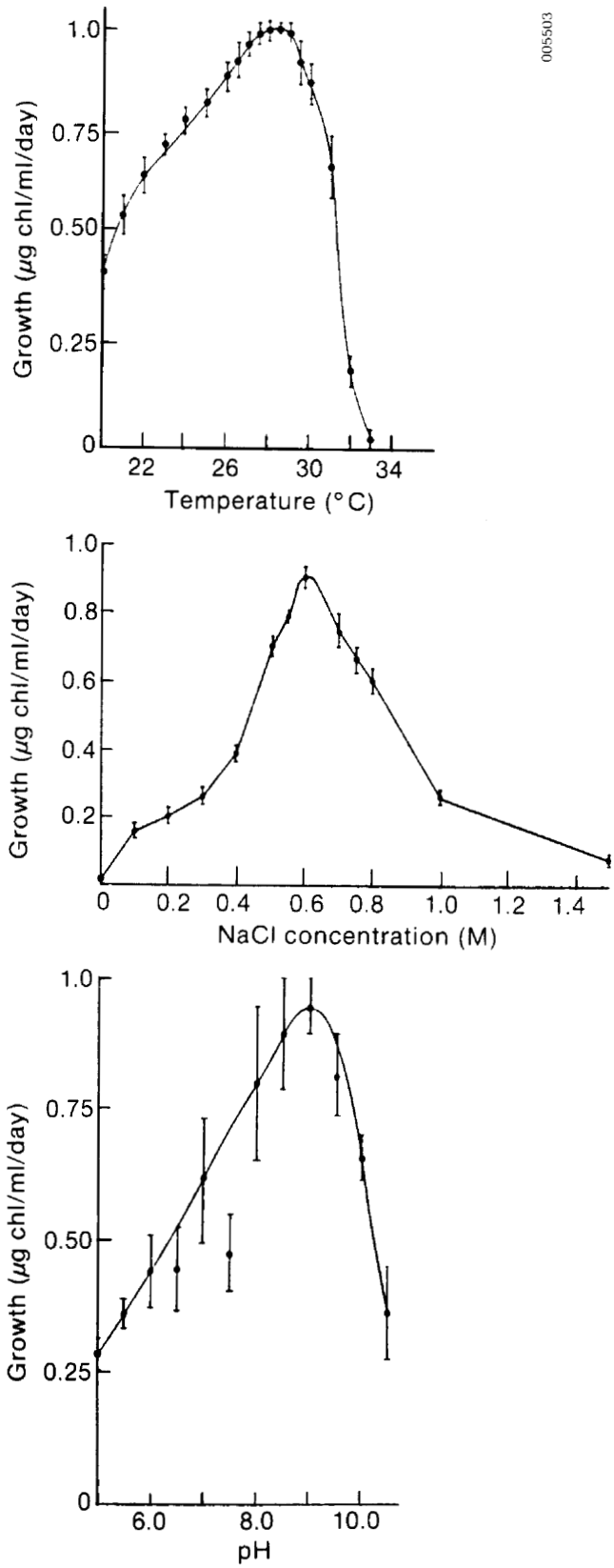

Figure 2-20. Growth Response of Nannochloropsis to Variable Temperature, Salinity, and pH

two outdoor experimental facilities: one is operated by the University of Hawaii, and the other by Microbial Products, Inc. (formally EnBio, Inc.) in California. Figure 2-21 is a schematic of the raceway in Hawaii, and Figure 2-22 is a diagram of the pond system operated by Microbial Products. The two systems differ primarily in depth: the ponds are operated at 20 cm and the raceways at 10 cm. The mixer types, harvesting methods, and media salinities also differ. In addition to the large experimental units, work is also conducted in scaled-down culture units (Figures 2-23 and 2-24).

The shallower raceway permits the use of foils, which affect systematic vertical mixing in the raceway (Figure 2-25). The foils are shaped like airplane wings, forcing the water flowing over them to spiral downward, creating a helical flow pattern downstream of the foil. The optimum angle of attack is approximately 23 degrees for a flow rate of 30 cm s^{-1} and culture depth of 10 cm. Foil arrays are placed 1.2 m apart. Photosynthetic efficiency increases after insertion of the foils 25%-40%. This enhancement is assumed to result from more efficient utilization of the flashing light effect. The flashing light effect is the increase in productivity that results from exposing microalgae to intermittent flashes of dark and light. A modeling effort describing the flashing light effect indicates that (1) at very low light intensity, there is no flashing light effect, (2) at very low cell densities there is no flashing light effect, and (3) the magnitude of enhancement from the flashing light effect increases with increased light intensity and increased cell density.

During 1984, outdoor culture experiments were conducted with a number of species. University of Hawaii researchers obtained excellent yields with the green flagellate Platymonas and marine diatom Chaetoceros. Both strains grow well at temperatures above 30°C, resulting in yields greater than 35 g/m^2 day sustained for periods greater than one month (Table 2-13). A major discovery was that diluting the Platymonas culture every third day enhanced production. In this production mode, daily yields averaged 46 g/m^2 day ash-free dry weight over a one month period (Figure 2-26). This represents a photosynthetic efficiency of 11% (based on photosynthetically active radiation). This yield is over twice the best long-term yields achieved in microalgal mass culture systems grown exclusively on inorganic nutrients. Nutrient starvation experiments indicated that Platymonas stored carbohydrates instead of the desired lipids. However, Chaetoceros, a diatom, was found to accumulate up to 55% lipid after a 4-5 day period of silica starvation. Yield optimization studies with Chaetoceros resulted in yields of 42 g/m^2 day using a 75% dilution rate every two days. The major constraint for the culture of Chaetoceros is a requirement for vitamins and silica and a susceptibility to predators.

In contrast to research in Hawaii, research conducted by Microbial Products focused on ways to reduce operating costs. Major cost reductions were made by increasing the number of harvest/media recycles, reducing the amount of trace elements added and optimizing the mixing speed and power requirements for the system. It was interesting that for this pond system, mixing speeds between 5 and 30 cm/s did not change biomass yields. These studies showed that it was possible to lower the production costs of Scenedesmus from $7.50 to $2.50 per dry pound. Input requirements for the two systems are contrasted in Table 2-14.

One interesting comparison between the Hawaii raceway system and the Microbial Products pond system was the water oxygen concentration. The Hawaii raceway system does not develop oxygen concentrations greater than 10-15 mg/L, whereas the Microbial Products pond system develops oxygen concentrations greater than 40 mg/L. This difference is due to the method of mixing; the airlift pump used in the Hawaii raceway system deoxygenates the water, whereas the paddle wheel used in the pond system does not.

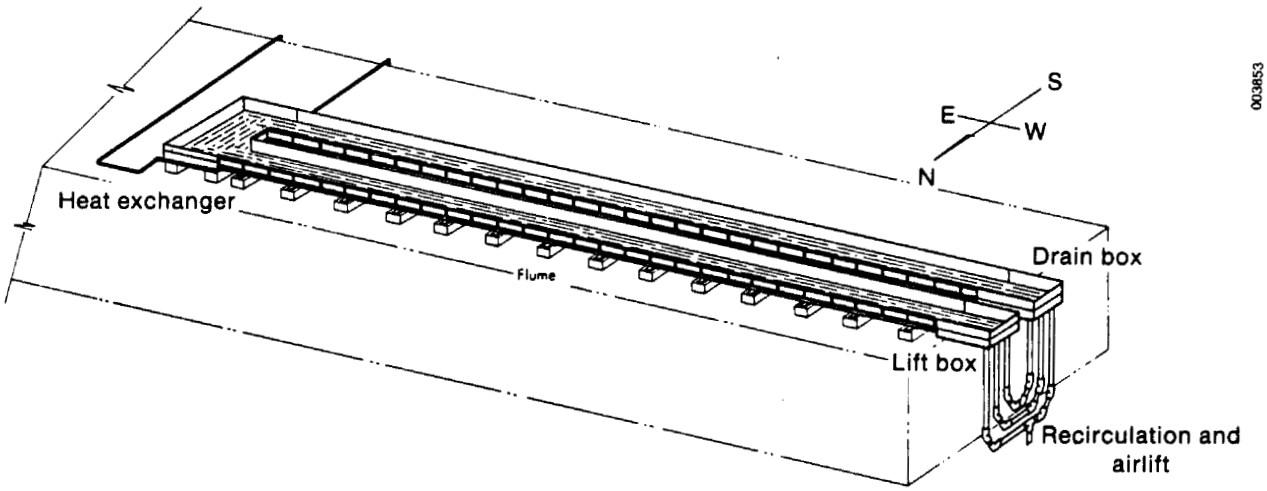

Figure 2-21. Algal Production Raceway, Including Location of Heat Exchanger, Lift Box, Drain Box, and Airlift System

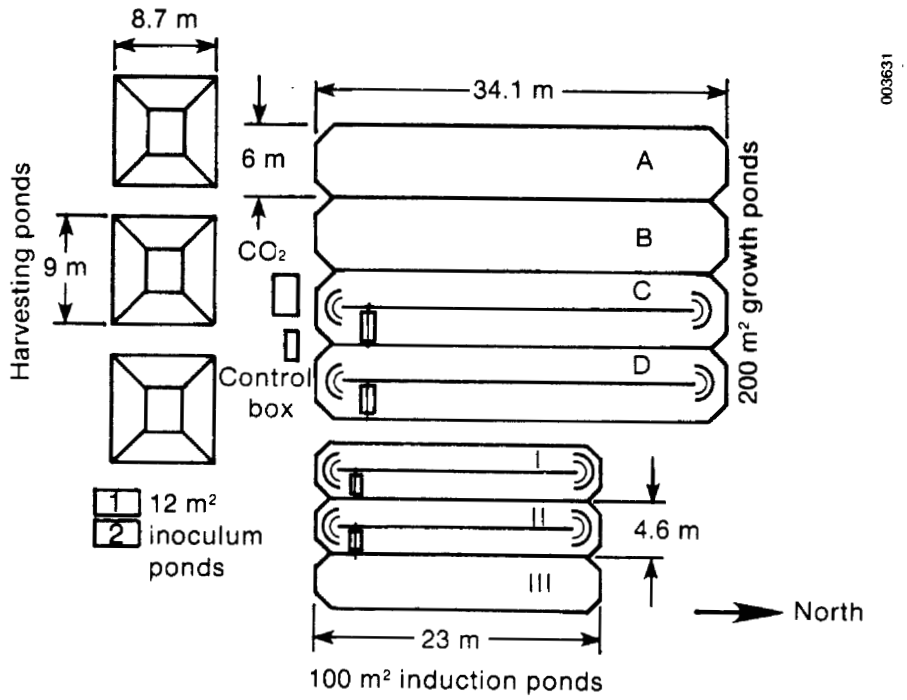

Figure 2-22. Pond System Operated by Microbial Products Co.

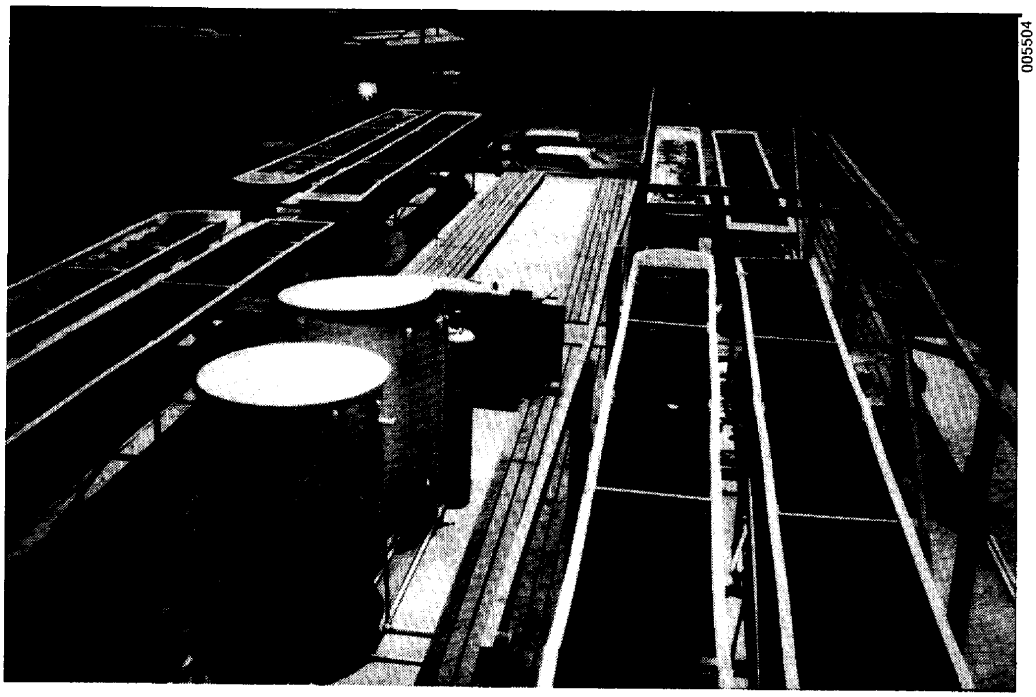

Figure 2-23. Small (8-m^2) Experimental Raceways Operated in Hawaii

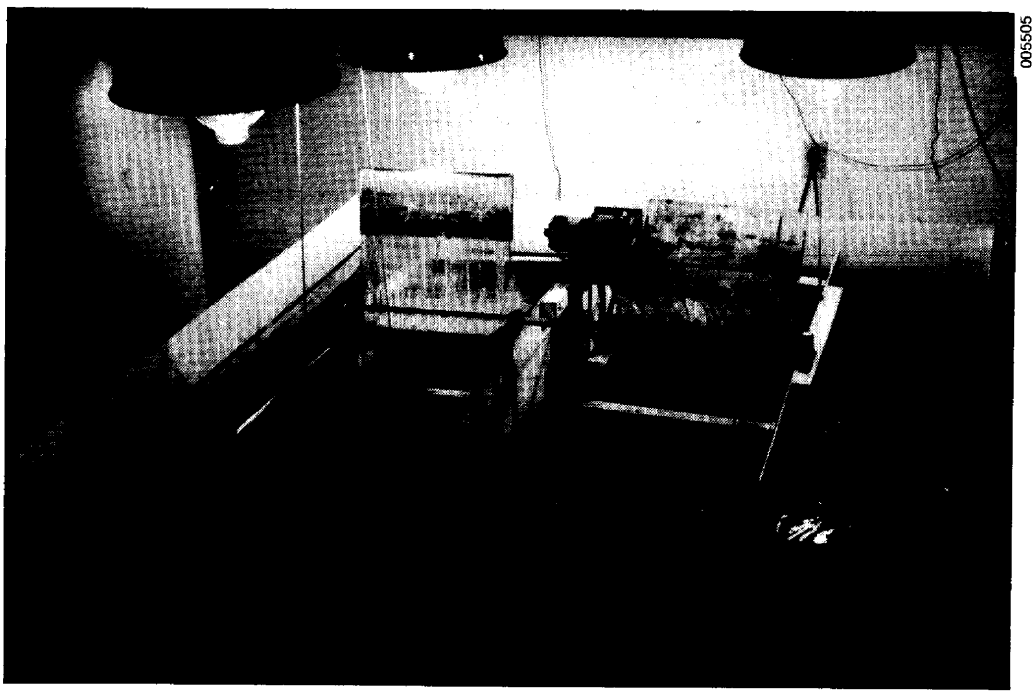

Figure 2-24. Indoor Ponds Used for Winter Studies by Microbial Products

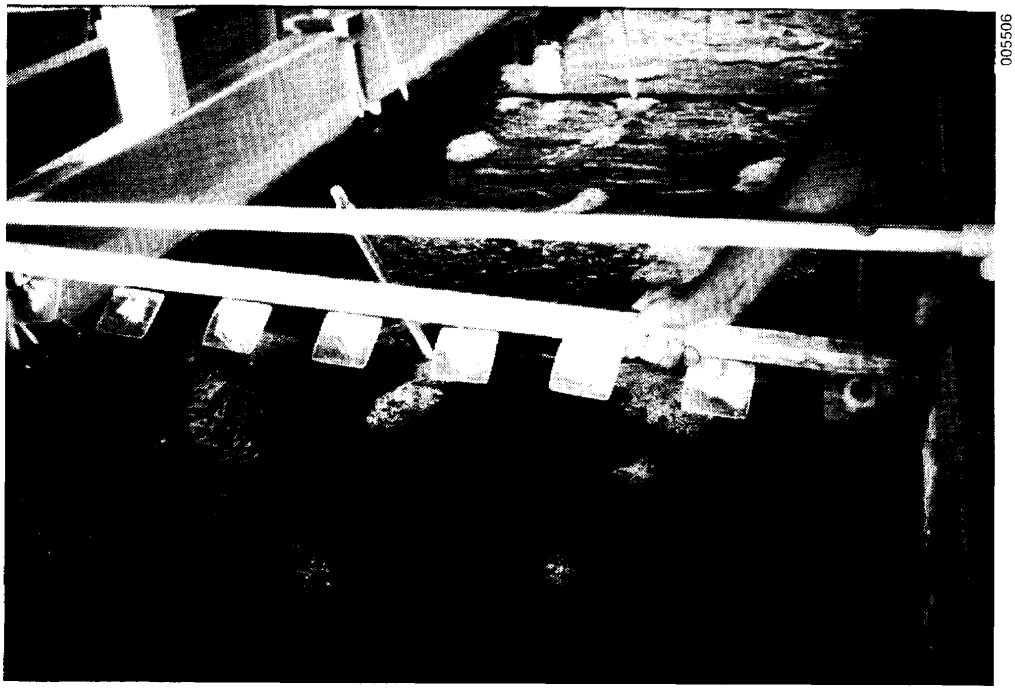

Figure 2-25. Foils Installed in the Hawaii Raceway System

Table 2-13. Performance Data from the Raceway and Pond Systems

	Raceway		Pond	
	Platymonas	Chaetoceros	Chlorella	Scenedesmus
Productivity ($g\ dry\ wt/m^2\ day$)	25-55	20-42	20-21	15-20
Lipid content (%)	15	15	22	20

This provides another criterion for species selection. It was determined that the Scenedesmus that did well in the pond was not inhibited at high oxygen concentrations, whereas the laboratory-screened Ankistrodesmus was very much inhibited. This helps explain why Scenedesmus out-competed the Ankistrodesmus in the Microbial Products pond system. Table 2-15 summarizes the major screening and culture accomplishments made in 1984.

2.4.1.4 Species Improvement: Microalgae

Species improvement comprises three areas of research: (1) lipid metabolism and regulation, (2) effects of light modulation on enhancing growth, and (3) the use of genetics to develop strains that allocate more fixed carbon to lipids.

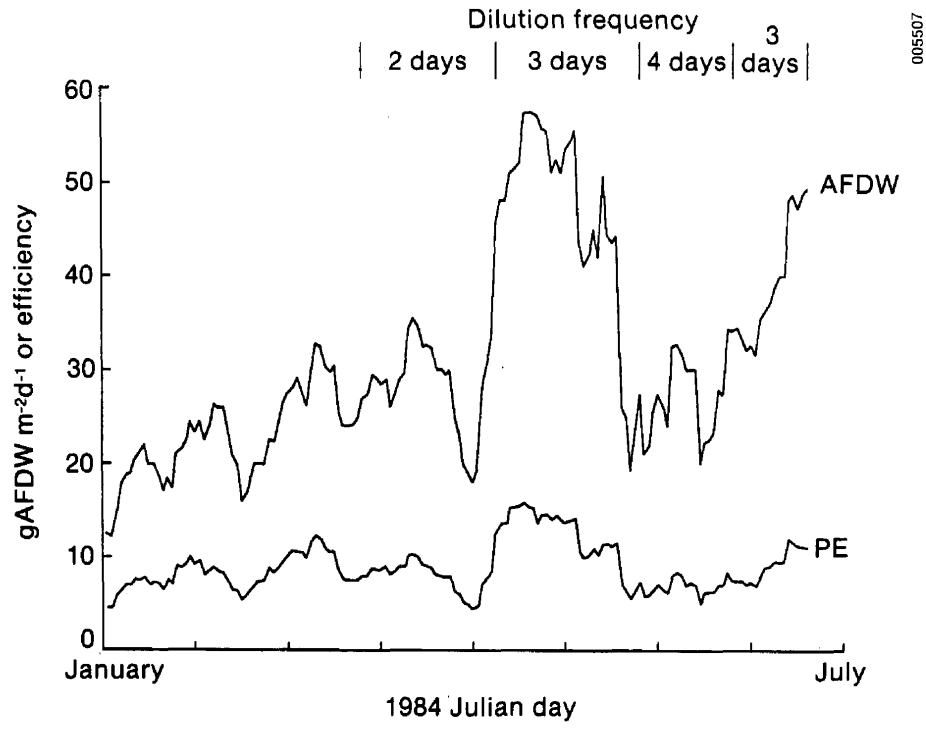

Figure 2-26. Long-Term Biomass Production Obtained with Platymonas

Table 2-14. Input Requirement for the Raceway and Pond System (resource input/kg dry biomass output)

	Raceway	Pond
CO_2 (kg)	2.5	3.0
Nitrogen (g)	85	120
Phosporous (g)	11	a
Energy (kcal)	200 (calculated 100% efficiency)	850 (actual)
Water (L)	210	90

[a]Not reported.

Lipid Physiology. Microalgae are seen as promising feedstocks for fuel production due to their extremely high productivity and their ability to produce biomass with a high lipid content. Lipids are a particularly attractive energy feedstock due to their high energy content and the potential for their direct conversion to liquid fuels similar to those currently in use. Unlike many other lipid-producing plants such as seed crops, high lipid content is characteristic of the entire algal biomass and is not restricted to a single portion of the plant.

Table 2-15. Screening and Culture Accomplishments

- From 320 strains of microalgae collected and isolated, 46 were classified as good growers.
- A screening protocol was established that most efficiently selects for strains that do well in outdoor mass culture.
- Five strains of microalgae were identified that accumulate over 40% lipids.
- Outdoor yields were improved 40% by growing thermophilic strains and developing better management techniques.

Under conditions suitable for rapid growth, algae produce approximately 10%-30% of their biomass in the form of lipids, principally polar lipids, that form an important constituent of cell membranes. When conditions are not favorable for rapid growth but light energy continues to be available, energy storage products are formed. These storage products can be utilized for growth when conditions once again become favorable. Both lipids and carbohydrates are formed as storage products. Some algae store energy in the form of starch granules, while others store energy as various forms of nonpolar lipids.

The induction of storage product formation represents a diversion of the products of photosynthesis from the synthesis of new cellular machinery to the synthesis of storage lipids or carbohydrates. Typically this induction of lipids is associated with a cessation of cell division, a breakdown of cellular membranes, and a reduction in the amount of cellular proteins. Figure 2-27 shows this relationship for Isochrysis that has been grown under nitrogen-deficient conditions. Chemically, lipids can be formed nearly as efficiently as other cell constituents, but in practice the lipid induction phase is frequently associated with a low overall efficiency of lipid production. While the lipid content of microalgae under nutrient stress can be very high (greater than 60% of the total cell biomass), the achievement of this high lipid content may require sufficient time because lipids are not being produced at a rapid rate.

The nature of the stress employed to promote lipid induction may be an important influence on the efficiency of lipid product accumulation. Nitrogen deprivation is the most commonly employed stress to induce lipid formation. The other effects of this stress may lead to decreased photosynthetic efficiency. It has been demonstrated that silicon deprivation can lead to rapid lipid formation, but the efficacy of this trigger is restricted to diatoms, which require silicon for the construction of their siliceous frustule. Temperature or salinity stress may also be effective in inducing lipid formation.

For lipid induction processes to be effective, cells must shift cellular metabolism to the production of lipids without seriously interfering with the overall energetic efficiency of the cell. It is likely that the efficiency of lipid product induction will vary both with the species and with the nature of the lipid trigger. The efficiency of the lipid formation process during the transition between active growth and storage product formation is very important and is presently being characterized.

To understand the time course of lipid synthesis, studies were conducted with Isochrysis and Ankistrodesmus. It appears that during the course of cultivating Ankistrodesmus in

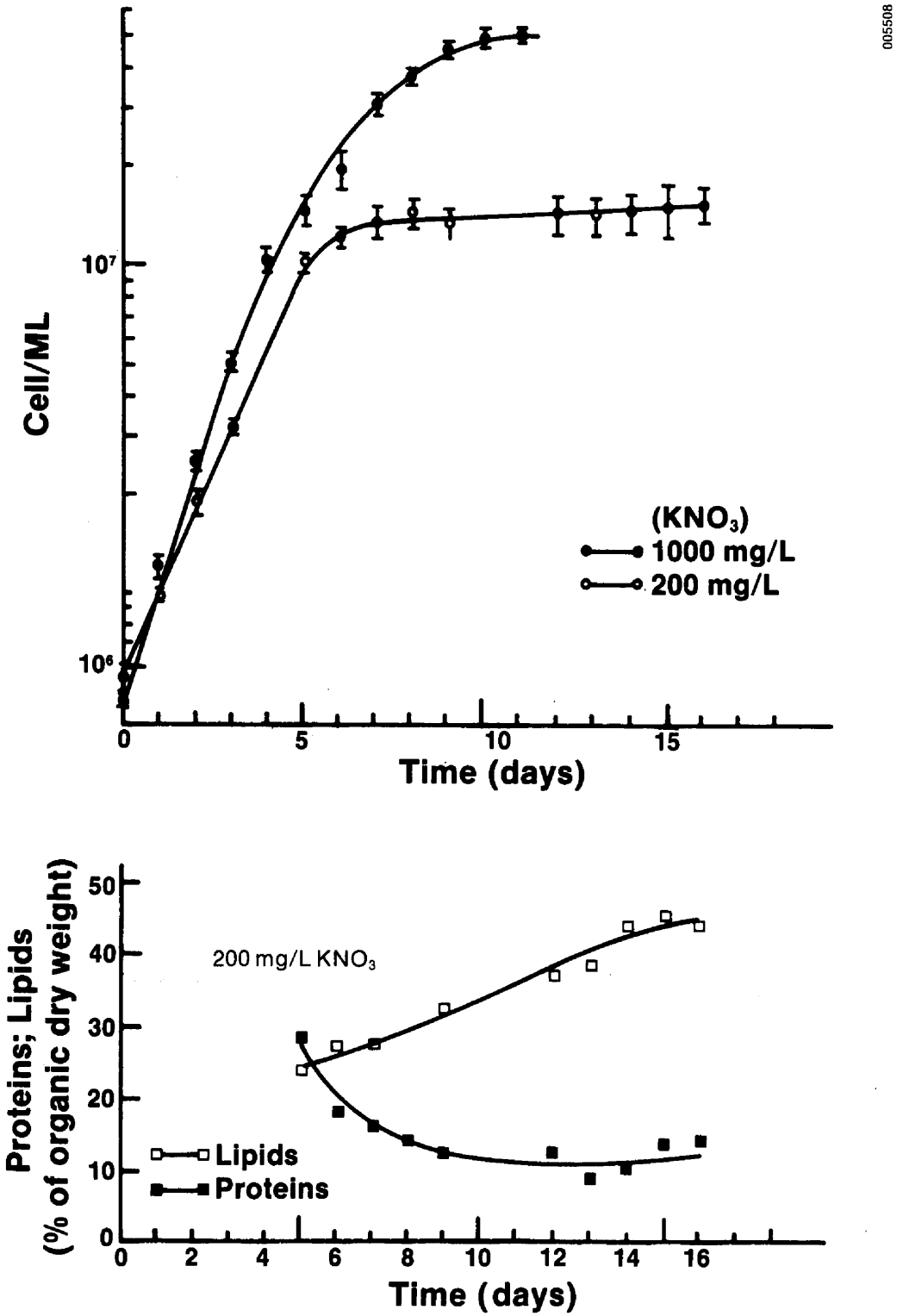

Figure 2-27. Cell Division vs. Lipid Accumulation Relationships for the Chrysophyte Isochrysis

nitrogen-deficient media, the cellular lipid concentration drops just prior to the initiation of rapid lipid synthesis (Figure 2-28). The lipid production phase is preceded by a burst in carbohydrate production.

A second set of studies was aimed at defining the relationship between the nutritional state of the cell and the efficiency of lipid production. Three stages of the culture period were initially defined: nitrogen sufficient (Pre-ND), postnitrogen deficient 1 (Post ND-1), and postnitrogen deficient 2 (Post ND-2). A culture of Chlorella SO1 was grown and allowed to progress through the three stages.

The first stage, designated as Pre-ND, represents a 36-h duration of rapid cell division and mass production immediately preceding nitrogen depletion. A high efficiency for the production of total algal mass was observed. The efficiency of carbohydrate/protein production greatly exceeds that of lipid production. As shown in Figure 2-29, most of the energy for lipid production is channeled to the synthesis of polar (structural) lipids during this stage of the culture growth.

The second stage, designated as Post-ND1, covered the first 144-h period immediately following nitrogen depletion. During the Post-ND1 period, a good efficiency of total mass production was maintained, but a significant increase in efficiency of lipid production occurs at the expense of carbohydrate and protein production (Figure 2-30). The most dramatic changes occur in the production efficiency of neutral lipids, which exhibited a sevenfold gain from 0.47% to 3.59%. In contrast, the production of polar lipids was almost completely shut off (Figure 2-30).

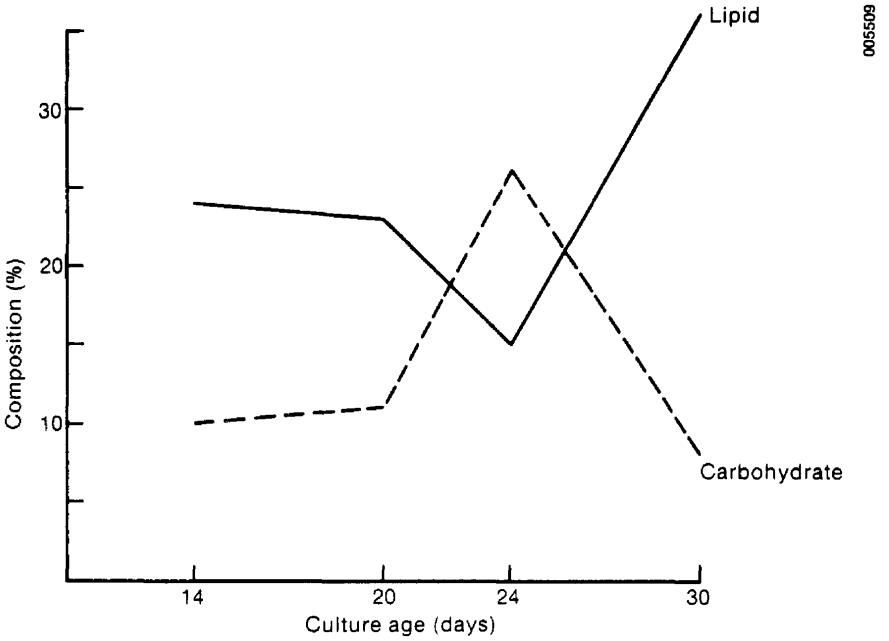

Figure 2-28. Time Course for Lipid Accumulation in the Green Alga Ankistrodesmus

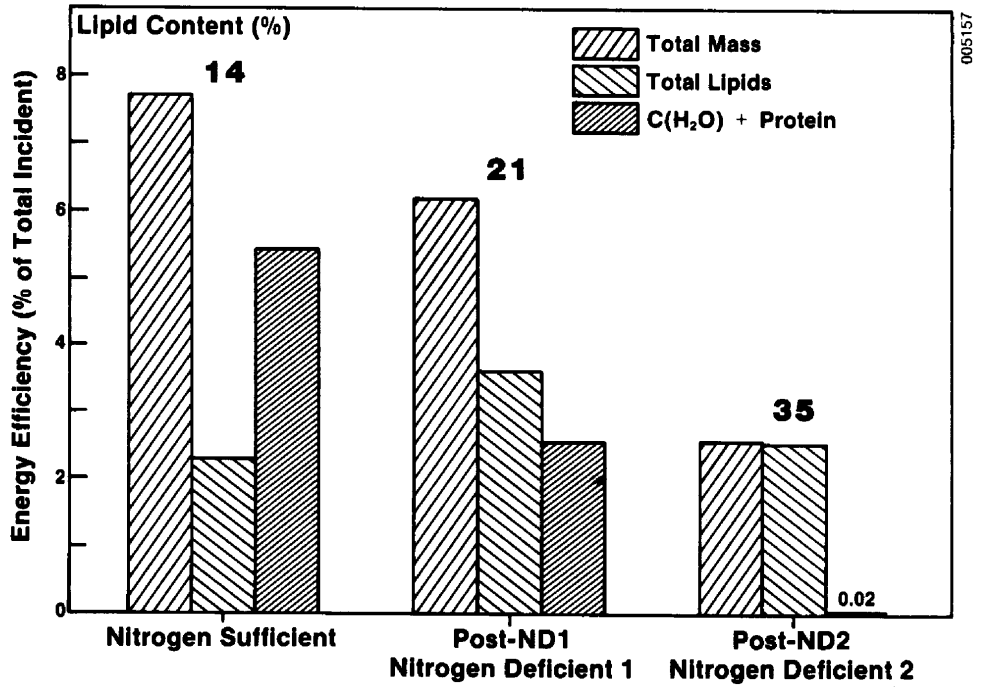

Figure 2-29. Total Lipid Accumulation vs. Energy Efficiency for Chlorella SO1

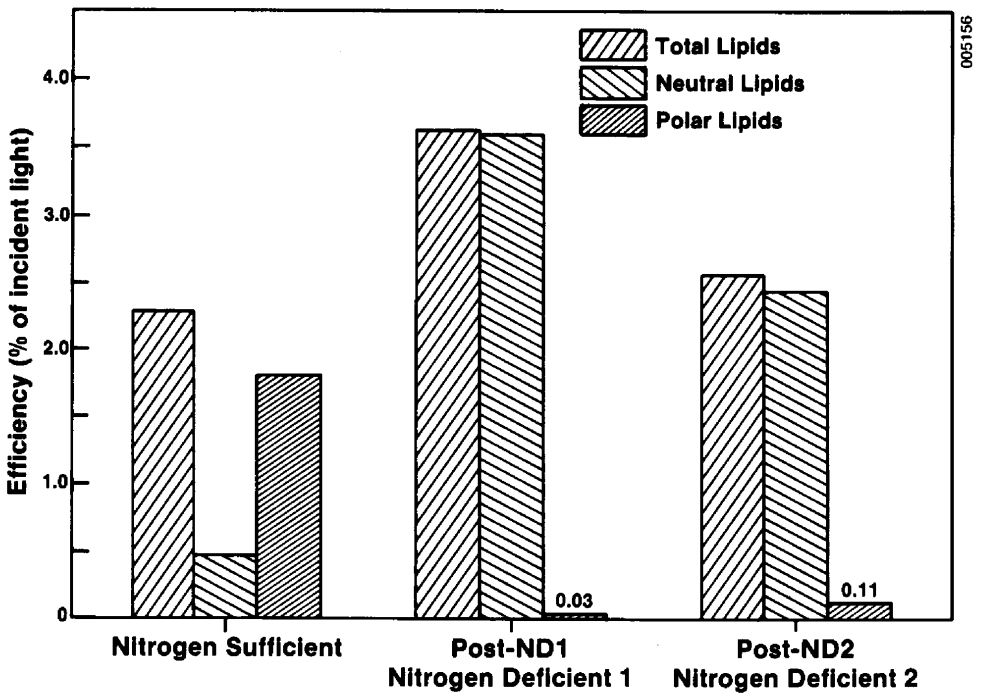

Figure 2-30. Yield Efficiency of Polar and Nonpolar Lipid of the Cell During Nitrogen Deprivation Stress

The third stage, designated as Post-ND2, represents a less productive stage after the culture has been subjected to an extended period of nitrogen deprivation. In spite of a significant loss of energy efficiency, the values for the production of total lipids and neutral lipids still exceed those obtained during the Pre-ND period.

From this study there appears to be a period during nitrogen depletion when the cell has an increased lipid synthesis efficiency. Studies are continuing to elucidate efficiency differences between species and to determine the effects of stresses other than nitrogen deprivation.

Growth Physiology. Modulation of light intensity can significantly improve microalgal photosynthetic efficiency. It has been demonstrated that high-intensity light is used much more efficiently when it is received for short periods followed by periods of darkness. As previously described, a series of wing-shaped foils has been introduced into flowing microalgal cultures to generate vortex mixing. These foils, which produced turnover at 1-2 cycles per second, were credited with approximately doubling the productivity of the culture. However, the cost of operating such a device may be significant, since the drag introduced by the numerous foils leads to increased energy consumption for pumping. Quantification of the productivity enhancement response to light modulations of various frequencies permits the calculation of the productivity benefit associated with various mixing regimes, which then can be compared with the costs associated with mixing.

Modulated light responses of microalgae have been investigated extensively, but the data collected do not permit the calculation of expected enhancements at cycle periods of 1 second or more, which are the periods which are most likely to be economically achieved in microalgal mass cultures.

Microalgal species differ significantly in their photosynthetic response to light intensity. Some species grow rapidly at low light intensities but are killed at even moderately high intensities, while other species can tolerate light intensity at or above that of full sunlight. It is likely that careful screening of species will lead to the identification of strains with desirable light response characteristics. Responses to light modulations may also differ between species, but interspecific differences have received no attention since almost all of the research performed to date has utilized the freshwater green alga Chlorella.

Mathematical characterization of the relationship between the frequency of light modulations and the resulting enhancement of photosynthetic efficiency has been completed for Phaeodactylum tricornutum preconditioned in dense, light-limited culture. This permits the prediction of photosynthetic efficiency enhancement as a function of the flashing light environment to which the cells are exposed (Figure 2-31). Cells preconditioned at low light intensity required less rapid modulations for photosynthetic efficiency enhancement than cells preconditioned at high light intensity.

Results such as these may help to explain why many previous investigators of the "flashing light effect" found that high-frequency modulations were required for enhancement. The preconditioning history of the cells is critical in this regard. Table 2-16 summarizes the major species improvement accomplishments made in 1985.

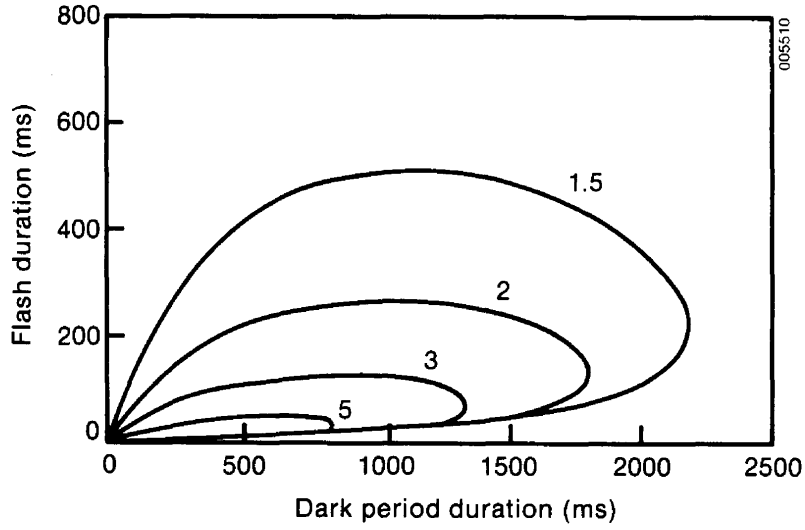

Figure 2-31. Relationship between Flash Duration, Dark Period Duration, and the Relative Rate of Photosynthesis in Phaeodactylum

Table 2-16. Species Improvement Accomplishments

• Lipid yield was found to be highest for a period of 3-5 days after the onset of nitrogen starvation in a green microalga.
• Microalgal cells that have been acclimated to the low light level of dense cultures were found to require less rapid modulations to enhance photosynthesis.

2.4.2 Engineering Research and Development

The objective of engineering research is to integrate biological concepts with engineering principles to develop a cost-effective microalgal culture technology. To accomplish this, engineering research is divided into two tasks: design coordination and component development.

2.4.2.1 Design Coordination

The objective of this subtask is to combine engineering criteria with biological perspectives to develop new approaches, novel systems, or design adaptations that stimulate research and development.

Three projects were initiated in FY 1984 to design and conduct cost analyses of three different production concepts. The designs are for an advanced open pond system, an advanced raceway system, and a closed continuous culture system. The designs, when complete, will incorporate features that will maximize production of a desired species at the least cost and will be operable on a scale necessary for fuel production. These designs will be competitively evaluated, and one or more scaled versions will be considered for construction, to be run as an experimental facility.

2.4.2.2 Component Development

Harvesting. One of the more significant problems in producing microalgae-derived oils is the concentration of dilute algal suspensions into a slurry for subsequent processing. The extent to which present technology for harvesting is economic for microalgae production depends on the final product value and the type of species used in the growth ponds. For example, existing microalgae facilities producing health-food and protein supplements use species such as Spirulina, which have a sufficiently large cell size and high enough product value to compensate for a high harvesting expense. Because some of the microalgal species studied in the Aquatic Species Program are much smaller than Spirulina and the value of the product fuel is not as high, an effective yet cheaper harvesting technology is required.

For most outdoor microalgae facilities, the solids concentration in the harvestable suspension ranges between 0.015% (150 mg/L) and 0.15% (1500 mg/L). For most uses, the desired solids concentration of the harvested biomass should be over 85% with the possible exception of where liquid solvents are used for extraction of lipids. Thus the required solids concentration factor from a production pond to the final product is in the range of three orders of magnitude and can reach a factor greater than 4000.

Concentrating the biomass solids involves liquid-solid separation (water constitutes the liquid in this case) in three major stages: (1) removal of free water to a level up to 10% solids; (2) removal of bound water (capillary, interstitial, etc). to levels between 13% and 25% solids, and (3) removal of hydration and cellular waters up to full dryness or commercial dryness of over 35% solids (Figure 2-32). Since the cost for each percent of

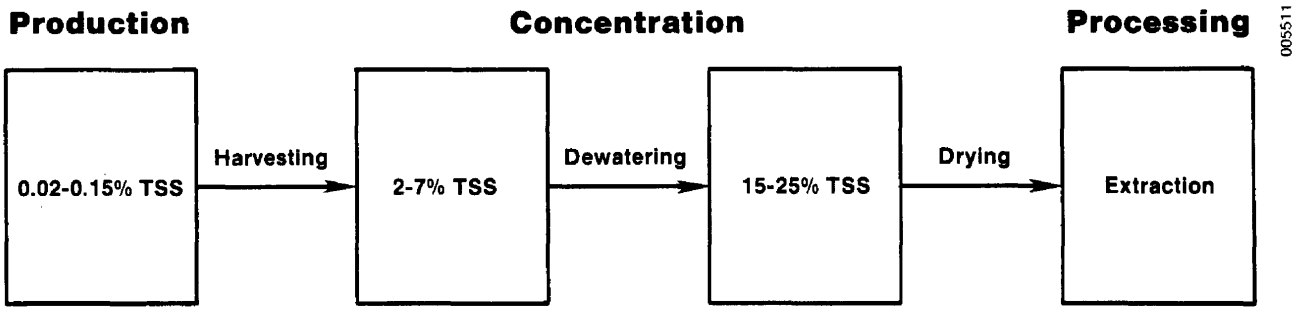

Figure 2-32. Scheme for Concentration of Microalgae from the Pond to Processing

water removed increases exponentially in each ascending stage, it is vastly uneconomical to skip a stage. For example, it is very costly to dry a slurry of 2% solids without applying thickening and dewatering techniques to produce a cake of at least 14%. Similarly, it will be very expensive to directly centrifuge pond suspension containing 0.03% solids to produce a cake of 18% solids without first separating the biomass to produce a slurry of 4%-8% solids.

The first stage of removing most of the free water from the pond suspension is therefore most essential, as concentrating factors between 100 and 300 are attainable at relatively reduced costs. During 1984, harvest research conducted in Israel emphasized autoflocculation as well as chemical flocculation followed by sedimentation or floatation.

Organic polymers as well as inorganic flocculants were used in standard jar tests to determine the algae flocculation potential and select the best flocculant for destabilization of Isochrysis galbana suspensions. The flocculation process in marine environment was found to be difficult, evidently due to high salt content of the culture medium and the high motility of the marine microalga I. galbana.

The effect of ionic strength (I) on the harvestability by flocculation of Isochrysis using ferric chloride and alum is illustrated in Figure 2-33. The algal flocs obtained by ferric chloride were much bigger and stronger than those obtained with alum. The marine system requires a large quantity of inorganic flocculant, and this may affect the quality of the algal product as it goes to processing. Several cationic polymers that are most efficient in fresh water were found to be ineffective for flocculation in the marine system. The application of low doses of ozone reduced the requirement for ferric chloride from 1.4 mM to 0.3 mM (Figure 2-34). This result suggests that a possible harvest scheme for a marine flagellate such as Isochrysis would be an ozone treatment before the application of a flocculant. A cost evaluation of this process is currently being conducted.

2.4.3 Technology Analysis

The technology analysis activities provide R&D guidance for the Aquatic Species program by analyzing and integrating the results of the biological and engineering research tasks. This in-house work is accomplished by conducting systems studies of the various research initiatives and identifying the R&D options that offer potential gains to the overall program objectives. There are two tasks in the activity: (1) resource assessment and (2) technical and economic evaluations.

2.4.3.1 Resource Assessment

The objective of this task is to assess and evaluate the availability and location of resources required to support microalgal oil production. A preliminary resource assessment of the American Southwest was completed during 1983. This assessment found that 33 million acres of land were potentially highly suitable for microalgal production. Of the required resources, carbon dioxide and water would seem to be most limiting to the development of the technology. Power plants are the single largest potential source of carbon dioxide, followed by cement plants and natural CO_2 deposits (Table 2-17). In contrast to natural deposits of carbon dioxide, power plants are widely distributed geographically. The flue gas, at atmospheric pressure, contains up to 16% carbon dioxide. It is felt that the CO_2 supply from power plants and natural deposits is more than sufficient to supply an extensive microalgae technology in the Southwest.

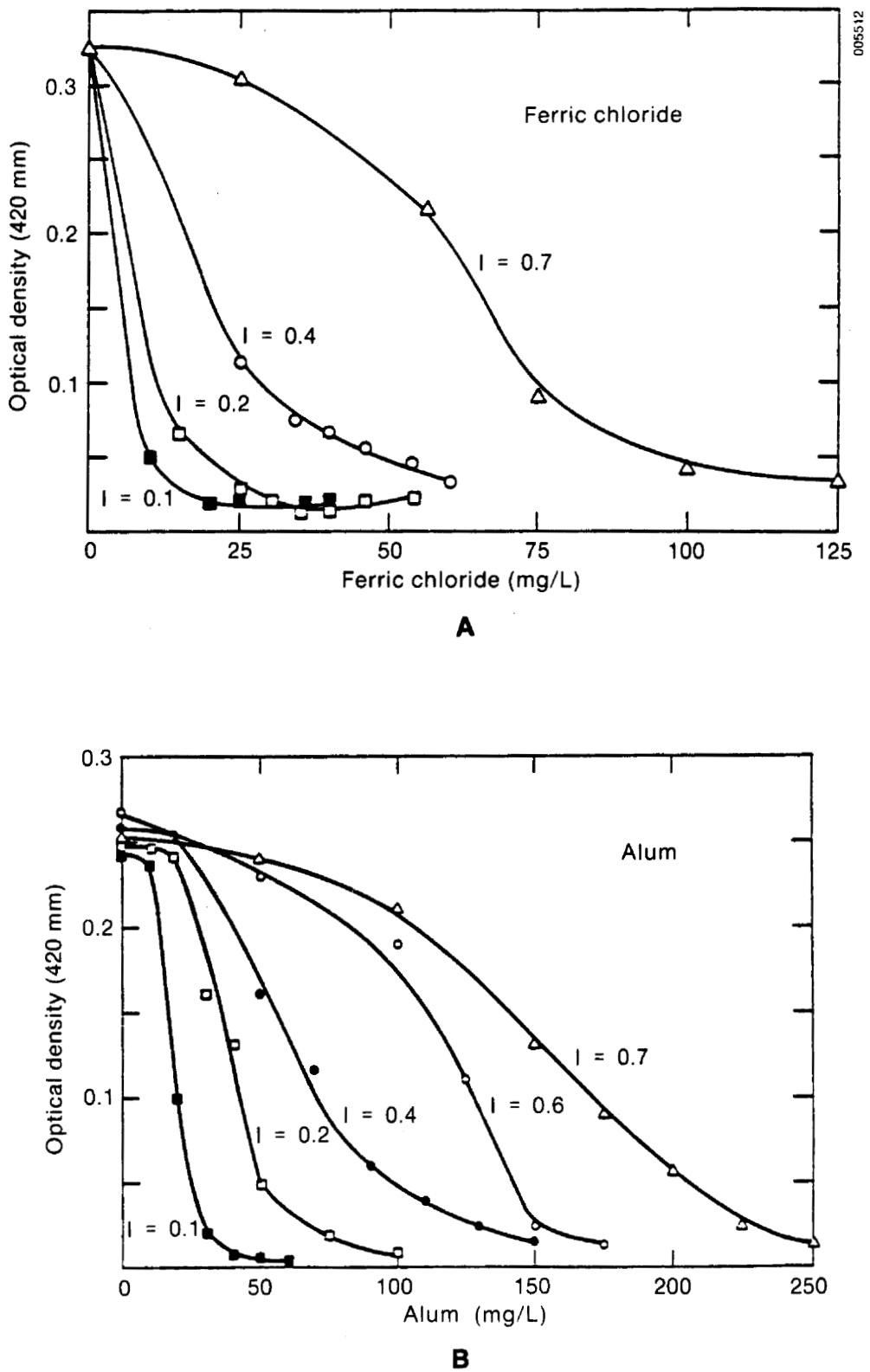

Figure 2-33. **Effect of Ionic Strength (I) on Flocculation of Isochrysis by Ferric Chloride (A) and Alum (B)**

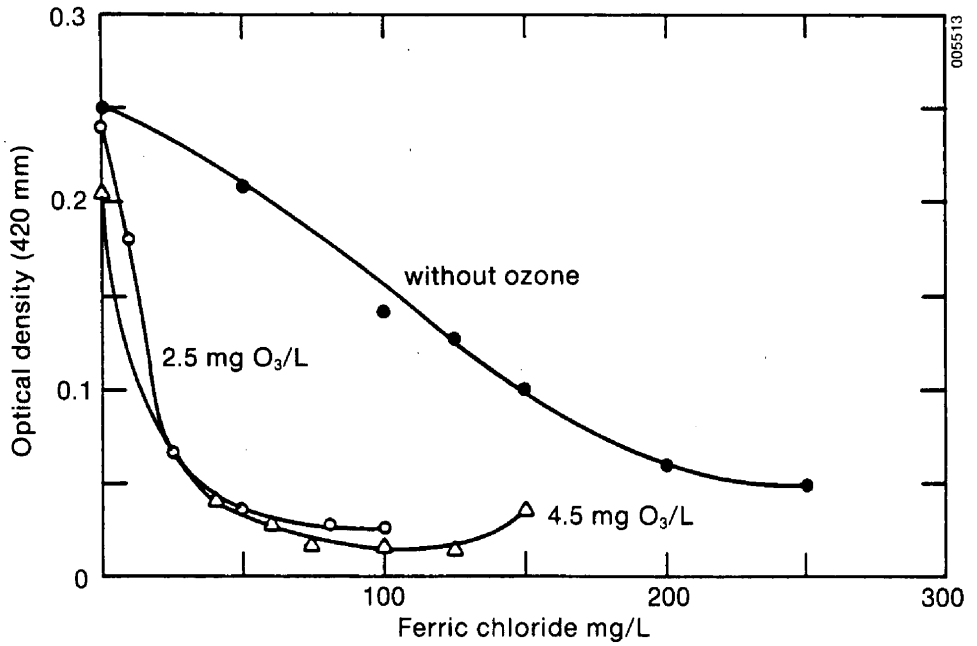

Figure 2-34. Effect of Ozone Pretreatment on the Improvement of Marine Microalgae Flocculation of Ferric Chloride

Table 2-17. Total Potential CO_2 Supply from Various Sources

Source	Amount (10^6 m^3 day)
Natural CO_2 deposits	38.7
Power plants	1546.9
Cement plants	75.0
Ammonia plants	20.3
Ethylene oxide plants	1.57
Ethanol plants	0.4
Natural gas processing	7.21
Hydrogen plants	10.4
Fluid catalytic cracker unit regenerator	3.6
Total	1724.0

Source: Anada et al. 1983.

The availability of saline water remains the biggest unknown. In an open system it is anticipated that 5 acre-ft/yr would be required to replace evaporative losses. While saline aquifers are found throughout the Southwest, the total volume these aquifers can supply on a sustained basis has not been determined. Since very little information is

available on the saline water resources of the desert Southwest, the quantification of this resource is a high priority activity. In addition to utilizing water from underground aquifers, it might also be beneficial to utilize seawater at coastal sites and waste irrigation runoff from agricultural areas. These options will be explored during the coming year.

2.4.3.2 Technical and Economic Evaluations

A complete technical and economic evaluation was conducted on the production of fuels from a microalgal feedstock. This effort was divided into two areas: feedstock production and subsequent conversion of the feedstock into fuels. An Algal Production and Economic Model (APEM) was developed to estimate capital and operating costs for mass culture facilities. This model estimates that if today's technology was applied on a large scale (e.g., 20-ha modules in a facility of 1000 ha), a microalgal feedstock suitable for conversion to fuels could cost $436/t (1984 dollars) (Table 2-18). Sensitivity analysis indicated that the production cost could be reduced to $224/t by a series of improvements such as increasing salinity tolerance, increasing photosynthetic efficiency, increasing lipid content, and decreasing losses from water evaporation and CO_2 outgassing (Figure 2-35). Based on these microalgal production cost estimates, integrated refinery options for conversion of the microalgae to high-energy liquid fuels were evaluated. This portion of the analysis is based on preliminary data for processes that were developed for feedstocks similar, but not identical to microalgae.

Table 2-18. Summary of Reference Production Facility Cost Contributions for Direct Cost and Capital Cost (1984 $)

Cost Category	$/t	Percentage of Category	Percentage of Total Cost
Capital Cost			
Culture system	36.0	50.3	
Harvester system	18.3	25.6	
Engineering fees	5.9	8.3	
Contingency	9.1	12.7	
Land	2.1	2.9	
Total capital cost	71.5	100.0	16.4
Operating Costs			
Labor and overhead	56.1	15.4	
Utilities	18.6	5.1	
Nutrients	206.8	56.8	
Water	17.8	4.9	
Operations	26.9	7.4	
Maintenance	37.9	10.4	
Total operating cost	364.1	100.0	83.6
Total feedstock cost	436.0		100.0

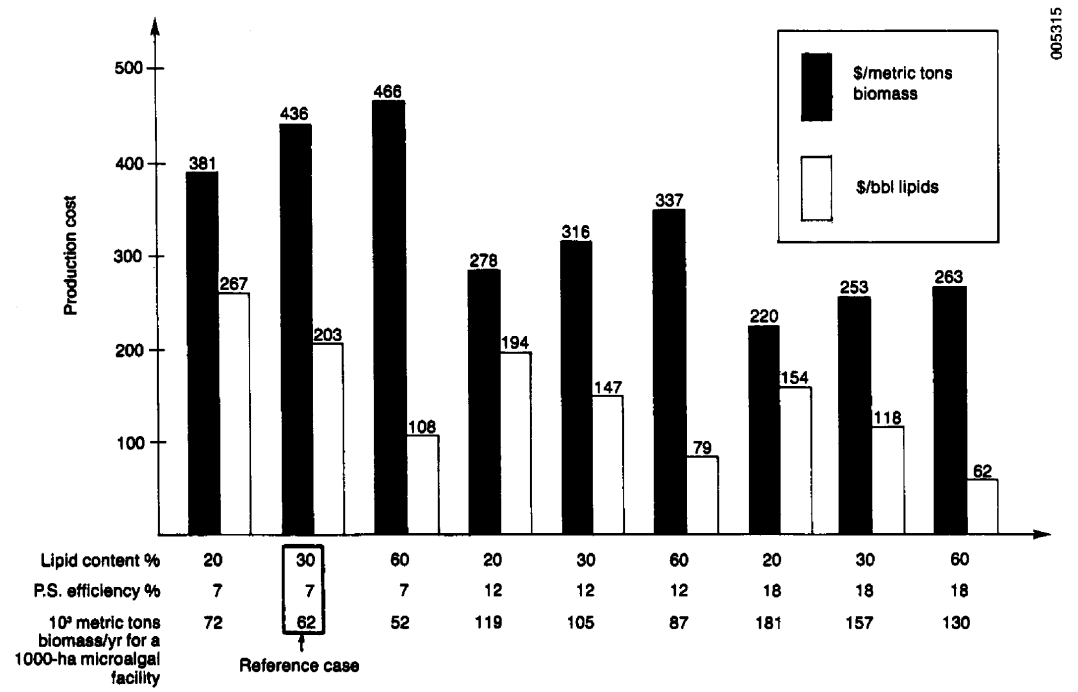

Figure 2-35. Biomass Production Cost Sensitivity to Photosynthetic Efficiency and Lipid Content

Of the three major algal components (lipid, protein, and carbohydrate), the lipid component was determined to have the greatest potential as a source of fuels to replace conventional hydrocarbons. Two processes were examined--one based on the conversion of triglycerides into methyl fatty esters, which are being extensively investigated as potential diesel fuel substitutes, and a catalytic reduction process for the production of hydrocarbons, primarily in the gasoline range. The estimated costs of these fuel products compare favorably with the projected costs of conventional fuels at the turn of the century, as long as the presumed improvements in lipid yields are achieved (Figure 2-36). Table 2-19 summarizes the major engineering and analysis accomplishments made in 1984.

Table 2-19. Engineering and Analysis Accomplishments

- Designs were commissioned for an advanced pond system, an advanced raceway system, and a closed continuous system.
- A technical and economic evaluation indicated that large-scale microalgae production for fuels is feasible providing (1) lipid yields from microalgae can be improved, (2) there is sufficient saline water for large-scale development, and (3) microalgal lipids can be converted to conventional fuels.

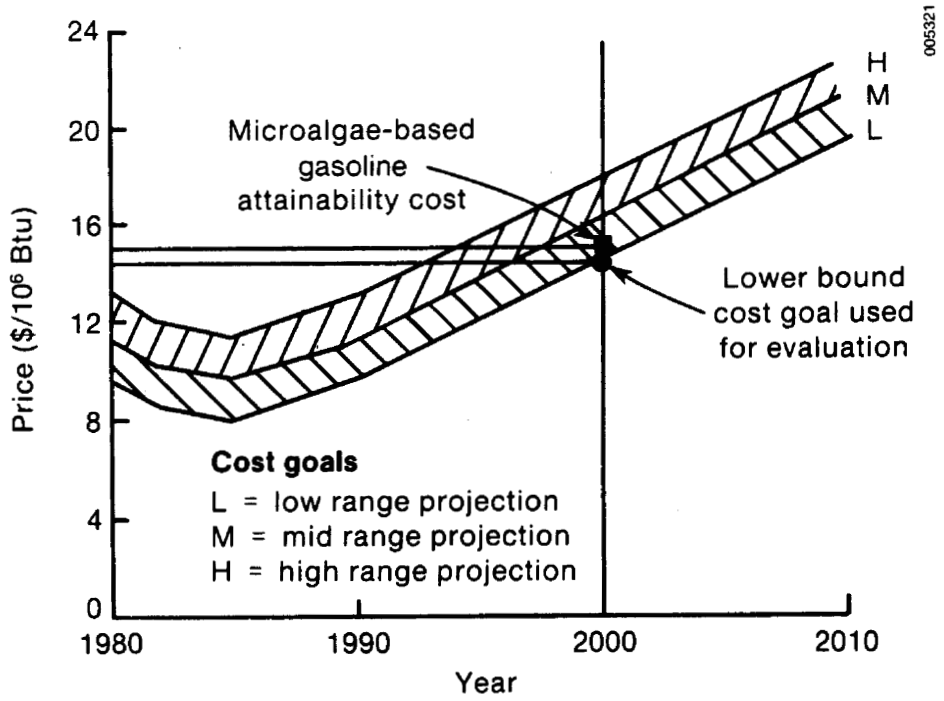

Figure 2-36. Gasoline Cost Goals and Attainable Gasoline Costs from Microalgae

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