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FY 1987 Aquatic Species Program Overview

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PREFACE

This report is an overview of the progress and research accomplishments of the Aquatic Species Program, field managed by SERI, during FY 1987. The Program receives its funding through the Biofuels and Municipal Waste Technology Division of the Department of Energy.

For further details, contact the SERI Biofuels Program Office (Donna Johnson, Aquatic Species Program Coordinator, 303-231-1472).

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SUMMARY

The goal of the Department of Energy/Solar Energy Research Institute (DOE/SERI) Aquatic Species Program is to develop the technology base to produce liquid fuels from microalgae at prices competitive with conventional alternatives. Microalgae are unusual plants that can accumulate large quantities of oil and can thrive in high-salinity water, which currently has no competing uses. The algal oils, in turn, are readily converted into gasoline and diesel fuels. The best site for successful microalgae production was determined to be the U.S. desert Southwest, with potential applications to other warm areas. A technical and economic analysis, *Fuels from Microalgae*, demonstrated that liquid fuels can be produced from mass-cultured microalgae at prices that will be competitive with those of conventional fuels by 2010. Aggressive research is needed, but the improvements required are attainable.

The four prime research areas in the development of this technology are growth and production, engineering design, harvesting, and conversion. Algae are selected for three criteria: tolerance to environmental fluctuations, high growth rates, and high lipid production. From 1982 to 1986, the program collected more than 3000 strains of microalgae that are more than twice as tolerant to temperature and salinity fluctuation than the initial strains. Productivity has been increased by a factor of two in outdoor culture systems since 1982, and lipid content has also been increased from 20% of body weight in 1982 to greater than 66% of body weight in 1987. Research programs are ongoing in lipid biochemistry and genetic engineering so that ultimately strains can be modified and improved to combine their best characteristics.

An outdoor test facility (OTF) is being built in Roswell, N. Mex. Using the six 3-m^2 ponds that have been built, researchers will perform controlled replicate experiments and screen species outdoors using saline groundwater. Large-scale experiments will be performed to compare pond liners, times and rates of mixing, carbon dioxide injection, and other engineering features in the two 0.1-ha (0.25-acre) ponds being constructed. Depending on the availability of funds, a 0.5-ha (1.25-acre) pond will be constructed in FY 1989 to study scale-up issues.

Research to date has demonstrated that all algae can be harvested at a cost of 0.5¢-1.5¢ kg^{-1} dry weight using water soluble flocculant polymers. Methods to reduce the costs of this harvesting even further are currently being examined. Conversion research is just beginning in the program. Since algal lipids cannot be used as a fuel directly because they contain 10% oxygen (crude petroleum contains essentially no oxygen), the algal lipids need to be extracted and converted into gasoline and diesel fuels.

Not only the oxygen concentration but the viscosity of the fluid needs to be decreased. Future program activities include screening and characterizing the algal strains collected and reducing the collection to the best 10-25 oil-producing strains by FY 1990. Research in lipid biochemistry, strain improvement, and genetic engineering will continue so that the quantity of oil produced by this technology is maximized. Construction of the OTF will be completed, and by the end of FY 1988, a year of production data on algae grown in the desert Southwest will have been collected. New harvesting and conversion projects will be initiated early in FY 1988 to further reduce the costs of producing liquid fuels from microalgae. Major analysis efforts in the upcoming year will be on resource and environmental assessments of the technology, with emphasis on carbon dioxide supply, brine disposal, and possible climatic impacts.

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1.0 INTRODUCTION

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. Resources such as biomass were often viewed as attractive solutions to the energy problem because of their non-depletable, renewable nature. While the first biomass sources considered were readily available, such as wood or corn, it was apparent that new biomass sources should also be developed, including aquatic species. The current U.S. Department of Energy emphasis is placed on technology for our future energy supplies and not commodities (DOE 1987).

The DOE/SERI Aquatic Species Program is designed to develop the technology base for large-scale production of lipid-yielding microalgae and for conversion of the lipids into liquid fuels. The region with the most promise for success was determined to be the U.S. desert Southwest, with other warm areas of the United States offering additional potential. This technology could potentially produce between 150-400 barrels oil $acre^{-1}$ yr^{-1}, depending on the growing season. An artist's conception of the facility is shown in Figure 1-1.

Microalgae are small, unicellular plants that range in size from 1 to 200 μm. Microalgae productivity rates are higher than those of most other plants. Table 1-1 shows the productivity rates of many other plants and indicates the order of magnitude greater productivity that we expect can be obtained in outdoor culture ponds. They are also unique organisms in that they can accumulate storage lipids in large quantities within their bodies (Figure 1-2). Historically, microalgae have been grown in mass culture for food production and waste treatment (Benemann et al. 1987), but the hope of producing an

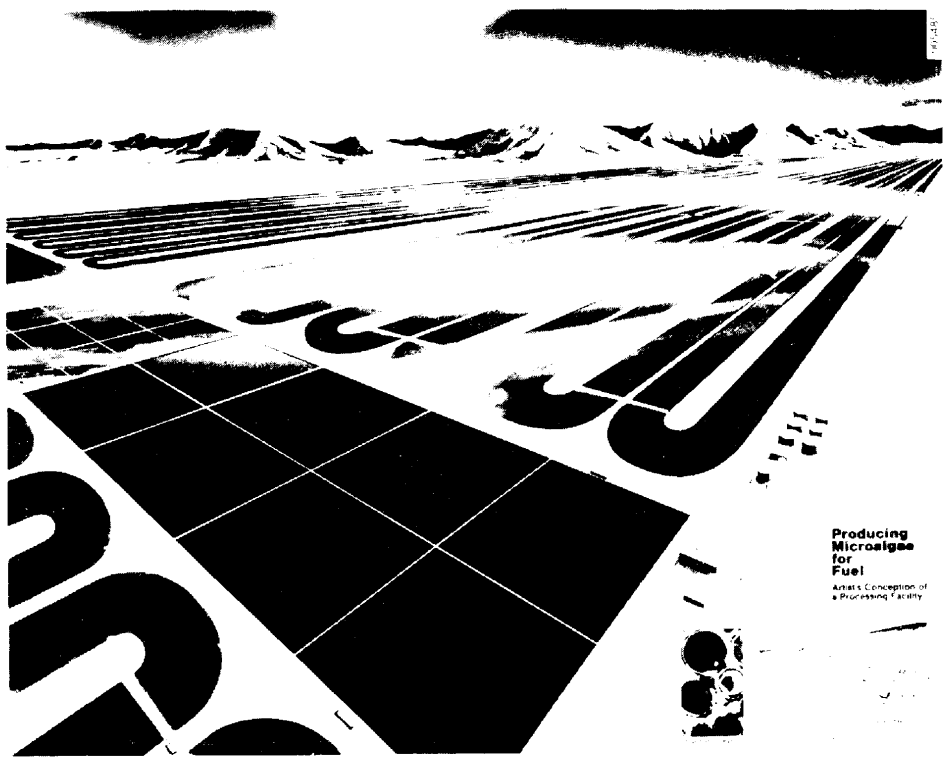

Figure 1-1. Artist's Concept of Microalgae Fuel Farm in the American Southwest

Table 1-1. Comparative Productivity Rates in Different Plant Communities (adapted from Whittaker 1975)

Plant Community	g m^{-2} yr^{-1}
Continental:	
Tropical rain forest	2200
Temperate deciduous forest	1200
Woodland and scrubland	700
Desert and semidesert scrub	90
Cultivated land	650
Marine:	
Open ocean	125
Continental shelf	360
Algal beds and reefs	2500
Microalgae ponds	12,500

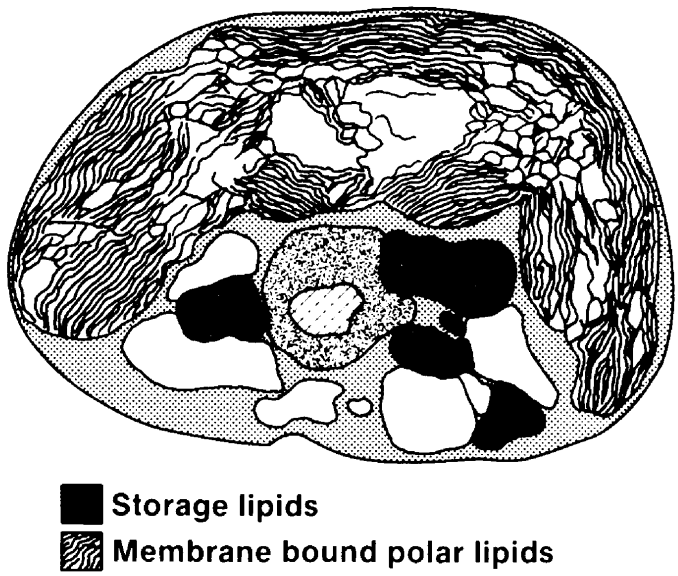

Figure 1-2. Micrograph of Algal Cell

abundant, low-cost source of protein has not been realized. The most promising early results of mass algae culture have been in the field of sanitary engineering, where microalgae are used to treat wastewater in oxidation ponds. From wastewater technology, application has been expanded to include protein production and treatment of irrigation water. Microalgae are being grown in Israel, Australia, Mexico, Taiwan, and the United States for high-value products for the health food market; these microalgae products include the alga *Spirulina* ($10,000/dry ton) and the vitamin beta carotene ($60,000/dry ton). Cultivating microalgae as a soil conditioner and as a food source for culturing fish and shellfish is increasing in importance.

Following the energy crises of the 1970s, the possibility of using algae as a source of energy received widespread attention. Microalgae can be grown in large outdoor ponds, using the resources of sunlight, saline water, nitrogen, phosphorus, and carbon dioxide to produce proteins, carbohydrates, and lipids (Johnson 1987). In the process, they can double their biomass three to five times a day. After a rapid growth phase, the algae can be transferred to induction ponds where, under nutrient limitation, many algae stop growth and division and use all their energy to make lipids as storage products for survival. Once the cells have accumulated lipids, they are harvested, and the water is recycled back into the growth ponds. The harvested cells are subjected to an extraction process to remove the lipids, primarily triglycerides with fractions of isoprenoids, phospholipids, glycolipids, and hydrocarbons. Lipids contain more oxygen and are more viscous than crude petroleum. The two most promising fuel conversion options are transesterification to produce fuels similar to diesel fuels and catalytic conversion to produce gasoline. While microalgal lipids represent the premium energy product, the energy trapped in the other biomass constituents can also be used; e.g., the cell residue after lipid extraction can be digested anaerobically to produce methane and carbon dioxide, which can be recycled for use in the algae production system.

2.0 GOAL AND OBJECTIVES

The goal of the Aquatic Species Program is to develop the technology base for large-scale production of oil-rich microalgae and methods to convert the microalgae lipids into gasoline and diesel fuels needed for industry and transportation. To achieve this goal, the objectives of the program are to:

- Provide a slate of microalgal strains and determine their required growth conditions for high, sustained lipid production under outdoor conditions
- Develop inexpensive, large-scale, outdoor mass culture technologies to grow microalgae
- Improve the methods to harvest microalgae so the process is inexpensive and efficient
- Evaluate and technically address resource requirements or limitations to grow microalgae in the desert Southwest of the United States
- Develop technologies for converting microalgae lipids into high-value liquid transportation fuels
- Transfer the technologies to the private sector for continued development and rapid commercialization by involving industry in the research process at the earliest possible time.

3.0 RESEARCH AND TECHNOLOGY DEVELOPMENT

The four main research areas critical to the development of microalgae technology for large-scale algal production and conversion into liquid fuels are (1) microalgae growth and production, (2) engineering design, (3) harvesting, and (4) conversion (Figure 3-1). The design of a microalgae mass culture system must be tailored to the characteristics of the culture organism while species must be selected that contribute to economic construction and facility operation. Microalgae must be selected that are environmentally tolerant, have high growth rates, and produce large quantities of lipids. In addition, the choice of a suitable species affects harvesting ease. The types of lipids that the algae produce will determine the conversion methods. Thus, all four areas are highly interactive. Each area of research and technology development and the major accomplishments in FY 1987 will be discussed in detail in the remainder of this report.

Approximately half of the research sponsored by the Aquatic Species Program is conducted in house at SERI, and the other half is subcontracted to universities and small businesses. Table 3-1 shows the funding breakdown by research areas for the FY 1987 budget of $1.7 million. Growth and production received 61% of the total budget; engineering design, 9%; harvesting, 3%; and conversion research, 6%. Analysis and resource assessment received 8% and management, the remaining 13%. A summary of the FY 1987 Aquatic Species Program active subcontracts is given in Table 3-2. This includes projects funded in FY 1986 and FY 1987.

3.1 Microalgae Growth and Production

As mentioned in Section 2.0, for microalgae technology to be successful, it is necessary to cultivate microalgae species that are tolerant to fluctuating temperatures and salinity, have high growth rates, and can produce large quantities of lipids. There are four subtasks in this area: species screening and characterization, lipid biochemistry, strain improvement, and genetic engineering.

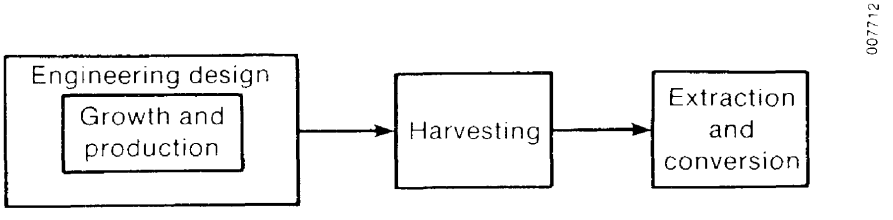

Figure 3-1. Four Stages Necessary to Produce Liquid Fuels from Microalgae

Table 3-1. FY 1987 Procurement Plan Summary For Aquatic Species Program

Task/Projects	Funding (1000 $)
Growth and Production	
Screening and Characterization	420
Lipid Biochemistry	185
Strain Improvement	190
Genetic Engineering	250
Engineering Design	155
Harvesting	50
Conversion	100
Analysis and Resource Assessment	130
Management	220
Total	1700

Table 3-2. Aquatic Species FY 1987 Active Subcontracts (FY 1986 and FY 1987 Funding)

	Title	Contractor	Date of Performance
1.	Optimization of outdoor culture	University of Hawaii	5/86 - 9/87
2.	Production of liquid fuels and chemicals by microalgae	Microbial Products	3/86 - 2/87
3.	Screening and characterizing oleagenous microalgae species from the Southeastern United States	Alabama A&M	2/86 - 1/88
4.	Characterization of hydrocarbon producing strains of microalgae	Scripps Institute of Oceanography	2/87 - 2/88
5.	Improvement of microalgal lipid production by flow cytometry	Oak Ridge National Laboratory	12/86 - 11/87
6.	Collection of high energy yielding strains of saline microalgae from the Hawaiian Islands	University of Hawaii	3/86 - 10/87

Table 3-2. Aquatic Species FY 1987 Active Subcontracts (FY 1986 and FY 1987 Funding) (Concluded)

	Title	Contractor	Date of Performance
7.	Genetic variation in high energy yielding microalgae	City College of New York (cost shared 70:30)	3/86 - 12/87
8.	Collection of high energy yielding strains of saline microalgae from southwestern states	Arizona State University (cost shared 75:25)	3/86 - 5/87
9.	The effects of fluctuating environments on the selection of high yielding microalgae	Georgia Institute of Technology (cost shared 80:20)	3/87 - 4/88
10.	Collection and selection of high energy thermophilic strains of microalgae	Montana State University	3/86 - 5/87
11.	Characterization of photosynthetic efficiency and growth for selected microalgae in dense culture	Martek	9/85 - 2/87
12.	Nutritional requirements for maximal growth of oil-producing microalgae	Jackson State University	9/86 - 12/87
13.	Chrysophycean lipids: Effects of induction strategy in the quantity and types of lipids	Selma University	1/87 - 1/88
14.	Biochemical elucidation of neutral lipid synthesis in microalgae	Montana State University	1/86 - 10/87
15.	Transformation and somatic cell genetics for the improvement of energy production in microalgae	University of Nebraska	3/87 - 3/88
16.	Biochemical elucidation of neutral lipid synthesis in microalgae	University of Nebraska	3/87 - 3/88
17.	Algal genetics	Neushul Mariculture, Inc. (cost shared 20:80)	2/86 - 1/87
18.	Design and operation of a microalgae outdoor test facility	Microbial Products	3/87 - 3/88

3.1.1 Species Screening and Characterization

By 1986, more than 3000 microalgal strains had been collected from diverse geographical locales and ecological niches. A major screening effort is now under way to reduce these to the best 10-25 strains that have the desired characteristics by FY 1990. The most promising strains collected are Bacillariophyceae (diatoms), Chlorophyceae (green algae), and Eustigmatophyceae (Eustigmatophytes). The program has identified several strains that tolerate severe environmental fluctuations in temperature and salinity. Strains of microalgae used by the program in 1982 exhibited temperature tolerances of 15°-25°C and salinity tolerances of 20-40 mmho cm^{-1}. With the intensive collection efforts nationwide, the program now has strains that can tolerate 10°-35°C and 10-85 mmho cm^{-1} (for comparison, seawater is approximately 35 mmho cm^{-1} salinity).

The screening and characterization of lipid-producing microalgae has been a subtask in the Aquatic Species Program since FY 1983. The following three areas have been emphasized in FY 1987: (1) characterizing warm-water strains, (2) initiating a cold-water strain collection and screening project, and (3) developing a screening protocol to be used in reducing the number of strains in the program from 3000 to 300 by the end of FY 1988.

Ten warm-water microalgae were characterized by SERI (Johansen et al., in Johnson and Sprague 1987), seven of which were added to the *Microalgae Culture Collection 1986-1987* (Barclay et al. 1986). The *Chaetoceros* strains had the greatest tolerance for high salinity. *Chaetoceros* 9 and 10 and *Navicula* 1 are considered the best new strains of the 10 characterized, and further study of these strains is under way.

Forty-nine strains from the desert Southwest (Sommerfeld et al., in Johnson and Sprague 1987), primarily diatoms and green algae, demonstrated growth rates exceeding one doubling per day, and five exceeded two doublings per day. The fluorescent dye Nile red was used to microscopically screen for intercellular lipid storage. Fluorometric quantification revealed that seven microalgal cultures yielded more than 200 mg L^{-1} triolein equivalent lipids, and four yielded greater than 400 mg L^{-1}. Preliminary growth optimization efforts with 15 of the strains indicate that the most rapid growth occurs when isolates are cultured with urea as the nitrogen source at 25°C under 500 $\mu E\ m^{-2}\ s^{-1}$ of light. For many of the strains, altering the nitrogen source and elevating the light intensity from 25 to 50 $\mu E\ m^{-2}\ s^{-1}$ had relatively little effect on growth rate. In addition, 55 strains were isolated in FY 1987 from Alabama and Mississippi and five were characterized (Tadros, in Johnson and Sprague 1987).

Cheng and Lewin (in Johnson and Sprague 1987) studied the production of lipids in 10 selected strains of marine microalgae that exhibit tendencies to float. During the past three years, 300 samples of surface sea-water strains were collected, and after a simple enrichment procedure, 80 different strains were isolated. All were tested for their salinity and temperature ranges for growth and their requirements for exogenous vitamins.

Six additional strains of the best-growing algae in the culture collection had their nutritional requirements defined (i.e., nitrate, nitrite, urea, phosphate, silica, and iron; Rhyne, in Johnson and Sprague 1987). Another study by Sriharan et al. (in Johnson and Sprague 1987) determined the effects of nutrient deficiency and temperature on fatty acid composition. The percentage of 16:1 and 18:1 fatty acids increased in the nutrient-deficient culture.

Mass-culture systems exhibit diurnal and seasonal fluctuations in key parameters affecting algal productivity and competitiveness: temperature, pH, oxygen, carbon dioxide, light, and nutrient availability. In species competition experiments with several strains of algae isolated by the Aquatic Species Program, certain parameters were more important than others in affecting species dominance: a diurnal cycle of high oxygen was more important than fluctuating temperature, which was more important than fluctuating pH. A computer model was developed that can predict the average diurnal and seasonal variations in pond environmental parameters (oxygen, pH, carbon dioxide, temperature, and light intensity).

Research efforts are continuing to increase the rates of productivity of microalgae to enhance yields of energy products. Research is being conducted in both laboratory and outdoor cultures to identify species and to develop culture management strategies that improve productivity rates. The level of productivity for microalgae shown in Table 1-1 has been met in the laboratory and in small outdoor test ponds on a short-term basis. For several months we have been able to sustain productivity rates at 70% of the target.

3.1.2 Strain Improvement

Conditions of high solar irradiance, pH, dissolved oxygen, salinity, and low carbon dioxide anticipated during the growth of algae may induce photorespiratory losses in phytoplankton. Algae have been screened for high specific growth rates under these conditions, with net production the primary concern. However, modifying conditions conducive to respiratory losses may result in even higher net production.

Photorespiration increased dramatically with pH, salinity, dissolved oxygen, and light intensity (Cohen, in Johnson and Sprague 1987). However, photosynthesis also increased faster than photorespiration at increasing light intensity. Thus, net production was still highest at the highest light intensity.

Experiments were performed to develop a computer model to predict the effects of environmental parameters on lipid production (Chelf et al., in Johnson and Sprague 1987). Factors examined included nitrogen concentration, silicon concentration, temperature, time, conductivity, and alkalinity. Measured characteristics included Nile red fluorescence and ash-free dry weight (AFDW). The multiple regression model from the ratio of Nile red fluorescence to AFDW has an R^2 of 89.34%. The most important variables in this regression model were nitrogen concentration and conductivity. This type of model may be used as a predictive model for complex biological systems.

Since carbon dioxide supply is a potentially costly part of algal lipid production, knowledge of minimum carbon requirements for maximum productivity is valuable. Carbon dioxide compensation points were examined in several strains of microalgae under nutrient-sufficient and nutrient-limited conditions (Chelf, pers. comm.). Two phases of carbon utilization were seen when cells were bubbled with air plus carbon dioxide and were preincubated. These two-phase curves were never seen with air-bubbled cells. The compensation points and the ratio of final inorganic carbon to alkalinity will be tested to see if they can predict conditions where carbon uptake will be less than optimal and to compare the carbon uptake ability of different species. Eventually we hope to correlate these data with the lipid production potential of microalgae under nutrient limitation.

Methods for increasing the reliability of using flow cytometry to produce high-lipid algal strains were developed this year (Solomon and Palumbo, in Johnson and Sprague 1987). Earlier results indicated that cells could be sorted with a flow cytometer on the basis of their lipid content after staining with a fluorescent dye that is specific for neutral

lipids. However, the resulting cultures often did not have higher lipid content than the parent cultures when measured many generations after the sort. Recent results suggest that by taking cell cycle differences into account, high-lipid daughter populations, when grown for a number of generations (at least up to three months), can retain their enhanced lipid levels. When lipid level was used as the sole criterion for sorting, sorts were successful and resulted in high-lipid daughter populations if they took place after the cells had stopped dividing. Exponentially growing populations were sorted successfully when the chlorophyll:lipid ratio was used for defining the sorting window. This procedure results in cell selection at all stages in the cell cycle and yields cells that are 25%-30% higher in lipid content than the average cell at that growth stage.

3.1.3 Lipid Biochemistry

The goal of the third major research area in microalgae growth and production is to increase the amount of lipid in each algal cell; our target is 60% lipid in outdoor cultures. Significant increases have been made in lipid quantity, from 20% of cell content in 1982 to 66% in the laboratory and 40% outdoors in 1987. We are able to induce lipid accumulation by removing nitrogen or silica (major nutrients required for growth) from the media. During this period of stress, some algae will begin to build up reserves of carbohydrates and others will accumulate lipids. In addition to nutrients, temperature, pH, inorganic carbon, and light can all affect the cell's lipid quantity.

Initial steps were taken to determine the contribution of neutral lipid synthesis to neutral lipid quantity (Guckert et al., in Johnson and Sprague 1987). Experiments involving pH indicated that manipulating pH and the inorganic carbon concentration increases neutral lipid accumulation in *Chlorella* at nonlimiting nitrate concentrations. These experiments provide evidence of a more universal mode of action of so-called neutral lipid "triggers" that can be exploited in the laboratory and perhaps outdoors. Various nutrient-deprivation regimes may all affect neutral lipid accumulation by disrupting the cell cycle.

To improve lipid yields in microalgae, we must understand the physiological and biochemical basis for partitioning photosynthetically fixed carbon dioxide into lipids. The rate of lipid synthesis and final lipid yield will depend on the availability of carbon for lipid synthesis and the actual levels and activities of the enzymes used for lipid synthesis. Conditions such as nitrogen deficiency that induce the accumulation of lipid by algae often drastically reduce the capacity for photosynthetic carbon dioxide fixation. Low lipid yields could result either from an absence of carbon skeletons or from low levels of enzymes. Improvements in lipid yield can be achieved only when the limiting factors have been determined.

Research efforts are continuing in order to determine the pathways of lipid biosynthesis in algal cells, especially in the cytoplasm, chloroplast, and mitochondrion. Each pathway possesses potential lipid triggers. Once the trigger is determined, it is expected that biochemical and genetic engineering techniques can be used to increase the lipid yield of promising algal strains.

Previous studies found that, in some algae, silicon deficiency induces an increase in the fraction of newly assimilated carbon that is partitioned into lipids and a decrease in the fraction that is partitioned into storage carbohydrate. Studies in FY 1987 (Roessler, in Johnson and Sprague 1987) focused on the enzymology of the carbohydrate and lipid biosynthesis enzymes. The storage carbohydrate is synthesized by two enzymes. One enzyme was not affected by silicon deficiency, but the activity of the other decreased 30%. Three enzymes present in the alga are involved in lipid synthesis. One of these

increased two-fold after silicon deficiency. The induction of the enzyme could be blocked by protein synthesis inhibition or gene transcription inhibitors. These results suggest that the increase in carbon allocation after silicon deficiency into lipids is probably due in part to reduced activity of the carbohydrate synthesis enzymes and increased activity of the lipid synthesis enzymes.

Since a commonly used lipid trigger, nitrogen deficiency, rapidly reduces photosynthetic capacity, it is useful to separate effects of nitrogen deficiency on photosynthetic efficiency from effects on carbon partitioning. Initial studies have therefore used two lipid-storing algae (Coleman et al., in Johnson and Sprague 1987). Lipid amount per cell increased during nitrogen deficiency and decreased with nitrogen addition. In two species, the increase in cellular lipid was not associated with an increase in lipid as a fraction of cellular dry weight. Nitrogen deficiency induces chlorophyll loss in all algae studied. The dependence in nitrogen-deficient cells of chlorophyll and chloroplast protein loss on high light intensities is consistent with photooxidative damage to the chloroplast. Based on these studies, photooxidative damage to the chloroplast may be a major factor limiting photosynthetic lipid yields in nitrogen-deficient cells.

3.1.4 Genetic Engineering

To date, no single microalgae strain has been found that exhibits environmental tolerance, high productivity, and high lipid yield. All three characteristics are necessary in one organism to meet program goals. For this reason, work has begun on developing genetic engineering methods so that by 1990, when the program has reduced its strains to the best 10-25, the methods to modify these organisms genetically will be available.

We are working in three areas of genetic engineering research: classical genetic manipulation methods, intraspecific genetic variability, and vector and protoplast fusion methodology. Each research area provides different parts of the total knowledge that we will need to genetically engineer a better organism.

It is likely that the long-term economic feasibility of using microalgae for fuel production will depend on the development of strains that have been genetically altered to improve lipid yields. The strategy to be used for developing improved strains depends on the patterns of genetic diversity found among the available wild-type strains in each species. Therefore, an investigation was undertaken of the genetic diversity in three types of microalgae with potential for oil production (Gallagher, in Johnson and Sprague 1987). Both gel electrophoresis and comparisons of the physiological traits were used. The former technique can be used to classify clones into discrete groups, and the banding patterns can be used as genetic markers in later manipulations. Examination of physiological traits yields information on continuous characteristics that are directly relevant to how different strains might perform in culture. In all three organisms, genetic diversity was found to be extremely high compared to terrestrial plants. This indicates that the genetic diversity in the species examined was underestimated.

During FY 1987, we also examined the genetic variability present within single species of microalgae (Johansen et al., in Johnson and Sprague 1987). Clones of a species sampled from different sites are often similar in their physiological response to conductivity, temperature, and nutrient stress. However, growth rates and lipid contents will vary significantly between clones, so that one or a few strains of a species can be designated as better candidates.

Intensive study has been directed toward establishing genetic diversity in *Chaetoceros muelleri* this year. This species was chosen for study because several clones have high

growth rates, broad salinity and temperature tolerances, and high lipid content. More than 200 clones of this species were isolated, and their genetic variability both within populations from a single site and between clones from widely separated sites was studied. Differences in allozyme banding patterns are evident even in clones isolated from a single collection. Correlations between morphological traits and physiological characteristics were noted.

The work on genetic diversity within single microalgal species has shown, at least among the species tested, that considerable genetic variability is present, even within clones isolated from the same sites. Such variability is desirable in that it indicates species have a large collective genome, and thus future genetic engineering efforts have a better chance of success.

The chloroplast genome of a model microalgal species was characterized (Meints, in Johnson and Sprague 1987) to determine whether these methods might be a diagnostic device for identifying specific *Chlorella* strains that are capable of biofuel production. If this were so, such genomes would be extremely useful for determining relationships between groups of algae and might determine the strategy for selecting partners in cell hybrid fusions. Unless a reasonably close relationship exists between fused partners, that stability of the hybrid is considered to be limited. Isolated chloroplast and nuclear genomic DNA from 13 strains of *Chlorella* were analyzed for relationships, and maps of the genomes were produced. Based on preliminary investigations it appears that algae can be easily grouped into specific classes according to their DNA restriction length polymorphisms; many or all algae could be analyzed in this manner, providing substantial information about algal relationships.

Two other genetic engineering methods being developed are fusing protoplast and finding suitable viral vectors for the algae (Meints, pers. comm). Protoplasts have been formed successfully from some algae cells using enzyme preparations. Work is continuing to regenerate the protoplasts to cell colonies. Viral vectors have been found, but none of them will attach to a free-living algal host. More than 250 algae were screened, but none of them were successful hosts for the viruses.

3.2 Engineering Design

Growth conditions in algae mass cultures can be divided into two categories: those dictated by the location of the culture, and those based on culture management strategy. Location-related variables include insolation, evaporation, rainfall, temperature, and wind velocity. Variables that can be managed include salinity, nutrient concentration, carbon dioxide concentration, culture mixing, culture aeration, and residence time of the population.

Growth conditions dictated by location will be among the prime considerations in siting the production facility. The DOE/SERI program has been based on the assumption that it will be necessary to locate a production facility in an area that receives large amounts of sunlight and has relatively warm temperatures. To have the best success, the facility must be located in an area that receives 5000 kcal m^{-2} d^{-1} and has more than 180 frost-free d yr^{-1}. This limits large-scale production to the southern United States (Figure 3-2).

Sunlight drives the production of biomass; therefore, ideal production systems will be located in areas that receive high insolation. It is also necessary to consider the trade-offs involved in achieving high insolation. If the costs of land, raw materials, or operation are significantly increased at a location with high insolation, siting solely by the solar input may be disadvantageous.

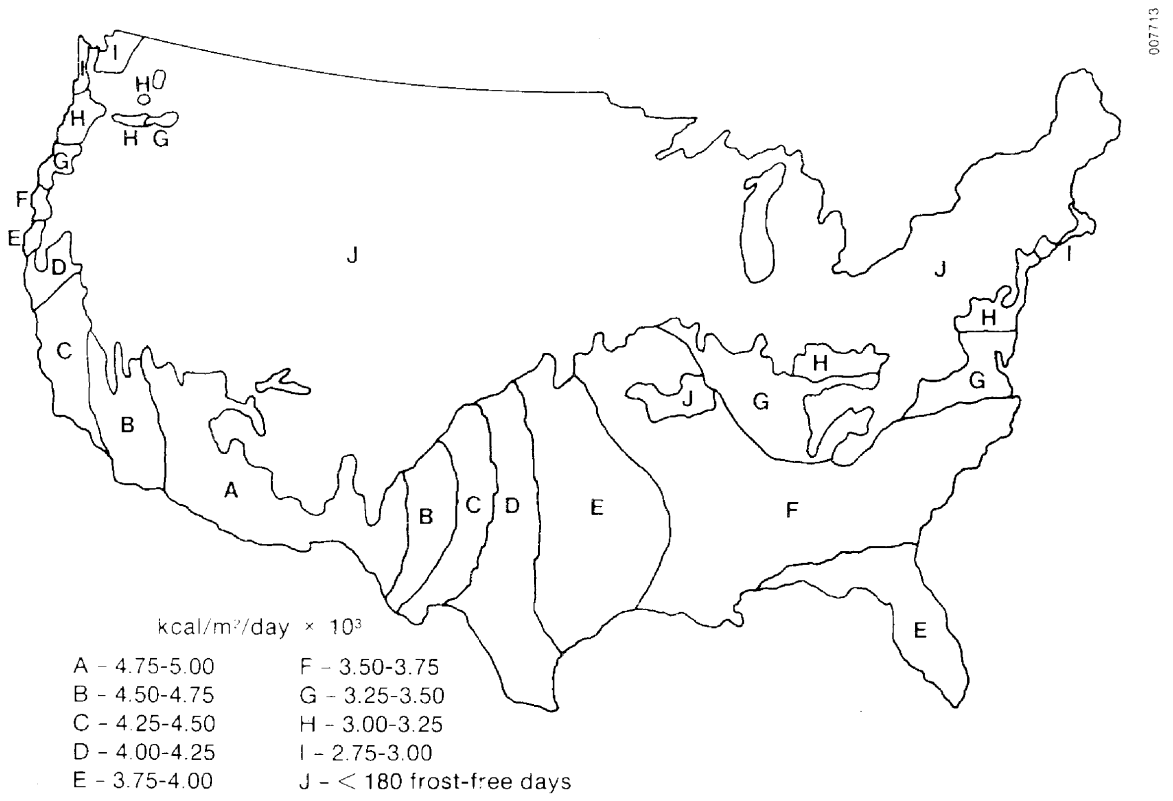

Figure 3-2. Solar Radiation in the United States Where There are More than 180 Frost-Free d yr^{-1}

Many other production conditions in outdoor systems are dictated by the engineering design and the management strategy. For example, if saline water is used, the salinity can be maintained at any given range governed by the algal species selected. Nutrient concentrations, carbon dioxide concentration, culture mixing, culture aeration, and culture turnover can also be selected.

The three designs examined in FY 1984 for the large-scale production of microalgae were open ponds, raceways, and enclosed tubes. The proposed costs for construction and operation of these three systems are shown in Table 3-3. Since all costs need to be kept to a minimum for the feedstock to be produced inexpensively, thereby producing an economic liquid fuel, open-pond systems were chosen as the facility plan for outdoor production (Figure 3-3).

Table 3-3. Capital Construction Costs for Three Different Algal Production Systems

Engineering Design	Cost ($/ha)
Open ponds	76,000
Raceways	161,000
Enclosed tubes	348,000

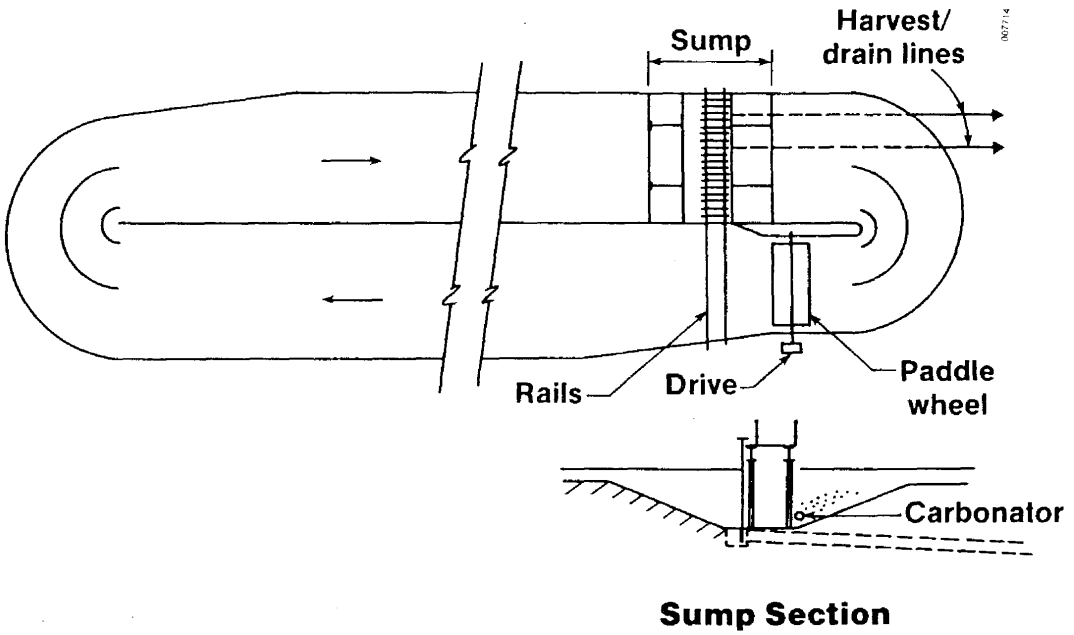

Figure 3-3. Open-Pond Design for Large-Scale Microalgae Production

Using the open-pond design, a microalgae outdoor test facility (OTF) is being constructed in Roswell, N. Mex., for the Aquatics Species Program. Microbial Products, Inc., of California is designing, constructing, and operating the facility (Weissman et al., in Johnson and Sprague 1987). The OTF will allow the program to evaluate outdoor production performance of microalgae and examine the problems and potential of scaling-up and operating large microalgae-production systems. This facility will allow the program to begin evaluations using saline groundwater in the desert Southwest.

During FY 1987 a small-scale system consisting of six 3-m^2 fiberglass open ponds was constructed and operated to evaluate the performance of algal species in terms of productivity and lipid content. The ease of operating and the low cost of constructing several of these small cultivation units makes them ideal for performing controlled, replicated experiments.

Since the ultimate goal of the Aquatic Species Program requires the production of large amounts of biomass, larger scale research systems are needed. Two larger open-pond systems, each 0.1 ha, were designed and are under construction. These will be used to compare the performance of low-cost earthen liners with the performance of expensive plastic membranes. Other scale-up problems will also be addressed in these and in a 0.5 ha demonstration open-pond system to be built in FY 1989 based on available funding. Biological and engineering assessments of microalgae production systems will be performed at the OTF. In addition, after several years of operation, a detailed economic analysis will be done to provide state-of-the-art economics for large-scale production.

Four 1.4-m^2 ponds were also constructed in the SERI greenhouse in FY 1987. The design for the ponds is similar to the OTF. The ponds are monitored for temperature, light intensity, and pH. These ponds will be used to supplement research at the OTF since many conditions can be controlled because the ponds are located indoors.

Navicula I was grown successfully in the greenhouse ponds for five weeks. The maximum production was 17 g m^{-2} d^{-1} AFDW. The ponds act as replicates with the exception of one pond, whose performance might be affected by its orientation to the sun. *Chaetoceros* is currently being grown in the ponds, and maximum production is about 10 g m^{-2} d^{-1}.

3.3 Harvesting

After the cells are grown, they must be separated from the water efficiently and economically. 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. Flocculation causes the cells to aggregate into larger clumps, which are more easily filtered or settle more rapidly. The ease of harvesting algae depends primarily on the organism's size, which determines how easily it can settle and be filtered. The most rapidly growing algal species are frequently very small and often motile unicells--the most difficult to harvest. Thus, it is necessary to maintain an effective interaction between the development of harvesting technologies and the selection of algal species for mass culture.

With current techniques and instrumentation, all microalgae can be harvested with polymers, although this is not economical. Polymer harvesting is technically feasible, but different algae need different polymers. The amount of polymer increases as the clarification requirement becomes more stringent, making it more cost effective not to require greater than 85% removal. With the most suitable polymers and appropriate application techniques, harvesting can be accomplished for polymer costs of 0.5¢-1.5¢ kg^{-1} dry mass, with removal efficiencies of 85%-95%. Currently, harvesting systems represent approximately 25% of the total capital investment in a microalgae mass culture facility. Polymers with higher rigid backbones are less affected by the salt concentration and are recommended as flocculants of microalgae in saline water.

Chemical flocculation aids harvesting regardless of the harvesting method used. Flocculant dose was reduced 75% by recycling the precipitant following flocculation back into the mixing-flocculation chamber. Using three flocculation cycles reduced the required chemicals and removed 90% of the microalgae from the water. By reducing the chemical dose the process is more economical.

3.4 Conversion

Analysis of fuel conversion options for microalgae biomass has demonstrated that the promise of microalgae for fuel production is best realized by using conversion processes based on cellular lipids. The two most promising fuel conversion options are transesterification to produce fuels similar to diesel fuels and catalytic conversion to produce gasoline. Although microalgae lipids represent the premium energy product, the energy trapped in the other biomass constituents can also be used; e.g., the cell residue after lipid extraction can be anaerobically digested for the production of methane and carbon dioxide.

We do not believe that the algal lipids can be used directly as a fuel either alone or blended with crude petroleum. Algal lipids contain approximately 10% oxygen; crude petroleum contains essentially no oxygen. The oxygenates would react at the high temperatures used in crude distillation and cause polymerization or other undesirable reactions.

Research is just beginning on the processes to extract and convert algal lipids into gasoline and diesel fuels. The first step in the process is the conversion of triglycerides into free fatty acids for transesterification. A naturally occurring enzymatic process within the algae, which accomplishes this conversion, was investigated. Promising species of lipid-producing microalgae were screened for the presence of this enzymatic activity. A regression model was developed to predict the effects of important variables on this process. Results indicate that silica concentration and temperature are important variables that control the conversion of triglycerides into free fatty acids. If the algae are left for 16-24 h at 20°C, more than one-half of the triglycerides are converted to fatty acids. This step may be an economical method since no chemicals are required.

4.0 FUTURE ACTIVITIES

Since the inception of the program, many improvements have been made in finding suitable species to produce fuels from microalgae. More than 3000 strains of microalgae were collected, and the collection program was terminated in FY 1986. A major effort between now and FY 1990 will be screening and characterizing these 3000 strains and reducing them to the best 10-25 strains. Research in strain improvement, lipid biochemistry, and genetic engineering will continue in FY 1988.

By the beginning of FY 1988, two 0.1-ha ponds will be constructed at the OTF. In FY 1989 a 0.5-ha pond will be built based on available funding. The OTF research for next year is to determine the performance of inexpensive earthen liners versus expensive plastic liners. An entire year of productivity data will give the first estimates of average production from these systems in the Southwest.

New harvesting and conversion research projects will begin in the first quarter of FY 1988. Innovative and improved methods for microalgae harvesting will be examined. In addition, research will begin to examine extraction and conversion methods to produce gasoline and diesel fuels from microalgae. Attention will be directed toward the identification of techniques by which these lipids can be extracted on a large scale and a detailed description of the characteristics of these lipids as they relate to their suitability as feedstocks for fuel conversion processes. Ultimately, conversion processes specifically tailored to the characteristics of microalgal lipids must be developed, either through the optimization of existing techniques or through the development of innovative conversion technologies. Such research activities require the production of algal biomass samples on a scale suitable for extraction and fuels characterization and will be obtained from the OTF. Samples from a number of promising species should be included since there are strong indications that the characteristics of lipids vary widely between taxa.

A major emphasis next year will be placed on assessing the resource requirements and environmental impacts of this technology. Assessment projects will be done on carbon dioxide supply, disposal of the brine generated from the algal ponds, and any possible impacts on climate from the ponds.

The net result of the research to date has been to reduce the projected price of gasoline derived from microalgae from \$18 gal^{-1} in 1983 to approximately \$7 gal^{-1} in 1987. However, many more developments are needed in the technology in the upcoming years to reduce the price of gasoline from microalgae to be competitive with fossil fuels by 2010.

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